

**SYNTHESIS AND THERMO-MECHANICAL CHARACTERIZATION OF
BARE AND COMPOSITE CHITOSAN FILMS**

by
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Abstract

In this present work, chitosan thin films were synthesized via two synthesis techniques: sol casting and spin coating. Subsequently, silver nanoparticles or carbon black particles were incorporated into the polymeric matrix at various loadings. Produced films were characterized by optical microscopy and profilometry to investigate the morphological properties of the films; Fourier transform infrared spectroscopy and X-ray diffraction to confirm the presence of chitosan in all films; and UV-vis to confirm the presence of the silver nanoparticles. In addition, thermal degradation and thermo mechanical analyses were conducted to establish the temperature range in which produced composites can be used. The average degradation temperature and the average glass transition temperature were determined to be 292 ± 5 °C and 82 ± 8 °C, respectively. Therefore these films may be used at temperatures up to 70 °C without any significant change in mechanical properties, and at temperatures up to 280 °C before experiencing degradation.

Resumen

Para este trabajo se sintetizaron películas delgadas de quitosán a través de dos técnicas: “sol casting” y “spin coating”. Luego de ello se procedió a optimizar su producción y a éstas se les añadió una matriz polimérica de nanopartículas de plata o de “carbon black” a diferentes concentraciones. Con el fin de caracterizar y estudiar las propiedades morfológicas de estas películas se utilizó microscopía óptica y perfilometría, espectroscopia infrarroja de transformada de Fourier y difracción de rayos X. Con esta última medición se corroboró la presencia de quitosán en todas las películas; y con espectroscopia de luz ultravioleta se confirmó la presencia de nanopartículas de plata. Adicionalmente, se hizo un análisis de degradación térmica y un análisis termo-mecánico a las películas para establecer el rango de temperatura en la cual estas podrían ser usadas. El valor encontrado de la temperatura promedio de degradación y de transición vítrea fue de 292 ± 5 °C and 82 ± 8 °C, respectivamente. Esto permite concluir que se pueden trabajar en estas películas a temperaturas inferiores a 70 °C sin que haya ningún cambio significativo en las propiedades mecánicas y que se puede llegar hasta 280 °C sin efectos de degradación.

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1. INTRODUCTION

Derived from chitin which is widely available in nature, chitosan is a non-toxic, inexpensive, biodegradable, and antibacterial material that can be synthesized into beads, films, gels, nanofibers, or nanoparticles. As this biopolymer is both multifunctional and cost-effective, it has moved to the forefront of research in environmental, biomedical, food and cosmetic applications. Environmental applications include adsorption of heavy metals from water and water filtration. Biomedical applications include biosensors, drug delivery, tissue engineering, medicines for hypertension and cholesterol, and wound dressing. However, it must be noted that the processing costs of chitosan for biomedical applications are higher due to the high purity required for these applications [1]. Food processing and agricultural applications include fertilizer, animal food additives, and edible coatings for fruits, vegetables, and seeds. One such edible coating, Nutri-save, was marketed commercially to reduce food spoilage as early as 1989. [2] Cosmetic applications include lotions and textiles. To meet the industrial demand generated by these applications, more than a dozen companies produce chitosan commercially. [3]

1.1. Motivation

1.1.1 Use of chitosan for heavy metal ions removal from water

Industrial processes around the world release toxic byproducts such as arsenic, vanadium, mercury, and chromium into the environment. These heavy metals seep into the ground water where they are ingested by plant and fish life, subsequently making their way into the food chain. Biomaterials with adsorption properties such as chitosan are an environmentally friendly and economical way to remove such substances from aqueous solutions. Porous beads and membranes possess high permeability, large surface area, chemical affinity with target pollutants,

and functionalization, making them good candidates to be considered as adsorbents in water cleaning operations.

Chitosan based adsorbents are among the most promising of these adsorbents due to their low cost and functional groups. Since chitosan is derived from chitin, the second most common polysaccharide on earth, it is both readily available and low cost. Chitosan is especially good for chelation of metals because of its hydrophilicity from the hydroxyl groups, the presence of highly reactive functional groups (NH_2 and OH), and the flexible structure of the polymer chain.

[4] In slightly acidic solutions, the amine group becomes cationic ($\text{R-NH}_2 + \text{H}_2\text{O} \rightarrow \text{R-NH}_3^+ + \text{OH}^-$), allowing chitosan to bond with negatively charged toxic metal ions including silver, chromium, vanadium, arsenic, and lead.

Once chitosan is synthesized into films or beads, the adsorption of heavy metals is measured and compared to the Langmuir model or the Freundlich model. The initial pH of the solution and the concentration of the solution are generally important factors in the maximum adsorption. However, the form and mechanical properties of the adsorbent also plays a large role in the process because an impermeable filter causes a large pressure drop, making the system less efficient.

In this study, both bare chitosan films and composite chitosan films containing carbon black, another potential adsorbent, will be synthesized. The mechanical properties of these composites are important when considered for sorption applications since they must not disintegrate when removing contaminants from wastewater. So far, there is no a systematic study on how the thermomechanical properties of the carbon-black bearing chitosan films may affect their structure and hence their adsorption capability.

1.1.2 Use of chitosan-based films in biomedicine

Due to their biocompatibility and mechanical properties, chitin and chitosan have also garnered wide-spread interest in biomedicine, especially in tissue engineering, drug delivery, cancer diagnosis, and wound dressing. Chitosan may be synthesized into nanofibers which may be used as scaffolds to cultivate cells since their form mimics that of the body's natural extracellular matrix. These biodegradable nanofibers can be loaded with drugs and ingested into the human body for controlled drug release. The form and morphology of the fibers allows control over the speed of the drug release. [5] Chitin and chitosan may also be synthesized into quantum dots, which emit light and permit cancer diagnosis via fluorescent microscopy. Chitosan is also suited to wound dressing because the polymers exhibit antibacterial and coagulative properties; it has been used to make bandages by the US armed services to stop bleeding in wounds since the functional group NH_2 acts as a coagulator and stops the flow of blood. [6]

In this study, both bare chitosan films and composite chitosan films containing silver nanoparticles, an antibacterial agent, will be synthesized. The thermal properties of these nanocomposites are of vital importance in potential bactericidal applications such as food protection and medical products, since the films may be treated at elevated temperatures for sterilization or processing. However, the thermomechanical properties of these composites have not been fully researched. [7]

An analysis of the thermo-mechanical behavior of chitosan films and chitosan-based nanocomposites will lend insight into the physical stability, chemical properties, and the durability when in use. The thermal properties allow us to predict the range of temperatures in which the polymer can be used as well as provide additional insights related to the polymer structures. Based on the above considerations, the main contributions of this research are to

provide a systematic evaluation of the synthesis parameters for bare and composite chitosan thin films, and an assessment of the structural, physical and chemical stability of these synthesized materials.

1.2. Objectives

1.2.1. Main

Determine the relationship between synthesis conditions and properties of pure chitosan and chitosan-based nanocomposite films.

1.2.2. Specific

- Determine the optimum conditions for synthesis of chitosan films.
- Develop an optimum protocol to disperse a nanometric phase in the chitosan matrix.
- Determine the effect of the type and concentration of the disperse phase (silver nanoparticles or carbon black) on the structural and functional properties of both the bare and composite films.

2. THEORETICAL BACKGROUND

2.1. The matrix: Chitosan

2.1.1. Origin

Chitin was first called “fungine” by Braconnot when he discovered it in fungi in 1811. When this same substance was later isolated in the tough exterior shell of May beetles, the name “chitin” was derived from the Greek word “chiton” which means “coat of mail”. [8] Chitosan, its derivative, was identified in 1859 by Rought. [4]

Chitin is the most abundant in nature second only to cellulose. Just as cellulose forms the structure of plant life, chitin forms the structure of many animal shells including lobsters (Figure 1), crabs, shrimp, krill, squid, butterflies, and some fungi. [5] Annual production of chitin in 2000 was estimated at 2,000 metric tonnes, with shrimp and prawn being the principal sources. [9]

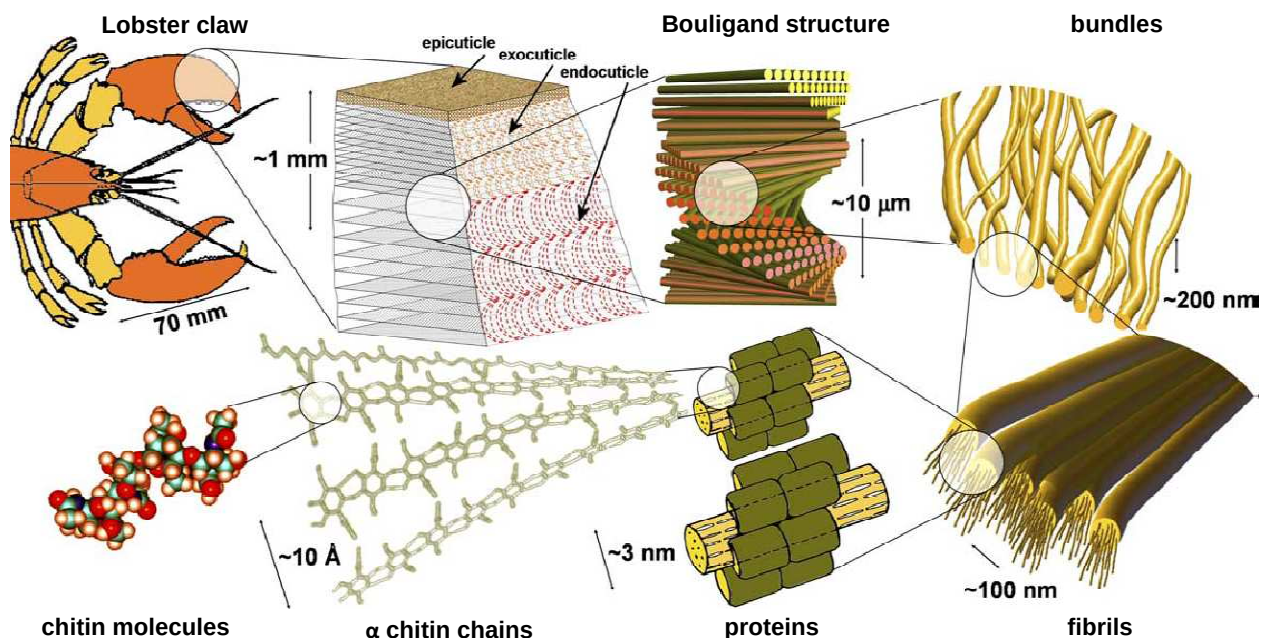


Figure 1 Origin of chitin from lobster [10].

Insects such as the Blue *Morpho* butterfly, green *Calloodes grayanus* beetle, and gold *Anoplognathus parvulus* beetle also owe their intense coloration to the chitin layers that make up their shells (Figure 2). [11]

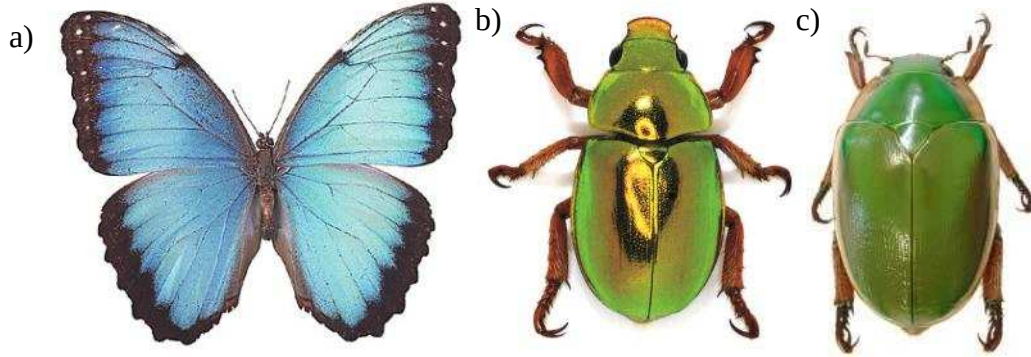


Figure 2 (a) *Morpho* butterfly [12], (b) *Anoplognathus parvulus* beetle [13] and (c) *Calloodes grayanus* beetle [14].

The crystalline ordering of chitin and the composite structure that chitin forms with proteins and CaCO_3 gives these exoskeletons their strength. The mechanical properties of dry chitin taken from various sources are tabulated in Table 1.

Source	UTS (MPa)	E (MPa)	ϵ (%)
Crab	36	1095	3.4
Prawn	21	1220	1.8
Beetle	80	2900	0.6

Table 1 Mechanical properties of chitin from various sources [15–17].

Chitin is formed of *N*-acetyl-D-glucosamine units linked by β - D-(1→4) bonds. Due to the hydrogen bonds between the polymer chains, chitin is a highly crystalline. To derive chitin from crustaceans according to the standard method, the shells are washed and crushed, demineralized with HCl, deproteinised with NaOH, and decolorized with KMnO_4 . On the other hand, the derivation of chitin from fungi, involved the washing, drying and crushing of the chitin and subsequent treatment with NaOH for deproteinisation. Finally, the chitin is extracted with LiCl

and DMAc and precipitated it in water. [1] Chitosan can then be easily formed by washing chitin in heated NaOH (40-50%). This alkaline treatment removes acetyl groups from the chain and reduces the molecular weight by breaking the bonds of the main chain via hydrolysis. [18] Removing the acetyl groups causes the polymer to be soluble in solutions below pH 6.3. A direct effect breaking the bonds between the carbon rings is that the molecular weight of chitosan (10,000 – 1,000,000 Daltons) is much smaller than that of chitin (1,000,000 – 2,500,000 Daltons). Generally, low molecular weight chitosan has a MW less than 50,000 Daltons, medium molecular weight chitosan has a MW between 50,000 and 150,000 Daltons, and high molecular weight chitosan has a MW greater than 150,000 Daltons.

2.1.2. Chemical structure

Since both chitin and chitosan are copolymers of acetylated and deacetylated groups, the percent of acetyl groups and the molecular weight are used to distinguish between the polymers. As seen in Figure 3, the polymer is loosely defined as chitin if more than 50% of the carbon rings have the acetyl group and as chitosan if less than 50% have the acetyl group. In most cases, the degree of deacetylation (DD) of chitin is around 15% and that of chitosan around 80%. The degree of deacetylation of the chitosan used in this study was certified to be 82% by the manufacturer.

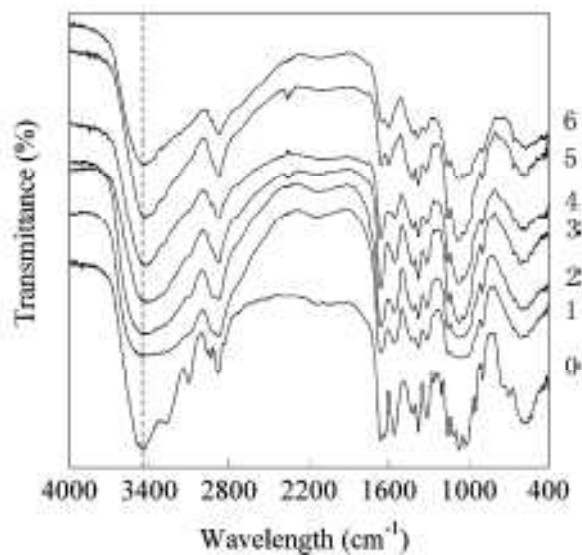


Figure 4 FTIR spectra of chitosan powder with increasing levels of deacetylation (sample 0 corresponds to chitin and sample 6 corresponds to chitosan) [21].

Wavelength (cm ⁻¹)	Absorption band	Comments
897, 1160	Antisymmetric C-O-C stretching of glycosidic link	
1030, 1070	C-O-C stretching of skeleton	
1420	-CH ₂ bending	Intensity changes with crystallinity
1550 - 1560	Amide II group (NH ₂) -NH bending	Disappears with crosslinking
1655	Amide I group (COCH ₃) Intermolecular and intramolecular hydrogen bonding of C=O with -NH and OH-6 groups Indicates in plane -NH bending, C=O stretching vibrations, CN stretching modes	Chitin
2865-1880	C-H stretching of glucose ring CH ₂ OH group CH ₃ in acetyl group	The C-H group intensity changes with degree of acetylation
3270, 3450	-OH stretching Hydrogen bonding Water interference	
3300	-NH peak	

Table 2 FTIR main bands of chitosan [22].

2.1.3. Crystalline structure

Chitosan owes its semi-crystalline structure to hydrogen bonds. In annealed chitosan, weak hydrogen bonds exist between the oxygen in the hydroxyl group (OH) and the oxygen in the hydroxymethyl group (CH₂-OH) and between the oxygen in the hydroxyl group and the oxygen in the carbon ring (C-O-C). These chains are further bonded together into sheets by direct hydrogen bonds between the nitrogen of the amine group and the oxygen of the hydroxymethyl group. The chitosan chains arrange themselves in an antiparallel fashion according to the space group is P2₁2₁2₁ as shown in Figure 5. [23]

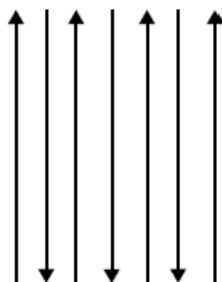


Figure 5 Antiparallel arrangement of chitosan chains [23].

Chitosan exhibits peaks at 10.7° and 20.2° (Figure 6a), although these peaks shift slightly with the degree of deacetylation. [21] It possesses a orthorhombic unit cell with the parameters or a = 0.867 nm, b = 0.892 nm, and c = 1.024 nm as shown in Figure 6b. [24]

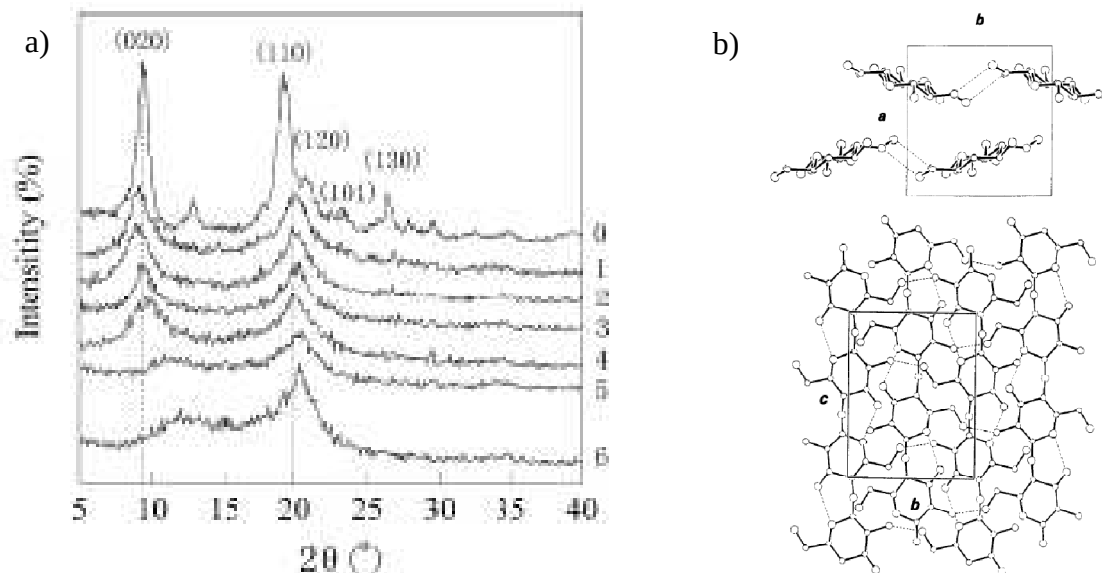


Figure 6 XRD spectra of chitosan powder with increasing levels of deacetylation (sample 0 corresponds to chitin and sample 6 corresponds to chitosan) [21], (a), and corresponding chitosan structure [24], (b).

2.1.4. Thermal properties

At the glass transition temperature, the mechanical properties of a substance change. The glass transition temperature of chitosan is under some debate as may be seen in Table 3.

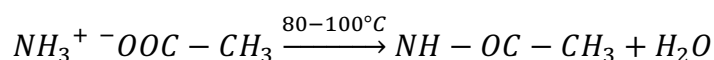
T_g (°C)	Method	Chitosan Morphology	Year	Reference
203	DMA, DSC	Film	2000	[25]
94	DMA	Film	2002	[26]
153	DMA	Film	2004	[27]
130 – 140	DSC	Powder	2004	[28]
196	DSC	Film	2005	[29]
85	DMA, DSC	Film	2007	[30]
170	DSC	Film	2010	[31]

Table 3 Reported T_g temperatures for chitosan.

The T_g may vary up to 20°C due to the film preparation (the acid used in preparation and the neutralization), properties of the chitosan (crystallinity, moisture content, and degree of deacetylation) [29], [26] and the characterization method used (DSC, DMA, or TMA) [32]. In

chitosan, the T_g has been attributed to the torsional oscillations at the glycosidic bonds between the two glucosamine rings and a corresponding change in the hydrogen bonding. [30]

Quijada-Garrido determined the degradation stages of chitosan via a thermogravimetric analyzer connected to a mass spectrometer. The water lost in the first step may be water bound to the polymer via hydrogen bonding or water liberated by the formation of an acetyl amine group from the amine group of chitosan and the acetyl group from the acetic acid as seen in the following equation [30]:



Temperature (°C)	Chemical released	% Weight change
125	Water	15%
190	Methane, ammonium	25%
260	Decomposition of main chain	40%
360	Formation of residue by remaining carbon	
525	Formation of methane	

Table 4 Stages of thermal degradation of chitosan [30], [33].

2.1.5. Applications

2.1.5.1. Arsenic Adsorption

Arsenic is released into the environment via natural sources such as volcanoes and geothermal wells and via human sources such as pesticides, mining, and manufacturing of pharmaceuticals and semiconductors. Arsenic pollution has been reported in USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, New Zealand, Japan and India. The World Health Organization (WHO) provisional limit is 10 ppb (0.01 mg/L), but countries including Bangladesh and China maintained the older limits of 50 ppb (0.05 mg/L). [34]

It takes two forms: trivalent arsenite and pentavalent arsenate. Of the two, arsenite is more soluble, mobile, and toxic to human health, having been shown to attack the liver, skin, lungs, and other internal organs. Fungi, yeasts, and bacteria can produce toxic forms of arsenate from

those available in the soil. [35] Pentavalent (+5) or arsenate species are AsO_4^{3-} , HAsO_4^{2-} , H_2AsO_4^- while trivalent (+3) arsenites include $\text{As}(\text{OH})_3$, $\text{As}(\text{OH})_4^-$, $\text{AsO}_2\text{OH}^{2-}$ and AsO_3^{3-} . As(V) predominates at pH less than 6.9 [34], while in the pH range 4 – 9, the predominate form of arsenate (As V) is H_2AsO_4^- . In contrast, the predominate species of arsenite (As III) at the same pH is H_3AsO_3^0 , which does not have a charge and cannot interact with the protonated amine group NH_3^+ . [36]

Chitosan beads may be used to remove both species of arsenic from water; at a pH of 5, the reported capacities were removed 1.83 mg As(III) / g chitosan beads and 1.94 mg As(V) / g chitosan beads. In the case of As(V), this sorption capacity may be due to the electrostatic interaction between the negative As(V) ion and the positive charge on the ion group of chitosan NH_3^+ . Hydrogen bonding or Van der Waal's force may explain the quantity of As(III) that is adsorbed by the chitosan. [37]

2.1.5.2. Bactericidity of chitosan

Bacteria may be classified as gram-positive or gram-negative. As the composition of their bacteria wall is different, a bactericidal material may have a different response depending on the type of the bacteria. The wall of a gram-positive bacteria contains a large amount of peptidoglycan and small amounts of protein. In contrast, the wall of a gram-negative bacteria contains various polysaccharides, proteins and lipids (including peptidoglycan). [38]

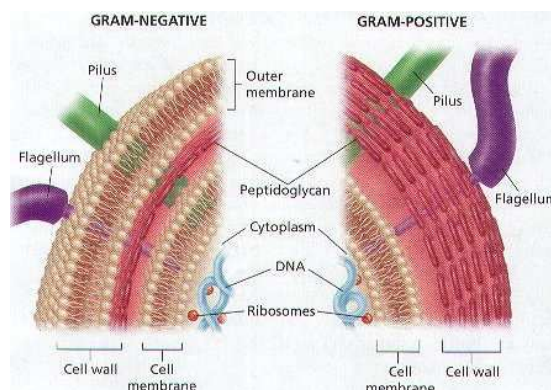


Figure 7 Gram negative and gram positive bacteria [39].

The antibacterial properties of chitosan were discovered in the 1980s and 1990s. Studies have shown it to be bactericidal (kills the bacteria) or bacteriostatic (stops the growth of the bacteria) against both gram positive bacteria and gram negative bacteria.[40] The minimum inhibitory concentration (or MIC) is the lowest concentration that will visibly inhibit the growth of chitosan. Values determined by various research groups are shown in Table 5. While the values may not be comparable between research groups due to non-standardized procedures, the MIC is a good indicator of the bactericidity.

Bacteria	MIC (ppm)	Reference (from paper)
<i>Escherichia coli</i>	20	[7]
	100	[50]
	468	[60]
	650	[49]
	1000	[31,61,62]
<i>Pseudomonas aeruginosa</i>	>200	[50]
	1700	[61]
<i>Staphylococcus aureus</i>	20	[7]
	100	[19]
	>800	[44]
	700	[61]
	>1250	[49]

Table 5 MIC of chitosan [40].

These antibacterial properties of chitosan have been attributed to three mechanisms. The first, and most widely accepted, states that the positively charged amine groups of chitosan interact with the negatively charged outer membrane of the bacteria, destabilizing it and altering its permeability. However, at high concentrations the chitosan chains may surround the bacteria instead of interacting electrostatically with its cell wall. The second asserts that chitosan chelates heavy metals and enzymes necessary to bacteria growth. The third affirms that the chitosan chain penetrates the cell and interferes with the DNA reproduction process. [38][40]

The bactericidity depends on the concentration and pH of the solution and the degree of deacetylation of chitosan. Below its pKa value (6.3), the chitosan disassociates into its ions, allowing electrostatic interaction with the bacterial wall. Therefore, with few exceptions, chitosan is not antibacterial at a neutral pH and its bactericidity increases at lower pHs. [41] Increasing the degree of deacetylation increases the bactericidity, since the number of positive groups increases and the electrostatic interactions with the bacteria increase.

2.2. The disperse nano-phase

2.2.1. Silver

Medicinal properties have been attributed to silver since around 400 BC, when Hippocrates wrote that silver had beneficial healing and anti-disease properties. The antibacterial properties of chitosan were discovered in the late 19th century. In 1884, German obstetrician Carl Crede noticed that treating newborns with eyedrops containing 2% silver nitrate prevented blindness due to bacteria conjunctivitis, which is contracted during delivery when newborns come into contact with sexually transmitted diseases in the birth canal. In 1893, Karl von Nageli also observed that bacteria died after 4 minutes of contact with diluted silver nitrate. [42] Currently, silver is used as an antibacterial agent in a wide variety of medical products. Commercial

products such as Acticoat, Bactigrass, Fucidin, SilvaSorb, SILVERCELL, AQUACEL, and PolyMem have been approved by the US Food and Drug Administration or European Food Safety Authority. [7]

On a nanoscale, silver exhibits enhanced bactericidity. Silver nanoparticles are usually produced using a salt precursor, a reducing agent, and a stabilizing polymer, however they can also be produced via γ -irradiation, microwave, and UV irradiation. Silver nitrate may be reduced to silver metal particles by irradiation with UV-light in the presence of a polymer such as PVP or HEPES.[43],[44] They may also be reduced by natural light in the presence of a polymer such as a SMA-POE copolymer.[45]

The color of the silver nanoparticles gives an indication of their size. This phenomenon is seen in Figure 8, in which a light yellow solution containing stable silver nanoparticles was achieved by reducing silver nitrate with boron hydroxide. However, further stirring causes the solution to turn violet and then gray in color, signifying that the nanoparticles have agglomerated and will settle out of the solution. [46] In this work, all of the solutions were violet, indicating agglomeration of particles.

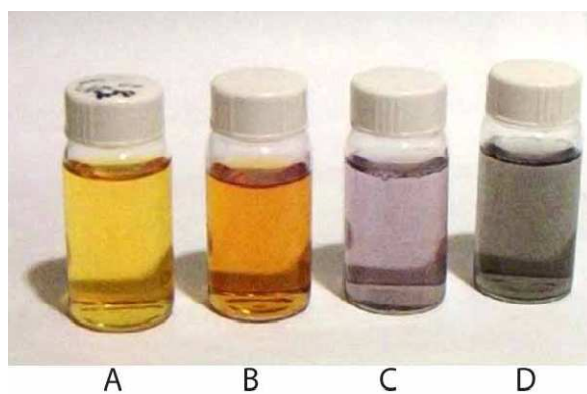


Figure 8 Change in color with crystal size of silver nanoparticles in (A) clear yellow, (B) dark yellow, (C) violet and (D) gray solution [46].

The size of the silver particle may be determined from the UV-vis spectra. For example, the clear yellow sol from Figure 8 exhibits the narrow plasmon centered on 400 nm as shown in Figure 9a; this plasmon corresponds to a particle size of 10-14 nm as tabulated in Table 6. In contrast, the violet solution from Figure 8 exhibits a plasmon with asymmetric broadening toward larger wavelengths as shown in Figure 9b, corresponding to a particle size of >80 nm.

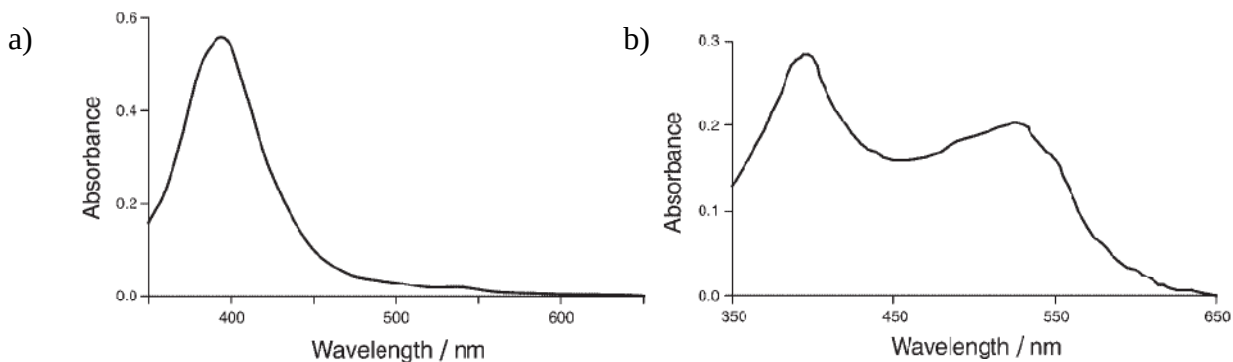


Figure 9 UV vis of clear yellow solution, (a), and violet solution, (b), shown in Figure 8 [46].

Particle Size (nm)	λ_{max} (nm)
10-14	395-405
35-50	420
60-80	438

Table 6 Particle size as determined by UV-vis [46].

The previous example illustrates the fact that a red shift of the UV-vis plasmon indicates particle growth. Similarly, a narrow plasmon indicates monodisperse Ag nanoparticles, whereas an asymmetric broadening toward larger wavelengths indicates particle agglomeration.

The size of the silver particle is very important to the application because the bactericidity increases when the silver particles are synthesized on a nanoscale. One proposed mechanism of bactericidity asserts that these particles attach to thiol-containing proteins in the bacterial cell wall. This would allow proteins and other intercellular constituents to leak out of the cell. Some studies show that the particles do not have a direct affect on anything inside the cell. [47], [26]

The silver nanoparticles may disturb the electrical gradient when they attach to the bacteria wall; they may also adsorb oxygen, limiting bacterial growth. [48]

Chitosan is non-toxic, biodegradable, has commercial applications in food protection [2] and bandages [6], and has been reported to be antibacterial. Therefore, a chitosan composite bearing silver nanoparticles with enhanced antibacterial properties is very likely to have applications in food protection and medical devices.

Furthermore, chitosan may be used both as a stabilizing polymer and a reducing agent for silver nanoparticles, avoiding the introduction of potentially toxic chemicals during the synthesis process. [49] When chitosan is used as the reducing agent with the salt AgNO_3 , the Ag^+ ions may be reduced by the $-\text{OH}$ group or the NH_2 . [7], [50], [51] In the case that silver particles are formed in the presence chitosan, an increase in reaction temperature or time increases the intensity of the UV-vis plasmon (Figure 10), indicating an increase in the number of particles. [7], [48], [50], [51] A base may also be added to the solution in order to reduce the silver nanoparticles. [47] None of the reports that used chitosan to reduce the silver nanoparticles indicated that light had an effect on the reduction of the particles.

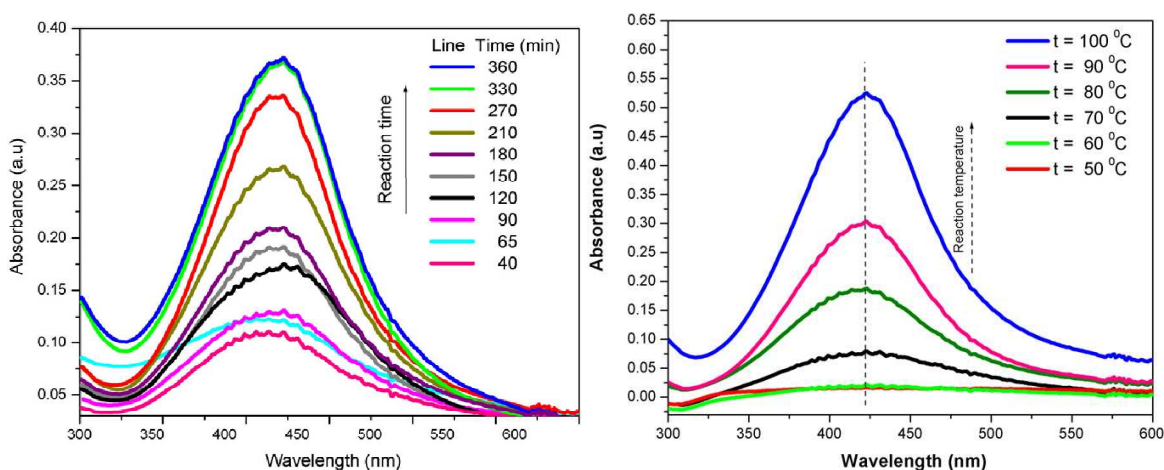


Figure 10 UV-vis spectra of silver nanoparticles reduced by chitosan as a function of a) time (temperature = 80°C) and b) temperature (time = 6 hours). In both cases $[Ag^+] = 3.33 \text{ mM}$, $[CS] = 0.33 \text{ mg/ml}$) [51].

Several research groups have reported the plasmon for silver nanoparticles reduced in a chitosan solution between 400 and 420 nm; these nanoparticles ranged from 10 to 13 nm in diameter [51],[50]. However, the plasmon for silver nanoparticles has also been reported at higher wavelengths (440-445 nm) [52],[53].

The thermal properties of these nanocomposites are of vital importance in potential bactericidal applications such as food protection and medical products, since the films may be treated at elevated temperatures for sterilization or processing. While both the degradation temperature and mechanical properties of chitosan films containing silver nanoparticles have been tested [7], the glass transition temperature has not been determined.

2.2.2. Carbon Black

Formed by the incomplete combustion of organic materials, carbon black was first used as an ink pigment in 300 A.D. However, since 1900 its most common use has been to reinforce rubber; in 2004, 5 million metric tons were produced for the tire market alone.

As may be seen in Figure 11, the smallest discrete of carbon black is an *aggregate* which is made up of nearly spherical *particles* bound together. Particle diameters may range from 20 nm to several hundred nm; aggregate diameters may range from 100 nm to several micrometers. These aggregates may become physically entangled to form *agglomerates* which range from 1 μm to several millimeters in diameter. To facilitate handling, the carbon black manufacturing process forms spherical agglomerates known as *pellets* which range from 0.1 mm to 3 mm in diameter.

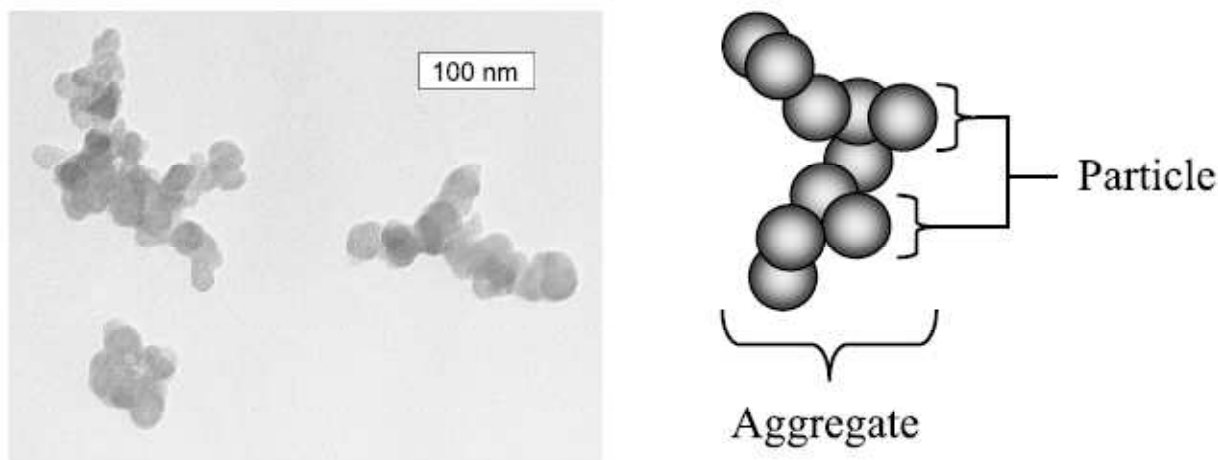


Figure 11 Transmission electron microscopy, (a), and schematic of carbon black [54], (b).

Carbon black manufacturing consists of reaction, filtration, pelletizing, and drying. Due to the processing and the raw materials employed, carbon black contains such elemental impurities as hydrogen (0.2 – 0.4%), oxygen (0.2 – 0.5%) and sulfur (1-2%) and other contaminants such as moisture, ash, and oily residues. In the furnace process, carbon black is produced from hydrocarbons (usually residual oils from refineries) at high pressures and temperatures between 2500 and 3400°C in an oxygen-depleted atmosphere. After “cracking”, or breaking of the hydrogen-carbon bonds, the carbon black is quenched to less than 1500°C and subsequently filtered in cloth bags to separate it from the gas byproducts. Since this carbon black is a very fine, lightweight powder that is difficult for customers to manage, it is formed into pellets by mixing in water and a pelleting aid. Finally, the pellets are dried in order to reduce the water content.

The mechanical properties of carbon black-polymer composites depend primarily upon the carbon black loading, the specific surface area, and the structure, or measure of the complexity in shape of the carbon black aggregates. Properties such as blackness, stiffness, and wear

resistance, hardness, viscosity, stress at high strain increase proportionally with an increase in these parameters.

The dispersion of the phase also increases the mechanical properties and may be differentiated into microdispersion (nm to μm) and macrodispersion (μm to mm). Microdispersion affects the interaction between the polymer and the carbon black and the formation of a network between the carbon black particles via van der Waals forces, while macrodispersion affects the appearance and the mechanical failure properties. Although the van der Waals forces between the carbon black particles increase the strength of the composite, they also increase the difficulty of dispersion.

Carbon black is designated by four characters, where the first is a letter indicating the curing rate (N or S), the second indicates the surface area, and the last two are arbitrarily assigned. N330, the carbon black used in this work, is one of the most common in the tire and mechanical rubber goods industries because of its good abrasion resistance, easy processing, and relatively good tensile and tear properties.

Carbon black may be crystalline (sp^2 hybridized) or amorphous (sp^3 hybridized); the crystalline carbon is composed of “three or four graphitic planes in a turbostratic arrangement with an interplanar distance slightly greater than that of graphite” [54]. In the XRD spectra, carbon black exhibits a slight peak at 24° corresponding to the (002) plane of the graphite structure.[50] [56]

Adsorption studies have identified heterogeneity in the surface energy of the carbon black, with the crystallite edges being the most active sites, followed by the amorphous region, and finally the planar surface. While carbon black modified with nitric and sulfuric acid removes heavy metals such as As(V), Cu(II) and Cd(II) from water, the literature does not suggest that as-received carbon black has an effect [55], [56], [57]. However, bare carbon black has been shown

to remove volatile organic compounds such as toluene and xylene [58], non-steroidal anti-inflammatory drugs such as naproxen and ketoprofen [59], and hormones such as progesterone [60]. Since chitosan also reportedly possesses adsorption properties, carbon black was chosen as a disperse phase to enhance the matrix response for absorption applications.

2.3. Literature Review: Synthesis and Applications of Chitosan Structures

As before mentioned, chitosan may be formed into beads, films, gels, sponges, nanofibers, and nanoparticles. This study will be focused on films because they are optimal for antibacterial coverings in medicinal and food applications. Chitosan films may also have applications in water filtering since they are hydrophilic and can be semi-permeable to gases such as O₂, CO₂ and water vapor; the permeability of chitosan films increases when they are wet. [61]

Among the various techniques available to synthesize chitosan thin films, spin coating and sol casting are among the most popular. In spite of this, a review of the literature only yielded one study that compared the two techniques; that study investigated the structural, antibacterial, and metal chelating properties of the films. The sol cast films were more crystalline than spin coated films, which may be attributed to the longer drying time of the sol casting films. [62]

Of the two techniques, sol casting permits greater scalability provides more reproducibility in film thickness, requires less precursor solution per film, and is less time consuming. However, spin coating provides greater control over the porosity of the films and lends itself to characterization techniques which require the film to be on a substrate such as profilometry, AFM and nanoindentation.

While various studies have investigated the thermomechanical properties of bare chitosan sol cast films, there is still debate over the glass transition temperature as explained in Section 2.1.4. Furthermore, none of these studies determined the glass transition temperature using the

technique thermomechanical analysis (TMA). The thermomechanical properties of the chitosan composites require further study as well; the glass transition temperature and degradation temperature of chitosan films containing carbon black and the glass transition temperature of chitosan films containing silver have not been determined. In addition, none removed the spin coated films from the substrate for characterization and therefore no studies compared the thermal properties of both spin coated and sol cast chitosan films. This work will determine the structural and thermomechanical properties of bare and composite spin coated and sol cast films; it will also compare the T_g of sol cast films determined via TMA with those reported in the literature using DMA and DSC.

2.3.1. Synthesis of bare chitosan films

Characterization of films synthesized by the sol cast-approach

Chitosan sol cast films are produced by dissolving 1-3% w/v chitosan in 1-3% v/v acetic acid. A controlled volume of this solution is then poured into a mold and allowed to dry at elevated temperatures (60°C). The film is placed in a dilute sodium hydroxide solution to neutralize any remaining solvent, and then dried flat. The morphology, structure, mechanical, thermal, sorption, and antibacterial properties of the films are then determined via various characterization techniques. The following section first summarizes these thermal and mechanical properties of bare sol cast chitosan films, focusing on the debate over the glass transition temperature of chitosan, and then proceeds to the reported antibacterial and metal chelating properties of these films.

In 2000, the T_g of chitosan films synthesized was set at 203 °C by Sakuri et al. using DSC and DMA spectra. [25] Two years later in 2002, Lazaridou and Biliaderis determined the T_g to be 95°C by DMA. At 15% moisture content, chitosan films exhibited a Young's Modulus of 1500

MPa, an ultimate strength of 100 MPa and an elongation of 45%. They also determined that the chitosan films become more ductile with increasing moisture contents and that the mechanical strength of the films depended upon the polymer concentration, moisture content, crystallinity, cross-linking, ageing and macrostructure. [26]

Chitosan sol cast films that had been aged for 3 weeks and that had a moisture content of 14% were synthesized and characterized by Suyatma (2005). These films exhibited an ultimate stress of 63.1 MPa and elongation of 7.2%; the T_g and T_{deg} were determined to be 196°C and 280-300°C respectively by DSC. [29]

Wu et al (2004) reported the T_β at 15-22° C due to the hydration of the -CH₂OH groups and the T_g at 153°C using DMA. The chitosan films also exhibited a bacteriostatic effect against *E.coli* and *S.aureus*. [27]

After presenting an extensive literature review on the reported T_g of chitosan, Quijada-Garrido et al. (2007) characterized chitosan sol cast films with the techniques DMA, DSC, and TGA-MS. By DMA, the T_β was determined to be -30°C and was attributed to movement of the side chains -NH₂, CH₂OH, and -NH-CO-CH₃; the T_g was determined to be 85°C and was attributed to the torsional oscillations at the glycosidic bonds between the two glucosamine rings and a corresponding change in the hydrogen bonding. DSC did not reveal information about the T_g . TGA-MS set the T_{deg} at 260°C and attributed it to the decomposition of the main chain with the production of H₂O, CO and CO₂. [30]

The mechanical and antifungal properties of chitosan sol cast films were tested by Martínez-Camacho et al in 2010. Films with a thickness of $40 \pm 10 \mu\text{m}$, exhibited a strain of $1.96 \pm 0.06\%$ and an ultimate strength of 2.5 MPa. The glass transition temperature was calculated to be 170°C via DSC. The chitosan films inhibited the growth of the fungi *Aspergillus niger*. [61]

Li et. al. tested the antibacterial properties capabilities of chitosan sol cast films. The films were found to reduce the *E.coli*; however, the chitosan molecules had to be dissolved in order to interact electrostatically with the bacteria. [62]

Foster and Butt found that although chitosan solutions inhibit growth of *E. coli*, *S. aureus*, and *S. epidermidis* by 98%, chitosan thin films are not antibacterial. They suggest that the polymer chains in the film do not interact with the bacteria cell wall. [63]

Characterization of films synthesized by the spin coated approach

Chitosan spin coated films are produced by first dissolving 1-3% w/v chitosan in 1-3% v/v acetic acid. A small volume of this solution is then placed onto a substrate which is subsequently spun at 1000-3000 rpm and dried on a hotplate. Most researchers do not apply various layers. Similar to the sol cast film, the film is then placed in a dilute sodium hydroxide solution to neutralize any remaining solvent, and then dried flat. These chitosan spun coated films have been fabricated for optical applications [64], sensors of heavy metals [65], biomedical applications [66], and antibacterial applications [62].

In 2001, Ligler produced spin coated chitosan films with uniform thickness for optical applications. A crosslinker was added to prevent dissolution in water and other solvents and a plasticizer was added to decrease cracking of the film when heated. Film thickness was controlled from 200 to 100 nm by varying the speed between 1500 and 3000 rpm respectively; higher speeds led to thinner films. The resulting single layer films possessed uniform thicknesses with a standard deviation of 5% within a given film. [64] Subsequently the effect of metal ions was tested on the optimum spin coated chitosan films. These blue films possessed a thickness of 110 nm and showed a reflectance peak at 385 nm. The color, film thickness, and the reflectance peak changed after being dipped in solutions containing Fe(III), Zn(II), Hg(II),

Mn(II), Co(II), Cu(II), Cr(VI), and Cd(II). The metal ion concentration showed negligible effects and the counter ions caused a reflectance shift in the opposite direction. Therefore, these films may be used to qualitatively test water for metal ions based on color change. [65]

In 2011, Li et al. tested the antibacterial properties and metal binding capabilities of chitosan/PEO films. Rapid solvent evaporation prevented the chitosan chains from arranging themselves into a semi crystalline structure; therefore no chitosan peaks were present in the XRD spectra. 80/20 and 50/50 chitosan/PEO films bound 26.47 mg Cr(VI) / g film. The spin coated films showed negligible effects against E. coli due to the very small quantity of chitosan. [62]

2.3.2. Chitosan based nanocomposites

Many composite films are based on chitosan. Chitosan films have been used as a matrix to host a wide variety of compounds ranging from ZnO and Ag for bacterial applications, to graphene oxide and carbon nanotubes for biosensors, to TiO₂ and magentite for water cleaning. They have been blended with biopolymers such as gelatin, starch, cellulose, keratin, alginate, silk and pectin and with synthetic polymers such as polyethylene oxide, polyvinylpyrrolidone, polyvinyl alcohol, and polyaniline. Finally, they have been functionalized with innumerable compounds. [67]

This work will be limited to bare chitosan films as discussed above and nanocomposite chitosan films containing silver nanoparticles and carbon black. The thermomechanical and bacterial properties of bare and silver bearing chitosan films and their precursor solutions will be explored in this section. Likewise, the thermomechanical properties of carbon bearing chitosan composites will be discussed.

Composites containing silver nanoparticles

Silver ions may be reduced to nanoparticles in an acidic chitosan solution without any other reducing agents. A chitosan composite containing silver nanoparticles (2 – 4 nm) was fabricated

by Sanpui in 2008 using chitosan as a reducing agent. The MIC and MBC for *E. coli* were 100 µg / mL and 120 µg / mL respectively. The composite destroyed all bacteria within 4 hours, and SEM imaging showed most of the bacteria were deformed and fragmented within 90 minutes. [47]

Similarly, in 2009 Wei et al synthesized both bare chitosan solutions and films with silver nanoparticle loading. Both the bare and NP loaded solutions were effective against the bacteria. The bare chitosan solution stopped bacteria growth (minimum inhibitory concentration or MIC) at 48, 24, and 12 µg/L for *E.coli*, *S.aureus*, and *B.subtilis* respectively. The chitosan solution loaded with silver nanoparticles killed bacteria (minimum bactericidal concentration or MBC) at 10 µg/L for *E.coli*, *S.aureus*, and *B.subtilis*. [50]

Modrzejewska (2010) reduced silver nanoparticles (60 nm) in a chitosan solution at 60 °C for 24 hours; the UV-vis spectra of the resulting solutions exhibited a plasmon around 425 nm. [48]

In 2010, Tran et al. tested silver nanoparticles synthesized using chitosan against various bacteria, yeast, and cancer cells. Time, temperature, and the concentration of chitosan and Ag precursor affect the size and speed of the silver nanoparticles. Reduction at 30 °C led to slow reduction rates and small nanoparticles, while reduction at $\geq 100^{\circ}\text{C}$ led to fast reduction rates and particle agglomeration. The MIC and MBC for the bacteria *E.coli*, *L.fermentum*, *S.aureus*, and *B.subtilis*, and the yeast *C.albicans* were 10 µg/mL and 2.5 µg/mL for the bacteria *P.aeruginosa*. The Ag nanoparticles also showed inhibitory effects on the growth of cancer cells. [51]

Sol cast films containing silver nanoparticles

In 2007 Long et al. and Chen et. al. both used gamma rays to reduce silver nanoparticles in a solution containing chitosan. [68],[69] Two years later, Yoksan and Chirachanchai used a similar technique to synthesize chitosan solutions containing silver nanoparticles ranging

between 7 and 30 nm in diameter. The initial AgNO₃ concentration, gamma ray dosage, and chitosan concentration affected the nanoparticle size; these nanoparticles did not precipitate out of the solution for up to three months. Thin films were sol cast from these solutions and tested against *E.coli* and *S.aureus* using the agar diffusion method (thin film discs were 2.8 cm in diameter). Bare chitosan films had no effect on bacteria growth, but discs of silver NP loaded chitosan films showed an inhibition zone of 0.354 cm for *E.coli* and 0.092 cm for *S.aureus*. The addition of silver nanoparticles (0.29% w/w) also increased the tensile strength from 67 MPa to 75 MPa and Young's modulus from 5719 to 6511 MPa. In solution, chitosan with silver nanoparticles stopped growth of *E.coli*, *S.aureus*, and *B.cereus* at a concentration of 5.64 µg/mL. [49],[70]

Wei (2009) also tested the bactericidal properties of the films made from the chitosan solutions containing silver nanoparticles. The sol cast films were tested using a novel technique; the bare chitosan films were not bactericidal after 3 cycles, but the chitosan films containing silver nanoparticles were bactericidal. [50]

In 2010, Li explored the antibacterial properties of chitosan sol cast films containing Zn and Ag nanoparticles. The antibacterial properties of blend films were significantly higher than those of the films without nanoparticles or with only one type of nanoparticles. The film containing 0.1% wt Ag and 10% wt ZnO exhibited the highest antibacterial properties while remaining translucent. [71]

That same year, Vimala et. al. synthesized porous chitosan films with silver nanoparticles using polyethylene glycol as a reducing/stabilizing agent and tested their antimicrobial, mechanical, and thermomechanical properties. The porosity increased the bacteria contact with the silver nanoparticles, thereby increasing the film antibacterial properties. A 5 mm diameter disc of the

film caused a growth inhibition ring of 20, 22, and 17 mm in diameter for *E.coli*, *Bacillus*, and *K.pneumoniae* respectively. However, pure chitosan films did not inhibit growth of the bacteria. The bare chitosan films (Young's Modulus of 1500 MPa, elongation at break of 5%) were stronger and more brittle than the chitosan films with silver nanoparticles (Young's Modulus of 584 MPa, elongation at break of 19%) or the porous chitosan films with silver nanoparticles (Young's Modulus of 795 MPa, elongation at break of 22%). The thermal degradation occurred at 489 °C for all films. As determined by thermal degradation, the chitosan films containing silver nanoparticles contained 8-11% silver. [7]

Chitosan based composites containing carbon particles

Carbon black chitosan composites have been synthesized via electrospinning and a spray layer by layer process for increased electrical conductivity. The mechanical properties of the electrospun films were tested, but the thermal stability was not determined for any of these composites. Notwithstanding, the thermal properties of chitosan composites bearing carbon will be important for applications in which they conduct electricity, in order to determine the composite's response to the temperature increase from the dispersed energy.

Bouvier et al (2009) synthesized chitosan thin films bearing carbon nanoparticles via spray layer by layer process. The increase in conductivity due to the incorporation of carbon nanoparticles allowed the chemo-electrical properties of the sample to be measured, giving this nanobiocomposite possible applications as a sensor for water and methane vapors. [72]

Carbon black was dispersed in chitosan nanofibers by Schiffman (2011) at loadings ranging from 2.5% to 62.5% w/w, in order to increase the conductivity of the resulting composites for applications such as electrodes, electromagnetic interference shielding, and sensors. Higher carbon black loadings caused an increase in the electrical conductivity of seven orders of

magnitude from $1.14 \times 10^{-8} \pm 9 \times 10^{-9} \text{ S cm}^{-1}$ for the bare chitosan fibers to $0.109 \pm 0.05 \text{ S cm}^{-1}$ for the nanofibers containing 62.5 wt% carbon black. An increase in the carbon black loading also decreased the elongation at break from $12 \pm 0.3\%$ for bare chitosan fibers to $0.28 \pm 0.03\%$ for chitosan nanofibers containing 2.5 wt% carbon black. An increase in the carbon black loading increased the noise to signal ratio in the XRD patterns. [73]

3. EXPERIMENTAL

3.1. Materials Selection

Biodegradable polymers used as matrices in composites include cellulose, starch, and chitosan; however, as mentioned in the introduction, chitosan is the only polycationic polymer, which lends it to antibacterial applications and metal chelation. The disperse phases for the nanocomposite therefore were selected to enhance these properties. The addition of silver nanoparticles, widely employed as an antibacterial agent, should increase the bactericidicity of the films. Similarly, the addition of carbon black as a disperse phase, widely use in the strengthening of polymers and recently used as an adsorbent [58], should augment the film's sorption of toxic heavy metals and volatile organic compounds from water.

All reagents were of analytical grade and were used without further purification. Required concentrations of chitosan powder ($(C_6H_{11}O_4N)_n$, 82% deacetylation, Sigma Aldrich), silver nitrate salt ($AgNO_3$, 99.9%, Alfa Aesar), and carbon black powder (C, particle size 30 nm, aggregate size 100 nm, Sid Richardson) or lamp black powder (C, particle size 134 nm, Fisher Science) were dissolved in high purity water (18 M Ω) and acetic acid (CH_3CO_2H , 99.7%, Alfa Aesar). Sodium Hydroxide (NaOH, 98%, Alfa Aesar) was dissolved in high purity water and used to neutralize the chitosan films.

3.2. Materials Synthesis

3.2.1. Chitosan matrix synthesis

The synthesis of chitosan bare and composite films was achieved by using two methods: sol casting and spin coating. Figure 12 summarizes both synthesis routes.

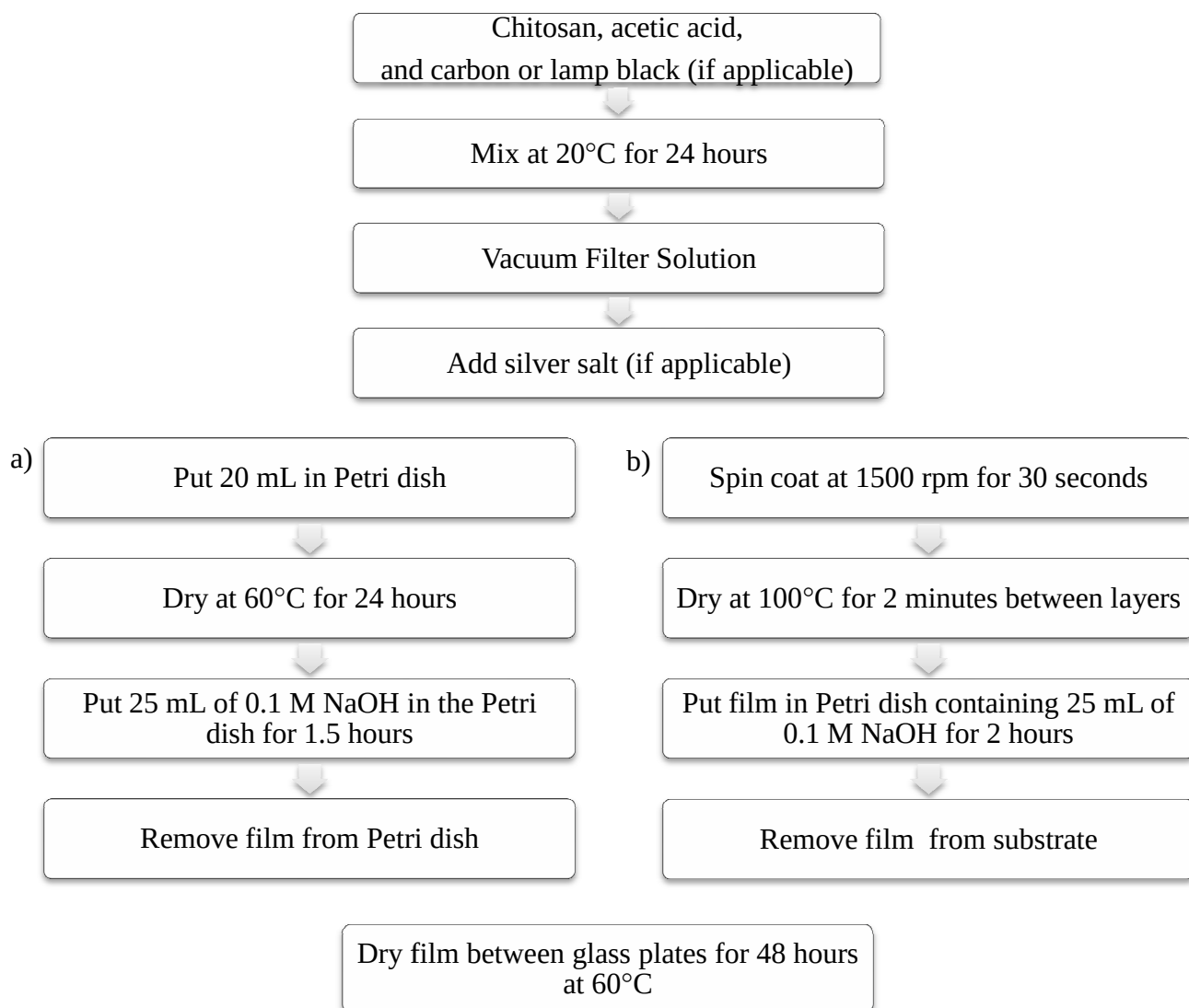


Figure 12 Synthesis method for sol cast, (a), and spin coated films, (b).

3.2.1.1. Sol cast chitosan films

Required amounts of chitosan (1% w/v) and acetic acid (1% v/v) were added to high purity water at room temperature conditions. Dissolution of the polymer was performed using a magnetic stirrer system at room temperature for 24 hours. At the end of the contact time, the solution was filtered was vacuum filtered using grade 4 cellulose filter paper (pore size 20-25 μm , Whatman). The resulting solutions were sonicated for 30 minutes and then appropriate amounts of the

solution (10 mL, 20 mL, or 30 mL) were deposited into Pyrex glassware with a diameter of 9 cm. These were put into an oven at 60°C for 24 hours until dry. The resulting films were neutralized with 0.1 M NaOH and removed from the Pyrex dish. Finally, to ensure that the films would dry flat, they were placed between two glass plates and dried in the oven at 60°C for 24 hours. The resulting film is shown in Figure 13a.

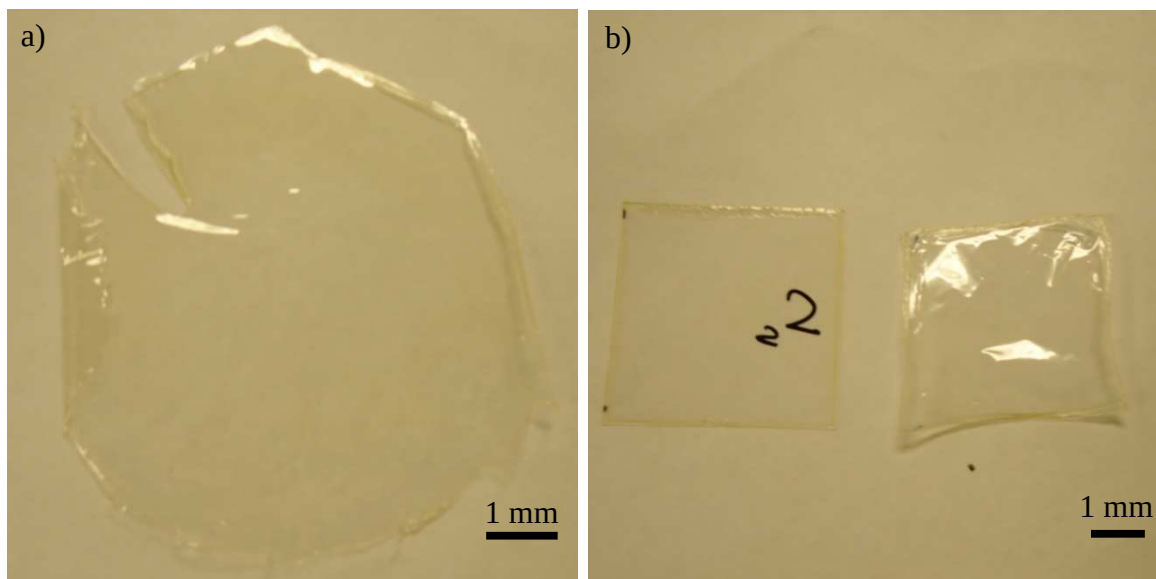


Figure 13 Bare sol cast (1% w/v chitosan, 20 mL solution) ,(a), and spin coated chitosan (1.75% w/v chitosan, 20 layers) films, (b).

3.2.1.2. Spin coated chitosan films

Required amounts of chitosan (1, 1.25, 1.5, 1.75, or 2% w/v) and acetic acid (1, 1.25, 1.5, 1.75, or 2% v/v) were added to high purity water at room temperature conditions. Dissolution of the polymer was performed using a magnetic stirrer system at room temperature for 24 hours. At the end of the contact time, the solution was vacuum filtered using grade 4 cellulose filter paper (pore size 20-25 μm , Whatman) and the resulting solutions were sonicated for 30 minutes to ensure that all bubbles were removed from the solution.

In order to determine how much polymer was removed by filtering, the filter paper was weighed before and after filtering. The difference between these two values was the amount of chitosan (CS) removed from the solution (Table 7); on average, filtering reduced quantity of chitosan in the solution by 0.016%.

Solution Parameters		Filter paper weight (g)		Calculations		
% CS before	mL	Before	After	g removed	% w/v CS after	% decrease in CS
1.50	100	0.2318	0.2802	0.0484	1.45	0.03
1.50	100	0.2280	0.2501	0.0221	1.48	0.01
2.00	150	0.2245	0.3050	0.0805	1.95	0.03
1.75	150	0.2303	0.2571	0.0268	1.73	0.01
1.75	150	0.2260	0.2471	0.0211	1.74	0.01
1.75	150	0.2244	0.2467	0.0223	1.74	0.01
1.75	150	0.2233	0.2496	0.0263	1.73	0.01
1.75	150	0.2231	0.2418	0.0187	1.74	0.01
1.50	100	0.2200	0.2716	0.0516	1.45	0.03
1.50	100	0.2190	0.2439	0.0249	1.48	0.02
1.50	150	0.2151	0.2462	0.0311	1.48	0.01
1.50	200	0.2193	0.2533	0.0340	1.48	0.01

Table 7 Chitosan removed from solutions by filtering

0.35 mL of the resulting solution was added drop-wise onto a clean glass substrate measuring 25x25 mm, dispersed with a spatula, and spin-coated at 1500 rpm for 30 seconds. As seen in Figure 14, a KW-4A Chemat Technology two stage spin coater (500 – 8000 rpm speed range, 3 – 60 seconds time interval) and a KW-4AH Chemat Technology hot plate (50 – 350°C temperature range, $\pm 2^\circ\text{C}$ resolution) were used. After each coating cycle, the films were dried for 2 minutes at 100°C to remove any remaining solvent. These spin-coating/drying cycles were repeated the required number of times (1, 5, 10, 15, or 20) to thicken the films. Produced films were then treated with 25 mL of 0.1 M NaOH for 2 hours to neutralize any remaining solvent, removed from the glass substrate, rinsed in de-ionized water for 5 minutes, then placed between two glass plates and dried at 60°C for 24 hours.

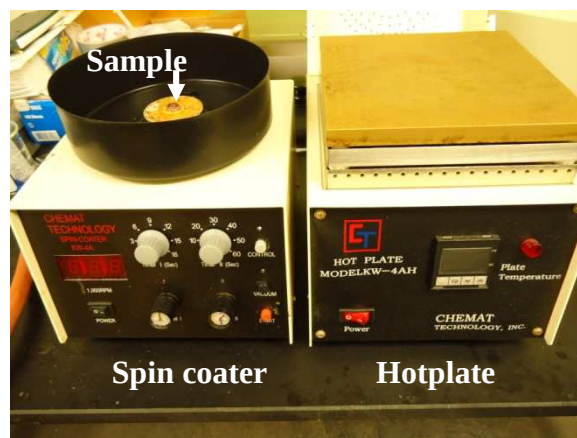


Figure 14 Chemat technology spin coater and hotplate.

Initially, five concentrations of chitosan in acid (1%, 1.25%, 1.5%, 1.75%, and 2%) were synthesized to determine the optimal concentration. The 1% films broke upon removal from the substrate. The 1.25% and 1.5% films could be removed from the glass substrates and did not contain many bubbles. Due to the viscosity, the 1.75% and 2.00% solutions contained bubbles inadvertently created when the solution was dispersed onto the substrate and the resulting films had a yellow tint due to the higher polymer concentration.

Subsequently films were synthesized using the optimal concentrations (1.25% and 1.5% w/v chitosan) with varying layers (1, 5, 10, 15, 20). The films of 1 and 5 layers could not be removed from the substrate and the films using 15 and 20 layers were yellowed and curled when removed from the substrate. The films synthesized with 1.5% w/v chitosan were slightly thicker than those synthesized with 1.25% w/v chitosan, which improves their mechanical properties. Therefore the optimal synthesis parameters were 1.5% and 10 layers.

Although the initial series to determine the optimum conditions for bare chitosan films was carried out using 25x25 mm glass microscope slides, the resulting films presented challenges in the characterization due to their size. Therefore glass substrates measuring 45x45 mm were used

for the composite chitosan films. The optimum parameters were determined in a manner similar to that of the smaller substrate. The volume, concentration, and number of layers were 1.3 mL solution, 1.75% w/v chitosan in 1.75% v/v acetic acid, and 20 layers respectively. All other parameters remained the same. The resulting film is shown in Figure 13b.

3.2.2. Chitosan based nanocomposite synthesis

3.2.2.1. Silver bearing chitosan nanocomposites

Chitosan composite films bearing silver nanoparticles were synthesized following the parameters developed for bare chitosan films with one additional step. A review of the literature revealed that adding AgNO_3 to a chitosan solution at 25°C led to slow formation of very small silver nanoparticles and that adding AgNO_3 to a chitosan solution at 90°C led to rapid formation of agglomerated silver nanoparticles. [51] In order to produce small particles, appropriate amounts of AgNO_3 (1, 3, and 5 mM) were added to the solution after filtration and stirred with a magnetic pill at 25 °C for 2 hours before sol casting or spin coating. The solutions were initially clear but changed in color as time progressed as shown in Figure 15. This change in color is attributed to the reduction of the silver ions by the hydroxyl and amine groups of the chitosan, resulting in the oxidation of the chitosan [50]. Resulting sol cast films are shown in Figure 16 and spin coated films are shown in Figure 17.

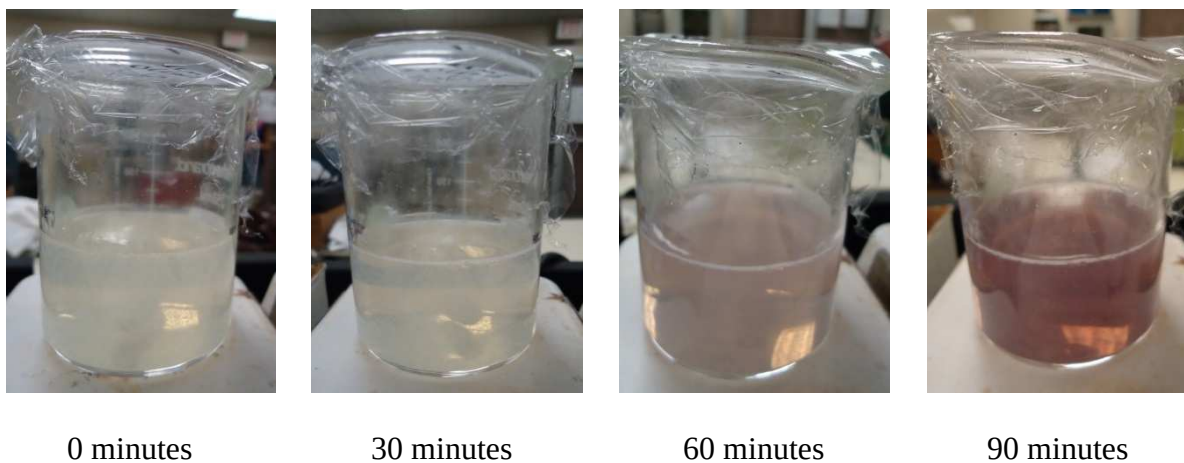


Figure 15 Color change of 1 mM AgNO_3 solution (1.5% w/v chitosan, 1.5% v/v AA, 25°C).

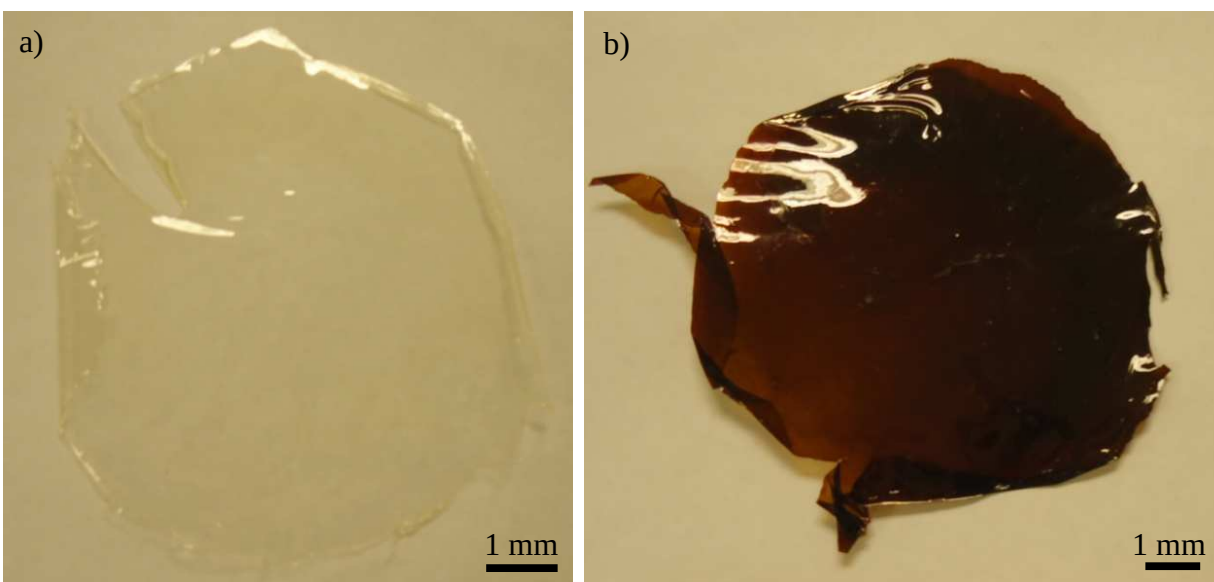


Figure 16 Sol cast chitosan films synthesized from a precursor solution containing (a) 0 and (b) 5 mM AgNO_3 (1% chitosan, 20 mL solution).

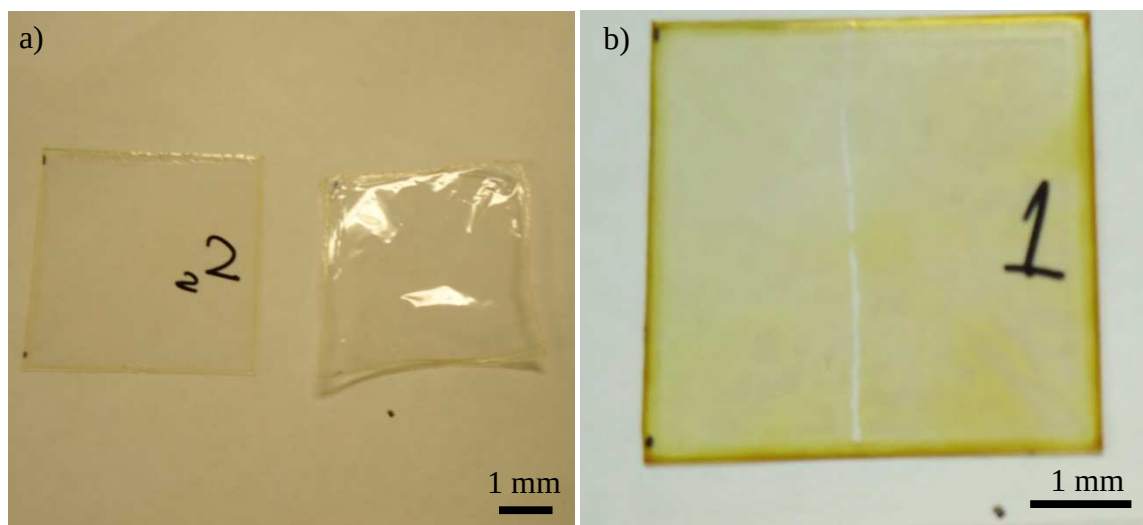


Figure 17 Spin coated chitosan films synthesized from a precursor solution containing (a) 0 and (b) 3 mM AgNO_3 (1.75% w/v chitosan, 20 layers).

3.2.2.2. Carbon black bearing chitosan nanocomposites

Chitosan composite films bearing silver nanoparticles were synthesized following the parameters developed for bare chitosan films with one additional step. Lamp black powder was added to the the solution in appropriate weight fractions (1, 3, and 5% carbon black / chitosan) before filtration for the sol cast films. Likewise, carbon black powder was added to the the solution in appropriate weight fractions (1, 3, and 5% carbon black / chitosan) before filtration for the spin coated films. Resulting sol cast films are shown in Figure 18 and spin coated films are shown in Figure 19.

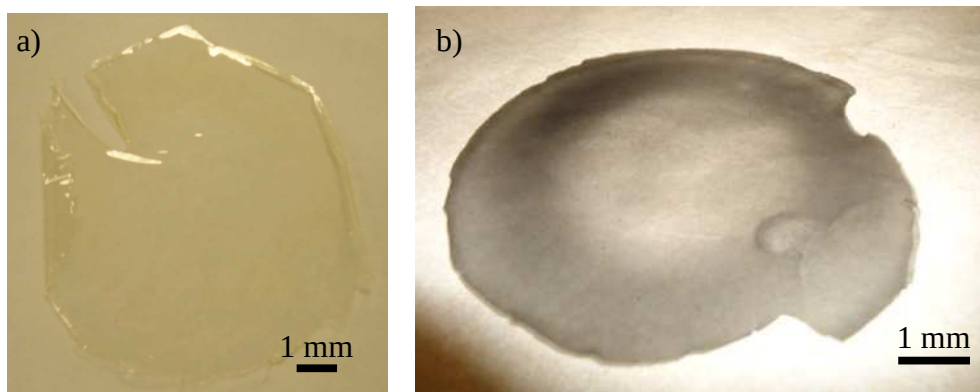


Figure 18 Sol cast chitosan films containing (a) 0% and (b) 1% w/w carbon black (1% w/v chitosan, 20 mL solution).

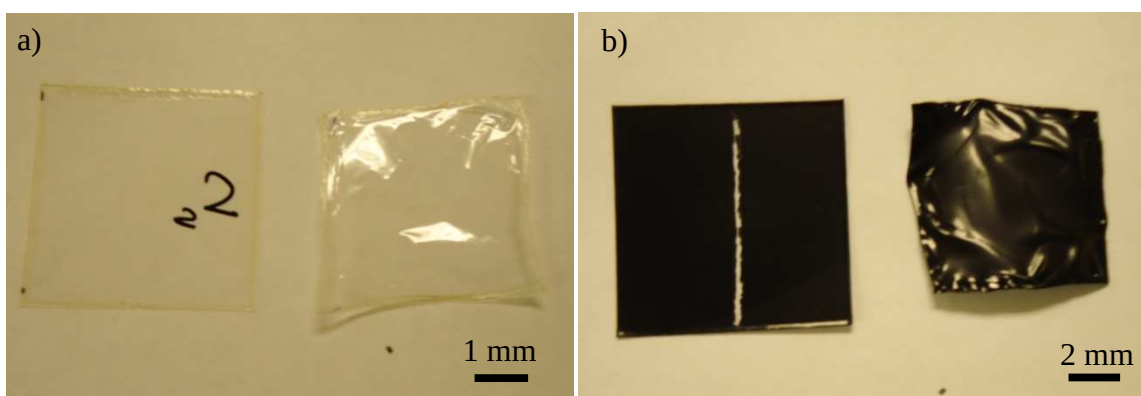


Figure 19 Spin coated chitosan films containing (a) 0% and (b) 5% w/w carbon black (1.75% w/v chitosan, 20 layers).

When carbon black (CB) was added to the solution, the viscosity of the solution increased and the solution had to be stirred during filtering. Due to this stirring, the filter paper usually broke and therefore could not be weighed after the filtering process. In the cases that the filter paper did not break, the solid removed from the solution included both chitosan and carbon black (Table 8); therefore the quantity of carbon black that was removed from the solution could not be directly calculated.

Solution Parameters			Filter paper weight (g)		Calculations
% CS before	mL	%CB	Before	After	g removed
1.50	40	1	0.2294	0.2458	0.0164
1.50	100	3	0.2297	0.3241	0.0944
1.50	100	5	0.2283	broken	broken
1.50	150	1	0.2285	0.2789	0.0504
1.50	150	3	0.2233	broken	broken
1.50	150	5	0.2315	broken	broken
1.75	150	1	0.2232	broken	broken
1.75	150	3	0.2303	broken	broken
1.75	150	5	0.2246	broken	broken
1.50	100	5	0.217	broken	broken
1.50	150	3	0.2182	0.2572	0.0390
1.50	150	7	0.2224	broken	broken
1.00	100	5	0.2173	0.2309	0.0136

Table 8 Carbon black and chitosan removed from solutions by filtering

3.3. Materials Characterization

3.3.1. Optical Microscopy

An optical microscope provides morphological sample information by focusing the image with refractive glass or quartz lenses. The image may be magnified up to 1500x with a resolution up to 200 nm. Current models also display a digital image on a computer screen via a charge coupled device. The microscope used in this work is shown in Figure 20; all images were taken at a magnification of 5x.

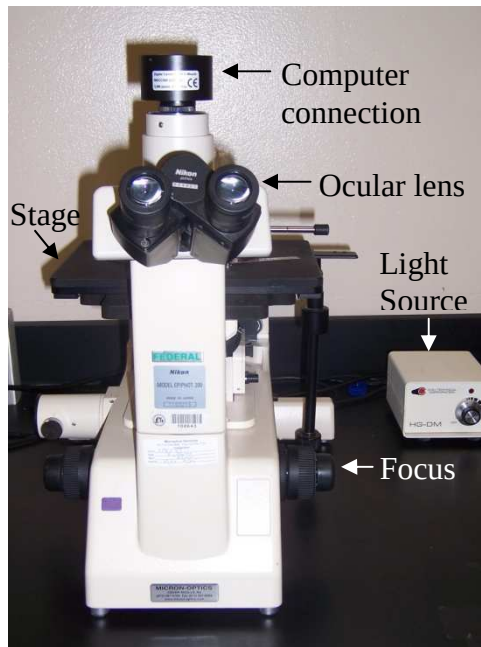


Figure 20 Nikon Epishot 200 microscope – Engineering Science and Materials Department at UPRM.

3.3.2. Atomic Force Microscopy

An atomic force microscope provides detailed sample information by scanning the surface with a cantilever beam. In the tapping mode, a cantilever beam oscillating near its resonance frequency lightly taps the sample. A laser which reflects off the cantilever beam sends feedback to the controller, which in turn controls the movement with piezoelectric elements for high accuracy. Both a topography image and a phase image may be obtained from the tapping mode. The phase image detects the phase lag between the signal driving the cantilever beam and the oscillations of the cantilever beam. In this way, it detects differences in material properties and provides a more precise image of composite materials. [74]

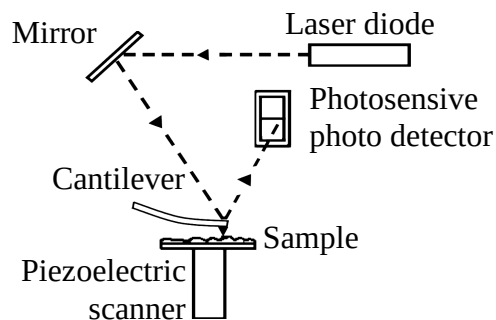


Figure 21 AFM detection [74].

The AFM software also provides a measure of roughness, however this measurement is dependent on the size of the image. Therefore the root mean square roughness for each film was calculated as the average of 5 roughness measurements per image from 3 – 30x30 μm images. Due to the fact that the samples must be deposited on a substrate in order to characterize them with the AFM, images were only taken of the spin coated films.



Figure 22 Veeco DiCP II AFM – Engineering Science and Materials Department at UPRM.

3.3.3. Profilometry

Profilometry provides precise information about the thickness of a film which is mounted on a substrate. This technique was only used for spin coated films since sol cast films were not attached to a substrate and could not be mounted in the profilometer. To analyze the film, first a

thin cut is made along the length of the film with a razor blade. The film is then loaded into the machine (Figure 23) and a needle is dragged across the incision three times. The film thickness is calculated as the difference in height of the film adhered to the substrate and the substrate.

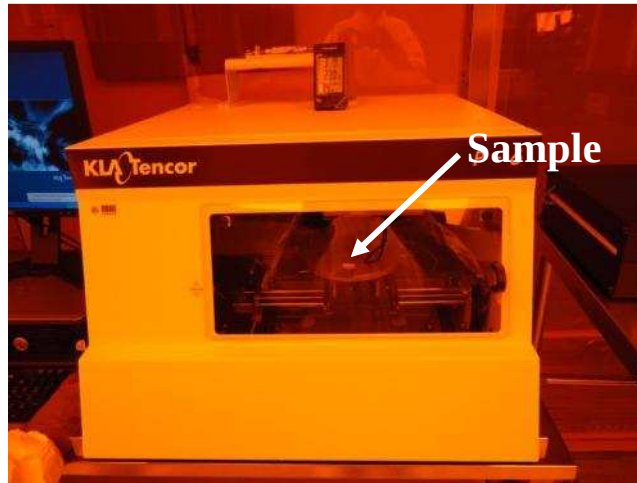


Figure 23 KLA Tencor Profilometer in clean room – Engineering Science and Materials

Department at UPRM.

3.3.4. X Ray Diffractometry

The X-Ray Diffraction spectra provides structural information including crystallinity, grain size, and phase composition. For compounds with regularly repeating lattice structures, the emitted x rays exhibit constructive interference (Figure 24a) and the diffracted beam is sensed by the detector. This occurs when the increase in path is a multiple of the wavelength as shown in Figure 24b.

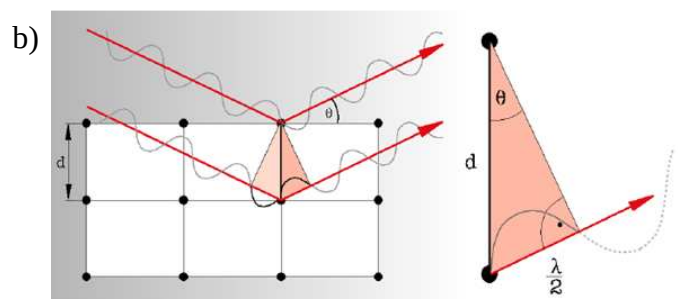
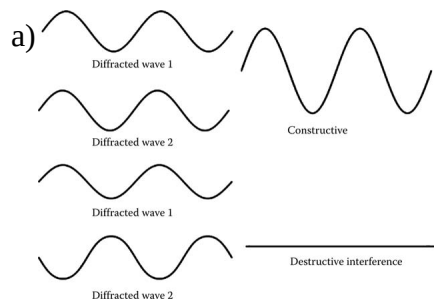


Figure 24 Schematic of constructive and destructive interference [75], (a), and diffraction [76]

(b).

This phenomena is described by Bragg's law and may be used to calculate the interplanar distance, d :

$$2d \sin\theta = n\lambda$$

where λ is the wavelength of the x-ray and θ is the Bragg angle.

The rays that diffract out of phase are cancelled out by each other out by destructive interference and therefore no peak occurs at that angle in the XRD spectra. However, incoming rays close to the Bragg angle are diffracted only slightly out of phase, and the ray that cancels it out completely is diffracted by a plane deep inside the crystal. This plane does not exist in very small crystals, so a signal is detected at angles near the Bragg angle and the width of the peak increases. On this basis, when the crystal is smaller than 100 nm, its size may be determined using Scherrer's law which is derived from Bragg's law:

$$\tau = \frac{K\lambda}{\beta \cos\theta}$$

where: K is the shape factor, usually set as 0.9

λ is the x ray wavelength, the average Cu $K\alpha$ wavelength is 0.1541 nm

β is the width of the peak at the half of the maximum intensity (FWHM), found with PowderX [77]

θ is the Bragg angle in radians

The lattice parameter, a , may be calculated as

$$a = d\sqrt{h^2 + k^2 + l^2}$$

where h , k , and l are the Miller indices of the selected plane.

In order to identify the material, the spectra was imported into Match! [78] and compared with the peaks of known chemicals in existing databases.

Cu-K α radiation and β Ni filter in a Siemens D500 powder x-ray Diffractometer were used to take all spectra. All spectra of chitosan and carbon black composite films were taken from 5 to 40 °C with a step of 0.01° and a dwell time of 1 second unless otherwise noted. To reduce noise, the composite films containing silver were taken over the range 35 to 45° with a step of 0.01° and a dwell time of 10 seconds.

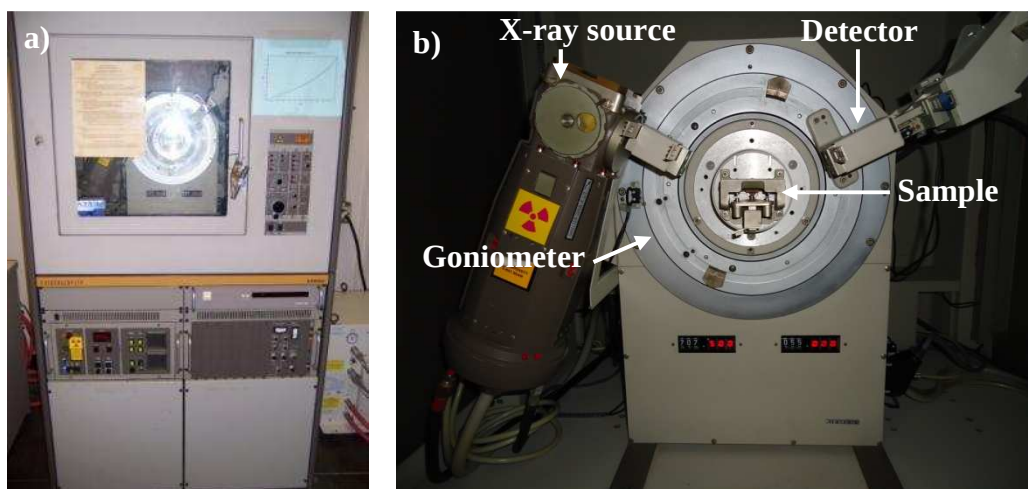


Figure 25 Seimens 500 XRD, (a), and detail of goniometer, (b)– Engineering Science and Materials Department at UPRM.

3.3.5. Ultraviolet Visible Spectroscopy

UV-vis uses light over the ultraviolet, visible, and near infrared spectrum, with the wavelength ranging from 200 to 800 nm. Shifts to higher wavelengths may indicate an increase in particles size or particle aggregation.

Beer's law is used to calculate the absorbance:

$$A = \log_{10} \left(\frac{I_0}{I} \right)$$

where A is absorbance, I_0 is the incident light at a given wavelength, I is the transmitted intensity.

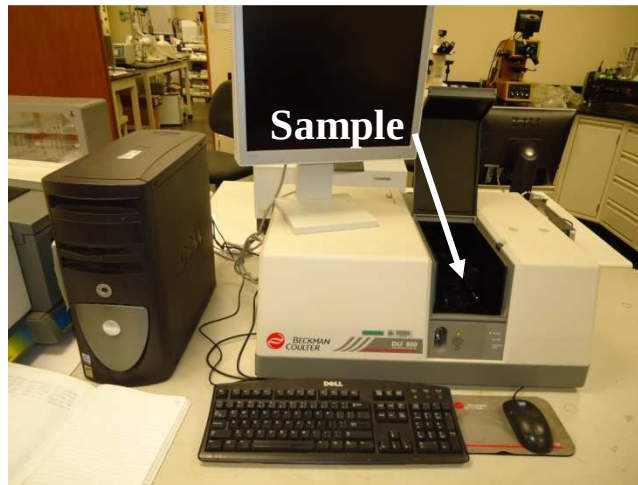


Figure 26 Beckman Coulter DU 800 UV/Visible Spectrophotometer – Engineering Science and Materials Department at UPRM.

3.3.6. Fourier Transform Infrared Spectroscopy

An FTIR emits light in the mid infrared regions to study the vibrations and rotations of the bonds present in a sample. The sample absorbs energy at the frequencies of the bonds between the atoms, allowing the identification of the sample (Figure 27).

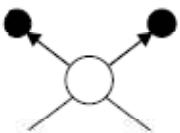
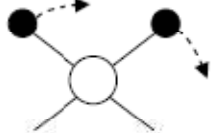
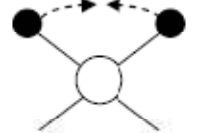
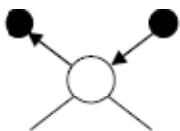
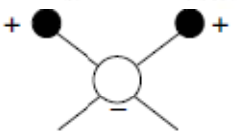
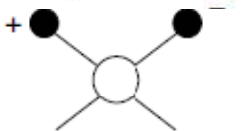
Stretching vibrations	Bending vibrations	
Symmetric stretch 	In-plane rocking 	In-plane scissoring 
Asymmetric stretch 	Out-of-plane wagging 	Out-of-plane twisting 

Figure 27 Stretching and bending vibrations. “+” indicates that the atom moves toward the reader and “-” indicates that the atom moves away from the reader [79].

Samples may be measured using transmittance or attenuated total reflectance (ATR). In transmittance mode (Figure 28), the beam from the source (I_0) passes through the sample to the detector. In ATR mode (Figure 29), the beam from the source reflects off of the sample and passes to the detector, thereby taking the spectra from the surface of the sample.

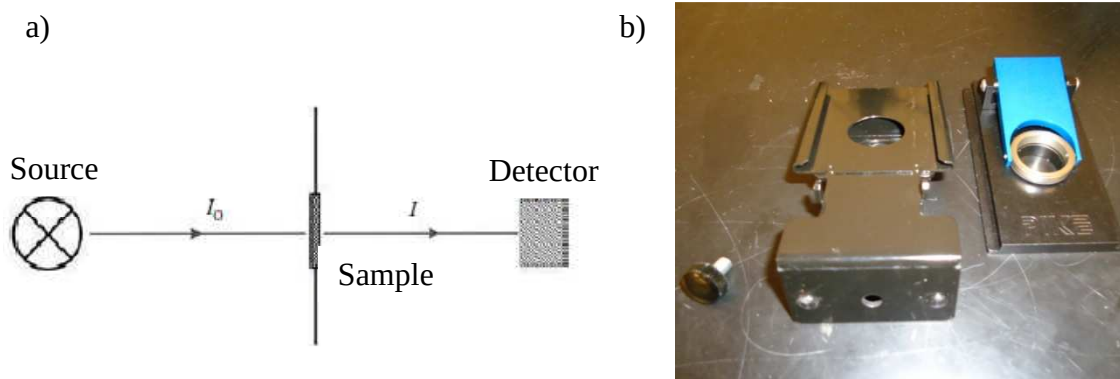
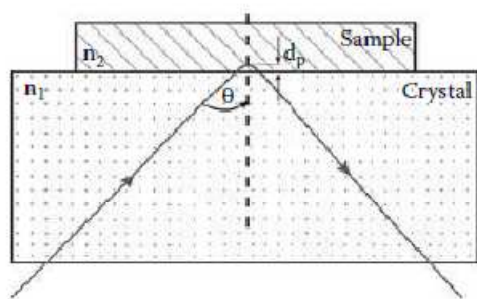


Figure 28 Schematic [80], (a), and universal sample holder, (b), used in transmittance mode.

a)



b)



Figure 29 Schematic [80], (a), and ATR sample holder, (b), used in ATR mode.

All spectra were taken in the spectral range of 4000 - 600 cm^{-1} by accumulation of 200 scans at resolution of 4 cm^{-1} in transmission mode unless otherwise noted. ATR was used in all cases because the thicker films exhibited saturation because the film thickness prohibited the light beam from passing through the film.

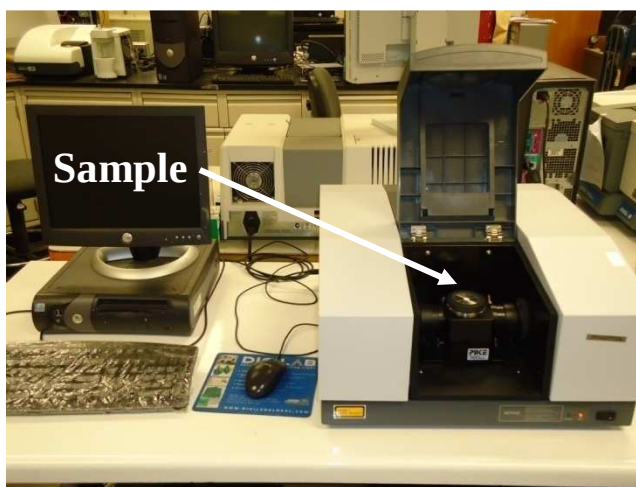


Figure 30 Shimadzu IRAffinity-1 FTIR – Engineering Science and Materials Department at UPRM.

3.3.7. Thermomechanical Analyses

A TMA measures changes in geometry as a function of temperature. As seen in the schematic in Figure 31a, the sample is subjected to a minimal load and the dimensions are measured while the temperature is increased. While in the schematic the sample is subjected to a compressive force, various sample geometries are available depending on the type of sample (Figure 31b). In this work, a tensile force is applied to the films.

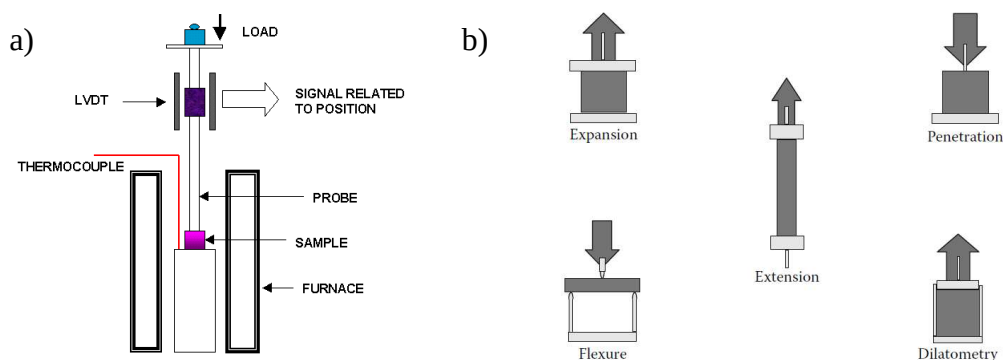


Figure 31 Schematic of TMA [81], (a), and sample geometries [82], (b).

These changes in sample geometry are caused by an increase in the free volume, which is the space that a molecule has for internal movement (Figure 31).

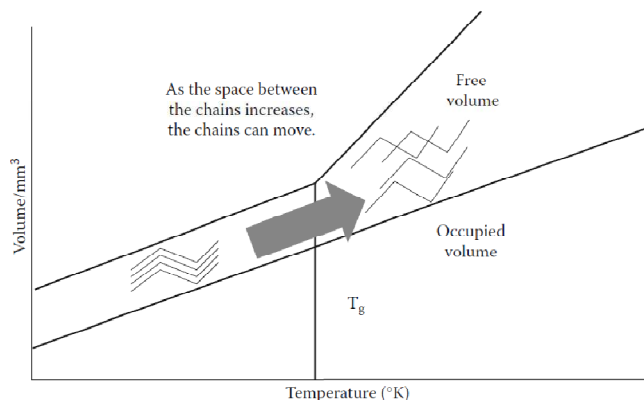


Figure 32 Free volume of polymers [82].

Expansion coefficients, glass transition and melting temperatures, solid-solid transitions, and swelling and elastic behavior may be extracted from the data. In this work, TMA will be used to

determine the glass transition temperature, T_g . The T_g occurs over a range of temperatures, since the length of the polymer chains contain multiple structures which are disrupted with various amounts of heat. [83] According to IPC standards, the T_g may be determined from a TMA spectra as the intersection of two tangent lines before and after the change in slope (Figure 33a).

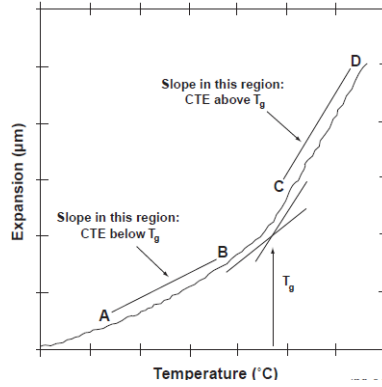


Figure 33 Methods to determine CTE and T_g from TMA [84].

Specifically, the T_g is the temperature corresponding to the intersection of lines AB and CD, where

- T_A – at least 10 – 25 °C above T_{initial}
- T_B – on the linear portion of the graph below the T_g
- T_C – on the linear portion of the graph above T_g
- T_D – 300°C

In this work, intersection of the tangent lines drawn before and after the glass transition temperature was considered to be the glass transition temperature. To increase precision, the TMA curve was fitted with a 6th order polynomial in Excel, and the first derivative was taken at $x_1=60$ °C and $x_2=160$ °C to provide the slope of the tangent lines.

$$y = Ax^6 + Bx^5 + Cx^4 + Dx^3 + Ex^2 + Fx + G$$

$$m = \dot{y} = 6Ax^5 + 5Bx^4 + 4Cx^3 + 3Dx^2 + 2Ex + F$$

Then, the intercept for each tangent line was calculated.

$$b_1 = y_1 - m_1x_1$$

Finally, the glass transition temperature was calculated as the intercept of these two lines.

$$y_1(x) = y_2(x)$$

$$m_1(x) + b_1 = m_2(x) + b_2$$

$$x = \frac{b_2 - b_1}{m_1 - m_2}$$

A sample curve is shown in Figure 34.

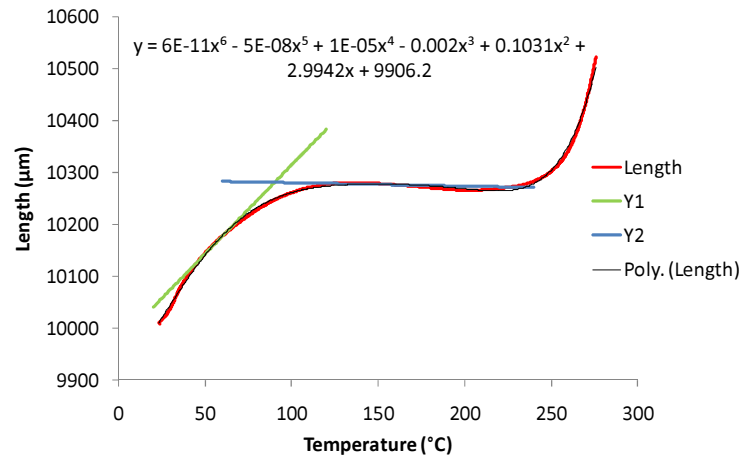


Figure 34 Method to determine T_g from TMA curve.

Unless otherwise noted, all tests were run in an air atmosphere, with a temperature ramp of 5 °C per minute from 25 – 275 °C. The force applied was 0.15 N in all cases. Samples were stored at room temperature prior to testing and were rectangular with dimensions 15 mm long and 5 mm wide. Three samples were run for each processing conditions.

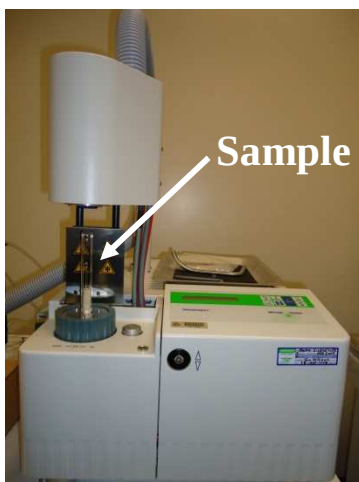


Figure 35 Mettler Toledo Thermomechanical Analyzer – Engineering Science and Materials Department at UPRM.

3.3.8. Thermogravimetric Analyses

A thermogravimetric analyzer may be used to determine properties such as degradation temperature (T_{deg}), sample purity, sample identification, solvent retention, reaction rate, activation energy, and heat of reaction. The equipment (Figure 36) consists of an electromagnetic mass balance, a resistive furnace, and a temperature sensor.

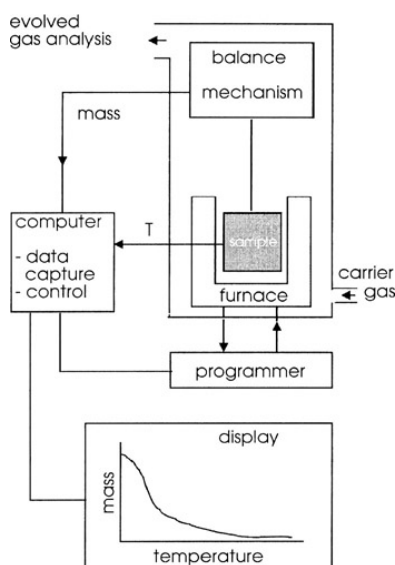


Figure 36 Schematic of TGA [85].

After the sample is placed on the balance, the mass and temperature are recorded as the furnace heats the sample until it decomposes into its original components. The resulting spectra gives the mass as a function of time or temperature. The first derivative of this spectra determines whether the process is exothermic or endothermic; a local minimum indicates the process was endothermic while a local maximum indicates it was exothermic. In this work, the T_{deg} was be calculated as the temperature corresponding to the minimum of the first derivative. (Figure 37).

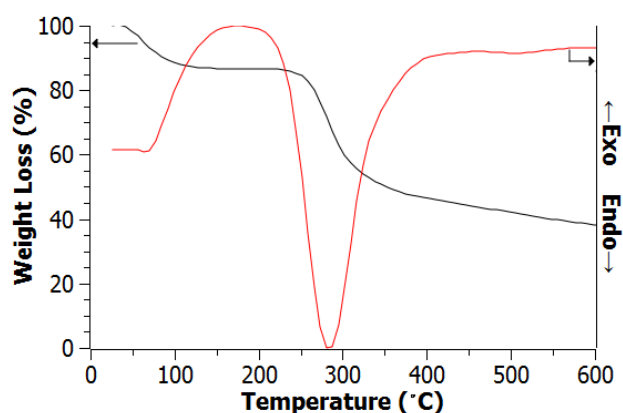


Figure 37 TGA spectra of weight loss and the first derivative.

The properties were evaluated on a Mettler Toledo TGA with a temperature ramp of 10 °C/minute from 25 to 600 °C in a nitrogen atmosphere. Samples were stored at room temperature prior to testing.

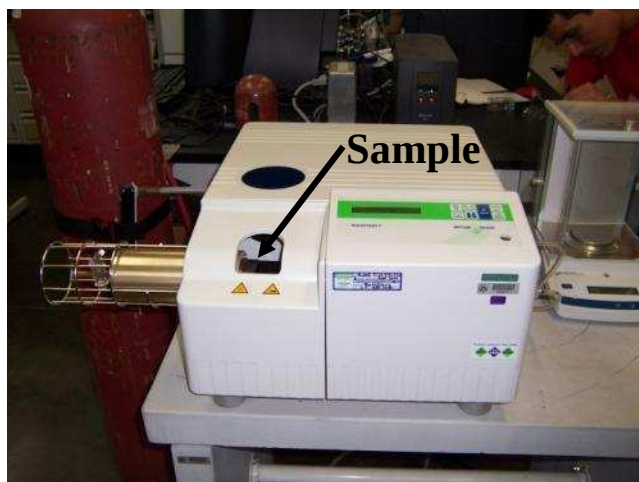


Figure 38 Mettler Toledo Thermogravimetric Analyzer – Engineering Science and Materials
Department at UPRM.

4. RESULTS AND DISCUSSION

4.1. Bare chitosan films

4.1.1. Structural Characterization

Structural analysis of produced composite materials was carried out to verify the development of polymeric matrix and the dispersoids. For this reason, X-ray diffraction and Fourier transform infrared spectroscopy were used to identify the crystalline phases and the functional groups of the polymeric matrix, respectively.

4.1.1.1. X-ray Diffraction Analyses

XRD analyses of the chitosan matrix confirmed the formation of the expected structures using both the sol casting and spin coating synthesis routes. Broad peaks centered on 10° and 20° corresponding to the structure of chitosan indicated a short-range molecular order. As seen in Figure 39a, the sol-cast film synthesized from 30 mL of precursor solution exhibits a more intense peak for the (020) plane than for the (110) plane than the powder spectra, which would suggest the rearrangement of the polymer chains during the synthesis process. This change in intensity of the peaks may be attributed to slow solvent evaporation during the synthesis process, allowing the polymer chains to organize themselves into a crystalline structure. In contrast, in the spin-coated film (1.75% chitosan, 20 layers) both peaks were less defined and the noise was increased. Li et al. credited this phenomenon to the rapid solvent evaporation which restricted movement of the polymer chains and prevented the formation of crystalline phases. [62]

Following similar reasoning, the varying intensities of the chitosan diffraction peaks in spin coated films synthesized at increasing concentrations (Figure 39b) and increasing number of layers (Figure 40) may also be attributed to the rapid solvent evaporation, which caused chains to remain in the arrangement that they assumed during the spin coating process.

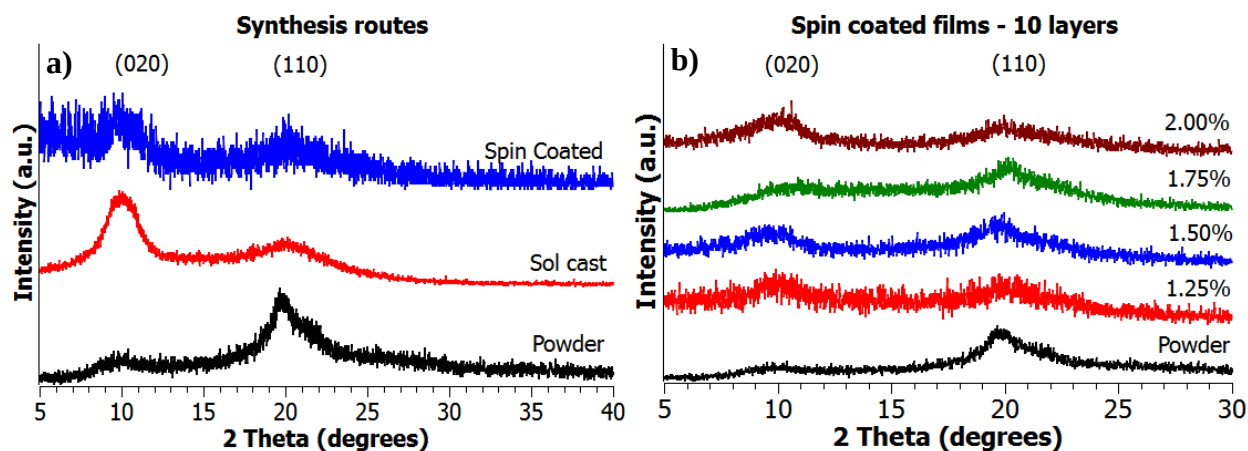


Figure 39 XRD spectra of chitosan films synthesized via sol-cast or spin-coating methods, (a), and spin-coated films produced at different concentrations of the precursor solutions, (b).

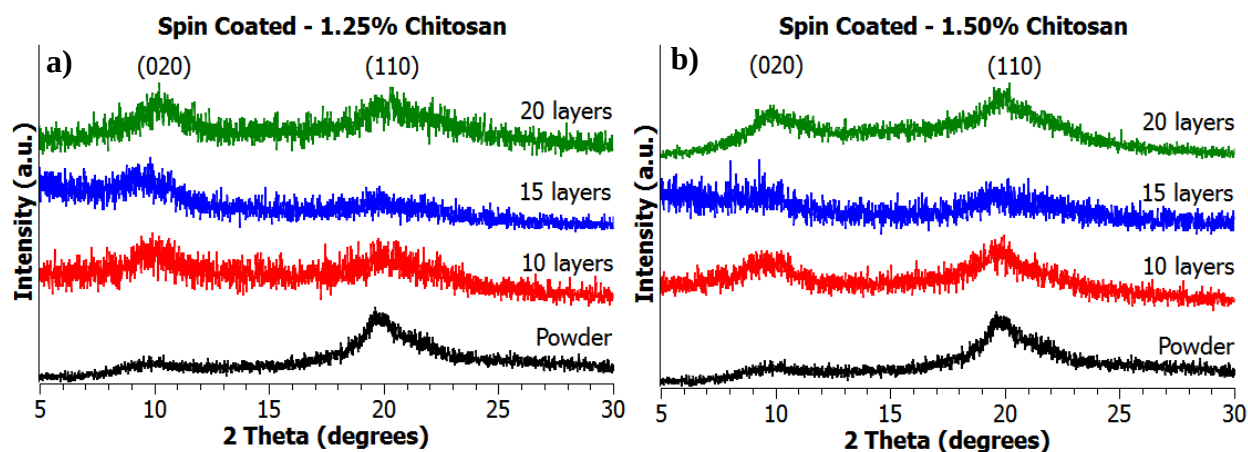


Figure 40 XRD spectra of chitosan spin coated films synthesized with increasing layers with chitosan concentrations of 1.25%, (a), and 1.50%, (b).

4.1.1.2. Fourier Transform Infrared Spectroscopy Measurements

The FTIR spectrum for precursor chitosan powder is shown in Figure 41a. The main bands at 895 and 1150 cm^{-1} (antisymmetric C-O-C stretching of glycosidic link), 1030 and 1070 cm^{-1} (C-O-C stretching of skeleton) were identified and assigned to the skeleton of the chitosan structure. The bands attributed to the functional groups were observed at 1475 cm^{-1} ($-\text{CH}_2$ bending), 1535 cm^{-1} (N-H bending of the NH_2 group), 1630 cm^{-1} (N-H bending of the NH_2 group and C=O

stretching in the COCH_3 group), 2865 cm^{-1} (C-H stretching of the glucose ring, the CH_2OH group, and the CH_3 in acetyl group) and 3350 cm^{-1} (N-H bending of the NH_2 group). The bands at 1630 and 3350 cm^{-1} were also attributed to hydrogen bonding. Obtained information was in agreement with previous reviews compiled by Kumirska [22] and Kasaai [86], who identified similar bands in chitosan. Figure 41b exhibits the FTIR spectra of bare chitosan sol cast films (20 mL) and spin coated films (1.75% chitosan, 20 layers). Both the spin coated and sol cast films exhibited the main bands found in the precursor chitosan powder, indicating that chemical structure of chitosan was not affected by the synthesis route.

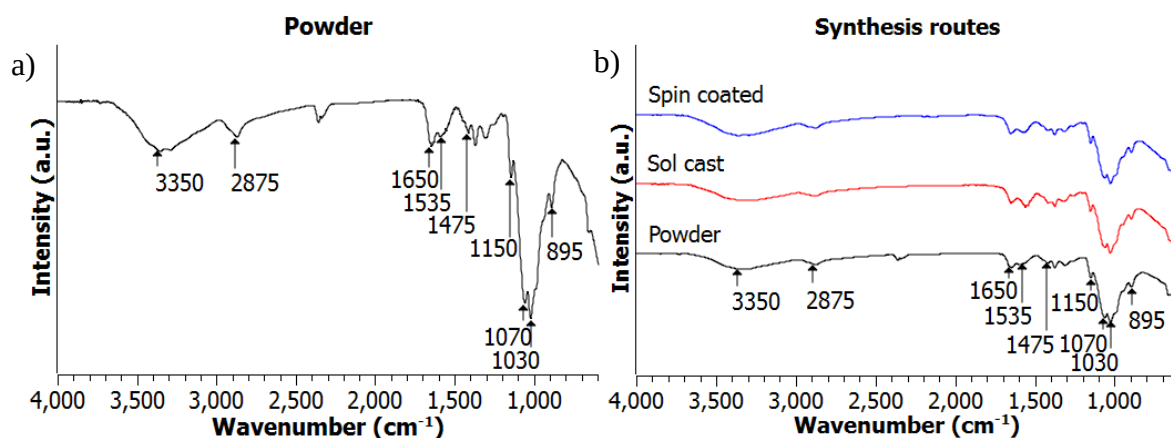


Figure 41 FTIR spectra of precursor chitosan powders, (a), and chitosan films synthesized via sol-cast or spin-coating routes, (b).

Figure 42 and Figure 43 show the FTIR spectra for bare sol cast and spin coated chitosan films synthesized at different thicknesses. There were no observed differences between the spectrum of standard chitosan powder and the spectrum corresponding to the sol cast films with varying thicknesses. Likewise, no significant differences were observed between the spectra of the bare chitosan spin coated films synthesized with increasing concentrations (Figure 42b) or increasing number of layers (Figure 43a, b). Accordingly, it may be concluded that an increase in film

thickness does not have remarkable influence upon the molecular structure of the polymeric matrix.

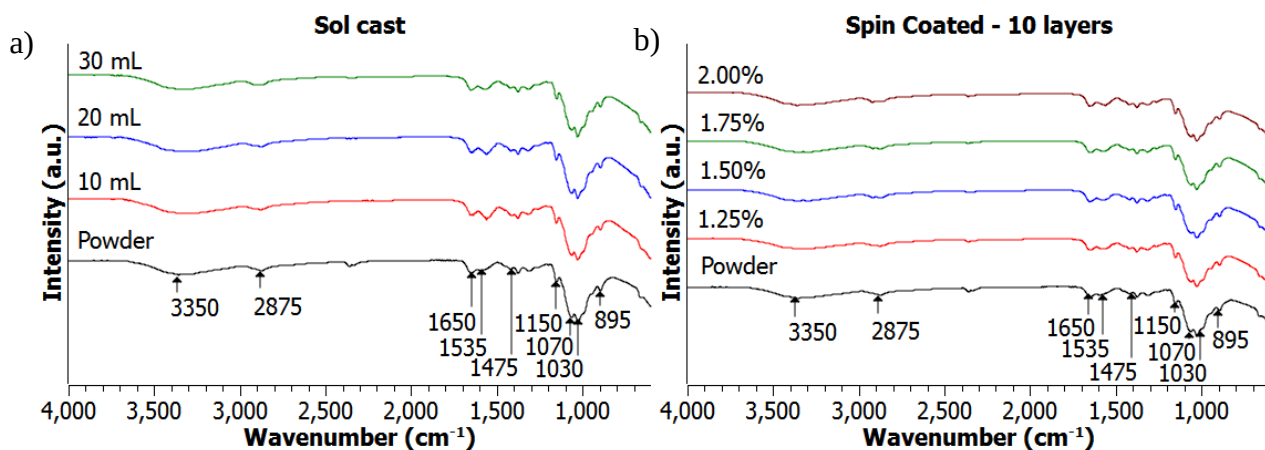


Figure 42 FTIR spectra of chitosan sol cast films with increasing thicknesses, (a), and spin coated films with increasing concentrations, (b).

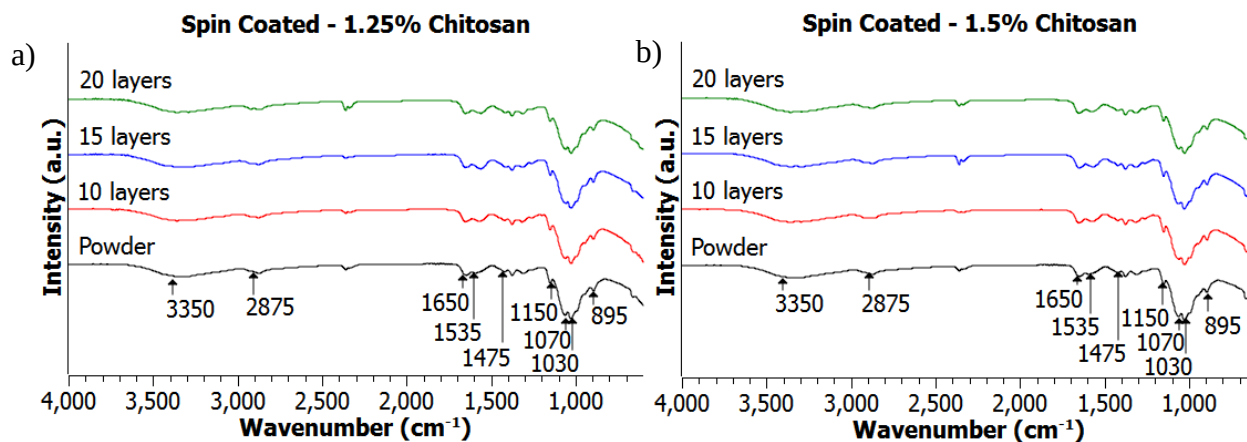


Figure 43 FTIR spectra of chitosan spin coated films synthesized with increasing layers with chitosan concentrations of 1.25%, (a), and 1.50%, (b).

4.1.2. Morphological Characterization of chitosan Films

4.1.2.1. Optical Microscopy

Optical microscope images of bare chitosan sol cast and spin coated films are shown in Figure 44.

The synthesis conditions were 20 mL of 1% w/v chitosan solution for the sol cast film and 20

layers and 1.75% w/v chitosan solution for the spin coated film. The images revealed that spin-coated films exhibited higher porosity than sol-cast films. This may be attributed to the partial dissolution of the first polymeric layers by the precursor solution and the rapid evaporation of solvent during the film thickening stages. A similar trend was observed in all other films synthesized via sol-cast and spin-coating approaches. This porosity may affect the XRD spectra of the spin coated films; the porosity would diffract the x-rays at arbitrary angles, increasing the noise to signal ratio.

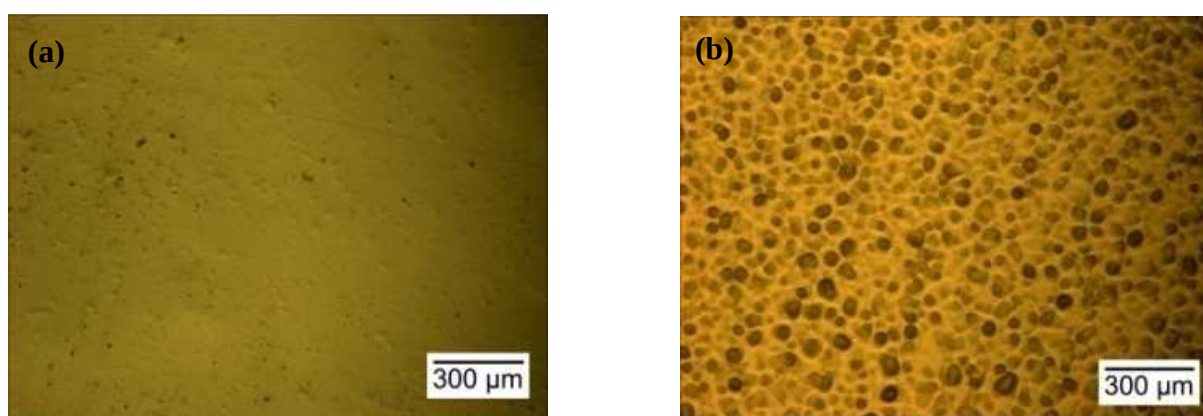


Figure 44 Optical images of sol-cast, (a), and spin-coated, (b), chitosan films.

4.1.2.2. Atomic Force Microscopy Analyses

AFM images of spin coated bare chitosan films are shown in Figure 45. These films were synthesized with 1.75% chitosan solution with 5 layers on the 25x25 mm substrate in order to be mounted in the AFM specimen holder. The image indicates that the chitosan spin coated films exhibit porosity even at a such a low number of layers. The roughness value was calculated three 30x30 μm images as the average roughness at five vertical lines; the average roughness value for bare chitosan films was 18.1 nm. AFM images for sol cast films are not available since samples must be attached to the substrate for measurement.

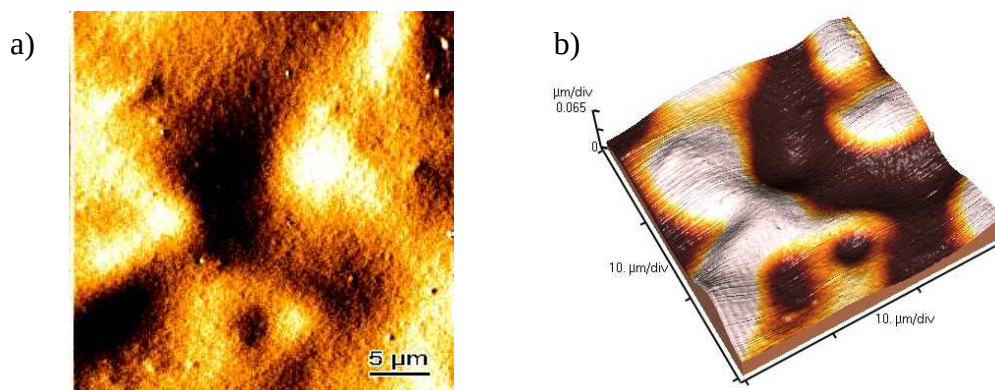


Figure 45 AFM phase, (a), and topography, (b), of bare chitosan film synthesized via spin coating with 1.75% w/v chitosan solution and 5 layers.

4.1.3. Films Thickness Measurements

Profilometry measurements for spin coated films with increasing numbers of layers and at increasing precursor polymer solution concentrations are presented in Figure 46a and Figure 46b, respectively. These measurements quantified the tendency which is visible to the naked eye: not only more layers but also higher chitosan concentrations increased the film thickness. Film thickness is critical to their mechanical strength; all films that were removed from the substrate without breaking were at least 10 μm thick.

The error bars indicate the standard deviation of the film thickness along the length of the film. The increase in standard deviation in films with 15 and 20 layers, Figure 46a, may be attributed to an uneven surface. This irregularity stemmed from the fact that the application of solution for each consecutive layer partially dissolved the layer below; furthermore this last layer took longer to dry as the film became thicker. In contrast the films with 1, 5, and 10 layers had relatively small standard deviation due to the smaller number of layers and the thinner film.

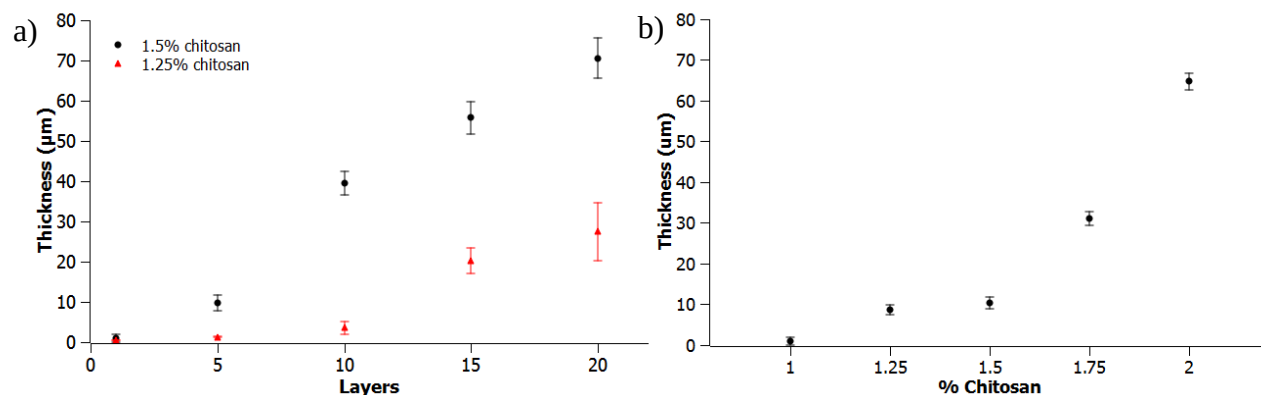


Figure 46 Thickness of spin coated films with various layers, (a), and concentrations of precursor solutions, (b).

4.1.4. Thermal characterization

4.1.4.1. Thermo-mechanical analyses

The TMA spectra for bare chitosan sol cast and spin coated films are shown in Figure 47; the corresponding glass transition temperatures of the sol cast film and the spin coated film were estimated at $81 \pm 3^\circ\text{C}$ and $74 \pm 5^\circ\text{C}$, respectively. These values are in agreement with those reported for bare chitosan sol cast films by Lazaridou (94°C by DMA) [26] and Quijada-Garrido (85°C by DMA and DSC) [30]. However, they are lower than the values established by other researchers: 153°C (DMA) [27], 170°C (DSC) [31], 196°C (DSC) [29] and 203°C (DMA and DSC) [25]. Reviewed works did not provide a T_g value determined by TMA.

The glass transition temperature of chitosan has been attributed to the torsional oscillations at the glycosidic bonds between the two glucosamine rings and a corresponding change in the hydrogen bonding. [30] The sol cast films exhibit a higher T_g than spin coated films which may be due their higher crystallinity (and therefore hydrogen bonding) as determined in by XRD.

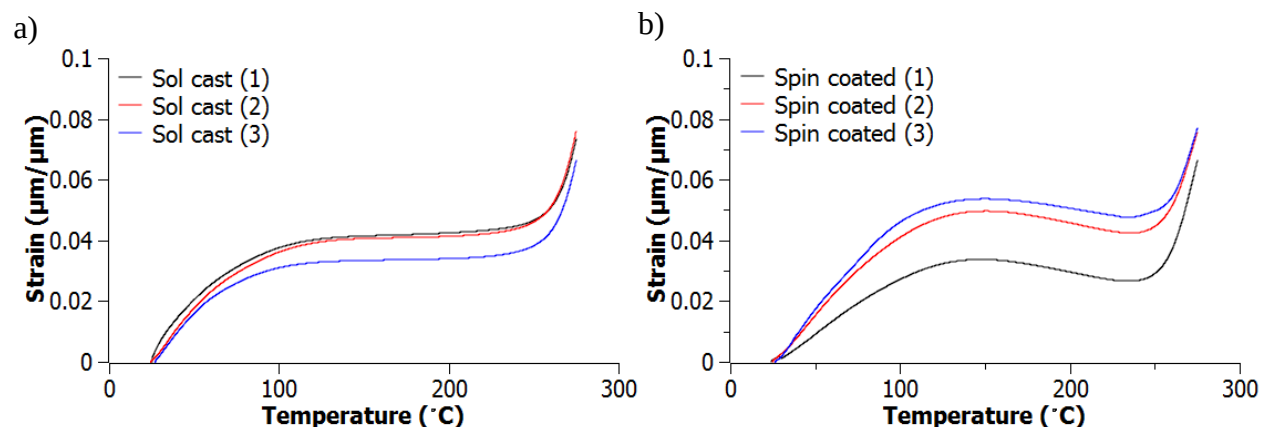


Figure 47 TMA spectra of bare sol cast, (a), and spin coated chitosan films, (b).

4.1.4.2. Thermogravimetric analyses

As a candidate for edible food coverings or for bactericidal applications in sterilized medical environments, chitosan films may be processed at elevated temperatures. On this basis, thermal degradation tests were run to determine the temperature at which bare chitosan films degraded. Figure 48 shows the thermal degradation curve corresponding to bare powder and bare sol cast and spin coated chitosan films in which degradation temperatures of 294, 281 and 292°C were established. These temperature values are relatively close to the temperature reported by Quijada-Garrido (260 °C). [30] The first derivative indicates that the process was endothermic, requiring an energy input to degrade the film. The weight loss at 125 °C may be attributed to the evaporation of water, and weight loss beginning around 290°C may be attributed to the degradation of the main chain. The weight loss beyond 320°C may be attributed to the formation of residue by the remaining carbon and the formation of methane. [30]

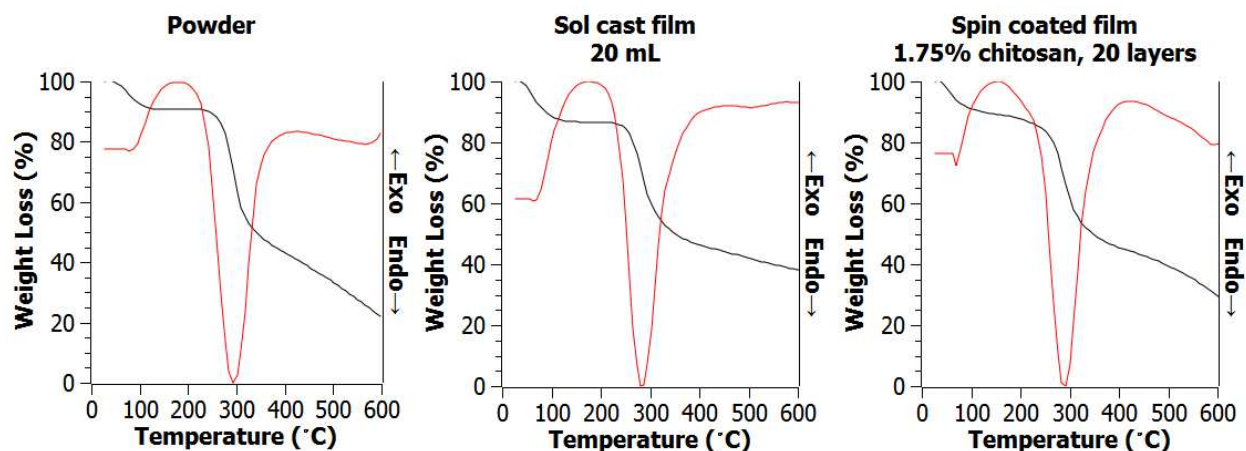


Figure 48 TGA spectra of chitosan powder, sol cast film and spin coated film.

In addition to the method of synthesis, the effects of chitosan concentration and number of layers of spin coated films on the degradation temperature were explored. The TGA spectra and the corresponding degradation temperatures are shown in Figure 49 and Table 9, respectively. Higher precursor polymer solution concentrations and increase in the number of layers produced, as expected, thicker films as confirmed via profilometry in Section 4.1.3. Taking this into account, it may be concluded that the thicker films degraded more slowly in the first stage (25 to 150°C) than the thin films. This occurrence may be attributed to the fact that the thicker films trapped more water inside the film and more energy was required to release the water from between the chitosan chains. In contrast, the thicker films had a more rounded shoulder in the second stage (150 to 300°C) indicating that more mass was lost before the onset of the degradation of the main chain.

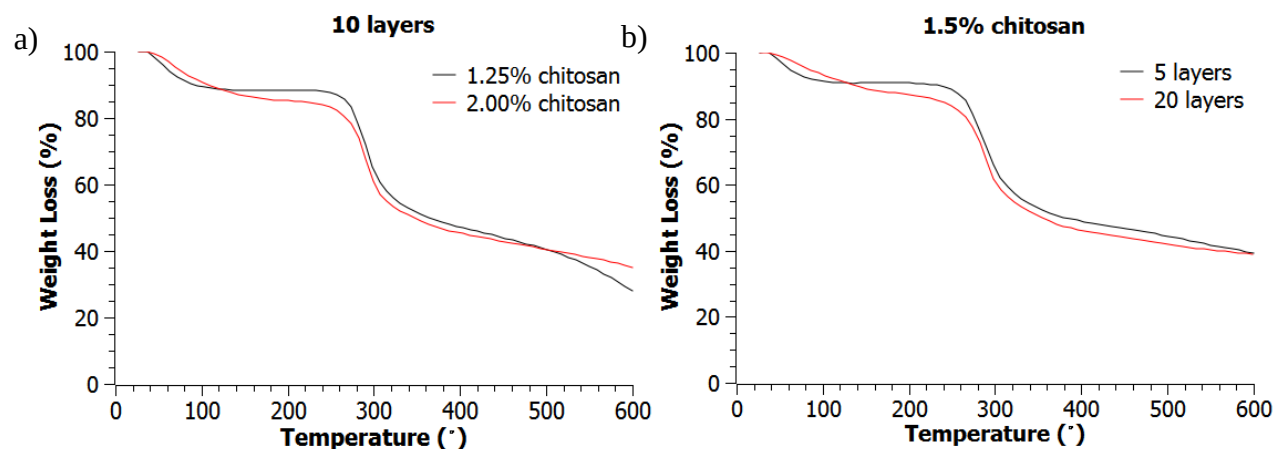


Figure 49 TGA spectra for chitosan spin coated films synthesized at different precursor concentrations, (a), and different numbers of layers, (b).

	T_{deg} (°C)
1.5% 5 layers	290
1.5% 20 layers	291
1.25% 10 layers	290
2.00% 10 layers	291

Table 9 T_{deg} of chitosan spin coated films

4.1.5. Conclusions

Bare chitosan films were synthesized via sol casting and spin coating techniques. The structural analysis indicated that sol cast films were more crystalline than spin coated films due to processing conditions; however, the chemical bonds within the polymeric chains in the films were not significantly affected by these processing conditions or by film thickness. An analysis of the film morphology revealed that the spin coated films are more porous than the sol cast films. Profilometry measurements confirmed that the thickness of spin coated films increased with the number of layers and the concentration of the precursor polymer solution. Thermomechanical analyses indicated that the glass transition temperature of the sol cast films ($81 \pm 3^\circ\text{C}$) is higher than that of the spin coated films ($74 \pm 5^\circ\text{C}$). Thermogravitational analyses set

the degradation temperature of the bare films at $289\pm4^{\circ}\text{C}$, and suggested that synthesis route does not exert a significant effect upon the T_{deg} .

4.2. Carbon black-bearing chitosan nanocomposites

4.2.1. Structural Characterization

4.2.1.1. X-ray Diffraction Analyses

XRD analyses of the chitosan-carbon black nanocomposites confirmed the formation of the expected structures synthesized by the sol casting (Figure 50a) and the spin coating (Figure 50b) synthesis routes. The broad XRD peaks centered on 10° and 20° corresponding to the (020) and (110) planes of chitosan were also encountered in the 1%, 3% and 5% weight fraction nanocomposites produced using both synthesis routes. As before, the spin coated films showed peaks of varying intensity, due to the rapid evaporation of the solvent. In this case, however, the spectra of the films containing carbon black agglomerates exhibited a lower signal to noise ratio, indicating that the carbon black disrupted the polymer's crystalline structure.

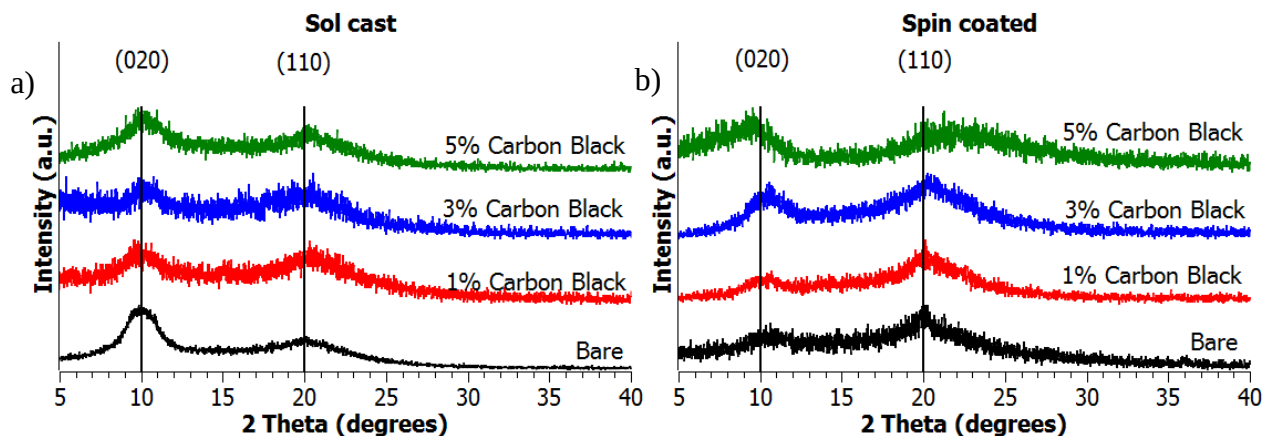


Figure 50 XRD spectra of sol cast, (a), and spin coated, (b), chitosan thin films containing carbon black.

4.2.1.2. Fourier Transform Infrared Spectroscopy Measurements

Figure 51 shows the FTIR spectra for bare chitosan and composite films containing 1%, 3% and 5% weight percentages of carbon black agglomerates produced by the sol casting and spin coating routes. The bands in the sol cast and spin coated films did not change with incorporation of carbon black, indicating that while there may have been a mechanical disruption of the chains, there was no chemical bonding between the chitosan chains and the carbon black disperse phase.

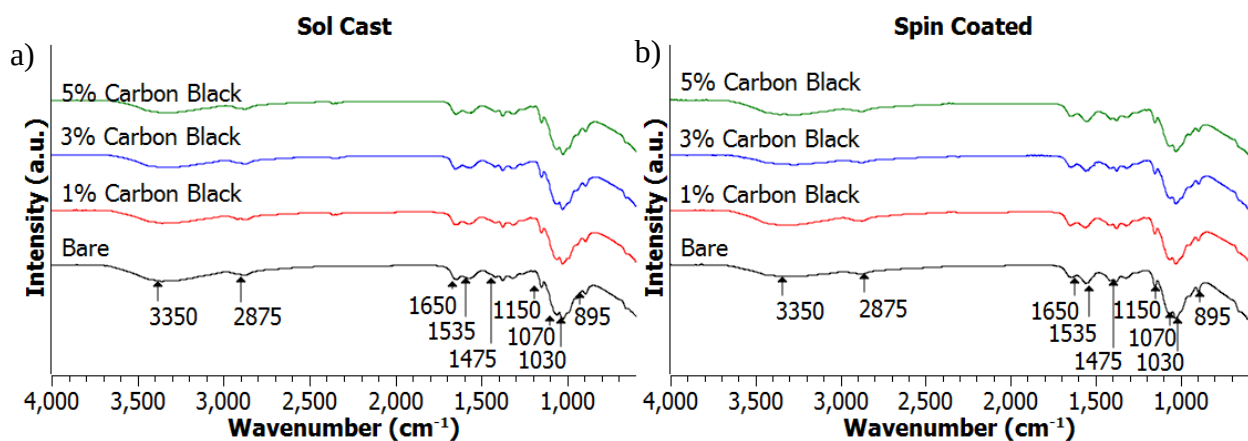


Figure 51 FTIR spectra for sol cast, (a), and spin coated, (b), chitosan films containing carbon black.

4.2.2. Morphological Characterization Analyses

4.2.2.1. Optical Microscopy

Composite chitosan films containing 1%, 3%, and 5% w/w carbon black were studied with optical microscopy at 5x magnification in order to qualitatively assess the film morphology and the dispersion of the carbon black agglomerates. Figure 52 shows the optical microscopy images corresponding to the sol cast and spin coated composites. Similar to the bare films, the spin coated films exhibited a remarkable porosity in comparison with the sol cast films, which showed a smoother surface. The porosity in spin-coated films was attributed to the partial

dissolution of the top layer by each subsequent application of solution and to the rapid solvent evaporation while drying on the hotplate. The film containing 3% carbon black shows greater porosity than the film containing 5% carbon black, which is likely due to the location in which the image was taken. The center exhibited pores, while the edges had exhibited “streaks”. This may be explained by the different shearing and centrifugal forces in each location during the spin coating process. In the center, the viscous forces are greater than the shear forces, forming a network containing pores; however at the edges, the shear forces are greater than the viscous forces, breaking the network and forming streaks. [87]

As defined in Section 2.2.2, the microdispersion is on the nanometric scale and affects the interaction between the polymer and the carbon black and the formation of a network between the carbon black particles via van der Waals forces. In contrast, the macrodispersion is on the micrometric scale and affects the appearance and the mechanical failure properties. In the carbon-black bearing chitosan films, a good macrodispersion of the carbon black can be observed for all weight fractions; even when there were carbon black clusters, they were homogenously distributed. Despite the agglomeration of carbon black aggregates and the resulting poor microdispersion, the particles were reasonably well dispersed within the polymeric matrix.

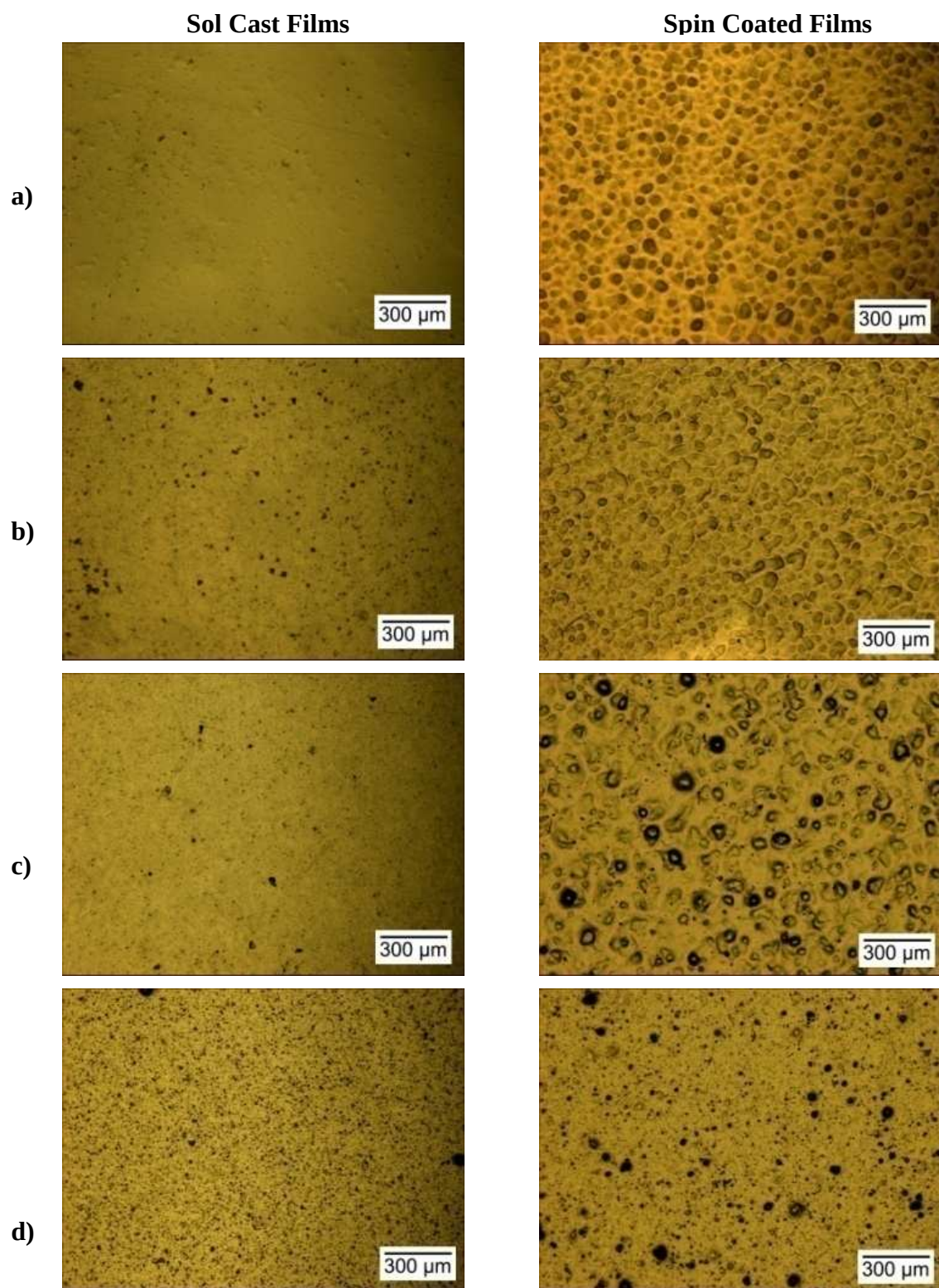


Figure 52 Optical images of chitosan sol cast and spin coated films containing (a) 0, (b) 1, (c) 3, and (d) 5% w/w carbon black.

4.2.2.2. Atomic Force Microscopy Analyses

AFM topography and phase images of chitosan films containing 0%, 1%, 3%, and 5% weight fractions of carbon black are shown in Figure 54. As in the bare films, the composite films were synthesized with 5 layers on a 25x25 mm substrate in order to be mounted in the AFM specimen holder. The roughness was calculated for each film according to the method explained in Section 3.3.2 and graphed in Figure 53. As expected, the roughness increased with an increase in the loading of carbon black. This increase may be attributed to the incorporation of more agglomerates due to higher loading and/or enlargement of agglomerate size.

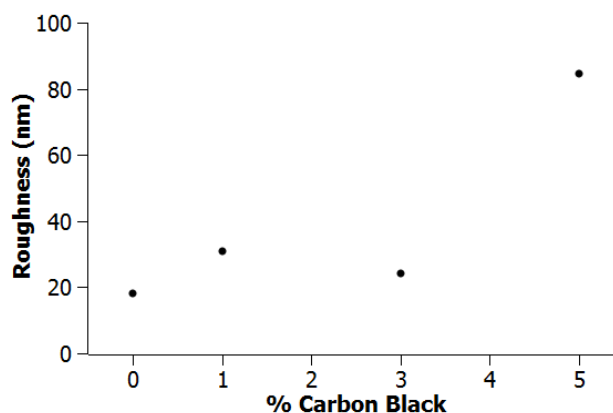


Figure 53 Roughness of spin coated films with carbon black.

The comparatively large aggregate diameter indicates that the carbon black, which is formed into pellets to facilitate customer handling, was not fully broken down during the magnetic mixing phase. More energy intensive mixing techniques such as ball milling or homogenization may be used to increase microdispersion of the carbon black within the polymer film.

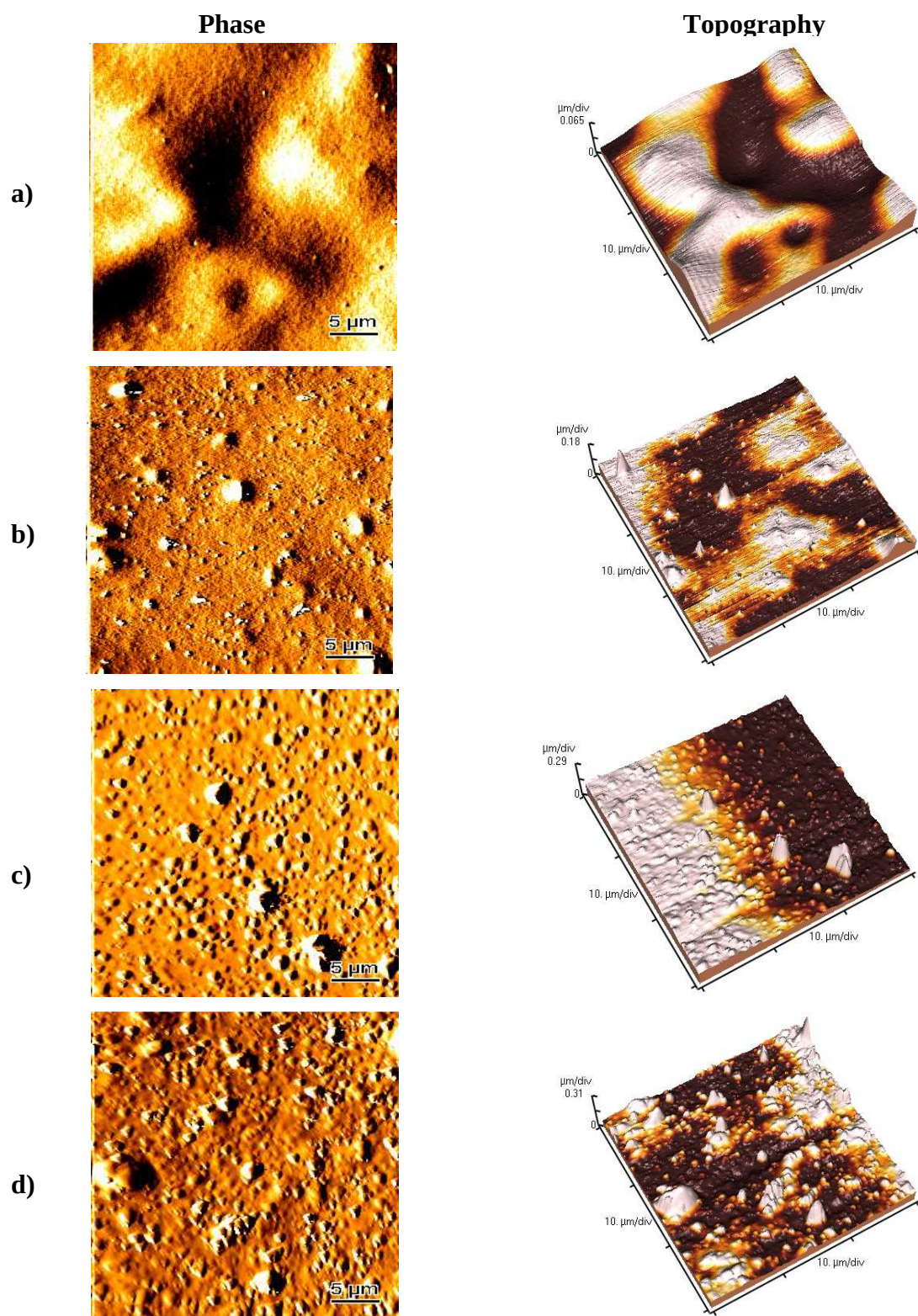


Figure 54 AFM of chitosan spin coated films containing (a) 0, (b) 1, (c) 3, and (d) 5% w/w carbon black.

4.2.2.3. Comparison of Film Thickness: Profilometer and Micrometer

Micrometer and profilometer measurements for spin coated films containing 0%, 1%, 3%, and 5% weight fractions of carbon black may be observed in Figure 55. Spin coated films were measured with both the profilometer when attached to the substrate and a micrometer when removed from the substrate; the thickness increased upon treatment with NaOH and removal from the substrate. Sol cast films were only measured with a micrometer, since they were not attached to a substrate.

Average sol cast film thickness ranged from 22 to 34 μm , and average spin coated film thickness as measured with the micrometer ranged from 60 to 99 μm . The thickness of the sol cast films is noticeably more homogeneous within a single film, showing that it is the superior synthesis method. An increase in the disperse phase was expected to increase spin coated film thickness since the addition of carbon black significantly increased the viscosity of the solution from which the films were synthesized; however it did not have a significant effect on film thickness.

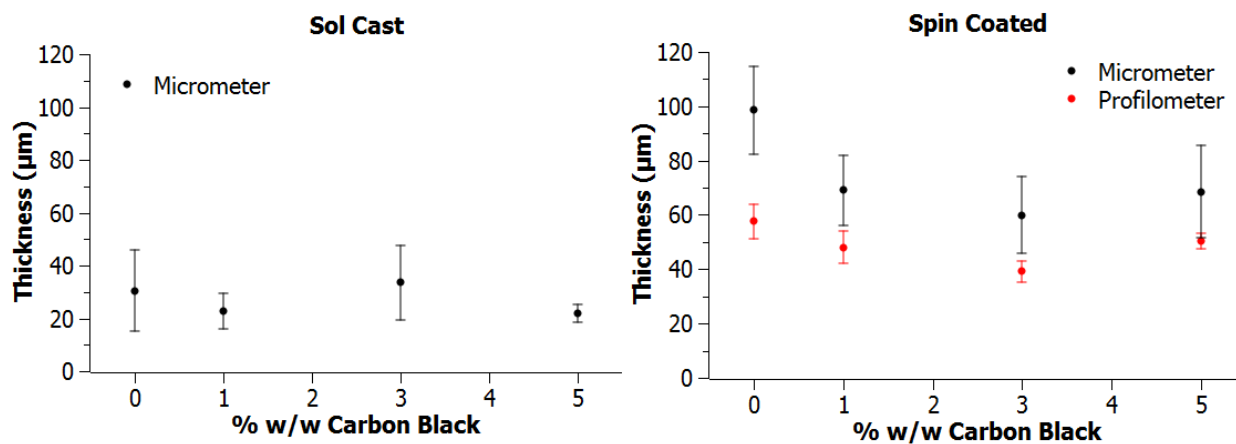


Figure 55 Thickness of chitosan films with carbon black synthesized via sol casting, (a), and spin coating, (b).

4.2.3. Thermal Characterization Measurements

4.2.3.1. Thermo-mechanical analyses

The TMA spectra for sol cast and spin coated films bearing 0%, 1%, 3%, and 5% weight fraction carbon black are displayed in Figure 56 and Figure 57 respectively. Three samples were run for each film to determine the reproducibility of the measurements; the resulting glass transition temperatures are summarized in Table 10 and graphed in Figure 58. The glass transition temperatures of the sol cast and the spin coated composite films ranged from 66 to 101°C. All spectra followed the same behavior.

While changes in the T_g may be attributed to the inhibition of the polymeric chain rotation due to the incorporation of the disperse phases within the chitosan matrix, no definite trend can be derived from the data due to the high standard deviation. As noted previously, the T_g occurs over a range of temperatures, since the length of the polymer chains contain multiple structures which are disrupted with various amounts of heat. [83] Therefore, it is conceivable that the calculated T_g varies between specimens from the same film. It is important to note that reviewed papers dealing with chitosan based composites did not carry out thermal stability test and thus the only possible comparison with the bare films.

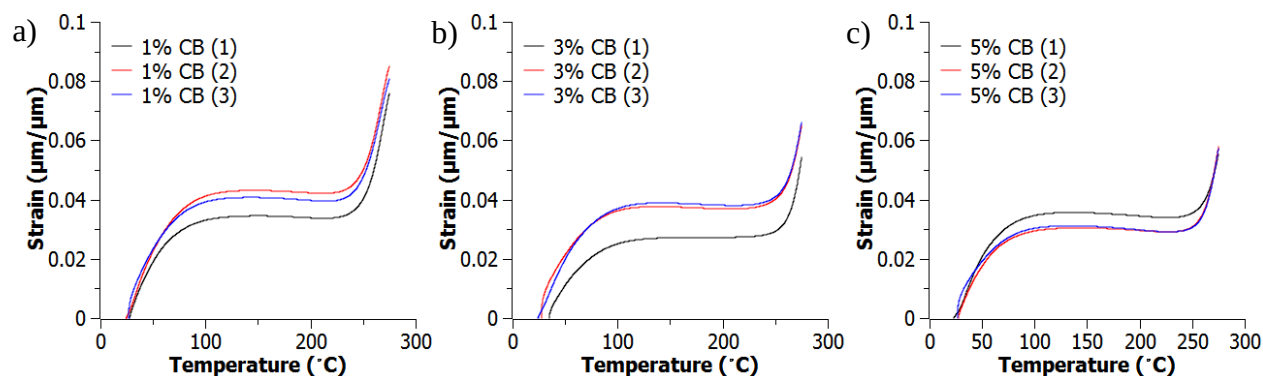


Figure 56 TMA spectra for chitosan sol cast films with (a) 1%, (b) 3%, and (c) 5% carbon black.

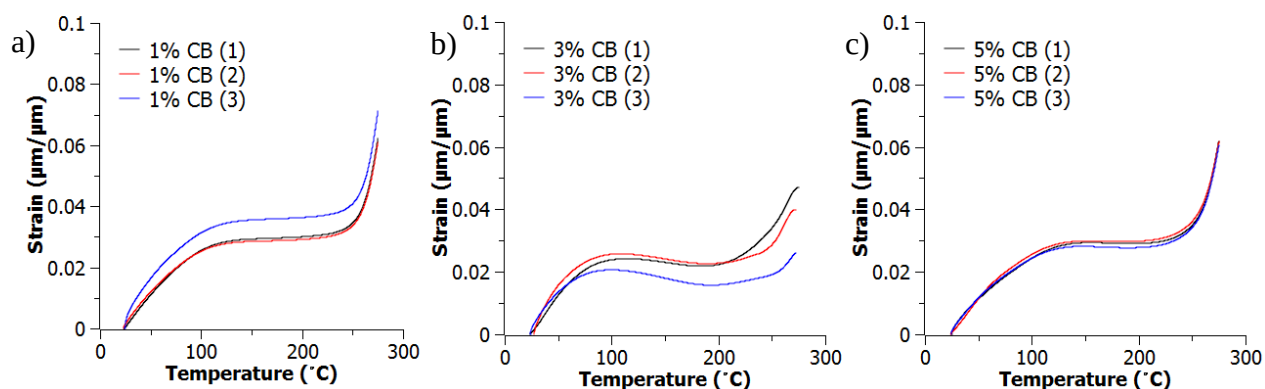


Figure 57 TMA spectra for chitosan spin coated films with (a) 1%, (b) 3%, and (c) 5% carbon black.

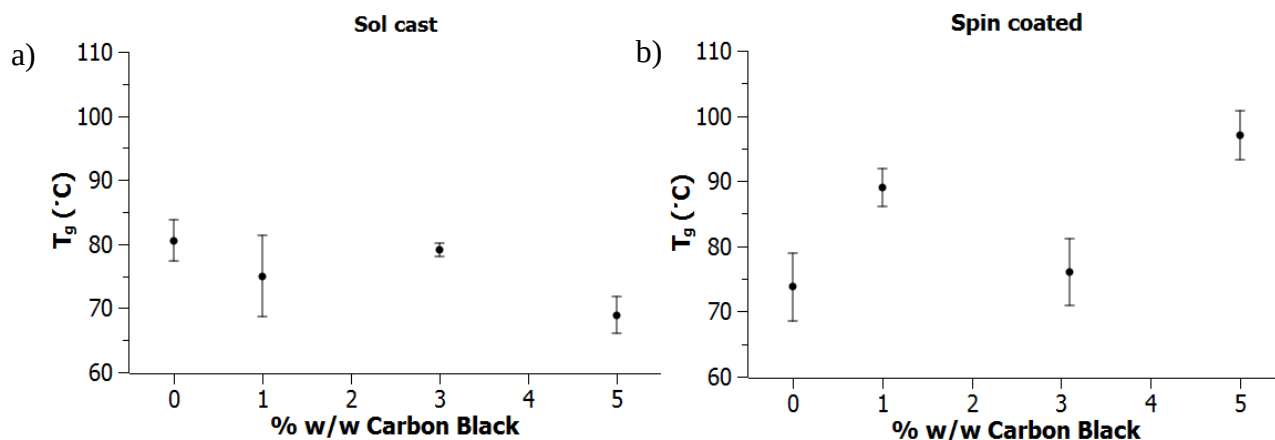


Figure 58 T_g of sol cast, (a), and spin coated, (b), chitosan films containing carbon black.

% w/w CB	T_g ($^{\circ}\text{C}$)	
	Sol cast	Spin coated
0	80.5 ± 3.2	75.1 ± 2.4
1	75.0 ± 6.4	89.4 ± 2.9
3	70.1 ± 1.1	75.8 ± 5.1
5	68.9 ± 2.9	97.0 ± 3.8

Table 10 T_g of chitosan films containing carbon black.

4.2.3.2. Thermogravimetric analyses

The thermal degradation curve of chitosan-carbon black composite sol cast and spin coated films are shown in Figure 59 and Figure 60, respectively; the corresponding degradation temperatures

are summarized in Table 11**Error! Reference source not found.** and graphed in Figure 61. The results showed that the produced composite films experienced a pronounced the greatest weight loss between 288 and 295 °C. No consistent trends are observed with an increase in the disperse phase, which may be attributed experimental error due to the small quantity of disperse phase. However, the average T_{deg} of the sol cast composite films ($293 \pm 4^{\circ}\text{C}$) was slightly higher than that of the bare film (281°C). While several works have investigated the dispersion of carbon nanoparticles in a chitosan matrix, none investigated the thermal properties of the resulting composites [72], [73]. However, the dispersion of carbon nanotubes in a polymer matrix has been reported to enhance the thermal stability of the resulting composites due to their excellent thermal conductivity. A thorough review of polymer nanocomposites revealed that an enhancement of the thermal stability usually occurs at lower nanoparticle loadings because at higher loadings the nanoparticles tend to agglomerate, reducing the contact area with the polymer and forming microparticles. [88]

From this study it may be concluded that these composite films may be used at temperatures up to 280°C without significant weight loss. While the proposed application of removal of heavy metals from water do not require such high temperatures, the thermal properties of these films will be important in other possible applications such as in electrodes, EMI shielding and sensors as mentioned in the literature review [73].

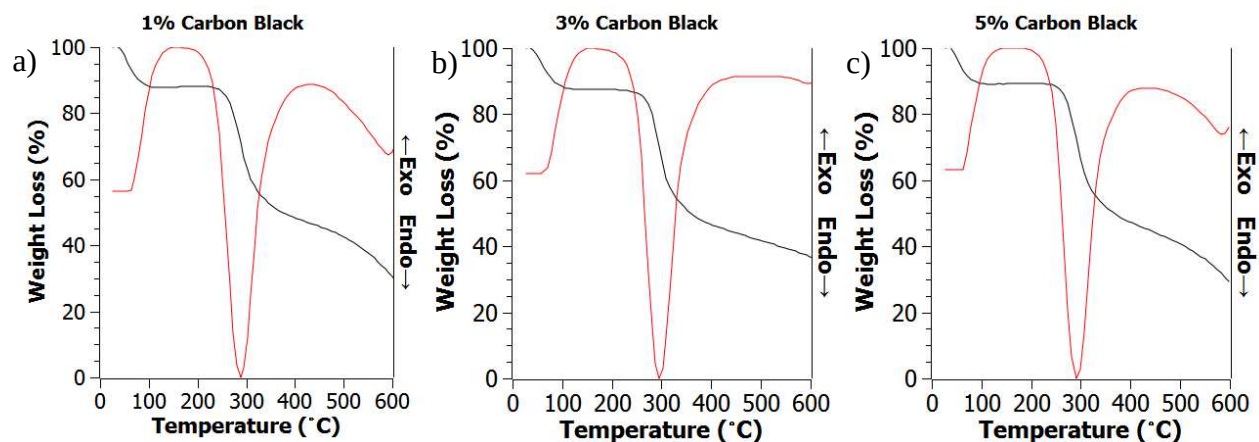


Figure 59 TGA spectra for chitosan sol cast films containing (a) 1%, (b) 3%, and (c) 5% w/w carbon black.

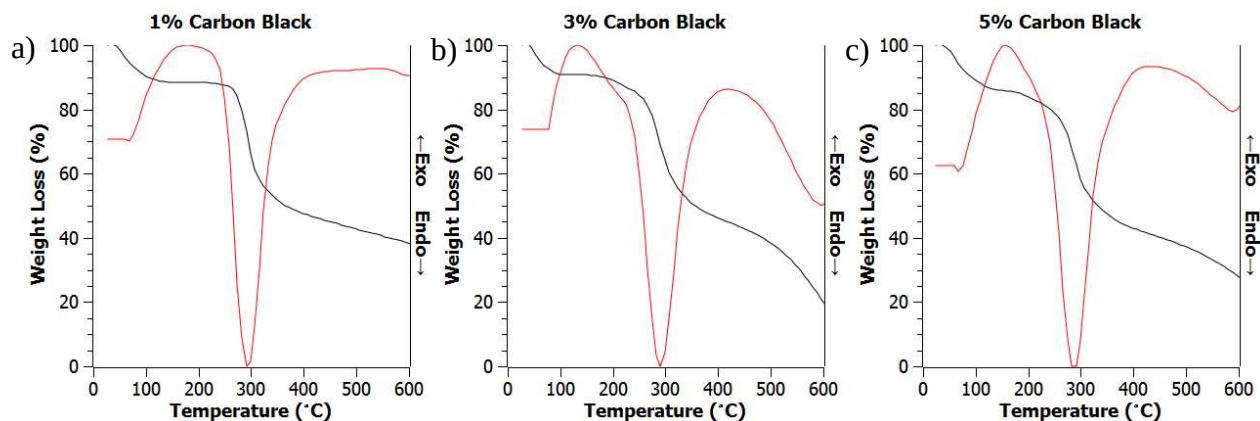


Figure 60 TGA spectra for chitosan spin coated films containing (a) 1%, (b) 3%, and (c) 5% carbon black.

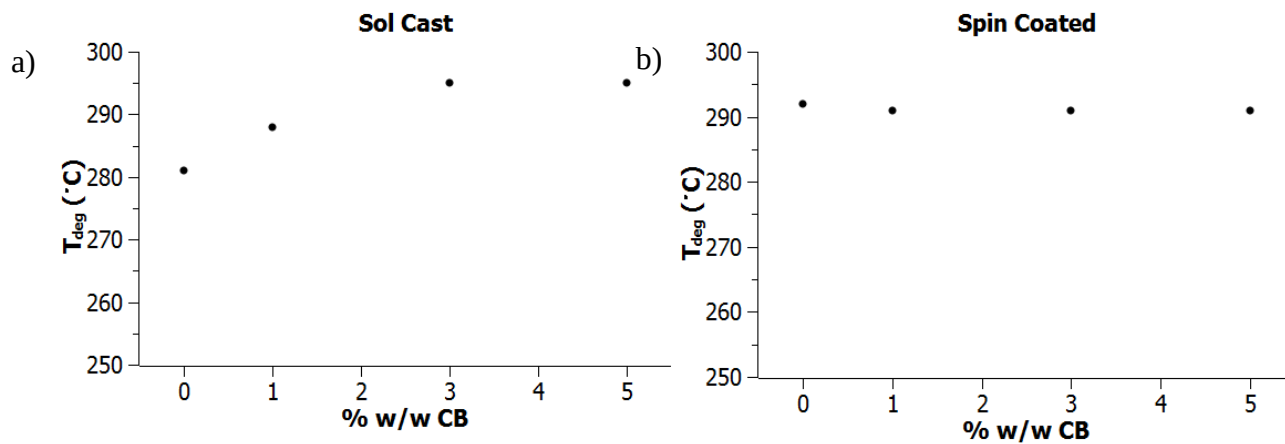


Figure 61 T_{deg} of sol cast, (a), and spin coated, (b), chitosan films containing carbon black.

CB concentration (% w/w)	T_{deg} (°C) in sol casted films	T_{deg} (°C) in spin- coated films
0	281	292
1	288	291
3	295	291
5	295	291

Table 11 T_{deg} of chitosan films containing carbon black.

4.2.4. Conclusions

Composite chitosan films containing 1%, 3%, and 5% w/w carbon black were synthesized via sol casting and spin coating. A structural analysis indicated that the carbon black was located between the polymer chains, disrupting the semi-crystalline polymer structure. However, FTIR spectrometry suggests that while there may be a mechanical disruption of the chains, there is no chemical bonding between the chitosan chains and the carbon black disperse phase. An analysis of the film morphology reveals that the spin coated composite films were more porous than the sol cast films. Optical microscopy and AFM indicate that the composite exhibits good macrodispersion but poor microdispersion. Due to the inclusion of the carbon black agglomerates, both film thickness and roughness increased with the highest loading of the disperse phase. Thermomechanical analyses indicate that the glass transition temperature varied from 66 to 101°C (or $81 \pm 10^\circ\text{C}$) and suggested that the inclusion of the disperse phase does not exert a significant effect upon the T_g . Thermogravitalational analyses indicated that degradation temperature of the carbon black bearing chitosan nanocomposite films ranged from 288 to 295°C (or $292 \pm 3^\circ\text{C}$), and that the T_{deg} of the sol cast films was higher than that of the spin coated films.

4.3. Silver-bearing chitosan nanocomposites

4.3.1. Structural and Optical Characterization

4.3.1.1. X-ray Diffraction Analyses

XRD analyses of the chitosan-silver nanocomposites synthesized via sol casting and spin coating are shown in Figure 62a and Figure 62b, respectively. The sol cast films show a very broad diffraction peak at 38° that can be attributed to nanocrystalline silver. The spin coated films did not exhibit any peaks; this may be attributed to the low concentration of AgNO_3 and the low reaction time and temperature involved with the synthesis of the films via spin-coating, leading to a low concentration of silver nanoparticles. However, the UV-vis measurements in the following section confirm the presence of silver nanoparticles both in the sol cast and spin coated films.

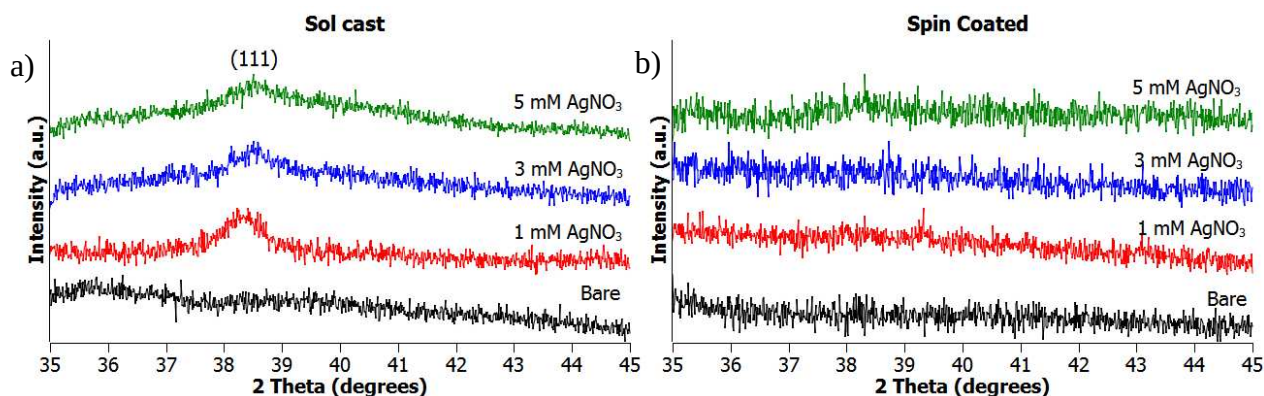


Figure 62 XRD patterns of chitosan sol cast, (a), and spin coated, (b), films with Ag nanoparticles.

4.3.1.2. Ultraviolet Visible spectroscopy measurements

The UV-vis spectra for Ag-bearing sol cast and spin coated films synthesized in presence of 0, 1, and 3 mM AgNO_3 are displayed in Figure 63 a and b, respectively. The films synthesized with 5 mM AgNO_3 are not shown because they exhibited saturation of the signal due to film thickness.

The sol cast films produced a narrow plasmon centered on 425 nm and the spin coated films produced a broad plasmon centered on 450 nm.

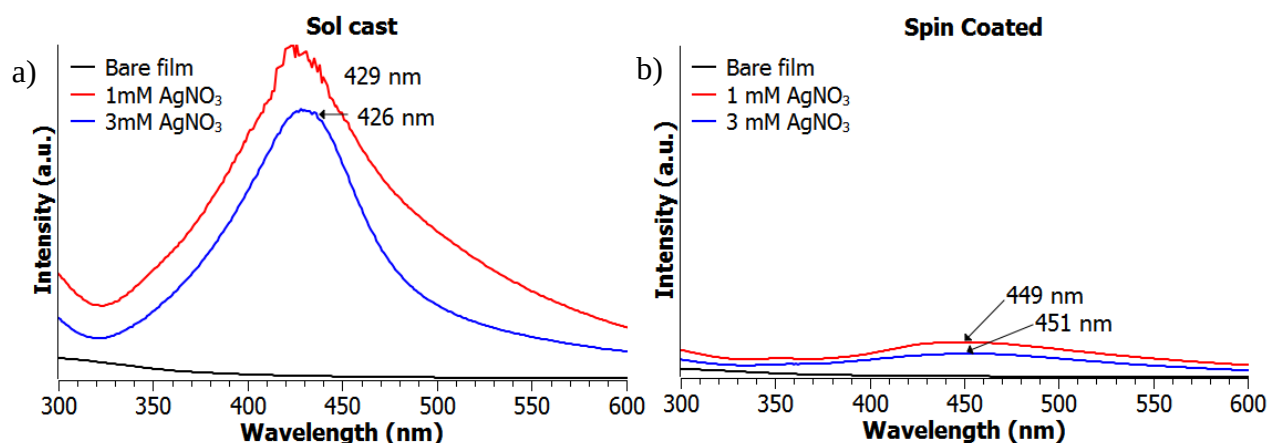


Figure 63 UV vis spectra of chitosan sol cast, (a), and spin coated, (b), film containing Ag nanoparticles.

Other research groups have reported the plasmon for silver nanoparticles reduced in a chitosan solution between 400 and 420 nm; these nanoparticles ranged from 10 to 13 nm in diameter [51],[50]. However, the plasmon for silver nanoparticles has also been reported at higher wavelengths (440-445 nm) [52],[53]. A narrow plasmon indicates monodisperse Ag nanoparticles, whereas an asymmetric broadening toward larger wavelengths indicates particle agglomeration. Therefore it may be concluded that both the sol cast and spin coated films contain silver nanoparticles; however, the silver particles in the spin coated films would be larger in size and in a smaller quantity than those in the sol cast films. The synthesis time, temperature and concentration affected the quantity of silver nanoparticles [51], suggesting that the 24 hours drying time at 60°C to synthesize the sol cast films may have contributed to the better formation and enhanced yield of silver. On the other hand, the 2 minutes drying time at 100°C between

layers of the spin coated films should not have been suitable for the complete formation of silver nanoparticles.

4.3.1.3. Fourier Transform Infrared Spectroscopy Measurements

Figure 64a and b show the FTIR spectra for bare chitosan and composite films synthesized with 1, 3 and 5 mM AgNO_3 via sol casting and spin coating, respectively. The bands in both the sol cast and spin coated films did not change with incorporation of silver nanoparticles, indicating that there is no chemical interaction between the chitosan chains and the disperse phase. This is not expected, since the functional groups of chitosan reportedly reduce the silver particles. In the literature, two research groups have reported a reduction in the band at 3400 cm^{-1} corresponding to $-\text{NH}$ and a new band at 1760 cm^{-1} . They attributed these changes to bonding between the silver and the amine group on the chitosan chain [7], [50]. Shifting in the bands corresponding to oxygen and nitrogen were considered due to an increase in bond length due to the coordination bond between the silver and the chitosan [7].

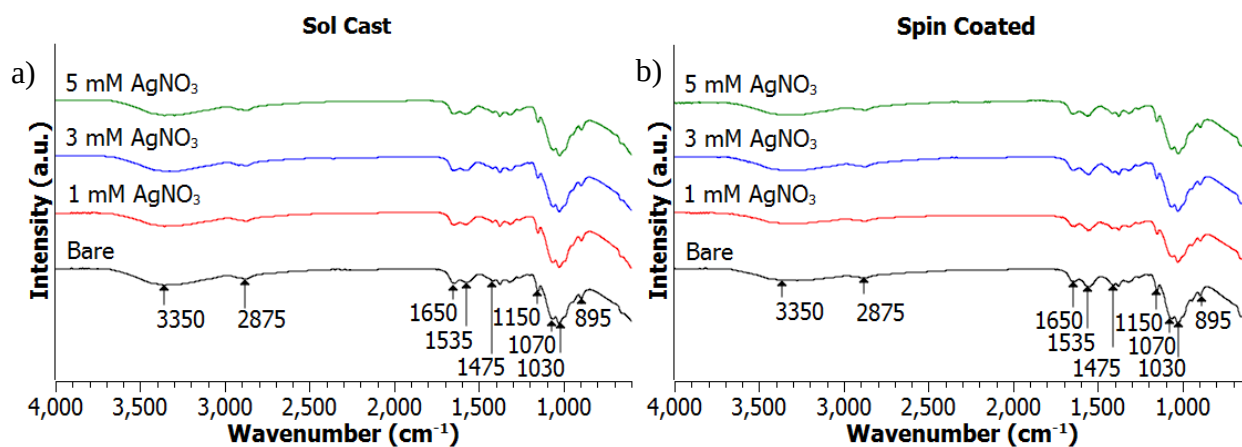


Figure 64 FTIR spectra of chitosan sol cast, (a), and spin coated, (b), film containing Ag nanoparticles.

4.3.2. Morphological Characterization

4.3.2.1. Optical Microscopy

Bare and composite chitosan films synthesized with 1, 3 and 5 mM AgNO₃ were studied with optical microscopy at 5x magnification in order to qualitatively assess the film morphology. Figure 65 shows the optical microscopy images corresponding to the sol cast and spin coated composites. Similar to the bare films, the spin coated films are more porous than the sol cast films due to the rapid solvent evaporation during the synthesis process and the partial dissolution of the top layer by each subsequent application of solution. The silver nanoparticles cannot be seen via optical microscopy, however at 100x (not shown) light is reflected by the films, giving the surface a slight brilliance that could be attributed to a fine deposit of metallic silver.

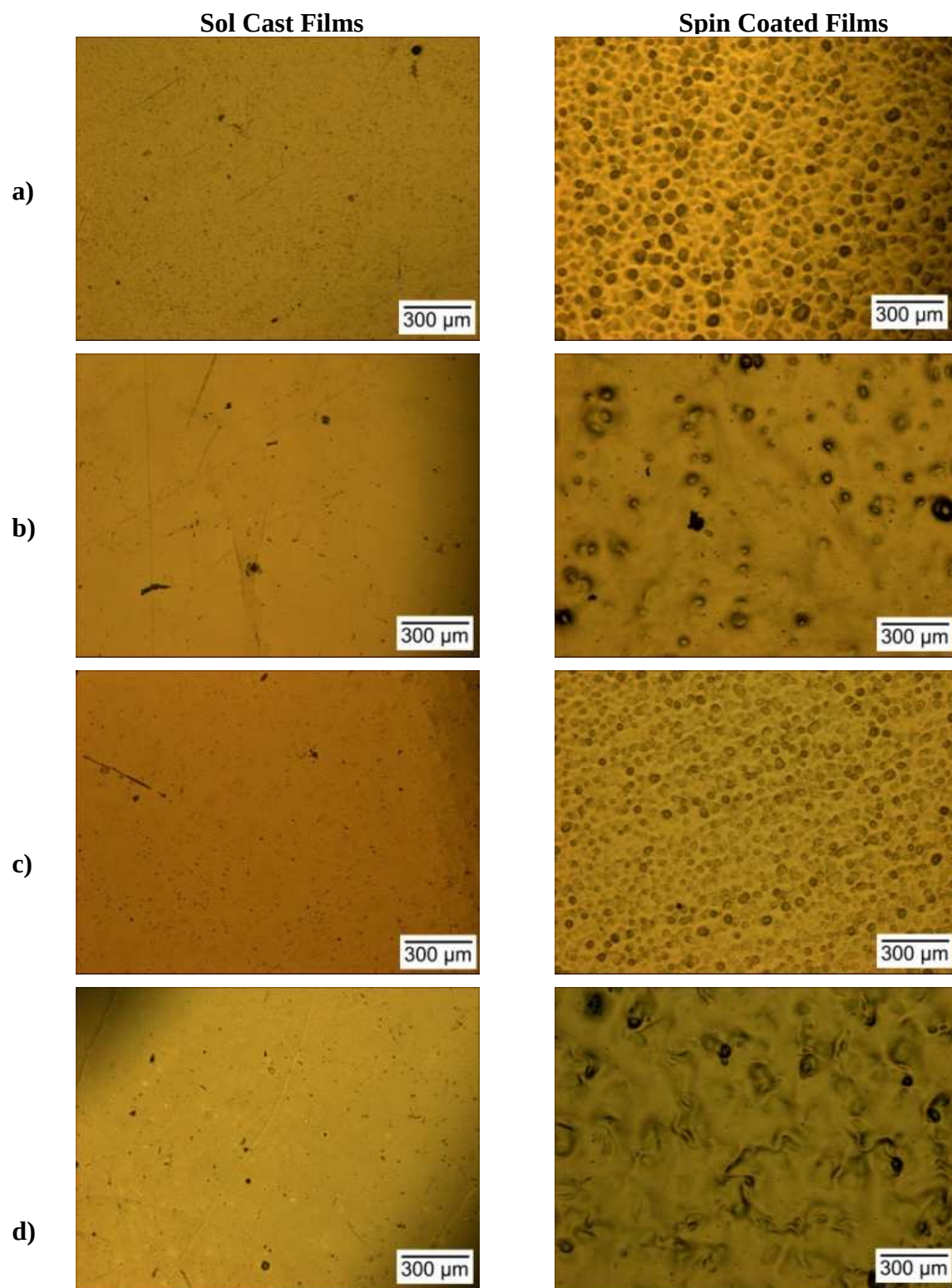


Figure 65 Optical images of chitosan sol cast films synthesized from solutions containing (a) 0, (b) 1, (c) 3, and (d) 5 mM AgNO_3 .

4.3.2.2. Atomic Force Microscopy Analyses

AFM topography and phase images of chitosan films synthesized with 1, 3 and 5 mM AgNO_3 are shown in Figure 67. As in the bare films, the films were synthesized with 5 layers on a 25x25 mm substrate in order to be mounted in the AFM specimen holder. The particles seen in the AFM images may be agglomerations of silver nanoparticles, however the bare film showed similar particles. The roughness was calculated for each film according to the method explained in Section 3.3.2 and graphed in Figure 66. No significant increase in the roughness of the films was noted with increasing silver particle loading; this may be attributed to the small quantity and size of particles formed.

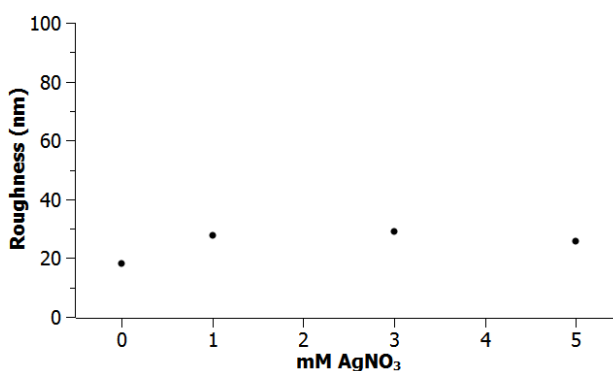


Figure 66 Roughness of spin coated chitosan films containing Ag nanoparticles

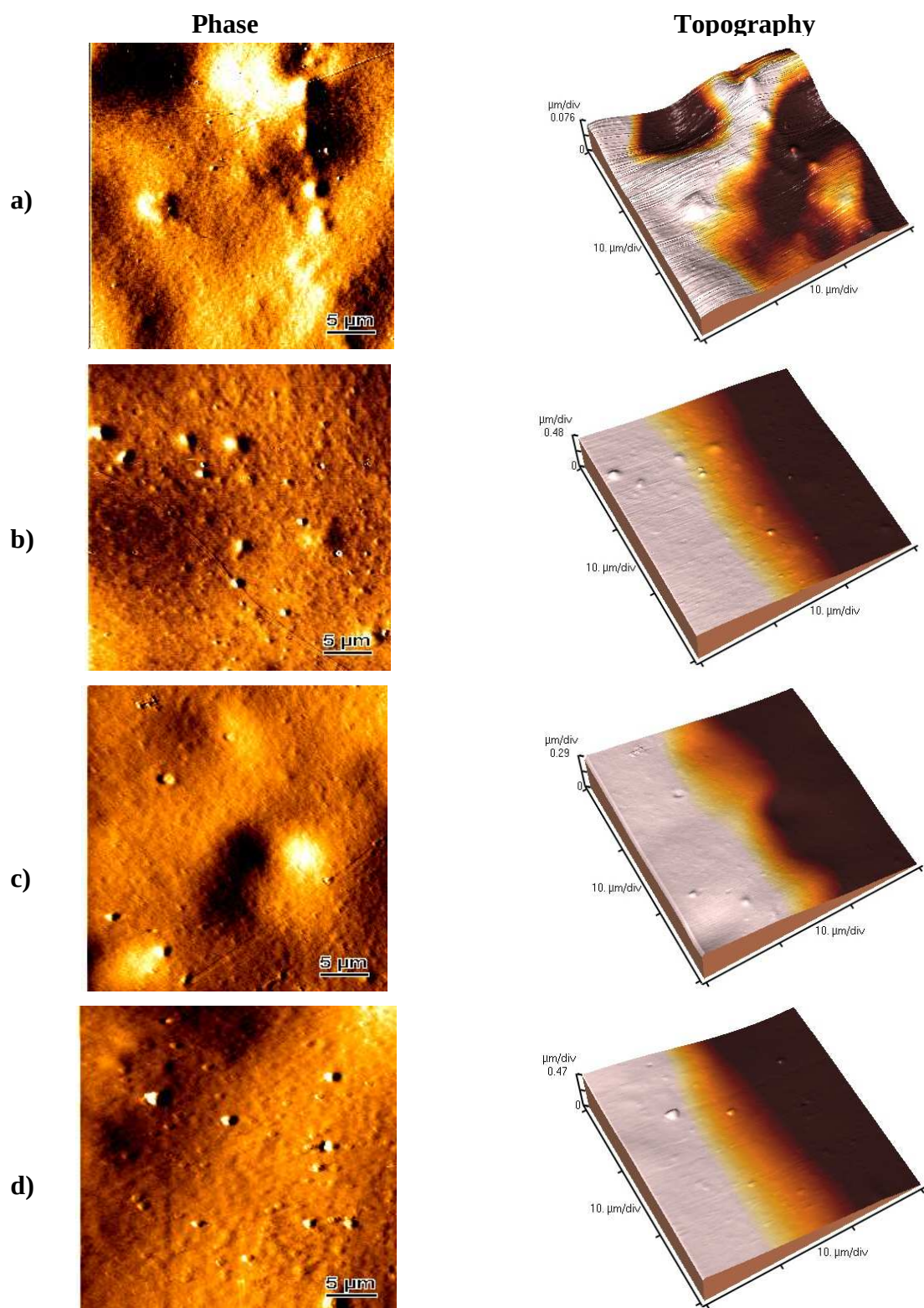


Figure 67 AFM of chitosan spin coated films synthesized from solutions containing (a) 0, (b) 1, (c) 3, and (d) 5 mM AgNO_3 .

4.3.3. Thickness measurements with profilometer and micrometer

Micrometer and profilometer measurements for sol cast and spin coated films synthesized from solutions containing 0, 1, 3, and 5 mM AgNO_3 may be observed in Figure 68. As with the chitosan films containing carbon black, spin coated films were measured with both a profilometer before removal from the substrate and a micrometer after removal from the substrate, while sol cast films were only measured with a micrometer. In almost all cases, the spin coated films increased in thickness after treatment with the NaOH and removal from the substrate. Average sol cast film thickness ranged from 27 to 35 μm , and average spin coated film thickness as measured with the micrometer ranged from 33 to 99 μm . The thickness of the sol cast films is noticeably more homogeneous within a single film and repeatable between various films; therefore, it may be selected as a superior synthesis method.

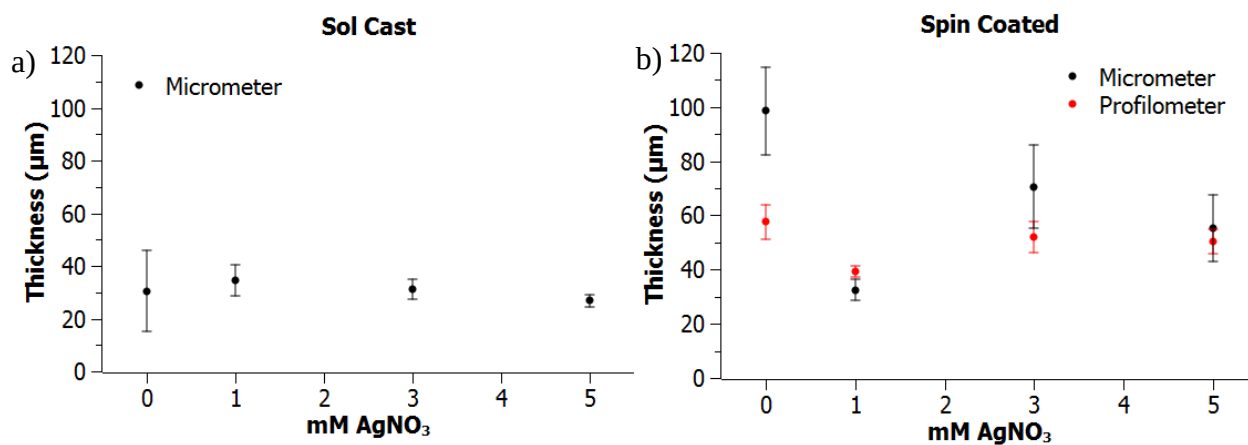


Figure 68 Thickness of chitosan films containing Ag nanoparticles synthesized via sol casting, (a), and spin coating, (b).

4.3.4. Thermal characterization

4.3.4.1. Thermomechanical analyses

The TMA spectra for sol cast and spin coated films synthesized with 0, 1, 3, and 5 mM AgNO_3 are displayed in Figure 69 and Figure 70, respectively. Three samples were run for each film to determine the reproducibility of the measurements; the resulting T_{deg} are tabulated in Table 12 and graphed in Figure 71. The glass transition temperatures of the sol cast and the spin coated composite films ranged from 68 to 100°C.

All spectra follow the same behavior, with the notable exception of the 3mM AgNO_3 spin coated film, where the slope at higher temperatures was less inclined. As determined by profilometry, this film is thinner than the others; as noted in the TGA, thinner films show a different degradation pattern. Since the number of silver particles is small as determined by XRD and UV-vis, it is reasonable that no significant trend should be seen.

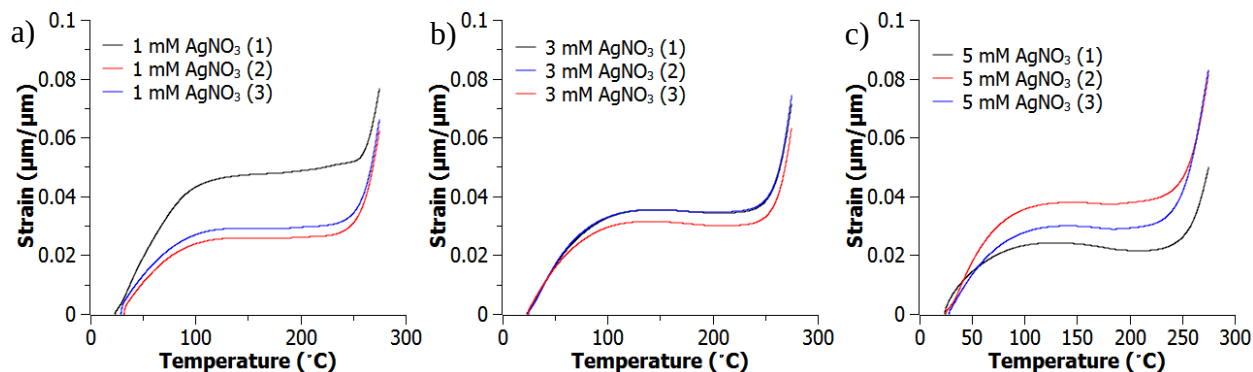


Figure 69 TMA spectra of chitosan sol cast films synthesized from precursor solutions containing (a) 1 mM, (b) 3 mM, and (c) 5 mM AgNO_3 .

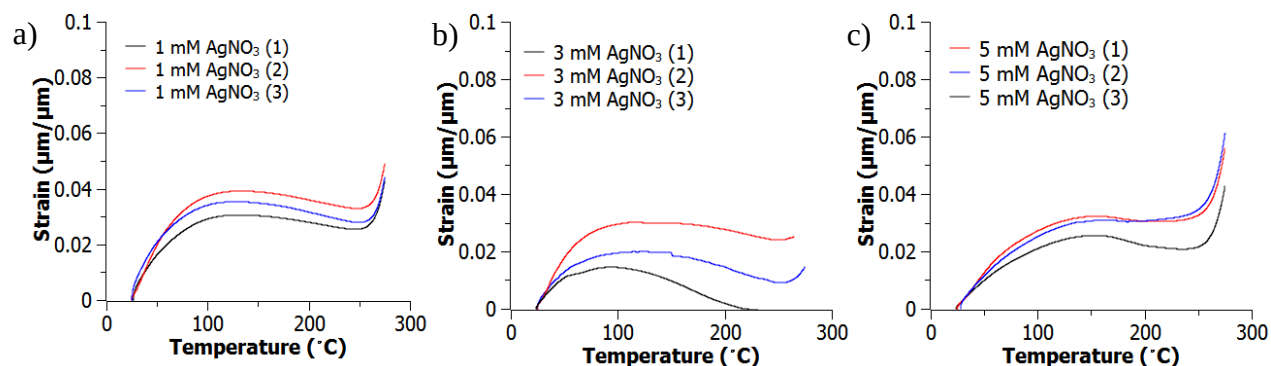


Figure 70 TMA spectra of chitosan spin coated films synthesized from precursor solutions containing (a) 1 mM, (b) 3 mM, and (c) 5 mM AgNO₃.

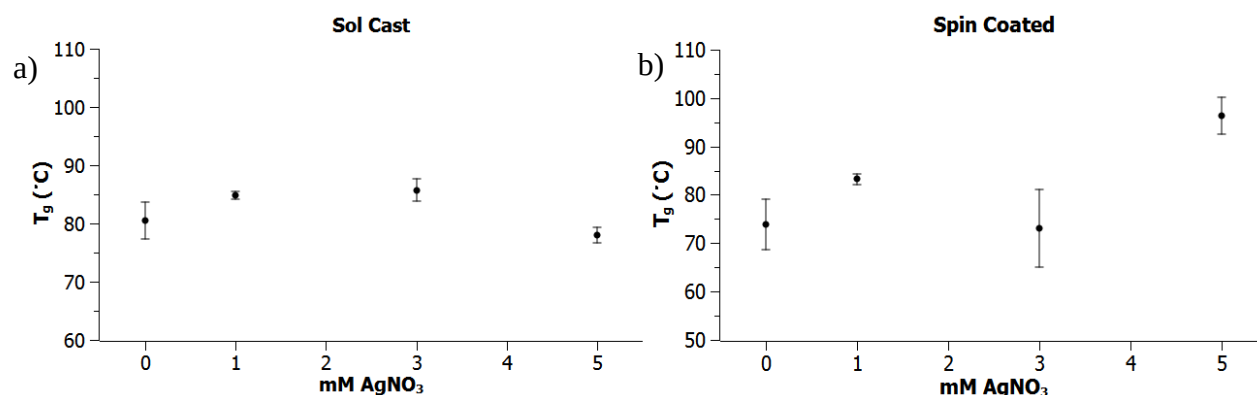


Figure 71 T_g of sol cast, (a), and spin coated, (b), chitosan films containing Ag nanoparticles.

mM AgNO ₃	T _g (°C)	
	Sol cast	Spin coated
0	81 ± 3	75 ± 2
1	85 ± 1	83 ± 1
3	86 ± 2	82 ± 8
5	78 ± 1	96 ± 4

Table 12 T_g of chitosan films containing Ag nanoparticles

4.3.4.2. Thermogravimetric analyses

The thermal degradation curve of chitosan-silver nanoparticle composite sol cast and spin coated films are shown in Figure 72 and Figure 73, respectively; the corresponding degradation

temperatures are tabulated in Table 13 and graphed in Figure 74. The results show that the produced composite films experienced the greatest weight loss between 282 °C and 299 °C.

As in the carbon black composite films, there was no definite trend in the degradation temperatures of the composite sol cast or spin coated films with an increase in silver nanoparticle loading; the average T_{deg} of the composites was $293 \pm 6^\circ\text{C}$ and that of the bare films was $286 \pm 8^\circ\text{C}$. This value is much lower than that of the chitosan films bearing silver nanoparticles synthesized by Vimala [7], but is similar to that of the bare chitosan films synthesized by Quijada-Garrido [30].

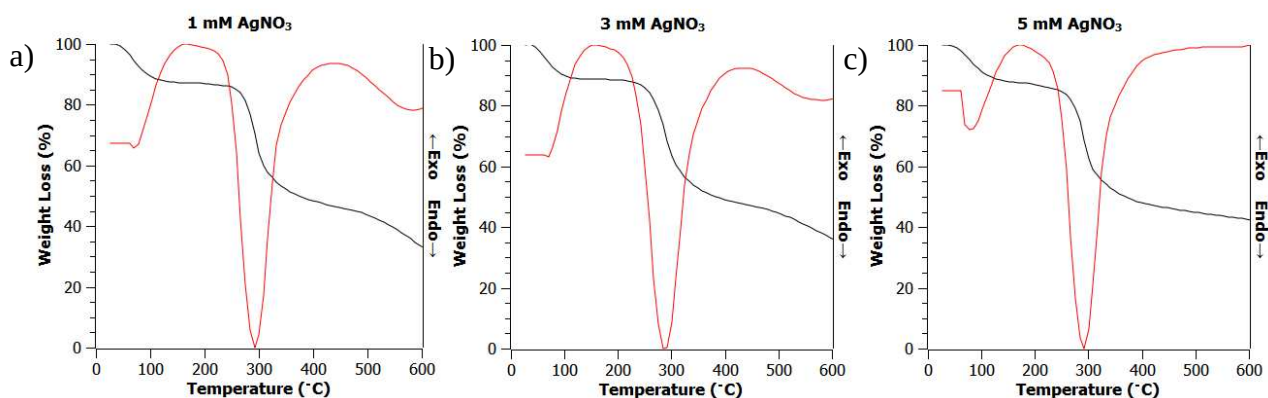


Figure 72 TGA spectra of chitosan sol cast films synthesized from precursor solutions containing (a) 1 mM, (b) 3 mM, and (c) 5 mM AgNO_3 .

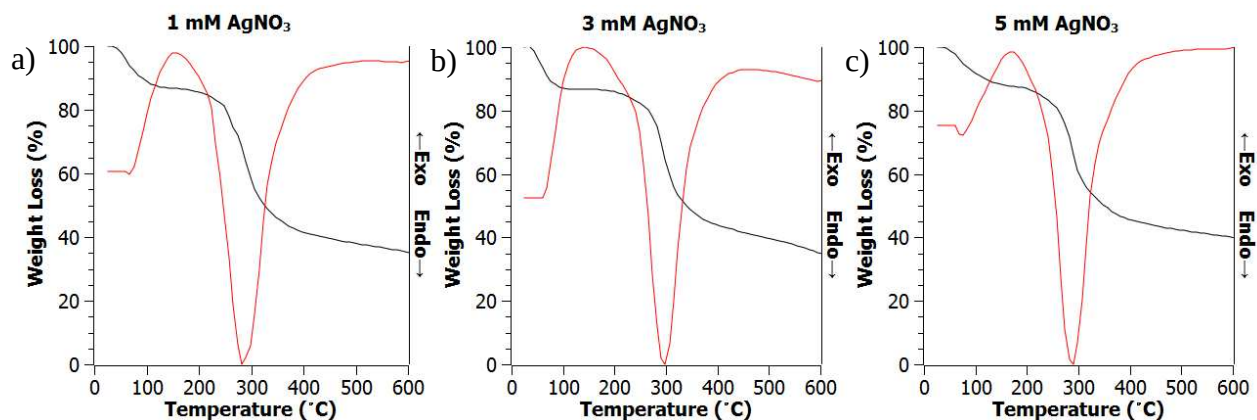


Figure 73 TGA spectra of chitosan spin coated films synthesized from precursor solutions containing (a) 1 mM, (b) 3 mM, and (c) 5 mM AgNO₃.

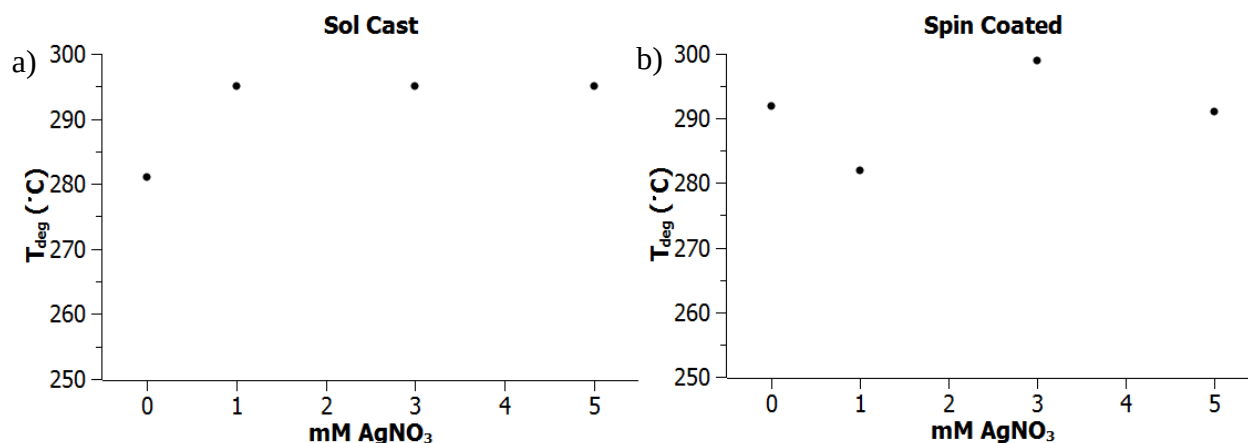


Figure 74 T_{deg} of sol cast, (a), and spin coated, (b), chitosan films containing Ag nanoparticles.

Ag concentration (mM)	T _{deg} (°C) in sol casted films	T _{deg} (°C) in spin-coated films
0	281	292
1	295	282
3	295	299
5	295	291

Table 13 T_{deg} of chitosan films containing Ag nanoparticles.

4.3.5. Conclusions

Composite chitosan films were synthesized with 0, 1, 3, and 5 mM AgNO₃ via sol casting and spin coating. An optical analysis confirmed the formation of silver nanoparticles in both the sol cast and spin coated films, and suggested that in the spin coated films small quantities of silver were formed and that the particles were agglomerated. A structural analysis confirmed the presence of silver nanoparticles in the sol cast films. FTIR spectrometry suggested that there was no chemical bonding between the chitosan chains and the silver particle disperse phase. An analysis of the film morphology reveals that the spin coated composite films were more porous than the sol cast films but the silver particles could not be seen. The roughness increased slightly

with the inclusion of silver nanoparticles, and the film thickness increased with the highest loading of the disperse phase. Thermomechanical analyses indicate that the glass transition temperature varied from 68 to 100°C (or $85\pm7^\circ\text{C}$) and suggested that the inclusion of the disperse phase does not exert a significant effect upon the T_g . Thermogravimetal analyses indicated that degradation temperature of the silver nanoparticle bearing chitosan nanocomposite films ranged from 282°C and 299 °C (or $293\pm6^\circ\text{C}$), and that the T_{deg} of sol cast films was higher than that of spin coated films.

5. Concluding Remarks

Bare and composite chitosan films were successfully synthesized via sol casting and spin coating. The thickness of the bare and composite films may be tuned by adjusting the synthesis parameters in both synthesis routes. Surface porosity and preferential alignment of polymer chains may be induced by selecting the appropriate synthesis route.

Microscopy techniques indicate a good macrodispersion but a poor microdispersion of the carbon black agglomerates. As suggested by the structural characterization, the inclusion of carbon black aggregates in the polyimide matrix inhibits the ordering of the polymeric chains. Optical spectroscopy indicates that silver nanoparticles may be reduced with chitosan.

While various studies have investigated the thermomechanical properties of bare chitosan sol cast films, there is still debate over the glass transition temperature and none of these studies determined the glass transition temperature using the technique thermomechanical analysis. In addition, none removed the spin coated films from the substrate for characterization and therefore no studies compared the thermal properties of both spin coated and sol cast chitosan films. Thermomechanical analysis results set the glass transition temperature of bare chitosan films at $78\pm4^{\circ}\text{C}$, that of the composite films bearing carbon black at $81\pm10^{\circ}\text{C}$, and that of the composite films bearing silver nanoparticles at $85\pm7^{\circ}\text{C}$. This indicates that the disperse phase may slightly increase the glass transition temperature of the composite films. These results agree with those of Lazaridou (94°C by DMA) [26] and Li (85°C by DMA and DSC) [30].

Thermogravimetric analysis indicates that the main polymer chain degrades at $286\pm8^{\circ}\text{C}$ for bare chitosan films, at $292\pm3^{\circ}\text{C}$ for composite films bearing carbon black, and $293\pm6^{\circ}\text{C}$ for composite films bearing silver nanoparticles. Therefore, it may be concluded that the incorporation of a disperse phase does not have a significant effect on the degradation of the

composite films. These produced chitosan nanocomposites may be used without any changes in their physical and structural stability for applications involving temperatures up to 70°C.

Appendix A – Synthesis and Characterization of Chitosan Beads

Introduction

As previously discussed in this work, chitosan is known to chelate heavy metals such as arsenic. Chitosan beads are optimal for this application due to their size and mechanical properties. On this basis, the synthesis and characterization was performed as elaborated in the following sections.

Literature Review: Absorption properties of chitosan beads

The following works discuss the capability of chitosan beads to remove toxic arsenic species from water. The absorption capacity of the composites reviewed in this section is listed in Table 14.

Adsorbent	Capacity from Langmuir model (mg As /g composite) pH in parenthesis		Reference
	As(III)	As(V)	
Chitosan beads	1.83 (5.0)	1.94 (5.0)	[37]
Iron coated chitosan flakes	16.1 (7.0)	22.5 (7.0)	[89]
Chitosan coated alumina	56.5 (4.0)	96.46 (4.0)	[36]

Table 14 Arsenic adsorption

In a study using pure chitosan beads, Chen et. al. found that at an initial pH of 5 the arsenic adsorption capacities for As(III) and As(V) were 1.83 and 1.94 mg As / g chitosan respectively. The beads were desorbed with H₂SO₄ and recycled up to 15 times. [37]

A study on the removal of arsenic from groundwater using iron–chitosan composites was conducted by Gupta et al [89] Both beads and flakes were synthesized with 13% iron. At a pH of 7, flake adsorption was 16.15 mg/g for As(III) and 22.47 mg/g for As(V) respectively. The bead adsorption was 2.32 mg/g for As(III) and 2.24 mg/g for As(V) respectively. The arsenic adsorption levels for both of these composites are higher than that of plain chitosan, indicating

that iron nanoparticles increase arsenic adsorption. Further investigation should be done to discover why the adsorption by the flakes is an order of magnitude higher than that of the beads. [89]

Boddhu coated beads of alumina with chitosan in an effort to increase efficiency since both chitosan and alumina adsorb arsenic. The resulting grains were roughly spherical with diameters between 100 and 150 μm . Although chitosan dissolves in acidic solutions, the chitosan composite does not; this property is desirable as filters must withstand flow and must be removed from the solutions intact. Adsorption was best modeled by the Langmuir model which assumes one layer of adsorption. Maximum arsenic adsorption was 56.5 mg As(III) / g chitosan and 96.5 mg As(V) / g chitosan, much higher than the values for pure chitosan. However, the fact that it can form hydrogen bonds with sodium hydroxide (NaOH) was used to remove the arsenic from the composite for recycling. [36]

Based on this information and the reported capability of carbon black to remove aqueous contaminants including arsenic as previously presented in this thesis, carbon black will be added to the chitosan based beads in order to increase the sorption capabilities of the resulting composites.

Materials selection

The only additional material used in the synthesis of the beads is glutaraldehyde ($\text{OHC}(\text{CH}_2)_3\text{CHO}$, 50%, Sigma Aldrich), which is the most common substance used to crosslink chitosan. Crosslinking prevents the resulting composites from dissolving in acidic solutions and increases the mechanical properties. The proposed mechanisms of cross linking are the Michael-type adducts with terminal aldehydes and the Schiff base formation as shown in Figure 75. [90]

The reaction scheme illustrates the synthesis of a polyacetaldehyde-terminated poly(2,6-dimethyl-1,3,5-trioxane) (PDMT-PA) through two main steps:

a) The first step involves the reaction of a poly(2,6-dimethyl-1,3,5-trioxane) (PDMT) intermediate with an amine group (NH₂) and acetaldehyde (CH₃CHO). The amine group reacts with the carbonyl carbon of acetaldehyde, forming a Schiff base intermediate (an imine).

b) The second step involves the reaction of the Schiff base intermediate with acetaldehyde. This step leads to the formation of a polyacetaldehyde chain terminated by a PDMT group, resulting in the final product, PDMT-PA.

Materials Synthesis

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synthesized with 2 M NaOH were the only ones that did not break when submerged in the GLA solutions, therefore they were the only ones fully characterized and are the results presented in the current work. After further testing, it was determined that beads synthesized at 1 M NaOH and 0.025 GLA were not affected by the stirring process. Therefore, the composite beads were synthesized with 1 M NaOH and 0.025 M GLA, in order to conserve reagents. The final synthesis route for the composite beads is summarized in Figure 76.

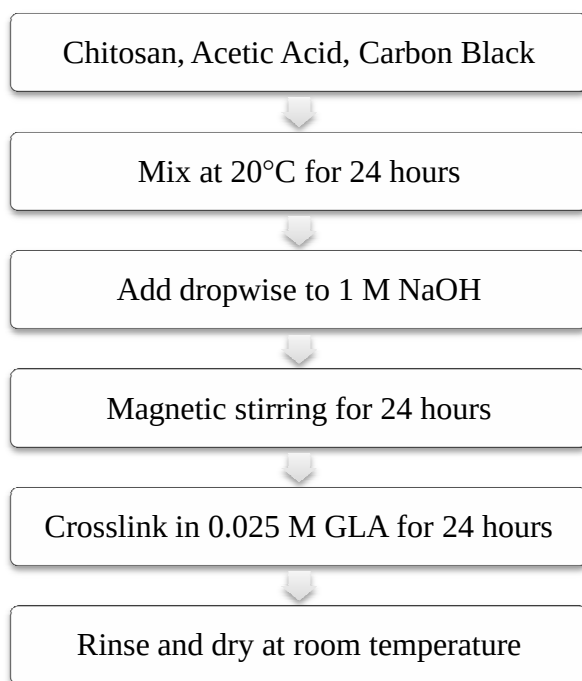


Figure 76 Synthesis method for chitosan beads

Materials Characterization

The stereomicroscope shown in Figure 77 was used to measure the size of the beads. Similar to the optical microscope, it provides morphological sample information by focusing the image with refractive glass or quartz lenses. However, the stereomicroscope only provides up to one tenth the magnification of an optical microscope and uses incident light illumination instead of

transillumination; this model provides magnification up to 11x. Current models also display a digital image on a computer screen via a charge coupled device.

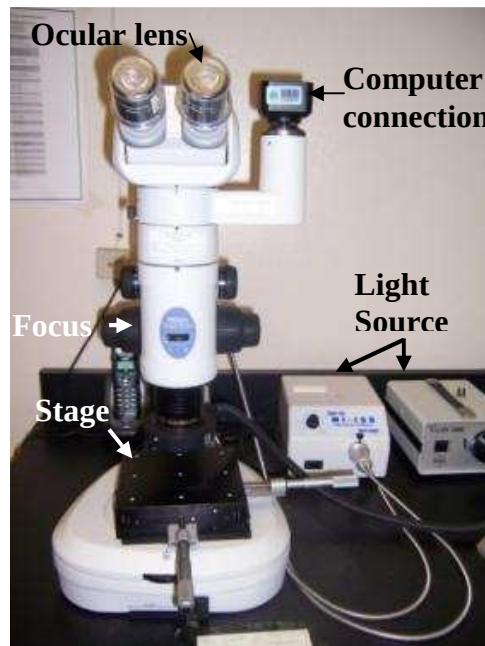


Figure 77 Nikon SMZ 1500 stereomicroscope – Department of Engineering Science and Materials at UPRM

Results

X Ray Diffraction analyses

XRD analyses of the chitosan matrix confirmed presence of chitosan in the synthesized beads. As seen in Figure 78, broad peaks centered and 20° corresponding to the structure of chitosan indicated a short-range molecular order. While the peak corresponding to the (020) plane that appears in the chitosan powder does not appear in the bare chitosan beads, it is present in the spectra of the composite beads as seen in Figure 78.

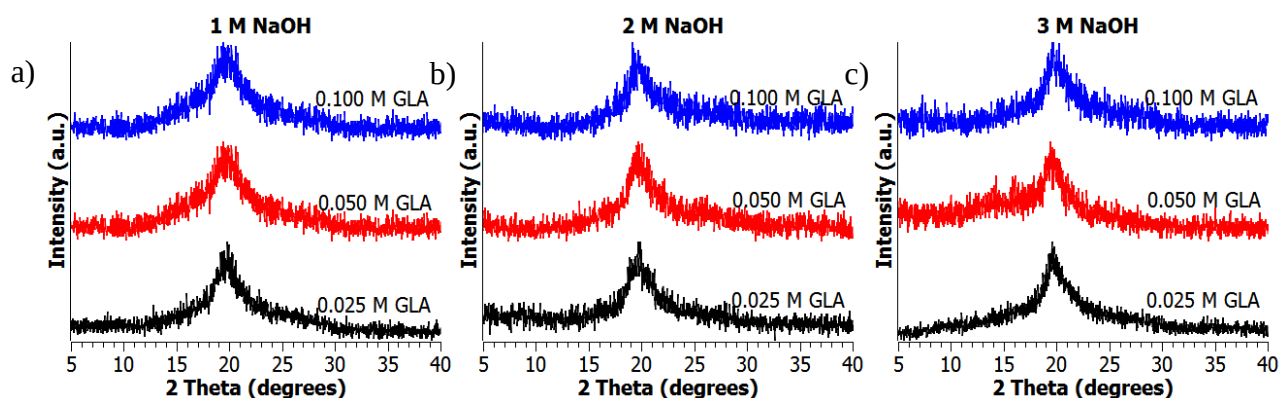


Figure 78 XRD of chitosan beads synthesized with 1 M NaOH, (a), 2 M NaOH, (b), and 3 M NaOH, (c).

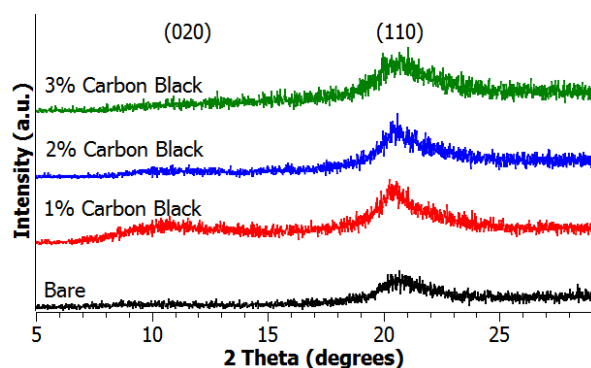


Figure 79 XRD of chitosan beads containing carbon black

Fourier Transform Infrared spectroscopy measurements

The Fourier Transform Infrared spectra shown in Figure 80 provide an analysis of the chemical bonds in the beads. The peaks corresponding to amide groups (3350 cm^{-1}) and the C-H bending (1475 cm^{-1}) changed slightly in intensity due to the crosslinking by GLA.

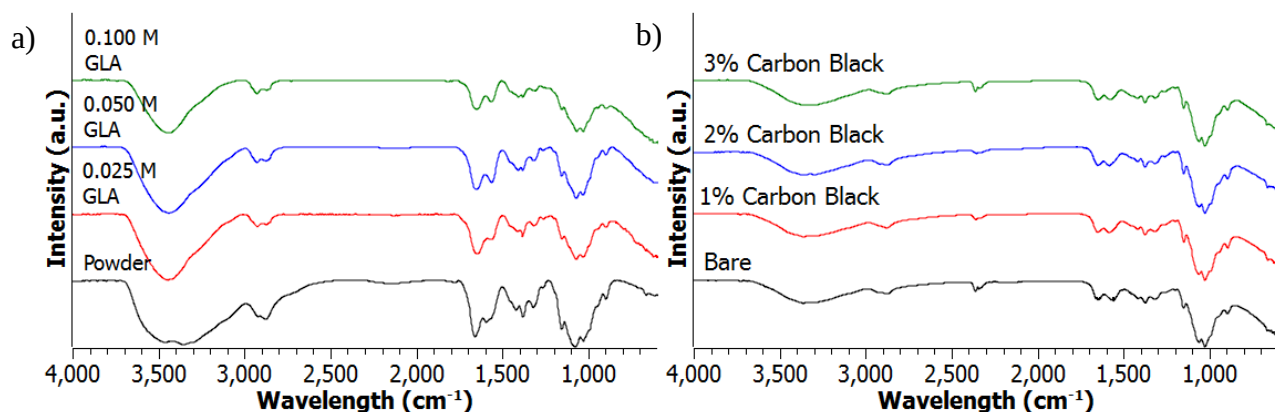


Figure 80 FTIR spectra of bare beads synthesized with 2 M NaOH (KBr pellet), (a), and b) beads containing carbon black (ATR), (b).

Optical Microscopy

Images of the beads were taken with a stereomicroscope and imported into GIMP [91] where a scale was imposed onto the image. Subsequently, 50 diameters were measured with the software Image J [92] and the histogram was created. The beads were relatively uniform; the mean diameter was 1.12 mm, 1.13 mm, and 1.17 mm for the beads synthesized with 2 M NaOH and 0.025 M, 0.050 M, and 0.100 M GLA respectively. Figure 81 shows the stereomicroscope image and the histogram for the beads synthesized using 2M NaOH and 0.025M GLA. Likewise, Figure 81 displays the images and the histograms of the bead diameters for the chitosan beads bearing carbon black. The average bead diameter for the composite beads were 1.6 mm, 1.7, 1.7, and 1.7 mm for 0, 1, 2, and 3% carbon black loading, respectively. These beads were larger due to the fact that they were synthesized without a hypodermic needle at the end of the tubing. Therefore it may be concluded that the size of the bead may be controlled based on the size of the tube and/or needle during the synthesis process.

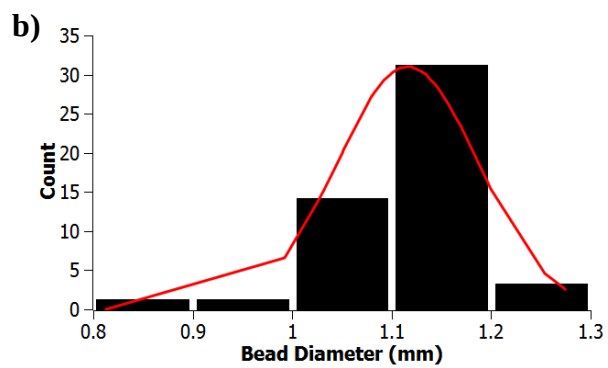
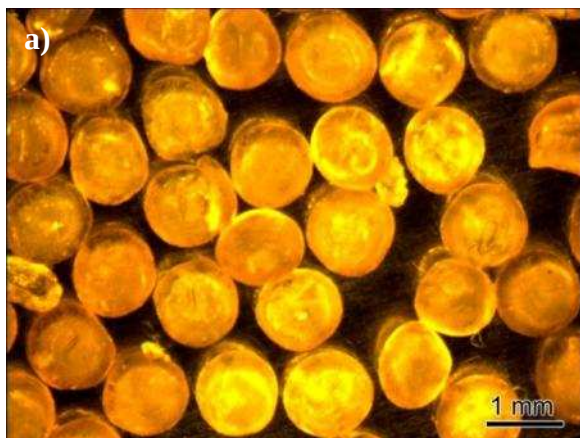


Figure 81 a) Stereomicroscope image and b) size distribution of chitosan beads

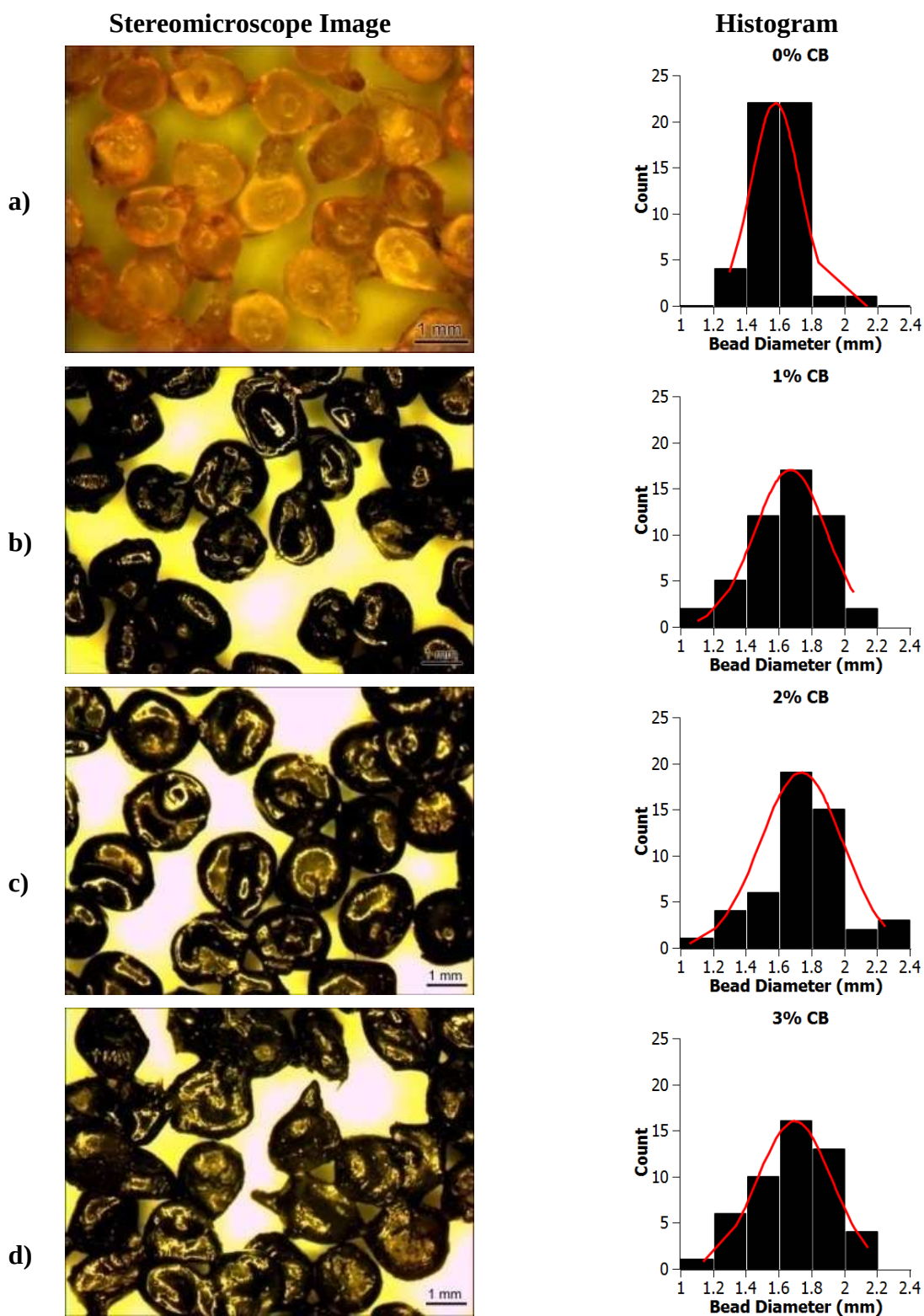


Figure 82 Sterioscope images and size distribution of beads containing (a) 0, (b) 1, (c) 2, and (d) 3% w/w carbon black.

Thermogravitalational Analysis

The TGA spectra for bare chitosan beads synthesized under varying conditions are shown in Figure 83. The spectra for chitosan beads containing 1, 2, and 3 % w/w carbon black are shown in Figure 84; the degradation temperature of the bare beads was 274°C and the average temperature of the chitosan beads bearing carbon black was 269±6°C. It may be concluded that the addition of carbon black does not significantly affect the degradation temperature as shown in Table 15. However, the method of synthesis may play a more significant role; the overall average degradation temperature of the beads (269±6°C) is slightly lower than that of chitosan films (292±5°C). It is also noticeable that there is no initial weight loss due to water, which may be attributed to the loss of water in the crystalline structure of chitosan when the beads are crosslinked. In summary, these beads may be used in applications up to 250°C without the degradation of the main polymer chain.

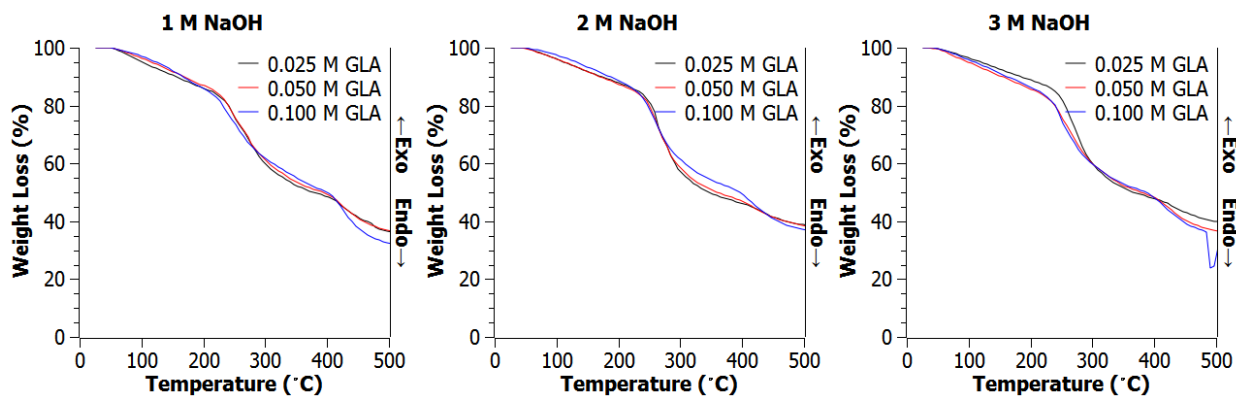


Figure 83 TGA spectra of beads synthesized using 2 M NaOH and 0.025 M GLA

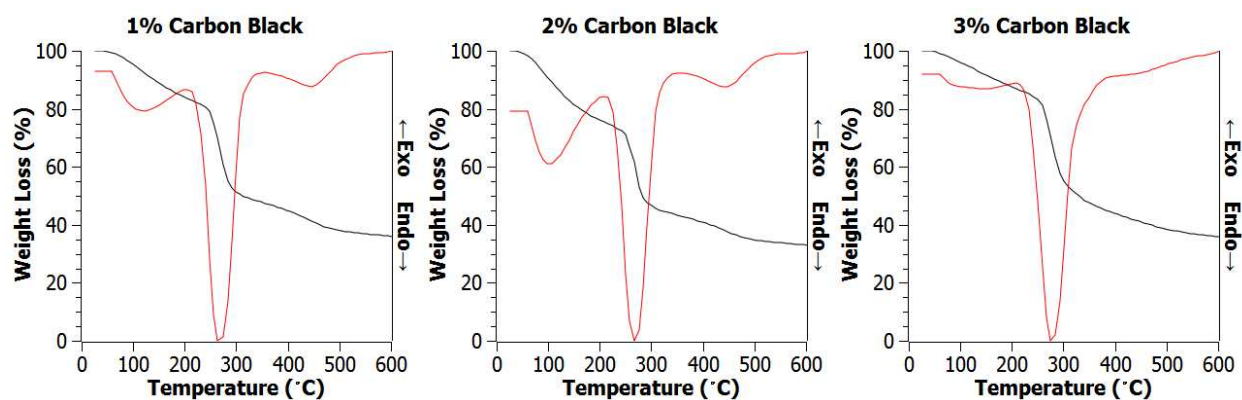


Figure 84 TGA spectra of beads containing carbon black.

% w/w CB	T _{deg} °C
0	274
1	264
2	266
3	275

Table 15 T_{deg} of chitosan beads containing carbon black.

Remarks

In summary, bare and carbon-black bearing chitosan beads with an average diameter of 1.7 mm were synthesized. The X-ray spectra of these beads exhibit the peaks corresponding to chitosan; likewise the FTIR bands correspond to the chemical structure of chitosan. Finally, since the degradation temperature of the beads was calculated to be $269 \pm 6^\circ\text{C}$, these beads may be used at temperatures up to 250°C .

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