Antibacterial Quaternary Chitosan / Alginate Polyelectrolyte Hydrogel Films with Silver Nanoparticles

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ABSTRACT

Polymeric materials have an increasing attention due to their cost effective, easy to use, bio degradable and bio compatible properties. Particularly chitosan and alginate are two natural polymers which form film by making polyelectrolyte complex to use as antimicrobial agent. In order to enhance the antimicrobial activity of chitosan quaternization of the polymer is one strategy.

In this regard, Quaternary chitosan was synthesized with glycidyltrimethylammonium chloride to fabricate polyelectrolyte bio composite film with alginate. Two different mililiters (10 mL and 20 mL) of silver nitrate solution were used to obtain a quaternary chitosan-alginate film loaded with silver nanoparticles. Polyelectrolyte hydrogel films were prepared by the simple method without using any other solvent. The characterization of the polyelectrolyte films was made by FT-IR Spectroscopy and the colour of the films changed from white to brown after adding silver solution, indicating that silver nanoparticles were loaded on the film. In addition, the immediate whitening of the film thrown into the PBS solution with the swelling test indicates that the film swells and releases silver nanoparticles into the environment.

The antibacterial and antifungal activity of four different kind of films (chitosan-alginate (V4), quaternary chitosan-alginate (V2), quaternary chitosan-alginate with 10mL silver (V1) and quaternary chitosan-alginate with 20 mL silver (V3)) were compared by the disk diffusion method and antimicrobial effect of silver was observed. The films have a hydrophilic character. In this study we fabricated a novel promising antimicrobial material which may use as wound healing or disinfection agent

especially against common pathogens *Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae).*

Keywords: Quaternary chitosan, Alginate, Silver Nanoparticles, Polyelectrolyte Film, Antibacterial, Antifungal, S. aureus, E. coli

ÖZ

Polimerik malzemeler, uygun maliyetli, kullanımı kolay, biyolojik olarak uyumlu ve parçalanabilir olduklarından dolayı artan bir ilgiye sahiptirler. Özellikle kitosan ve aljinat, antimikrobiyal ajan olarak kullanmak üzere polielektrolit kompleks film oluşturan iki doğal polimerdir. Antimikrobiyal aktiviteyi artırmak için de bir strateji kitosanın kuaternarizasyonudur.

Bu bağlamda, aljinat ile polielektrolit biyokompozit filmi üretmek için glisidiltrimetilamonyum klorür ile kuaterner kitosan sentezlendi. Gümüş nanoparçacık yüklü kuaterner kitosan-aljinat filmi elde etmek için iki farklı mililitrelerde (10 ml ve 20 mL) gümüş nitrat çözeltisi kullanıldı. Polielektrolit hidrojel filmler, başka bir çözücü kullanılmadan basit yöntemle hazırlanmıştır. Polielektrolit filmlerin karakterizasyonu FT-IR Spektroskopisi ile yapılmış ve filmlerin renginin gümüş çözeltisi eklendikten sonar beyazdan kahverengiye değişmesi, gümüş nanopartiküllerin filme yüklendiğini göstermiştir. Ayrıca şişme testi ile PBS solüsyonuna atılan filmin hemen beyazlaması, filmin şiştiğini ve ortama gümüş nanopartiküller saldığını gösterir.

Dört farklı film çeşidinin (kitosan-aljinat (V4), kuaterner kitosan-aljinat (V2), kuaterner kitosan-aljinat ile 10 mL gümüş (V1) ve kuaterner kitosan-aljinat ile 20 mL gümüş (V3)) antibakteriyel ve antifungal aktivitesi disk difüzyon yöntemi ile karşılaştırıldı ve gümüşün antimikrobiyal etkisi gözlendi. Filmler hidrofilik bir karaktere sahiptir. Bu çalışmada özellikle yaygın patojenler *Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis), Escherichia coli (E. coli), Klebsiella*

pneumoniae (Kpneumoniae)'ye karşı yara iyileştirme veya dezenfeksiyon maddesi olarak kullanılabilecek umut verici yeni bir antimikrobiyal materyal ürettik.

Anahtar Kelimeler: Kuaterner kitosan, Aljinat, Gümüş Nanopartiküller, Polielektrolit Film, Antibakteriyel, Antifungal, *S. aureus, E. coli*

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LIST OF ABBREVATIONS

AgNPs	Silver Nanoparticles
C. albicans	*Candida albicans*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
FT-IR	Fourier Transform Infrared Spectroscopy
K. pneumoniae	*Klebsiella pneumoniae*
MHA	Muller Hinton Agar
PBS	Phosphate Buffered Saline
S. aureus	*Staphylococcus aureus*
SDA	Sabouraud Dextrose Agar

Chapter 1

INTRODUCTION

1.1 Antibacterial Polymers

Antibiotics have been used as antibacterial since the nineties, although they have provided great benefit to humanity against bacteria, the resistance of some bacteria has led scientists to seek new ways. Thus, they discovered that metal ions and natural polysaccharides extracted from plants and various living things can be used in antibacterial studies [1]. Polymers lead to new discoveries every day in the fight against bacteria due to many advantages such as their porous structure, ability to make cross or ionic bonds, allows to workable in a wide pH range, being suitable for modification and easily synthesized. But most of all, they can be used together with other antibacterial material and possibility of increasing the antibacterial activity, which paves the way for future studies.

1.2 Chitosan

Chitosan is a natural linear polysaccharide, cationic polymer which is found in the structure of shellfish like shrimp and crab. On account of, it's non-toxic, biodegradable nature and its antibacterial properties due to the amino group it contains, it is used in the field of medicine [2]. It is also chemically stable and has a haemostatic character [3]. The studies have shown that chitosan accelerates wound repair and scar prevention. So, it has plenty using range such as; packaging material, wound healing, drug delivery and edible film. Besides, it is suitable for chemical modification and can be processed in different ways.

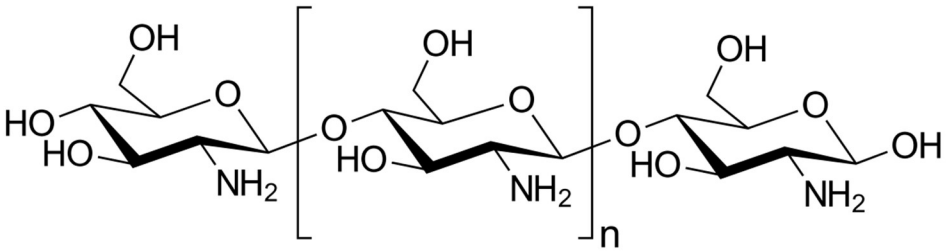

Figure 1: Chemical structure of Chitosan [4].

1.3 Chemical Modifications of Chitosan to Improve Antimicrobial Action

It has been reported that the antibacterial ability of natural polysaccharides improved by quaternarization [5]. Therefore, making chitosan quaternary with an ammonium salt group will increase its antibacterial properties. Also with quaternarization, chitosan becomes hydrophilic and its solubility increases at both low and high pH, unlike chitosan, which is soluble at low pH [5].

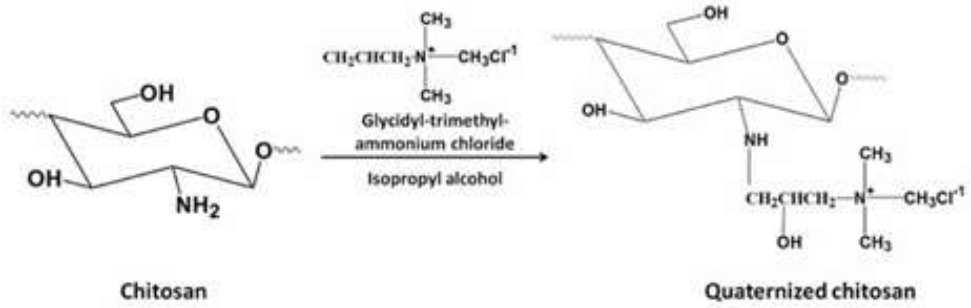

Figure 2: Quaternarization of chitosan with glycidyltrimethylammonium chloride [6].

1.3 Alginate

Alginate is also another natural polysaccharide, anionic polymer which can be obtained from brown algae. Due to its non-toxic, biocompatible, biodegradable and hydrophilic character it has widely using area especially to form gels and films. However, they do not have antibacterial activities. Therefore, making polyelectrolyte

complex with protonated amine groups of chitosan with alginate's carboxylate groups provides many advantages to the structure. Such as, mechanical stability and strength, swelling tendency and various application field. Wound dressings, tissue engineering and drug release is the most suitable biomedical application for it [7].

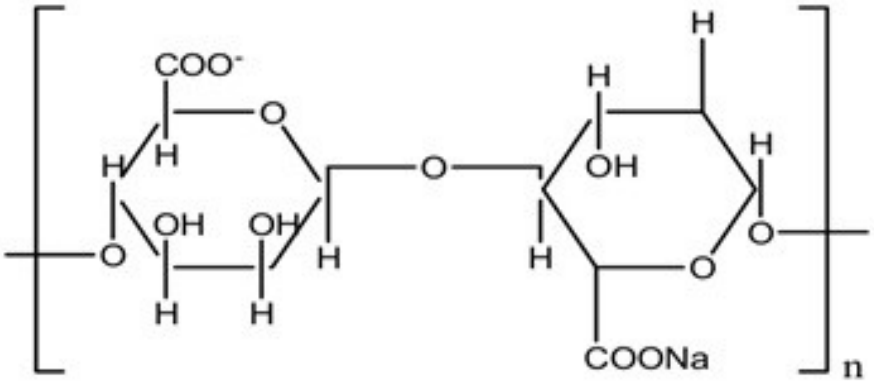

Figure 3: Chemical structure of sodium alginate [8].

1.4 Silver Nanoparticles

Many metal ions such as zinc, copper, silver are known to have antibacterial properties. Especially, silver ions have an effective activity against bacteria however, they also have high toxicity. Apart from that, it is safe for humans when used in small amounts, and also the reduction in particle size causes silver ions to pass through the cell membrane of bacteria and affect the cell division, which leads to the direct death of the bacteria [9]. To inhibit their toxic character they need a substance to make it stable in the structure. In which case it is best for application is to use biodegradable and biocompatible carbohydrate based polymers such as alginate, chitosan, gelatin collagen etc. [10]. And also chitosan, as a stabilizer, and sunlight containing UV light are used to accelerate the reduction of silver ions. The colour change after exposure to sunlight indicates the formation of silver nanoparticles [3]. Especially, in tissue engineering, drug delivery and wound healing, which is the using area of alginate and

chitosan, the release of silver nanoparticles from polyelectrolyte complex which has a hydrophilic character will allow hydration of the wound and better healing.

1.5 The Aim of the Study

The aim of this thesis is to synthesis quaternary chitosan and make silver nanoparticle containing polyelectrolyte bio-composite film with alginate by applying complexation method and to test antimicrobial activity of the films by disc diffusion method against gram positive and gram negative bacteria.

Chapter 2

EXPERIMENTAL

2.1 Materials

Chitosan (Medium Molecular Weight) (Sigma-Aldrich, Germany), Glycidyltrimethylammonium chloride (Sigma-Aldrich, Germany), Sodium Alginate (Sigma-Aldrich, Germany), Silver Nitrate Solution (0.1M) (Philip Harris), isopropyl alcohol (Sigma-Aldrich, Germany), distilled water, acetic acid (MERCK), ethanol (MERCK), acetone, Muller Hinton Agar (MHA) (HiMedia), Sabouraud Dextrose Agar (SDA) (HiMedia), *Staphylococcus aureus, (S.aureus) (ATCC 29213), Escherichia coli (E. coli) (ATCC 25922) Klebsiella pneumonia (K.pneumonia) (ATCC 11706), Enterococcus faecalis (E.faecalis) (ATCC), Candida albicans (C. albicans) (ATCC 10231) Rhodotorula (ATCC 20837)*

2.2 Method

2.2.1 Synthesis of Quaternary Chitosan

0.5 g Chitosan was placed into a round bottom flask and 5 mL of isopropyl alcohol was poured over it. The flask placed in a gasoline bath and a condenser was placed on top of it to obtain back the evaporated isopropyl. Temperature of the heater set at 80ºC and magnetic stirrer used to stir the reaction. After 30 minutes, 7.5mL of glycidyltriammonium chloride was added into solution. The reaction took place in 8 hours at a temperature of 80ºC and 2 days at room temperature with stirring. At the end of the reaction yellowish viscous solution obtained [11]. The flask decanted 3 times with ethanol, then filtered. In order to increase the drying rate, it was filtered

with acetone for the last time. After that, it left to dry at room temperature. Finally, cream solid quaternary chitosan was obtained.

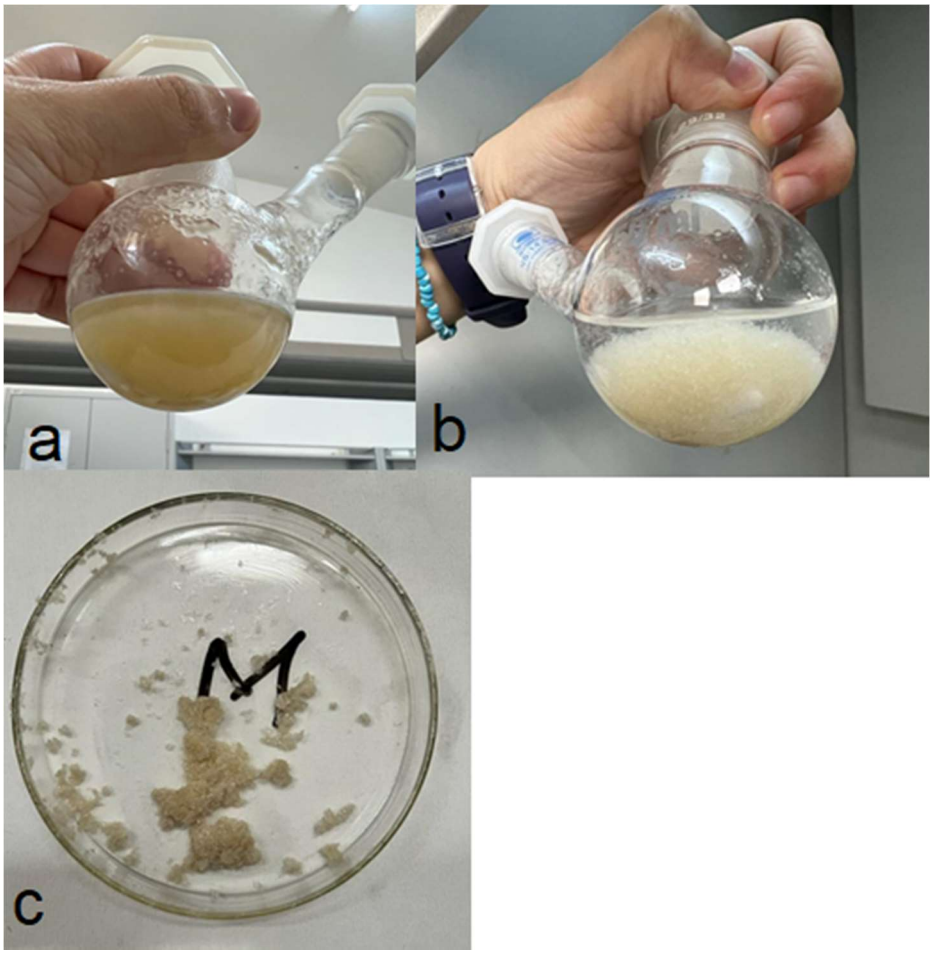

Figure 4: Synthesis of quaternary chitosan. (a) Yellowish viscous solution at the end of the reaction. (b) Cream coloured quaternary chitosan precipitated in ethanol. (c) Dried cream coloured solid quaternary chitosan.

2.2.2 Preparation of Biocomposite Films

First of all, chitosan 1% (w/v), quaternary chitosan 1% (w/v) and alginate 2% (w/v) solutions were prepared. Therefore, 1 g of Sodium Alginate was weighed and dissolved in distilled water to obtain 2% 50 mL Alginate solution. Then, 0.25 g of Chitosan was weighed and dissolved in 100% acetic acid to obtain 1% 25mL of chitosan solution. Finally, 0.25g of quaternary chitosan was weighed and dissolved in distilled water to obtain 1% 25mL quaternary chitosan solution. Also, ultrasonic bath

was used to increase the dissolution rate. Afterward, 2 petri dishes were weighed, numbered and noted. 7.5 g of Alginate solution and then 7.5 g of Chitosan solution (1:1) were weighed into the petri dish numbered V_4. V_4 was a colourless, viscous homogenous mixture. Then the solution of 22.5g of quaternary chitosan and 22.5g of alginate (1:1) were added into a beaker. The beaker was mixed at 1400 rpm for about 10 minutes until a homogeneous white mixture was obtained. After that 15g of this mixture was poured into the petri dish numbered V_2. Petri dishes were placed in an incubator to dry the homogenous mixtures. The homogenous, colourless films were obtained by drying at 40ºC for one day and at 65ºC for the second day. Finally, the films were weighed and weights of the films were found by subtracting the weights of the petri dishes.

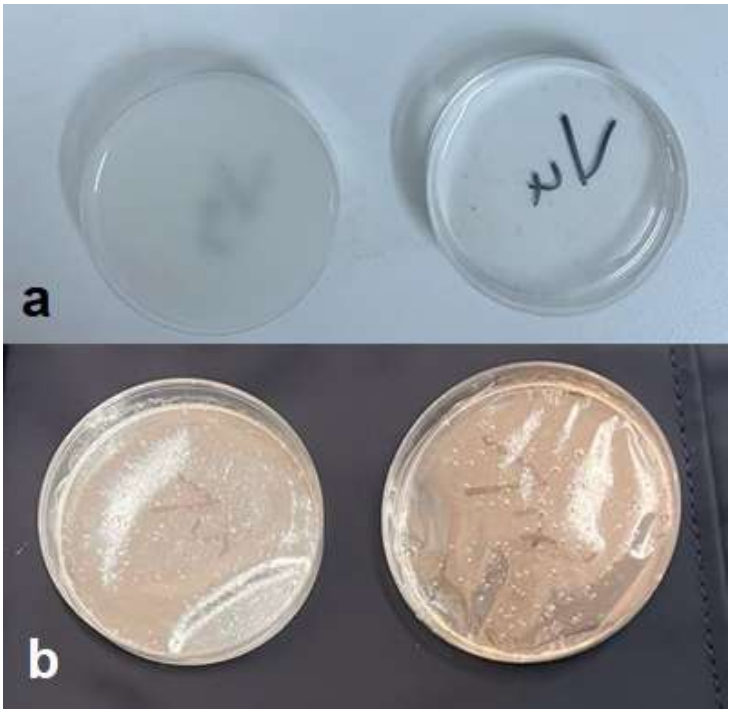

Figure 5: Preparation of bio composite films. (a) On the left homogenous white quaternary chitosan-alginate mixture (V_2) and on the right chitosan-alginate mixture (V_4) before incubation at room temperature. (b) After incubation at 65ºC, homogenous, colourless films are seen; on the left quaternary chitosan-alginate film (V_2) and on the right chitosan-alginate film (V_4).

2.2.3 $AgNO_3$ Loading to Biocomposite Films

Based on information from previous studies the antimicrobial activity of quaternary chitosan is higher than chitosan. Accordingly, quaternary chitosan was chosen to add silver nanoparticles. Two homogenous, colourless quaternary chitosan-alginate films were obtained by the same method as mentioned above and numbered V_1 and V_3. For the film V_1 10 mL of (0.1 M) $AgNO_3$ solution and for the film V_3 20 mL of (0.1M) $AgNO_3$ was added on them. Instantly, the colour of the colourless films changed into white and they began to swell. The films were exposed to light at room temperature for 24 hours to absorb the silver nitrate and the colour change from white to dark brown was also observed [12]. In similar studies, the colour change shows the formation of silver nanoparticles [3].Eventually, the films were then placed in an incubator at 37.5^{o}C for 5 hours to get rid of excess liquid remaining on them and then they left at room temperature for two days to dry completely. Afterward, the weight of the films was found by same method mentioned above.

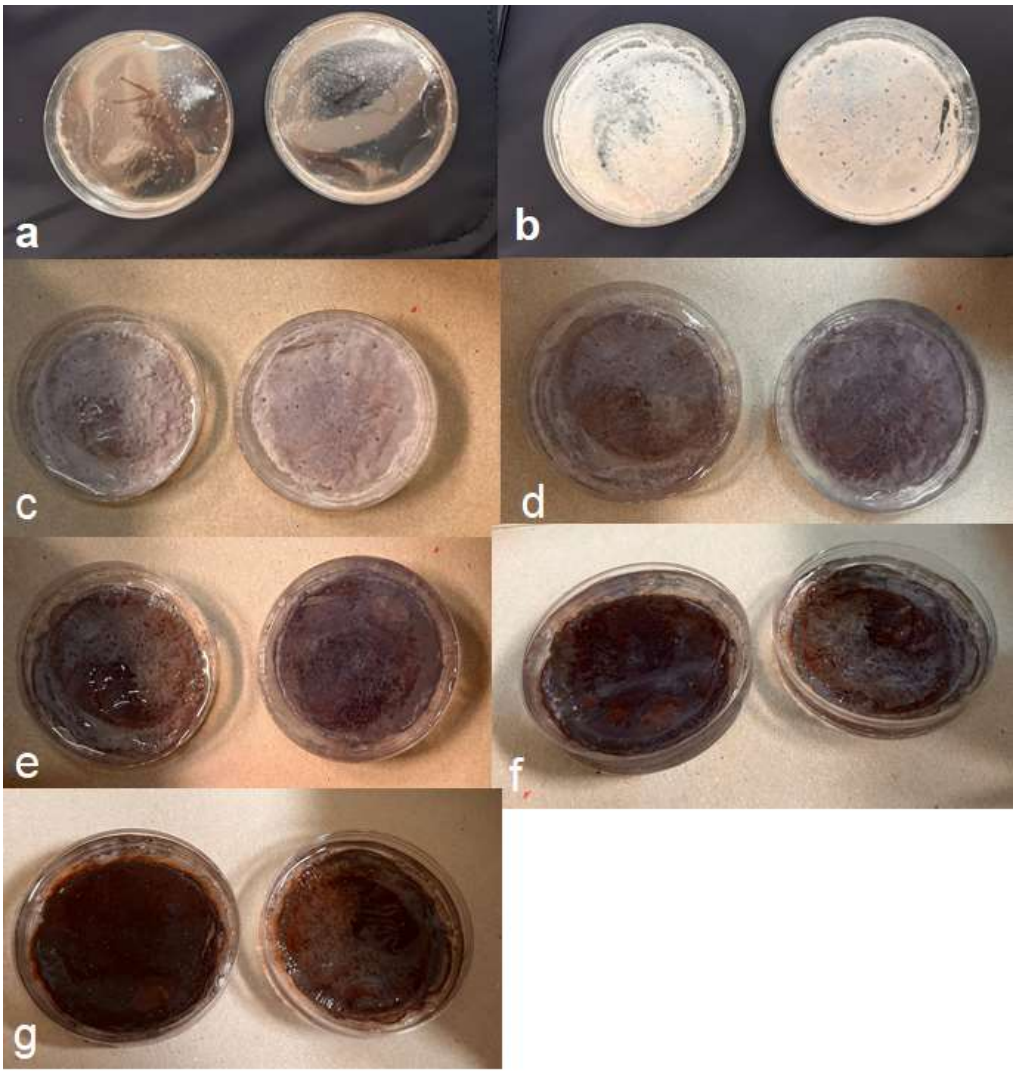

Figure 6: Silver nanoparticles loaded biocomposite films on the left (V1) and on the right (V3). (a) After incubation at 37.5ºC homogenous colourless films. ((b) 1 min (c) 20 min (d) 40 min (e) 60 min (f) 2 hours (g) 24 hours) later after adding $AgNO_3$ solutions.

2.3 Chemical Characterization

2.3.1 Weigh and Thickness

TW423L SHIMADZU analytical balance was used to weigh the masses of the films and the thickness of the films measured by XCAN LCD digital electronic ernier calliper.

2.3.2 FT-IR Spectrum

Perkin Elmer FT-IR spectrum two (UATR 2) was used to identify the functional groups in structure of the films and used polymers.

2.3.3 Swelling Measurement

Swelling test was applied to measure how much water the films could absorbs. For this test two films, V_2 (Quaternary Chitosan - Alginate film) and V_3 (Quaternary Chitosan - Alginate Films with 20 mL Ag^+) were used. Both films were tested in Phosphate Buffered Saline (PBS) [2].

2.4 Antimicrobial Activity Assays

2.4.1 Antibacterial Activity Assays

Kirby-Bauer Disc Diffusion method was used to detect antibacterial activity as reported previously [13]. Therefore, four different films which have same shape and equal weight with a diameter of 5.8 mm were placed on the 0.5 McFarland bacteria inoculated Muller Hinton Agar plates. Then they were putted into the incubator at 37ºC for 24 hours and the inhibition zone (mm) was measured with an electronic digital calliper. Four bacteria *Staphylococcus aureus, (S. aureus)*, *Escherichia coli (E. coli) Klebsiella pneumoniae (K. pneumoniae*), *Enterococcus faecalis (E. faecalis)* were selected and 5 mcg Ciprofloxacin antibiotic disk was used as the positive control group.

2.4.2 Antifungal Screening Assays

Antifungal Disc Diffusion method was used for antifungal activity screening *Candida albicans (C. albicans)* and *Rhodotorula sp.* were used as fungal agents to screen antifungal activity [14]. To cultivate yeast each measurement Sabouraud Dextrose Agar (SDA) was used. Firstly, 1mL of 0.5 McFarland yeast was added into 20 mL of SDA solution and poured into a petri dish for each fungal strain. Then four different

circular films which have equal amount and exactly the same shape with a diameter of 5.8 mm were placed on the agar media and incubated at 37^{o}C for 24 hours. Finally, the inhibition zone (mm) was measured with an electronic digital calliper and recorded.

Chapter 3

RESULTS AND DISCUSSION

3.1 Fabrication of Biocomposite Films

Four different kinds of films were prepared by this study. Synthesis of quaternary chitosan was a pioneer for all steps. One of the key points was to obtain a homogeneous mixture by stirring quaternary chitosan and alginate and then pouring it into the petri dishes. In addition, the colour change from white to brown after silver nitrate solution was added to the films was an indication of the formation of silver nanoparticles (12).

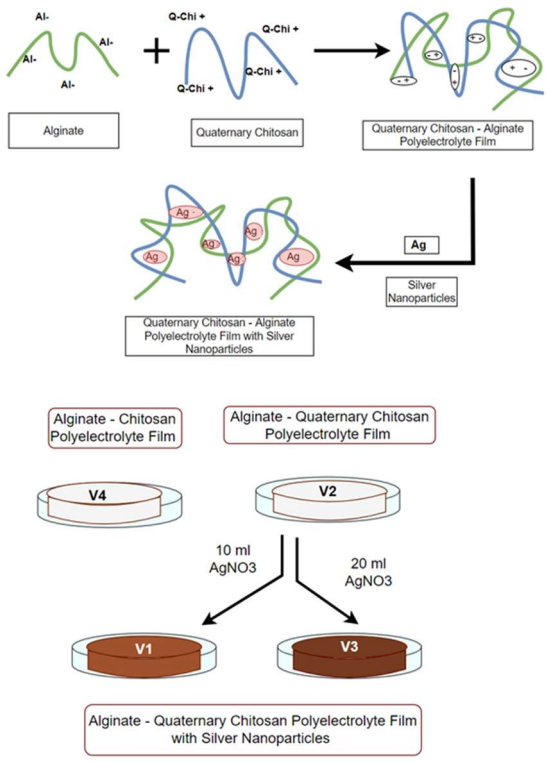

Figure 7: Synthesis scheme of the polyelectrolyte film.

3.2 Weigh and Thickness

At first empty petri dishes were weighed then mixtures poured into them. When the films were dried they measured again. By subtracting the weight of empty petri dish, from the total amount of film and petri dish, the weights of films were found.

For the films which include Ag^{+} nanoparticles, dry quaternary chitosan- alginate film was used and 0.1 M 10 mL and 20 mL of $AgNO_3$ solution poured on V1 and V3 films respectively.

Table 1: Weights of different films.

Name of the Film	**Empty Petri Dish (g)**	**Petri Dish + Film (g)**	**Film (g)**	**$AgNO_3$ (ml)**	**Final Film (g)**
V_1	4.103 g	4.343 g	0.24 g	10 ml	0.38 g
V_2	4.103 g	4.335 g	0.23 g	-	0.23g
V_3	4.407 g	4.648 g	0.24 g	20 ml	0.42g
V_4	4.414 g	4.602 g	0.19 g	-	0.19 g

The percent of silver nanoparticles loading capacity of the films can be found with the following equation.

$$AgNPs\ \text{Loading Capacity}\ \% = \frac{\text{Wfinal film} - \text{Wfilm}}{\text{Wfilm}}\ \text{x}\ 100$$

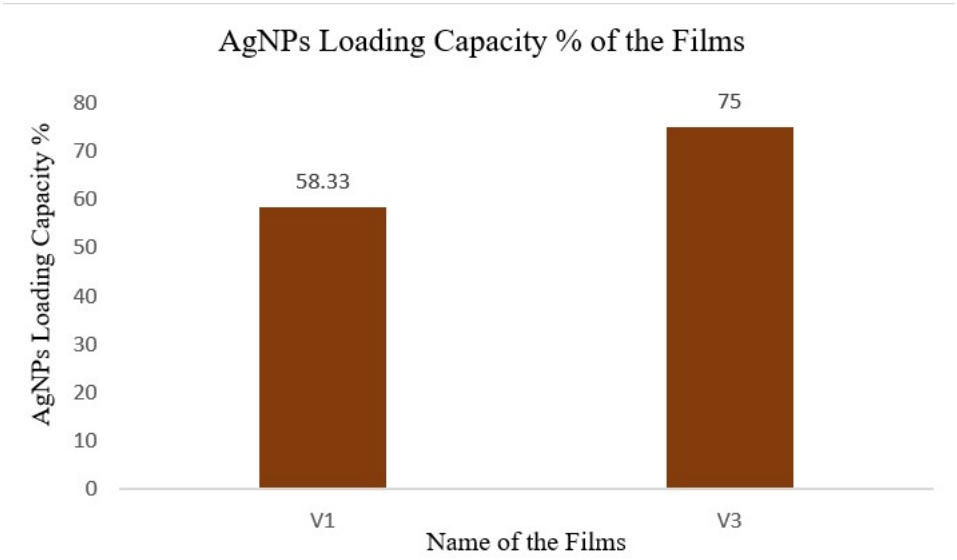

Figure 8: AgNPs Loading Capacity % of the Films.

The mass of the V_1 film increased by 0.14g, while the mass of the V_3 film increased by 0.18g. With the calculation made, the percent of silver loading capacity of V_1 and V_3 films was found 58.33% and 75% respectively.

The thickness of the silver containing V_1 and V_3 films was 0.8 mm, while the thickness of V_2 and V_4 films was 0.3 mm. It is thought that the 0.5 mm difference is caused by the silver particles that enter between the ionic bond occurred by quaternary chitosan and alginate.

3.3 FT-IR Characterization

Fourier transform infrared spectroscopy (FT-IR) was used to identify the functional groups in structure of the films and used polymers. FTIR spectra of alginate, chitosan, the film V_4 (alginate-chitosan film), quaternary chitosan, the film V_2 (quaternary chitosan- alginate film), the film V_1 (quaternary chitosan-alginate with 10 mL silver), and the film V_3 (quaternary chitosan-alginate with 20 mL silver) are shown in figures 9a, 9b, 9c, 9d, 9e and 9f respectively.

FT-IR spectrum of alginate: The broad peak at 3228 cm^{-1} represents O-H group, at 2982 and 2906 cm^{-1} peaks are related with the –CH stretching. The strong peak at 1596 cm^{-1} is due to the C=O stretching and C-O stretching shows at 1027 cm^{-1} [15].

FT-IR spectrum of chitosan: Broad stretching peak band centred at 3286 cm^{-1} represents to N-H and O-H group vibrations. Peaks at 2915 and 2879 cm^{-1} represent –CH stretching in chitosan. C=O stretching, N-H bending, and C-N stretching observed by the presence of the peaks at 1648, 1573 and 1375 cm^{-1} respectively. 1027 cm^{-1} and 1064 cm^{-1} peaks are indicated C-O stretching [16]

FT-IR spectrum of the film V_4: The film is made up of chitosan and alginate and also the spectrum confirms this as well. The peak at 3265 cm^{-1} is due to the O-H group in the both of the polymers structure. 2921 cm^{-1} shows the –CH stretching. C=O stretching, N-H bending and C-O stretching observed by the presence of the peaks at 1641, 1545 and 1065-1024 cm^{-1} respectively. And also at 1407 cm^{-1} N-H asymmetric bending observed.

FT-IR spectrum of quaternary chitosan: The peaks a corresponded to the chitosan. There are two biggest differences between the spectra. One of them is the decrease in the intensity of the peak due to the conversion of the N-H primary amine peak at 1561 cm^{-1} to the secondary amine in the structure. Second, the increasing peak at 1477 cm^{-1} is belong to the N-H due to the asymmetric bending, which is not detected in chitosan's spectrum [11].

FT-IR spectrum of the film V_2: The film V_2 is consists of quaternary chitosan and alginate. On the other hand, the film V_4 is made up chitosan and alginate. The main

difference between the films is that the peaks at 1545 cm^{-1} (N-H primary amine bending) and at 1641 cm^{-1} (C=O stretching) showed at one sharp peak at 1593 cm^{-1} due to existence of quaternary chitosan. Also at 1409 and 1026 cm^{-1} peaks are shows the existence of the N-H bending and C-O stretching respectively.

FT-IR spectrum of the film V_1 and V_3: Both the structure of films are exactly the same the difference between them is only 10 mL of silver nitrate solution. If we compare these films with the film V_2 the main difference of the spectrum is at the broad peak at 1400-1300 cm^{-1}. This due to the existence of N=O symmetry stretching with N-H bending [17]. Nitrate remained in the environment with the addition of silver nitrate solution.

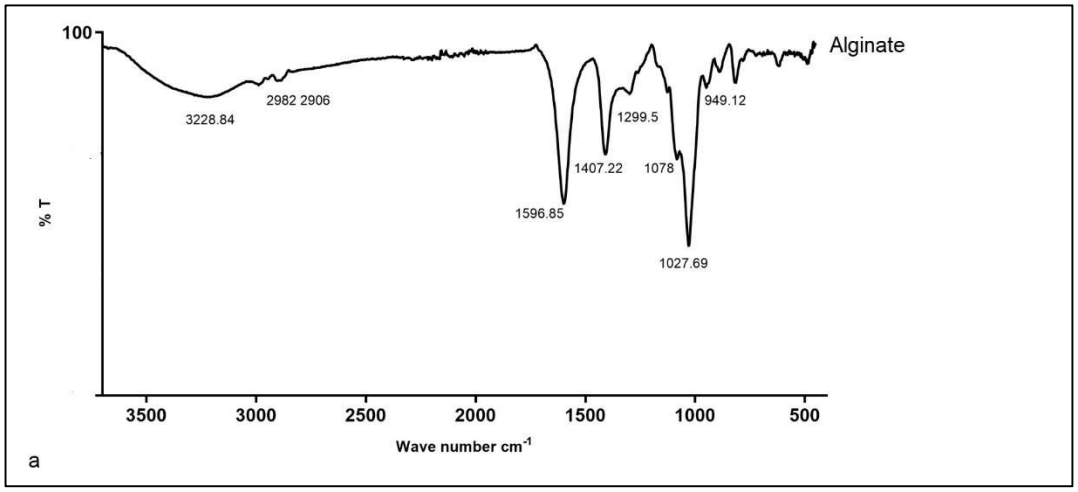

Figure 9a: FT-IR spectrum of alginate.

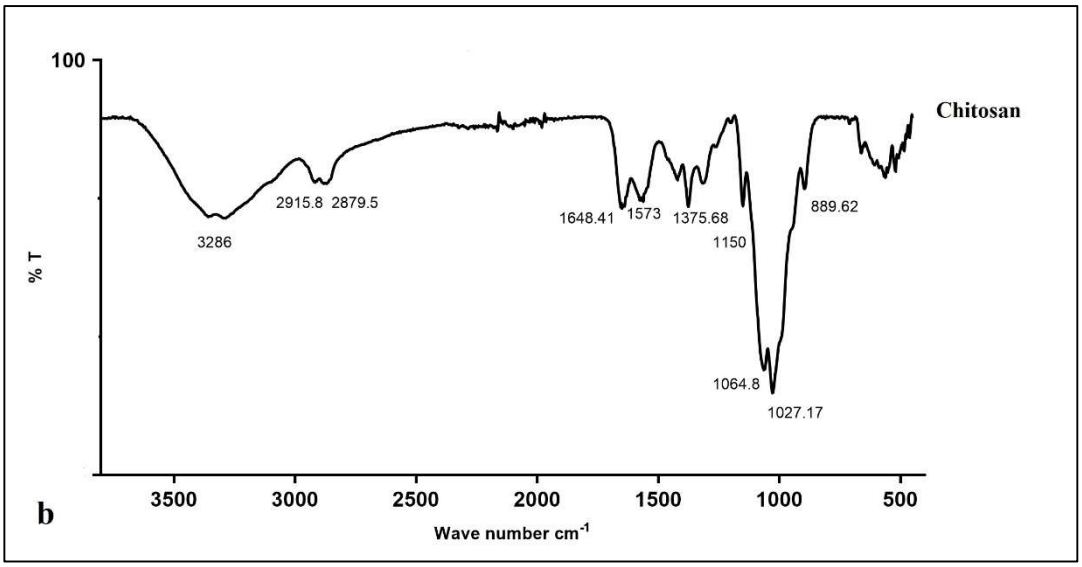

Figure 9b: FT-IR spectrum of chitosan.

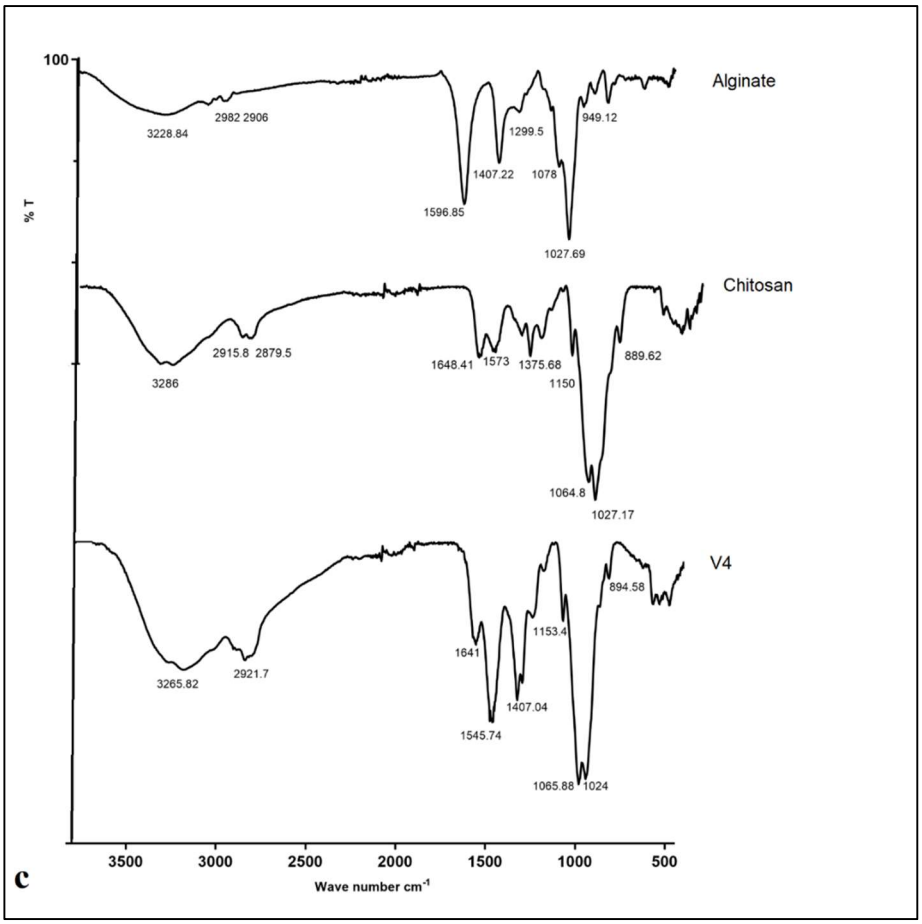

Figure 9c: FT-IR spectrum of film V_4.

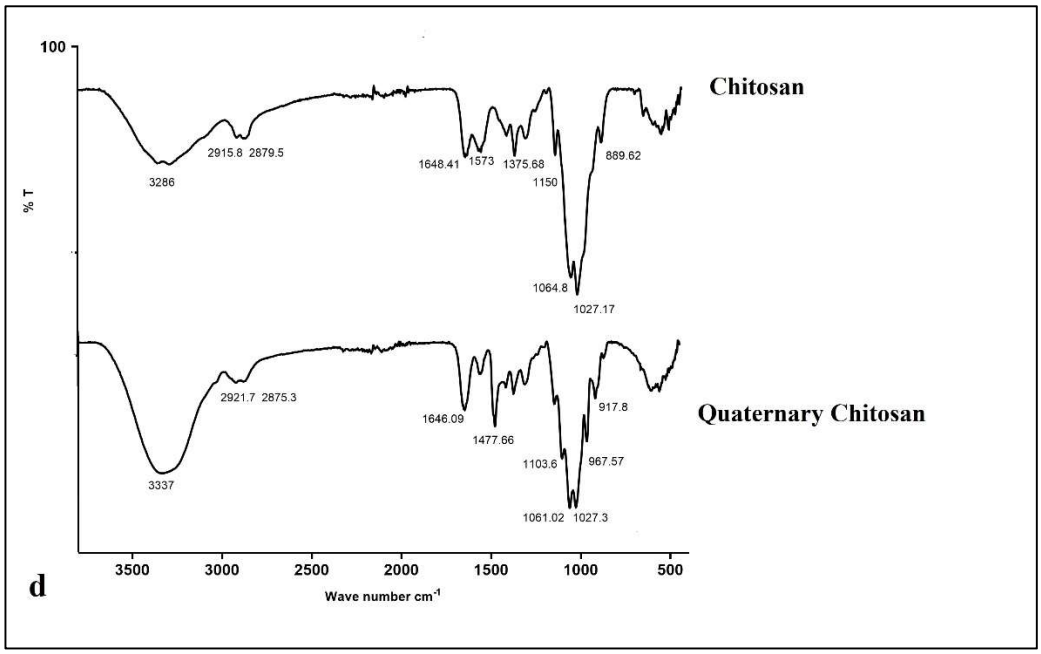

Figure 9d: FT-IR spectrum of quaternary chitosan.

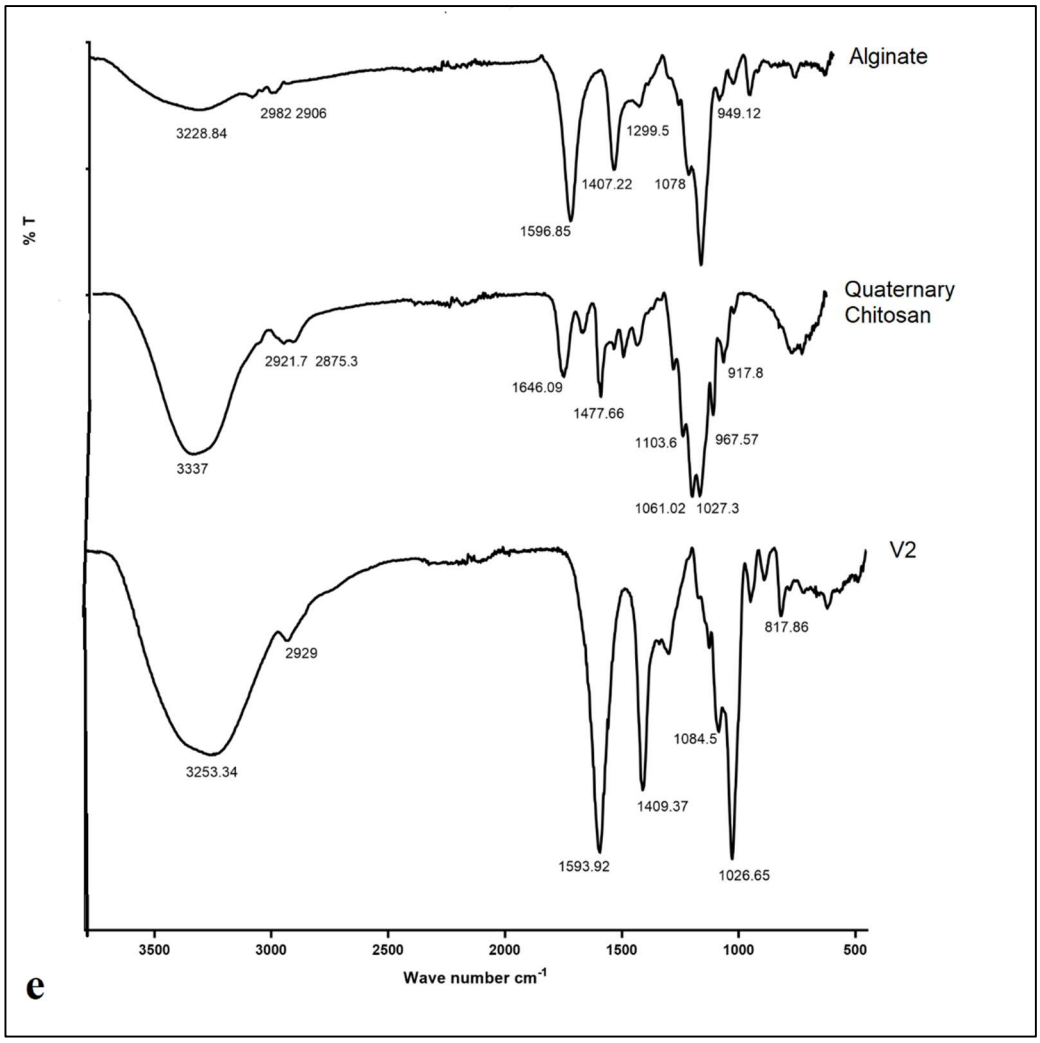

Figure 9e: FT-IR spectrum of film V_2.

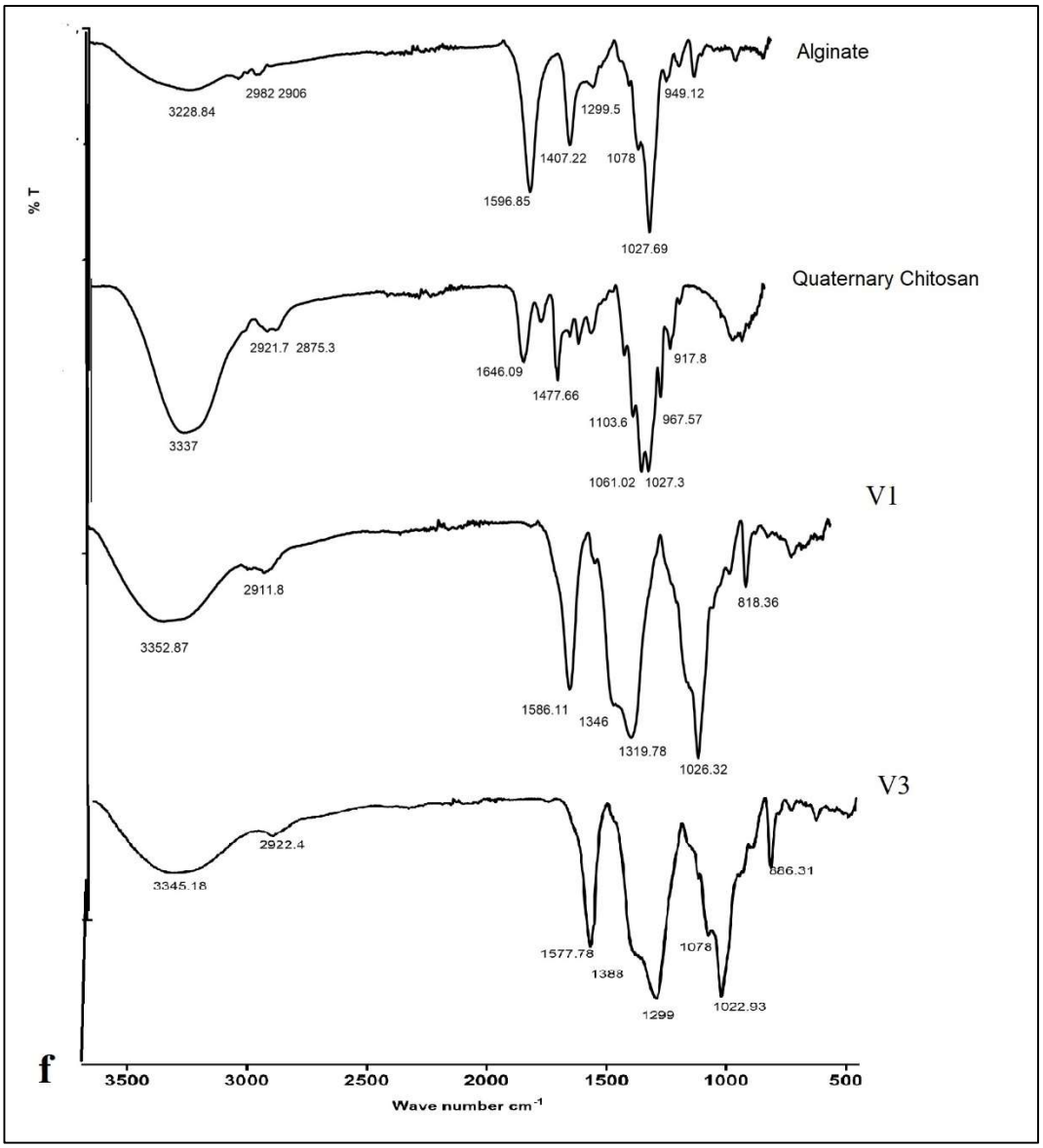

Figure 9f: FT-IR spectrum of film V1 and V3.

3.4 Swelling Test

First the dry sample of V_2 was weighed and found 0.06 g and it putted into 20 mL PBS solution. After one minute the film was weighed and it was 0.32 g; then again it put into the solution. However, after two minutes, the film dispersed in the solution. On the other hand, for film V_3 the initial dry film was 0.025 g and it putted into the 20 mL PBS solution. The swelling film was weighed 4 times. As soon as the film putted into the PBS solution it began to whiten and after 6 minutes it turned into brownish colour.

This may be due to the film releasing the silver in the first 6 minutes and then absorbing the silver again. By using the following formula, the swelling degrees of V_3 film were found 424 %.

$$\text{Swelling degree \%} = \frac{W1-W0}{W0} \text{ x } 100\%$$

W_0 = Weight of dry film; W_1 = Weight of the last film after immersed in PBS at pH 7.4 [3].

Table 2: Weights of V3 film after immersed in PBS.

Swelling Test of V3 in PBS	
Time (min)	**Weight (g)**
0	0.025
1	0.041
3	0.056
6	0.094
10	0.131

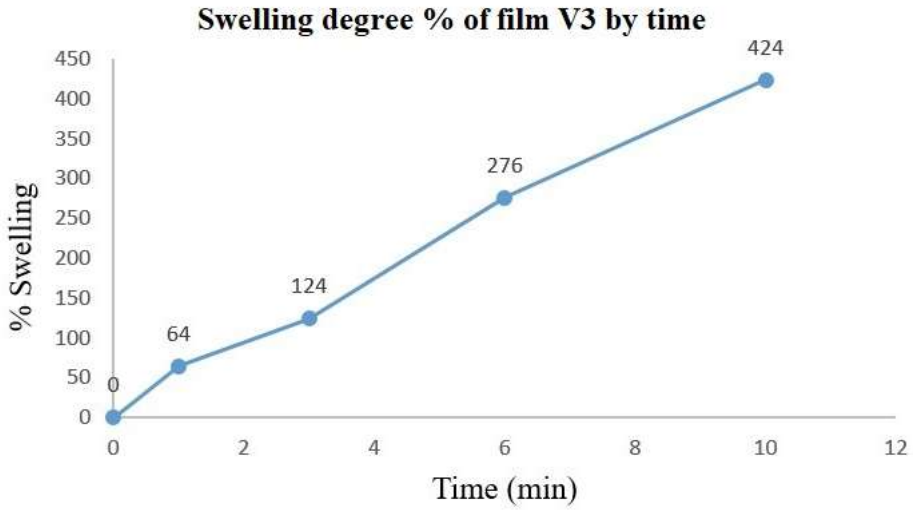

Figure 10. Swelling degree of film V_3 by time.

3.5 Antimicrobial Activity

3.5.1 Antibacterial Activity

Disc Diffusion method was used to detect antimicrobial activity. For antibacterial susceptibility four bacteria has been chosen such as; *Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis), Escherichia coli (E. coli), Klebsiella pneumonia (K .pneumoniae).*

Table 3: Inhibition zone in mm of films against bacteria.

Name of the Bacteria	V1 (mm) (Quaternary Chitosan – Alginate +10ml $AgNO_3$)	V2 (mm) (Quaternary Chitosan - Alginate)	V3 (mm) (Quaternary Chitosan –Alginate + 20 ml $AgNO_3$)	V4 (mm) (Chitosan – Alginate)
S. aureus	13.5 mm	-	14.5 mm	-
E. faecalis	11.5 mm	-	11.9 mm	-
E. coli	11.0 mm	-	13.9 mm	-
K. pneumoniae	11. 6 mm	-	12.0 mm	-

For *Ciprofloxacin*, which is the positive control group, the inhibition zone was measured as 26.6 mm for *S. aureus*, 17.0 mm for *E. faecalis*, 35.6 mm for *E. coli* and 14.6 mm for *K. pneumoniae.*

The film V1 formed 13.5 mm, 11.5 mm, 11.0 mm and 11.6 mm inhibition zone against S.aureus, E. faecalis, E. coli and *K. pneumoniae* respectively. On the other hand, the film V3 formed 14.5 mm, 11.9 mm, 13.9 mm and 12.0 mm zones against *S.aureus, E. faecalis, E.coli* and *K. pneumoniae* respectively.

It has been reported that the making chitosan quaternary is both effective on gram negative and positive bacteria [5]. However, as a result of the positive charges on the quaternary chitosan form ionic bonds with the negative charges of the alginate. No

significant antibacterial activity was found as the free charge may not be present in the polymer. (V_2). This is also applies to the film (V_4) which is made up of chitosan and alginate.

When the values found for *S. aureus and E. coli* were compared with another silver containing alginate chitosan film literature, the measured zone of inhibitions were found to be proportional [3]. All the inhibition zones were found to be greater than 10 mm.

As expected, the V_3 film, in which more silver solution was added, formed a wider inhibition zone than the V_1 film against all bacteria. The V_3 film showed the widest zone for *S. aureus* (14.5 mm) followed by *E. coli* (13.9 mm). Besides, the zones were measured almost the same for *K. pneumoniae* (12.0 mm) and *E. faecalis* (11.9 mm).

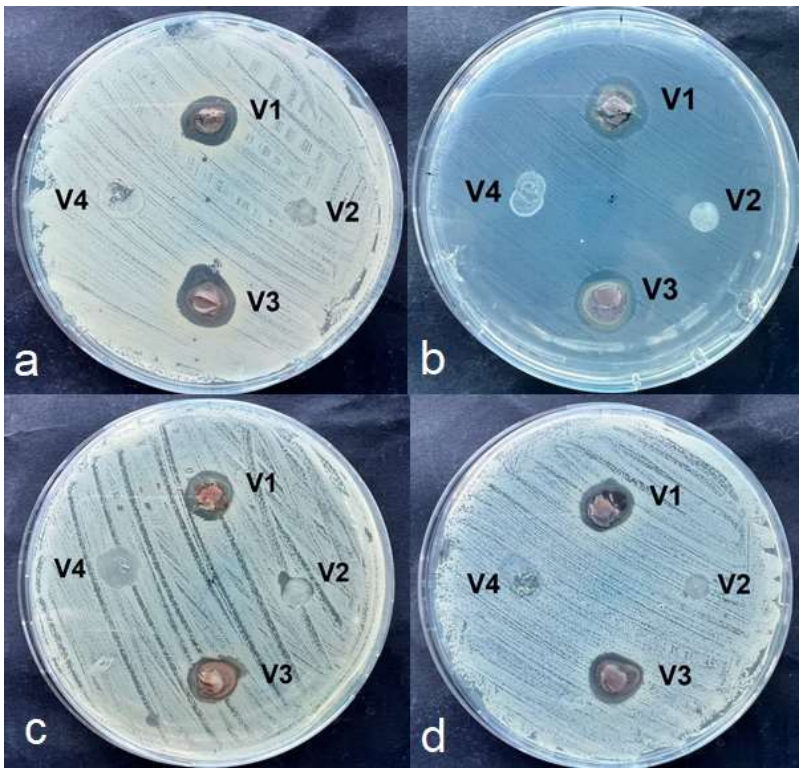

Figure 11. Antimicrobial activity against (a) *S. aureus* (b) *E. faecalis* (c) *E. coli* (d) *K. pneumonia.*

3.5.2 Antifungal Activity

For antifungal susceptibility 2 yeast such as *Candida albicans (C.albicans)* and *Rhodotorula sp.* was used.

Table 4: Inhibition zone in mm of films against yeast.

Name of the Yeast	V1 (mm) (Quaternary Chitosan – Alginate +10ml $AgNO_3$)	V2 (mm) (Quaternary Chitosan - Alginate)	V3 (mm) (Quaternary Chitosan – Alginate + 20 ml $AgNO_3$)	V4 (mm) (Chitosan – Alginate)
C. albicans	8.5 mm	-	11.2 mm	-
Rhodotorula sp.	8.4 mm	-	13.7 mm	-

As with the antibacterial activity, the V_3 film created more zones than V_1 film. The greatest antifungal activity was against Rhodotorula sp. (13.7 mm) of film V_3. The reason why the transparent zone formation was not observed may be due to the fact that the medium was prepared differently and the silver was released into the environment until the yeast reproduced. Zone formations can be seen more clearly when viewed from the front surface of petri dishes.

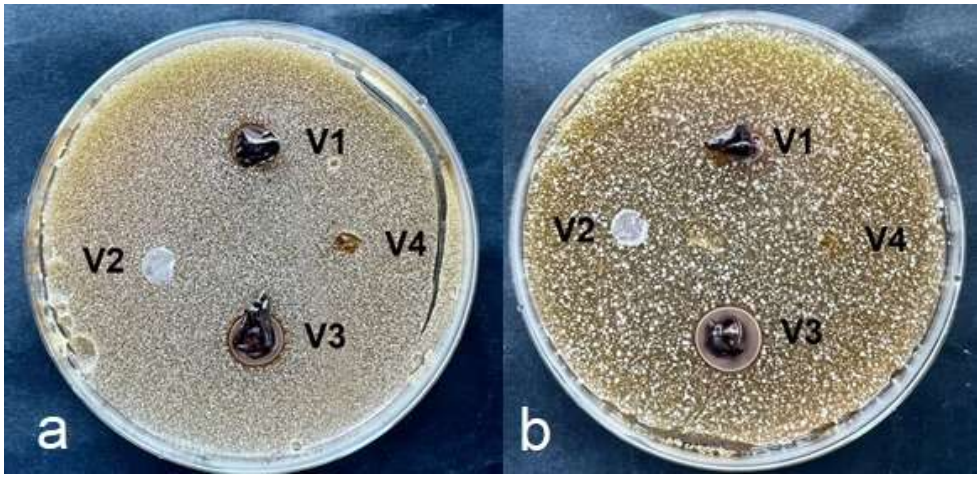

Figure 12. Antifungal activity against (a) *C. albicans* (b) *Rhodotorula sp.*

The pH of Muller Hinton agar (MHA) and Phosphate Buffer solution (PBS) is around 7.4 and the swelling test is proof that silver is released from the polymer. So, the silver has an antibacterial effect on this agar. Perhaps the acidic pH of SDA agar may have affected the antifungal activity. If antifungal activity is also performed on MHA, maybe the inhibition zone formed by the silver containing films can be found to be wider.

Chapter 4

CONCLUSION

Water-soluble polymer was obtained by quaternary chitosan synthesis. Especially at pH 7.4, synthesized quaternary chitosan alginate film swells in two minutes and the gel structure of the film disintegrates. It indicates that the films were synthesized by the FT-IR characterization. The silver nanoparticle loaded quaternary chitosan alginate polyelectrolyte films have antibacterial and antifungal activity. As expected, increasing the amount of silver also increased the activity against both gram positive and gram negative bacteria.

Future studies may involve re-synthesization of the films considering silver toxicity. This will pave the way for applications in many fields. Synthesis of, quaternary chitosan after the formation of alginate and chitosan films is also possible in futuristic perspective which may provide antimicrobial activity.

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