Preparation of Antibacterial Chitosan based Cryogel / Silver Nanoparticles Composites

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Approval of the Institute of Graduate Studies and Research

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Chemistry.

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We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

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ABSTRACT

In this thesis, four different types of cryogels were synthesized by using different concentrations of glutaraldehyde cross-linker and silver nitrate. Cryogel containing the highest concentration of cross-linker resulted in the formation of non-homogeneous cryogel structure due to localized viscosity increase around cross-linking agent. In other cryogels where cross-linker concentration was kept at 1% homogeneous mixture was achieved easily.

Reduction of silver nitrate into silver nanoparticles was confirmed due to the observation of colour change from yellow to brown. Cryogel which contains no amount of silver nitrate did not go under any colour change.

Drying and water retention capacity evaluation of cryogels showed that concentrated cross-linker containing cryogel had the highest water retention capacity wheras cryogel without any silver nitrate had the lowest water retention capacity.

Antibacterial activity of cryogels were tested by evaluation of inhibition zone against *E. coli* and *S. aureus*. Maximum inhibition zone of 16 mm in *E. coli* and 15 mm in *S. aureus* was observed by the cryogel containing the highest concentration of silver nitrate.

Keywords: Chitosan, Glutaraldehyde, Silver Nanoparticle, Antibacterial, Inhibition, *E. coli, S. aureus*

ÖZ

Bu tezde, farklı konsantrasyonlarda gümüş nitrat ve glutaraldehit çapraz bağlayıcı kullanılarak dört farklı kriyojel üretilmiştir. En yüksek miktarda çapraz bağlayıcı içeren kriyojelde çapraz bağlayıcı miktarına bağlı olarak artan viskozite sonucu homojen olmayan bir yapı görülmüştür. Çapraz bağlayıcı konsantrasyonunun %1 olarak kullanıldığı diğer kriyojellerde homojen yapı kolaylıkla oluşmuştur.

Gümüş nitratın gümüş nanopartiküle dönüşümü kriyojellerin sarı renkten kahverengi renge dönüşmesi ile onaylanmıştır. İçerisinde gümüş nitrat bulunmayan kriyojelde herhangi bir renk değişimi görülmemiştir.

Kriyojellerin kuruma hızı ve su tutma kapasitesi ölçülmüş olup çapraz bağlayıcı miktarı en fazla olan kriyojelin su tutma kapasitesi en yüksek ölçülürken içerisinde gümüş nitrat bulunmayan kriyojelin su tutma kapasitesi ise en düşük olarak ölçülmüştür.

Kriyojellerin *E. coli* ve *S. aureus* bakterilerine karşı olan antibakteriyel aktivitesi inhibisyon alanına bakılarak ölçülmüştür. *E. coli* (16mm) ve *S. aureus* (15mm) için en büyük inhibisyon alanının en yüksek miktarda gümüş nitrat içeren kriyojel tarafından oluşturulduğu gözlemlenmiştir.

Anahtar kelimeler: Kitosan, Glutaraldehit, Gümüş Nanopartikül, Antibakteriyel, İnhibisyon, *E. coli, S. aureus*

ACKNOWLEDGMENT

I would like to thank to my supervisor Prof. Dr. Mustafa Gazi and my co-supervisor Asst. Prof. Dr. Mümtaz Güran for their continuous support and great guidance.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

DNA	Deoxyribonucleic Acid
E.coli	*Escherichia coli*
GA	Glutaraldehyde
FT-IR	Fourier Transform Infrared Spectroscopy
MHA	Mueller Hinton Agar
S. aureus	*Staphylococcus aureus*
WHO	World Health Organization

Chapter 1

INTRODUCTION

1.1 Microorganisms and resistance problem

Microorganisms are present everywhere, from the air we breath to surface we eat our food on. Human body is also a great host for many different types of microorganisms. Commonly found gram-negative bacteria *Escherichia coli* causes inflammatory bowel diseases in humans and gram-positive bacteria *Staphylococcus aureus* can be easily isolated by human nasal swab inoculation [1,2]. Increased presence of bacteria in human body often leads to pathological conditions which often requires immediate medical attention for the protection of human health. Antibiotics are very popular but at the same time very misused antibacterial products. Uninformed, negligent and misuse of antibiotics has lead to development of antibiotic resistance by many bacterial species (WHO). As a result, scientists have been trying to come up with new ways to eliminate bacteria.

1.2 Metal ions used as antibacterial agents

Chemistry is a popular field when it comes to use of chemicals as well as development of new multifunctional materials for antibacterial use. Many metals are known to exhibit antibacterial properties. Silver, zinc, copper, nickel and lead can be given as example to metals with antibacterial effect [3,4]. Accumulation of metals may cause damage in bacteria cell wall, metals may be carried into the bacteria cell where crucial components become damaged or metals may also inhibit bacteria growth occuring in a resistant biofilm structure [3,5]. In a study which the antibacterial effect of various

metals against bacteria growing in a biofilm layer was tested showed that Ag^{+}, Hg^{+2} and TeO_3^{-2} metals had the highest toxicity against tested bacteria species *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* [5].

Silver is an important metal that needs to be mentioned exclusively because of huge amount of implementation of silver ions for use in medicine. Antibacterial contamination concerns are very important when it comes to recovering process during a basic illness. Silver has various action mechanism against bacteria cells which allows them to be good antibacterial materials. Penetration of peptidoglycan cell wall, inhibition of cell respiration cycle, interruption of replication by binding to DNA and inhibiting metabolic pathways are examples to effects of silver on bacteria [6]. Products that contain silver as an antimicrobial agent are very useful and most of these materials exist as a patented product. In one patented product, biliary duct implant was coated with silver nanoparticles to prevent biofilm formation (6). In another patented product, silver ions were used to aid wound healing [7].

1.3 Antibacterial polymers

Polymers are another example of antibacterial products which falls into the scope of chemistry. Polymers are macromolecular structures that are made up of covalently bonded repeat units. Polymers can exist in the nature in their unique way, also called as natural polymers or they can be manufactured by scientists, also known as synthetic polymers. There is an abundance of polymeric materials and each polymer possesses unique properties that can effect or shape its' use eventually. Teflon, nylon, rubber and polyvinyl chloride are examples to synthetic polymers that have found popularity for use in our daily life. Natural polymers are often sourced by many different living organisms. Deoxyribonucleic acid (DNA) is a huge natural polymer that is found in

every living organism which consists of a nitrogenous base and a sugar-phosphate backbone as its repeat unit. Alginate, cellulose and chitin are natural polymers that play a role in many plants or sea animals as a structural support.

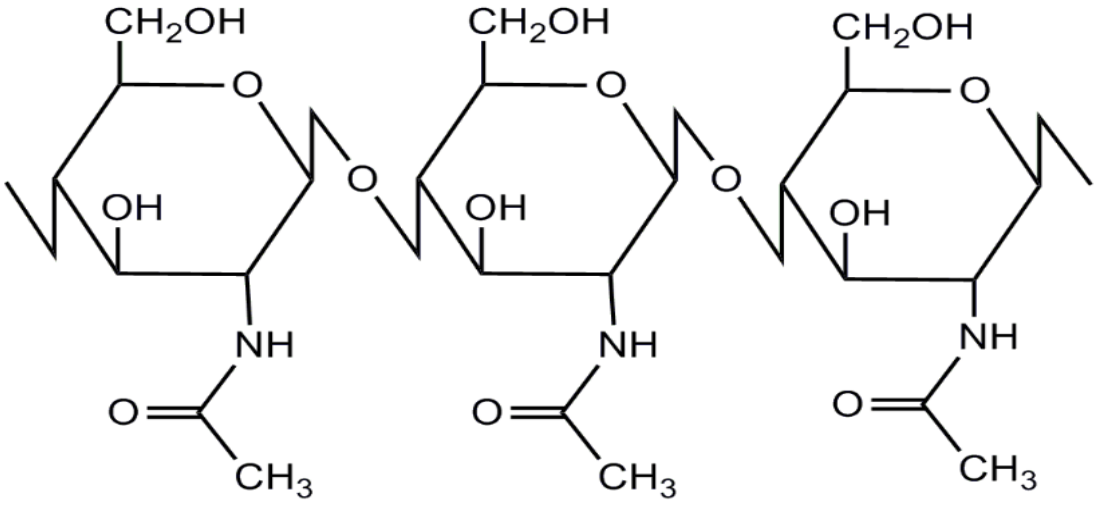

Figure 1: Structure of chitosan precursor; chitin

Antibacterial activity of many polymers have been studied extensively and their efficiency as an antibacterial agent and their action mechanism was found to be effected by properties such as structural resemblance to naturally occuring antimicrobial peptides, molecular weight of the polymer, presence of ionic groups and resistance to degradation [8]. Generally, polymeric materials have the potential of intense modification upon desire which allows them to be very efficient to manufacture and effective on use. Fine tuning possibility of polymers allows them to be combined with other potential antibacterial materials in order to achieve increased antibacterial activity.

1.4 Chitosan

Chitosan is a semi-synthetic polymer obtained by the deacetylation of natural polymer chitin. Treatment of chitin with sodium hydroxide results in deacetylation of the amine groups. As a result, linear structure of polymer contains repeat units alternating between acetylated and deacetylated form randomly. Chitosan has huge variety of medical and commercial uses such as incorporation into the bandages for wound healing, agricultural use as a biopesticide, paint ingredient in industry, drug delivery systems and as an ink in bioprinting systems [9].

In a study, chitosan was combined with silk and Ag or Sr nanoparticles to form a cryogel at -20^{o} C in order to test potential antimicrobial activity and osteoinductivity property. Silver nanoparticle loaded cryogels was found to exhibit higher antibacterial ratio against *E. coli* and *S. epidermidis* when compared to other cryogels [10].

In another study, chitosan was used to produce carboxymethyl chitosan (CMC) cryogels containing different amount of silver nitrate concentration at 10 and 5 mmol/L. Sodium borohydride was used as a silver reducing agent and brown coloured final cryogel products were obtained. Antibacterial activity study for these cryogels using disc diffusion method indicated the increase in initial silver nitrate concentration also increased the inhibition zone against *E. coli* [11].

Chitosan as an antibacterial agent has found many uses due to it being biodegradable, biocompatible, and lack of toxicity to humans. These properties and chemical structure of chitosan allow further modifications on the structure. Chitosan can be cross-linked to form a gel, it can be used to form beads and films. Cross-linking process is utilized

to connect different chains of polymers. Cross-linking process can occur in two ways; chemical cross-linking or physical cross-linking.

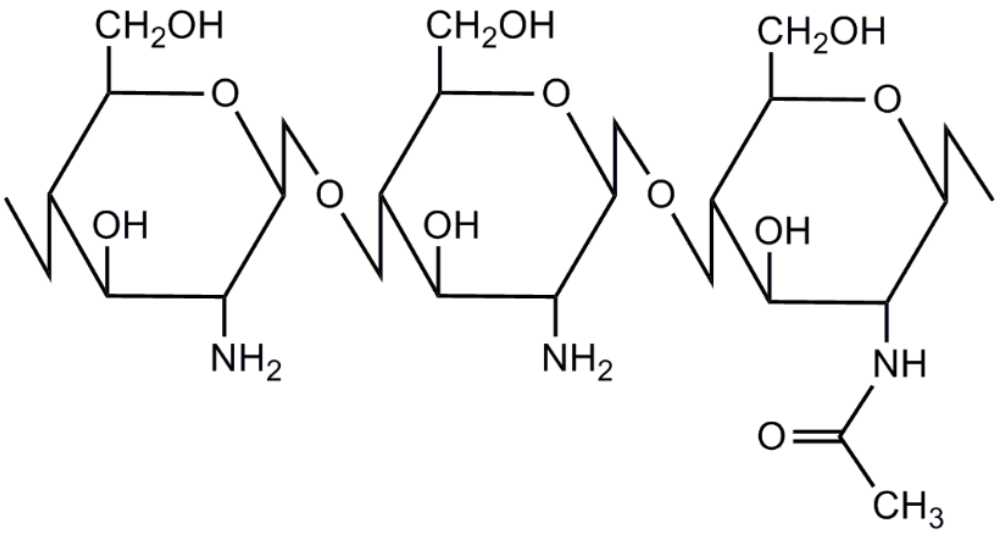

Figure 2: Structure of chitosan

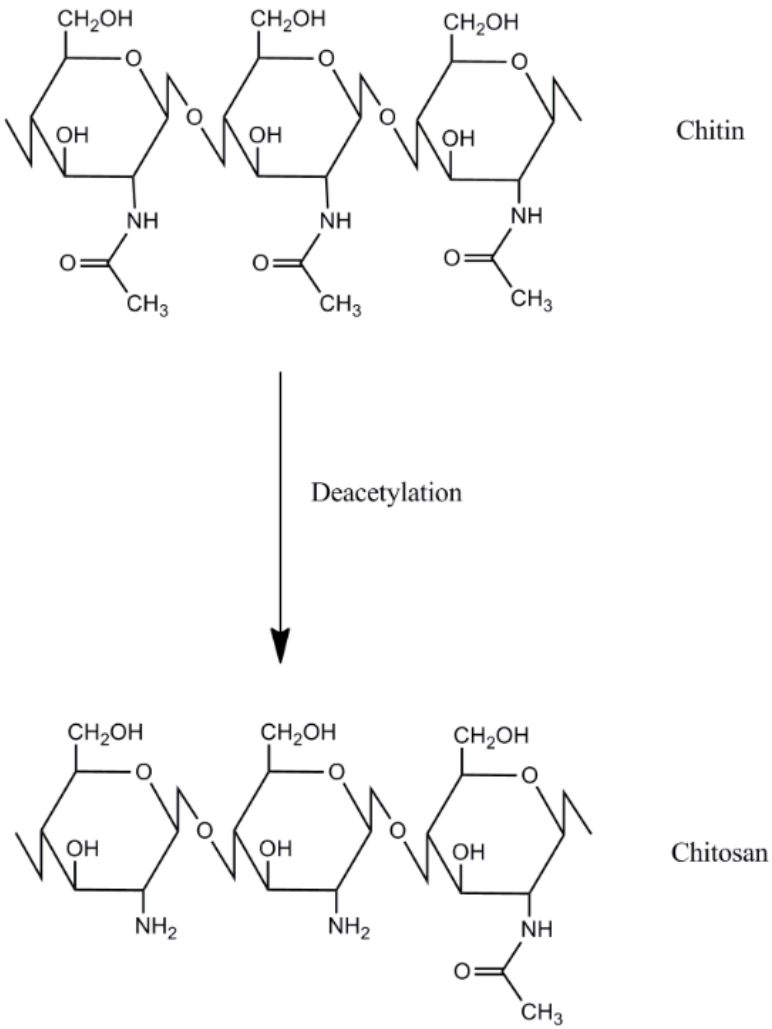

Figure 3: Chitin deacetylation to obtain chitosan

1.5 Physical and chemical cross-linking

Physical cross-linking refeers to the weak interaction holding together different polymer chains. Formation of sodium alginate gels can be achieved through ionic bonds introduced by calcium ion addition to the reaction mixture. Physical cross-linking often brings structural weakness and reduced mechanical stress to the material. Chemical cross-linking occurs via covalent bond interaction between different polymer chains. Formation of chemical cross-link is usually facilitated by an energy input such as heating, UV light exposure, increasing pressure and change in pH. Chemically cross-linked polymers exhibit higher structural strength, mechanical and

thermal stability when compared to physical cross-linking thus making recycling of many chemically cross-linked polymers very challenging. Glutaraldehyde, epichlorohydrin and ethylene glycol dimethacrylate are commonly used chemical cross-linking agents.

1.5.1 Glutaraldehyde as a chemical cross-linking agent

Glutaraldehyde (GA) reacts with amine groups via its carbonly reactive ends to provide chemical cross-linking. This property makes it a good candidate as a cross-linking agent for chitosan based polymers due to the presence of amine groups in chitosan backbone. In fact, literature contains many studies using these 2 reagents to obtain a specific polymeric material. In a study, effect of GA cross-linking on swelling ability of chitosan hydrogel was tested. Researchers added 1.0 mL, 1.5 mL, and 2.0 mL of 0.1% GA cross-linker to their 7.5% (w/v) chitosan dissolved in 2.0% acetic acid solution to obtain hydrogels with different cross-link density. It was found that the presence of cross-linker reduced overall swelling ability of hydrogels compared to initial no cross-linker containing hydrogel. In addition, swelling capacity of cross-linked hydrogels showed increase in swelling ability as the cross-link density increased [12].

1.6 Cryogels

Cryogel is a type of polymeric gel produced at subzero temperatures via the application of controlled freeze thawing procedure to the polymer mixture. Freeze thawing takes place in two steps: freezing of polymer mixture and application of increased temperature. Each cycle consists of these two steps. Introduction of high porous structure is achieved by this method. During the freeze thawing process, solvent becomes a crystalline structure while the polymer backbone remains unchanged. During each cycle, freezing and melting of solvent found between the polymer

backbone creates air pockets, also called as pores. For a successful freeze thawing cycle application of correct temperature in order to achieve complete frozen state of solvent is required.

Increaed porosity also increases the available surface area of the designed polymer. As a result, cryogel systems are very popular in tissue engineering and biomedical applications [13]. Chitosan cryogels have also been prepared and used for various applications. In one study, researchers were able to produce injectable chitosan cryogel microsphere scaffolds for use in tissue implanting. Combination of varying concentrations of chitosan with GA as a crosslinking agent allowed the production of microspheres with similar pore sizes. However, increased concentrations of chitosan also lead to increase in particle size which had a direct effect on the injection ability of the cryogels [14].

1.7 Aim of the Thesis

The aim of this thesis is to synthesize GA cross-linked silver nanoparticle containing cryogels and determine their water retention capacity, swelling behaviour and antibacterial activity.

Chapter 2

EXPERIMENTAL

2.1 Materials

Chitosan (medium molecular weight) (Sigma-aldrich, Germany) , glutaraldehyde (%25) (Sigma-aldrich, Germany) , silver nitrate solution (0.1M) (Philip harris) , acetic acid (glacial) (Merck-Germany) , mueller-hinton agar (HiMedia) , distilled water , *Escherichia coli* (ATCC 29922) and *Staphylococcus aureus* (NTCT 12493).

2.2 Method

2.2.1 Synthesis of silver nanoparticle loaded cryogels

Chitosan was dissolved in 1% (v/v) acetic acid solution using a magnetic stirrer to obtain a 1.5% (w/v) chitosan concentration. Then, 20mL of chitosan solution was mixed with 10mL 0.01M or 1mL 0.1M silver nitrate solution seperately under stirring in which silver nitrate was added slowly by a dropper. Following the adition of silver nitrate, mixture was heated up to 95^{o}C and was kept at this temperature for 15 minutes. Immediately after heat exposure, gluteraldehyde cross-linker at 1% (v/v) and concentrated concentration was added to the solutions and mixed throughly. Next, mixture was left to cooldown until it reached room temperature, poured in clean glass petri dishes to achieve a uniform spread and was transferred to the freezer where freeze

thawing cycle would take process. Freeze thawing procedure was done in 3 cycles in which each cycle took 24 hours.

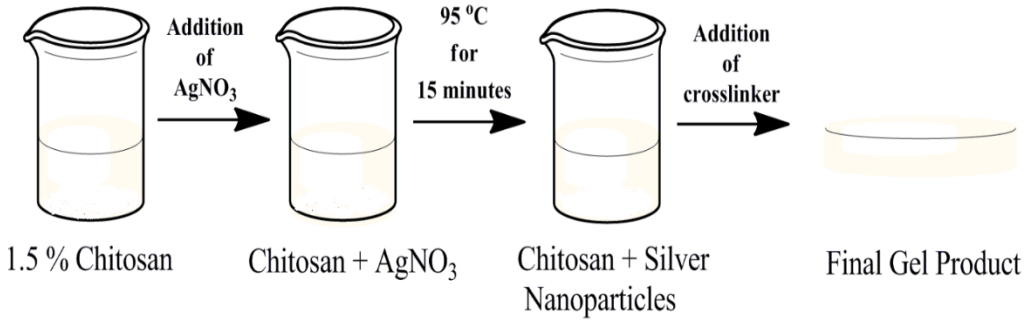

Figure 4: Illustration of cryogel synthesis procedure

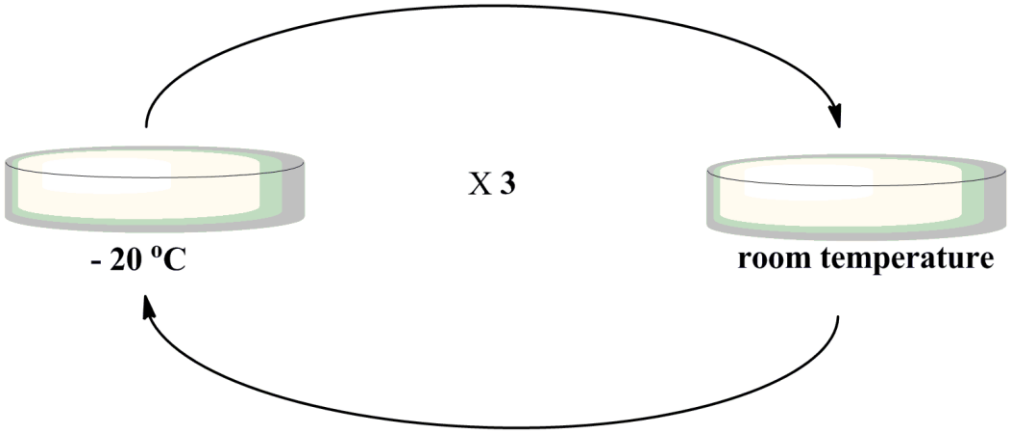

Figure 5**:** Explanation of freeze thawing procedure

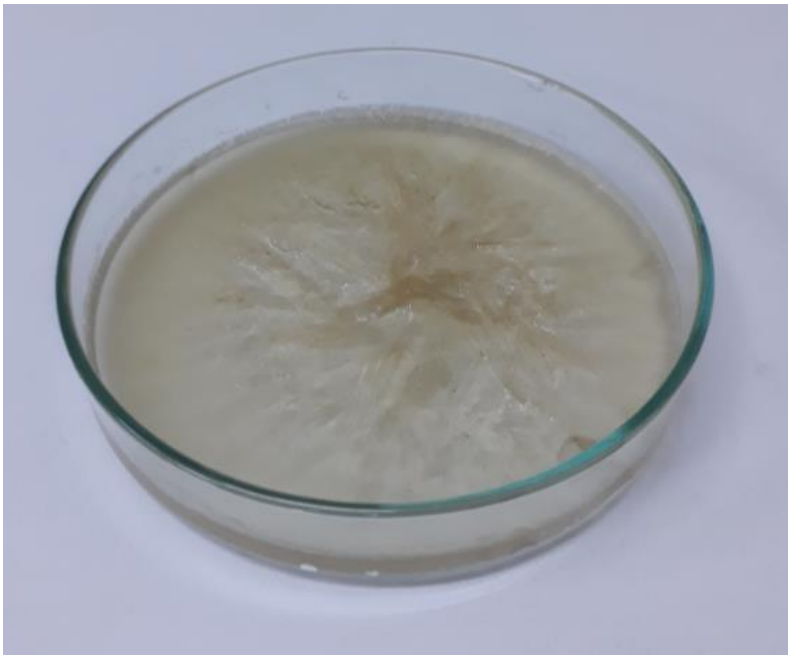

Figure 6: Cryogel sample after freeze thawing process

2.2.2 Drying and water retention capacity study

Introduction of porous structure to the synthesized polymer cryogels as a result of freeze thawing process leads to the increased amounts of water holding capacity for cryogels when compared to other materials. Water content and drying ability of the synthesized cryogels were tested by putting wet cryogels with a known initial mass into a 40C oven and removing them out at specific time intervals to measure their mass until it reaches a constant mass and decrease in the measured mass is no longer observed. Time intervals for drying study was chosen as 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180 and 210 minutes. Following formula have been used for the calculation of water retention (wr):

$$Wr = ((Mt - Md) / Ms)) \times 100 \quad (1)$$

Where Mt is the mass of cryogel sample at given time interval, Md is the mass of completely dry cryogel and Ms is the mass of initial fully swollen cryogel.

2.2.3 Swelling study

Swelling behaviour of the synthesized cryogels is an indication of structural integrity for cryogels and it was measured to understand the porous structure formed as a result of cryogel formation. Parts from each cryogel with a known initial mass was dried out completely prior to the use in swelling study. Then, each sample was placed in a pH 7.4 phosphate buffer and was kept at room temperature during the swelling study. At specific time intervals, cryogels were carefully removed from the buffer solution and their masses were measured using a calibrated balance. Immediately after they were placed back into the buffer solution until further measurement. Time intervals for swelling study was chosen as 5, 10, 15, 20, 25, 30, 45, 60 minutes and last measurement was taken at 24 hour mark. Following formula have been used for calculation of the % swelling:

$$\% \text{ swelling} = ((Mw-Md)/Md) \times 100 \quad (2)$$

Where Mw is the mass of wet cryogel and Md is the initial dry cryogel.

2.2.4 Characterization

2.2.4.1 FT-IR

Characterization of the used materials and the synthesized cryogels were done by using a Perkin Elmer FT-IR spectrum two (UATR 2). All cryogels were used in dried forms.

2.2.5 Antibacterial activity assay

Antibacterial activity potential of the synthesized cryogels against *S. aureus* and E. coli was tested by measuring inhibition zone after agar diffusion assay. Prior to the antibacterial testing, cryogels were cut out in a circle shape to resemble antibiotic disks and washed with distilled water to remove any unreacted materials. For the preparation of bacterial cultures, fresh bacteria was inoculated onto blood agar one day before the experiment. Then, newly grown *S. aureus* and *E. coli* colonies were used to make a

solution with 0.5 McFarland turbidity. Next, with the help of a sterile swab, this bacterial solution was spread on Mueller-Hinton agar (MHA) throughly. After that, circle cryogel cutouts were placed on these agars which was followed by the bacterial inoculation at 37^{o}C for 24h in an incubator. Ciprofloxacin was used as a positive control in this experiment.

Chapter 3

RESULTS AND DISCUSSION

3.1 General comparison of synthesized cryogels

In order to achieve homogeneous distribution of samples, glass petri dishes were used as mentioned previously. For cryogel 1, use of high concentration of GA lead to the formation of non-homogeneous clumps inside the mixture thus preventing cryogel from spreading evenly onto the petri dish. For the tests where cryogel 1 was used, an area with high visible amount of homogeneous structure present was cut out and used. In other cryogel samples smooth and homogeneous surface was achieved at the end of freeze thawing procedure thus making them easier to use in experiments. Colour change is an indicator of reduction of silver found in silver nitrate to silver nanoparticles. In all silver nitrate containing cryogel samples visible colour change of cryogels from yellow to brown was observed. In cryogel 4 which contains no silver nitrate there was no visible colour change indicating the colour change is related to the silver nanoparticle formation. Addition of 2mL 1% GA was decided experimentally during the production of cryogels. GA was added slowly into the each mixture under constant stirring and the structure of mixture was observed for each cryogel sample during the addition of cross-linker. After the addition of 2mL cross-linker, visible increase in viscosity indicated the increase in internal resistance caused by the bond formation between GA and chitosan thus no more cross-linker was added. In the case of cryogel 1, increased concentration of GA in the mixture lead to formation of seperate high viscous areas instead of forming a homogeneous structure.

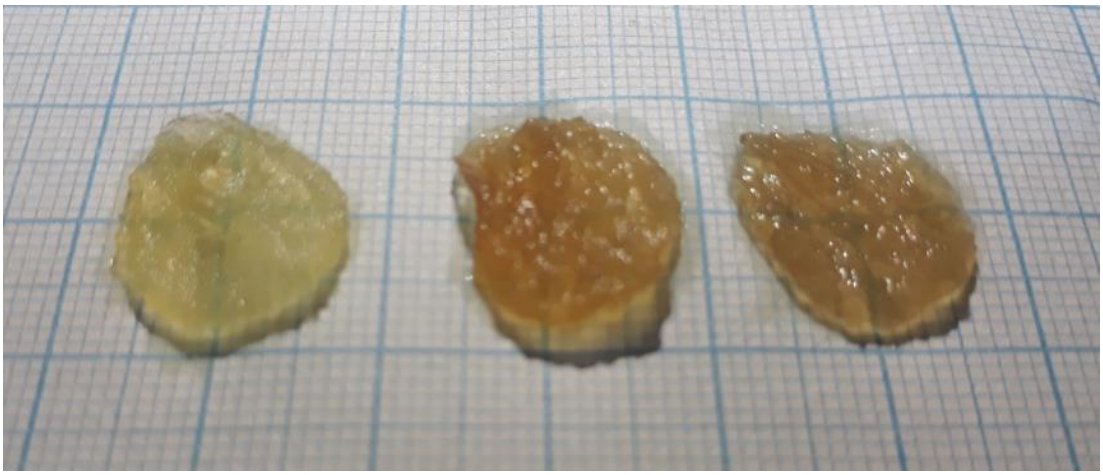

Figure 7: Indication of silver nanoparticle formation as a result of colour change (left: no silver, middle: 0.01M 10mL silver nitrate, right: 0.1M 1mL silver nitrate)

3.2 Drying and water retention

The effects of cross-linker concentration and silver nitrate concentration on drying speed and water retention ability of the synthesized cryogels were investigated. Table 1 shows the difference between each cryogel and Figure shows the water retention ability of cryogels.

Table 1: Contents of different cryogels

Name	Contents
Cryogel 1	0.01M (10mL) AgNO3 + Concentrated GA (2mL)
Cryogel 2	0.01M (10mL) AgNO3 + 1% GA (2mL)
Cryogel 3	0.1M (1mL) AgNO3 + 1% GA (2mL)
Cryogel 4	no AgNO3 + 1% GA (2mL)

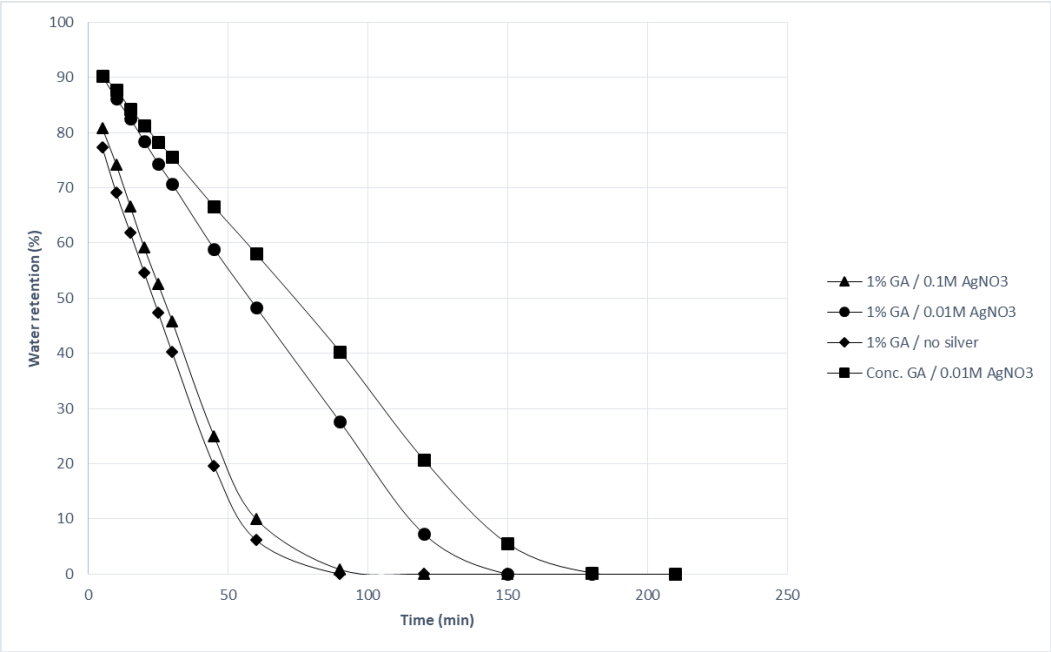

Figure 8: Water retention (%) capacity of cryogels vs time

Out of four tested cryogels, Cryogel 1 had the highest water retention capacity at any given time during the test. Minimum water retention capacity for Cryogel 1 was reached after 210 minutes wheras for Cryogel 2, 3 and 4 it was 150, 120 and 90 minutes respectively. These results indicate the effect of cross-linker concentration and silver nitrate concentration on water retention capacity. In Cryogel 1, use of highest amount of cross-linker introduced a structural resistance to water loss when compared to other cryogels. Cryogel 4 had the lowest amount of water retention capacity due to the lack of silver nanoparticles in the polymer structure. Presence of silver nanoparticles provides an interaction with free water molecules in the structure thus increasing water retention capacity. Cryogel 2 has higher water retention capacity when compared to Cryogel 3. As the only difference between these 2 cryogels is silver nitrate concentration, we may assume that introduction of silver nanoparticles by using different volumes of silver nitrate had a clear effect on their water retention capacity.

Cryogel 2 initially had higher amount of water introduction into the structure during the gelation process thus water retention capacity increased accordingly.

3.3 Swelling

Swelling capacity of cryogels were tested using a pH neutral phosphate buffer at room temperature. All cryogels reached constant mass after 60 minutes. This is as a result of limited space between the polymer structure caused by the drying method. Collapsed structure prevented the water uptake of all the cryogel samples resulting in a relatively short swelling behaviour. However, using the data obtained from drying study gives us % swelling for cryogel 1, 2, 3 and 4 as 2612.5, 2633, 1614 and 1840 respectively.

3.4 FTIR

FTIR spectra of chitosan, GA, Cryogel 2, 3 and 4 are shown in figures 9a, 9b, 9c, 9d and 9e respectively.

FTIR spectrum of chitosan: Broad stretching peak band centered at 3290 cm^{-1} represents O-H group vibrations. Peaks at 2926 and 2866 cm^{-1} represent –CH stretching in chitosan. Amide groups of chitosan C=O stretching, N-H bending and C-N stretching can be observed via the presence of the peaks at 1645, 1555 and 1376 cm^{-1} respectively. Finally the peaks at 1028 cm^{-1} indicates C-O and C-O-C stretching vibrations. FT-IR results are in correlation with literature information [15].

FTIR spectrum of GA: Peak around 1643 cm^{-1} indicates C=O stretching. C-H stretching can be observed by the peak at 2960 cm^{-1}. O-H stretching vibration peak at 3308 cm^{-1} is visible.

FTIR spectrum of Cryogel 2,3 and 4: Loss of peak at 1557 cm^{-1} which corresponds to N-H bending of chitosan indicates that crosslinking of chitosan by GA occurred through amine groups. Schiff's base reaction between C=N (imine bond) can be observed by the sharp peak at 1634 cm^{-1} further confirming the crosslinking reaction [16].

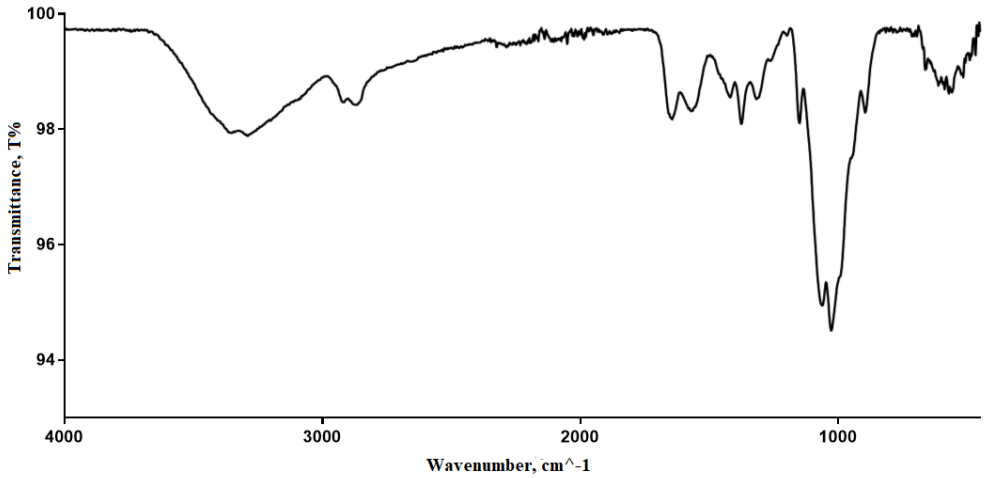

Figure 9a: FT-IR spectra of chitosan

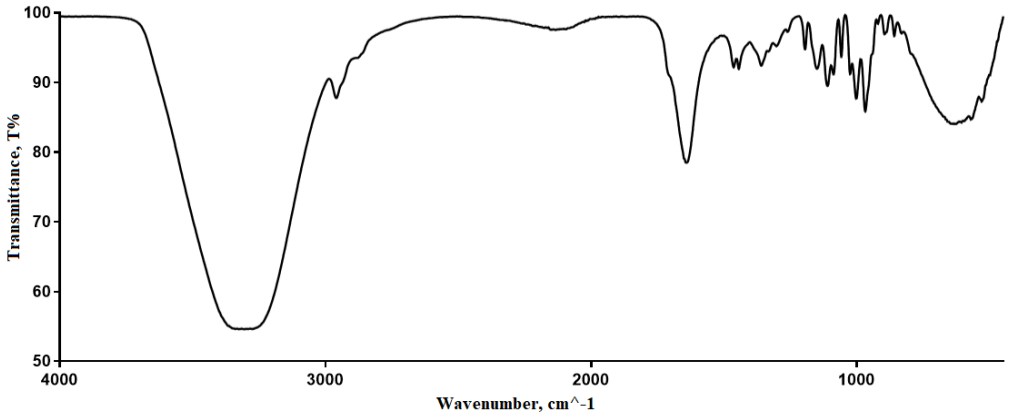

Figure 9b: FT-IR spectra of GA

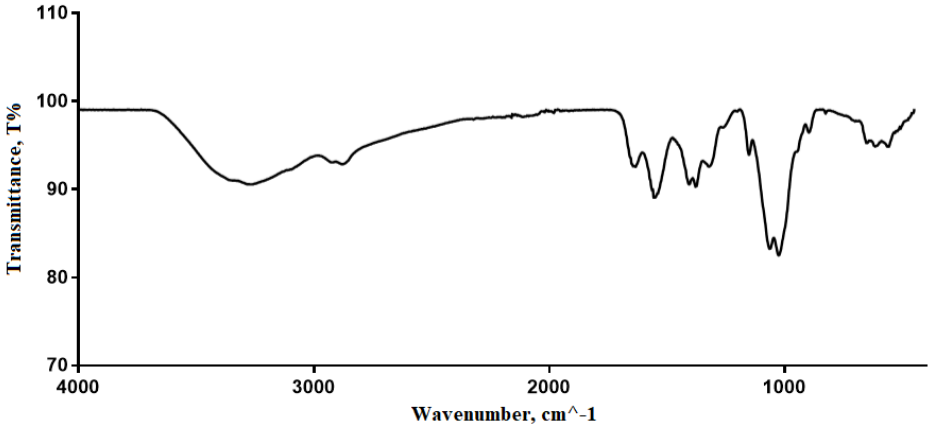

Figure 9c: FT-IR spectra of cryogel 2

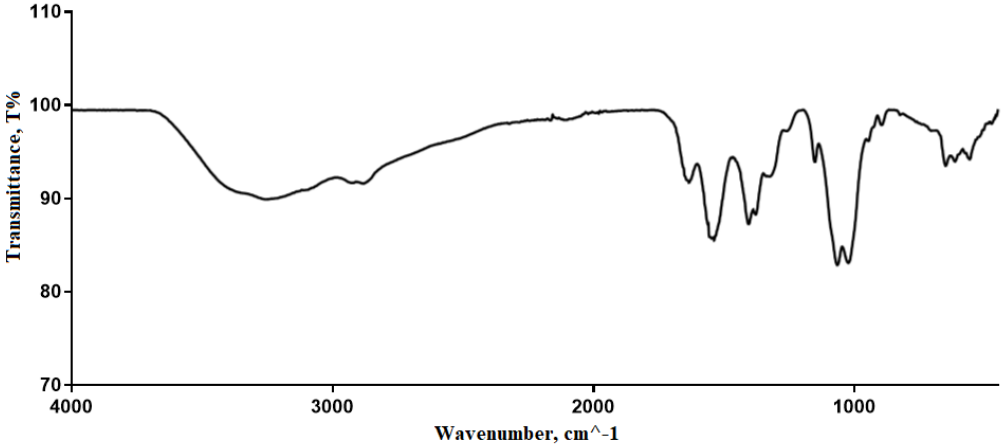

Figure 9d: FT-IR spectra of cryogel 3

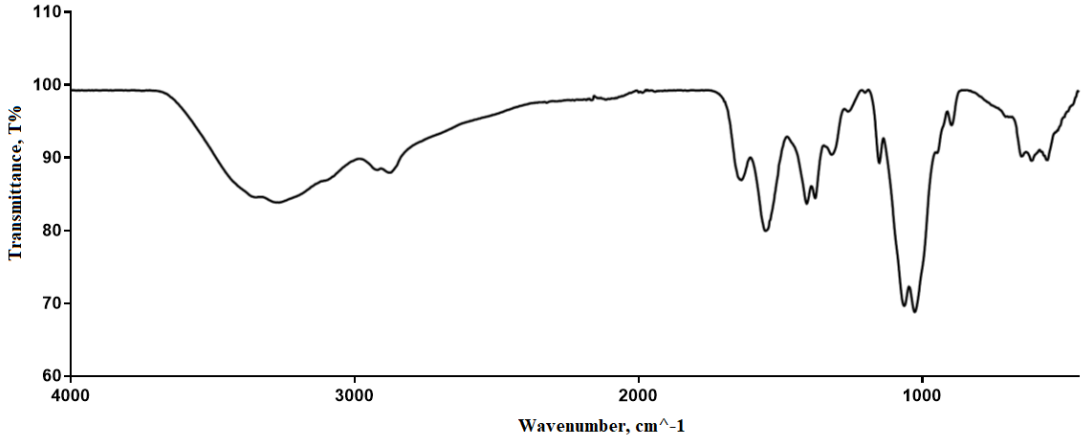

Figure 9e: FT-IR spectra of cryogel 4

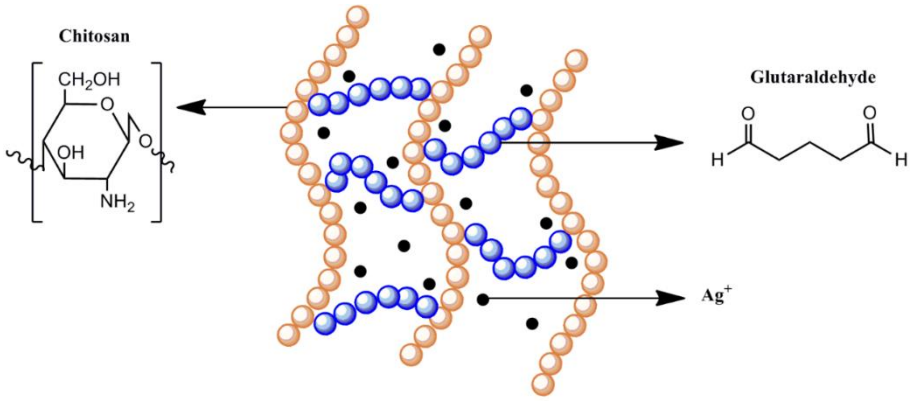

Figure 10: Schematic illustration of synthesized cryogel network

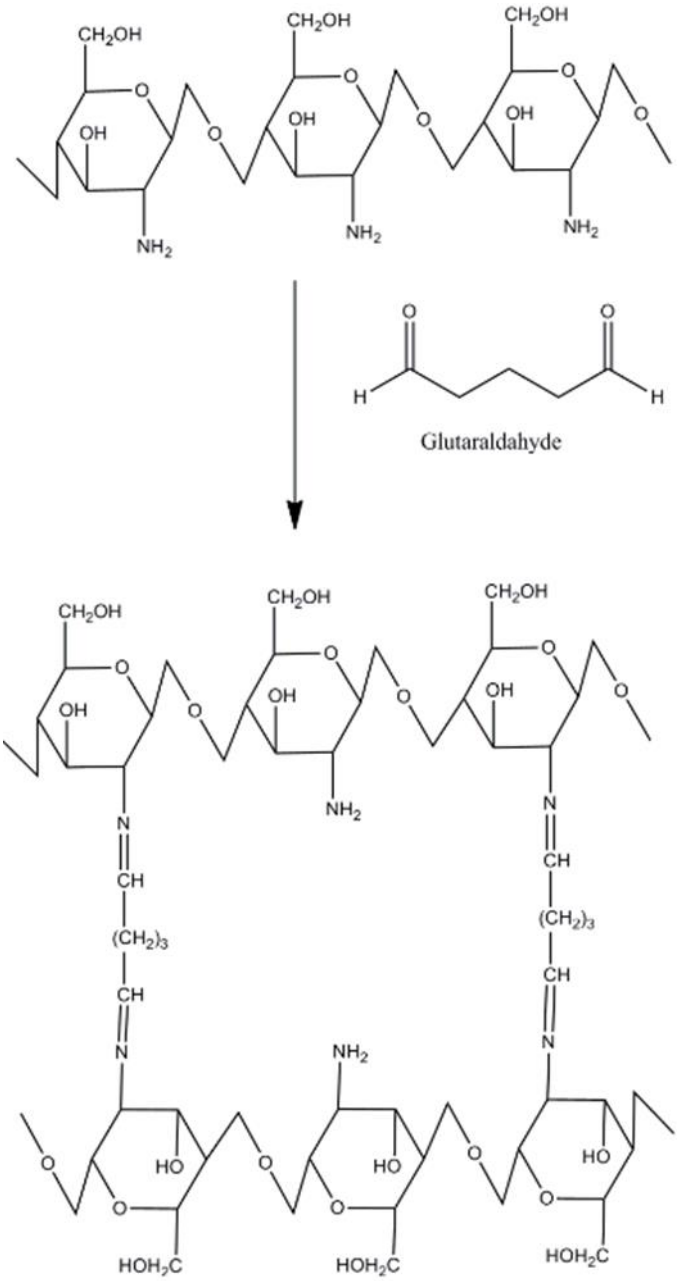

Figure 11: Chitosan and glutaraldehyde reaction

3.5 Antimicrobial activity

Antimicrobial activity of four different cryogels were determined by measuring the inhibition zone (mm) formed by the cryogel sample on bacteria inoculated agar plates after incubation at 37^{o}C for 24 hours. Table 2 shows the inhibition zone of each cryogel against two different types of bacteria.

Table 2 : Inhibition zone (mm) of cryogel samples against bacteria

	E. coli inhibition zone (mm)	*S.aureus* inhibition zone (mm)
Cryogel 1	14	15
Cryogel 2	15	14
Cryogel 3	16	15
Cryogel 4	13	13

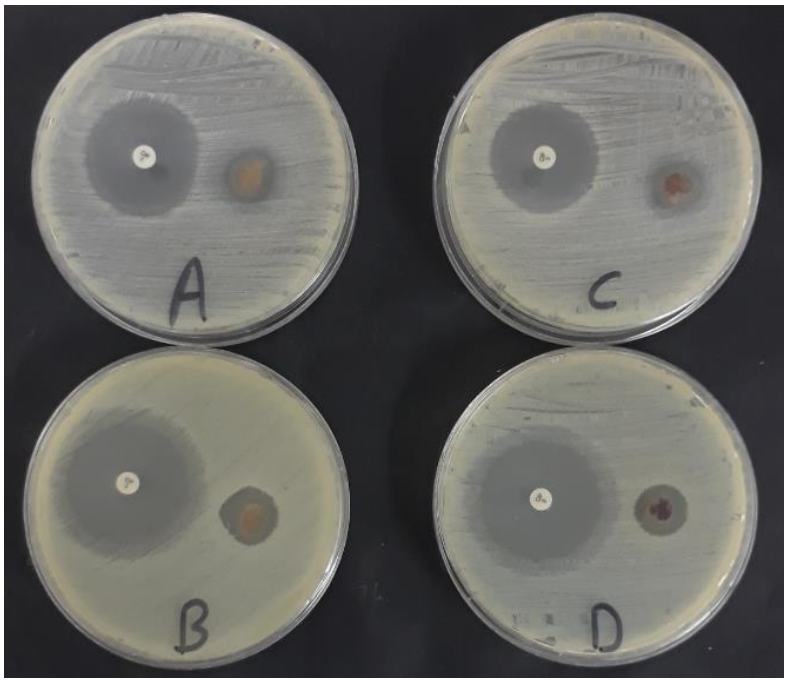

Figure 12: Antimicrobial activity of Cryogel 2 against A: *S. aureus* (14mm) and B: *E. coli* (15mm). Antimicrobial activity of Cryogel 1 against C: *S. aureus* (15mm) and D: *E. coli* (14mm). Ciprofloxacin disk inhibition zones are seen on the left side of the agar plates.

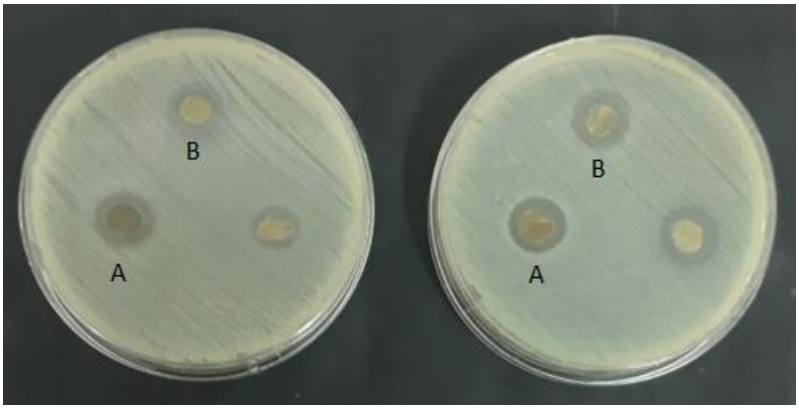

Figure 13: Antimicrobial activity of Cryogel 3 (A) against *S. aureus* (15mm) (left) and *E. coli* (16mm) (right). Antimicrobial activity of Cryogel 4 (B) against *S. aureus* (13mm) (left) and *E. coli* (13mm) (right)

Ciprofloxacin as a positive control group was used against both bacteria species. Inhibition zone of control group was 50 mm for *E. coli* and 35 mm for *S. aureus.* Our results showed that antimicrobial activity of synthesized silver nanoparticle containing cryogels did not differ much between the two tested bacteria species. Antimicrobial

effect of cross-linking agent GA was observed on Cryogel 4 at 13 mm. Addition of silver nanoparticles into the cryogel structure enhanced antimicrobial activity for Cryogel 1, 2 and 3. Highest inhibition zone was observed by Cryogel 3 at 16 mm against *E. coli.* For *S. aureus* highest inhibition zone was caused by Cryogel 1 and 3. Compared to Cryogel 2, antimicrobial activity of Cryogel 3 was slightly enhanced by increasing the concentration of silver nitrate. Silver is a strong antimicrobial agent and incorporation of it into the chitosan structure in the form of silver nanoparticles proved to be effective. Similar inhibition zones were observed in other studies where the effect of silver nanoparticles was tested [17,18]. Conditions that cause antimicrobial activity was further proved by these researchers using a DNA clevage method [17]. Effect of silver nanoparticles depend on the inhibition of intracellular ATP, disruption of plasma membrane and reaction with sulfur groups to bind with DNA. Formation of similar inhibition zones in two different bacteria species can be explained by the similarity of mechanisms that are targeted by silver nanoparticles.

Chapter 4

CONCLUSION

Cryogel 2, 3 and 4 were structurally in better homogeneous shape compared to Cryogel 1 because of the introduction of non-homogeneous mixture by the addition of concentrated GA during cross-linking. This non-homogeneous cryogel mixture containing increased amount of GA resulted in the excessive water intake capacity. Other cryogel samples were homogeneous thus allowing them to be utilized in other experiments easily.

Formation of silver nanoparticles with the help of high temperature (95^{o}C) proved to be effective for synthesized cryogels. Reaction mixture of cryogels containing silver nitrate started as a pale yellow colour which was then converted to dark yellow and brownish colour at the end of heat application period. This colour change is an indication of silver nanoparticle formation as the silver found in silver nitrate becomes a silver ion which then takes an electron from the medium to become reduced to silver nanoparticle.

Design of swelling experiment could be improved by using a freeze dryer to prevent cryogel structure from collapsing as opposed to oven drying method which resulted in non optimal conditions for swelling.

Presence of antibacterial activity for the synthesized cryogels indicates a potential environmental antibacterial use for cryogel samples especially in a water rich environment as the porous structure of cryogels proved to be efficient water containing molecules.

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