Anti-Bacterial Properties of Boron Cross-linked Chitosan/Poly (Vinyl Alcohol) Hydrogels Containing Zinc Oxide Nanoparticles

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ABSTRACT

Chitosan is a hydrophilic and versatile polysaccharide possessing functional groups such as carbonyl and amine groups which are areas for antimicrobial activity. PVA has high degree of swelling in aqueous solutions and mostly blended with chitosan because of its good tensile strength and elastic nature. Six chitosan- PVA hydrogel samples were prepared by varying the amounts of chitosan and PVA, blending them with equal amounts of boric acid (cross-linker), soaking them in sodium hydroxide overnight, washed several times with distilled water, freeze- thawed at -20^0 for 6hrs and $+ 37^0$ for 2hrs respectively for 5 cycles, then dried in an oven and later analyzed. During the hydrogel preparations, the first three samples were blended without zinc oxide nanoparticles and the last three with equal amounts of zinc oxide nanoparticles. Swelling percentages of samples 1- 3 were evaluated in 2 ways: (1) soaking a known amount of each dried sample in distilled water while varying time. (2) soaking another known amount of dried samples in different pH buffer solutions for 24hrs. Its kinetics showed that: as the pH increases, their swelling % increases and starts dropping as the pH gets more basic and that the higher the amount of PVA in a sample, the higher its swelling % in water with increased time.

The bacterial analysis of the samples were carried out in order to determine the effect of an increased amount of chitosan and of zinc oxide nanoparticles on bacterial growth. The LB agar medium was used for the culturing of the E.coli bacteria which is a gram negative bacteria possessing a thinner peptidoglycan membrane. The sample with the highest amount of Chitosan showed more inhibition than the others and the addition of nanoparticles to this sample showed an even more

greater inhibition towards the bacteria. Finally the Scanning electron microscopy (SEM) and FTIR of the prepared hydrogels were done inorder to determine the surface structures of the hydrogels and the various functional groups present in each hydrogel respectively.

Keywords : Chitosan-PVA hydrogel, Zinc oxide nanoparticles, Boric acid cross linker, Antibacterial properties, Swelling properties.

ÖZ

Kitosan-PVA hidrojel örnekleri, değişik miktarda kitosan ile PVA'nın sırasıyla -20 °C'de 6 saat + 37 °C 2 saat süreyle ilk dondurma/çözülme döngüyle hazırlanmıştır. Bu örneklerin, 5 donma/çözülme döngülüyle hazırlanmış olması sonucundaki kristallindeki artışla son derece esnek hidrojeller oluşmuştur. Eşit miktarda borik asitle çaprazbağlanmış çeşitli örnekler gece boyunca NaOH çözeltisiyle muamele edilmiş ardından hidrojel memran 5 kez saf suda durulanarak temizlenmiş ve 24 saat + 80 ° C'de bir fırında kurutulmuştur.Çinko oksit nanopartikülleri içermeyen numunelerin Şişme yüzdeleri iki yolla belirlendi: Birincisi, değişik zaman aralığında bilinen miktarlardaki kuru örneklerin saf su içerisideki, ikincisi ise farklı tampon çözeltilerindeki 24 saatlik etkileşimleridir. Kinetiğinden pH arttıkça % şişmede artış gözlenirken, pH ın oldukça yüksek, bazik şartlarında ise düşüş gözlenmektedir. Sudaki örneklerinden PVA miktarının artması PVA'ün sudaki çözünebilmesi özelliğinden, kitosanın ise çözünmeme özelliğinden dolayı şişmeyi arttıracaktır.

ZnO nanopartikülleri içeren ve içermeyen örnekler için antibakteriyel özellikler, öncelikle kitosanın ve PVA miktarlarının bakteri çoğalmasına etkisi ile, ikinci olarak bu örneklerin nanopartikül içermesi durumundaki bakteri çoğalmasını önleyici etkisi olup olmadığıyla belirlenmiştir. LB agar ortamı daha ince bir peptidoglikan membran sahip olan bir Gram negatif bakteridir ve E.coli bakteri kültürünün oluşturulması için kullanılmıştır. Yüksek miktarda kitosan içeren örneklerin diğer örneklere göre, bakteri çoğalmasına karşın daha fazla önleyici etkisi olmuştur ve bu önleyici etki nanopartikül ilavesiyle artış göstermiştir. Son olarak hazırlanan örneklerin analizleri SEM ve FTIR ile yapılmıştır.

Anahtar Kelimeler: Kitosan-PVA hidrojel, Çinko oksit nanaopartikülleri, Borik asit çaprazbağlayıcı, Antibakteriyel özellikler, Şişme özellikleri

DEDICATION

To my late dad Mr. Nanfang Valentin and to my lovely mum Mme Hangue Lisette.

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Special thanks to the almighty God for the strength he bestowed on me, to my family for their moral and financial support, to my supervisor Associate Prof Dr Mustafa Gazi for all the advices and assistance he provided during the realization of this work, to Dr Imge Kunter for her support during the antibacterial evaluations, to Mosab Abureesh for his help and guidance and to all my friends for their moral and intellectual support.

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NOMENCLATURE

PVA	Poly vinyl alcohol
SEM	Scanning electron microscope
ZnO	Zinc oxide nanoparticles
LB	Luria Broth
NaOH	Sodium hydroxide
$ZnSO_4$	Zinc sulfate
E.coli	*Escherichia coli*
FTIR	Fourier transform infrared

Chapter 1

INTRODUCTION

1.1 Aim

Evaluating the antibacterial properties of chitosan and Polyvinyl alcohol with and without nanoparticles in order to analyze the effect of chitosan and zinc oxide on bacterial growth. The addition of chitosan to PVA hydrogel increases the biocompatibility as well as the antibacterial properties of the hydrogel since chitosan possesses free amino.

1.2 Hydrogels

Hydrogels are materials which are either obtained or derived from synthetic or natural polymers, exhibiting a three-dimensional (3D) structure. They don't dissolve in water but rather retains it due to their structure thereby increasing in size (1– 2). Hydrogels are either obtained by covalently cross-linking linear polymers or simply by non-covalently cross-linking heterogeneous polymers (3). They possess a stable and modifiable structure, biocompatibility, easily swells in water (hydrophilicity) and non- toxicity are reasons why researchers used them in various fields such as in antibacterial purposes, medicine, food stuffs and in controlled drug delivery systems (1). Their physicochemical properties don't only depend on their structure, degree of cross-linking or molecular structure but also on the amount of water they can absorb.

1.3 Chitosan

Chitosan is a hydrophilic polysaccharide, a versatile D-glucosamine obtained from chitin N-deacetylation, produced industrially from shells of crustaceans and as well known to be a copolymer of N- acetyl glucosamine and of glucosamine (4). It has properties such as: non-toxicity, biocompatibility, biodegradability, binding capacity, bioactivity, capacity in assimilating metals, antimicrobial activity, capacity in binding to fat as well as possessing antibacterial properties either alone or associated to other natural polymers (5). Chitosan possesses a broad range of spectrum towards bacterials and fungi favoring the death of bacterials at a quicker rate. The bacterial cell membrane content leaks out when the positively charged molecules of chitosan reacts with its negatively charged cell membrane by causing their destruction (6). Chitosan is known to be poorly soluble that is it doesn't really swell. Many cross linkers are used in the production of chitosan hydrogels such as; glutaraldehyde, glyoxal, Quinone, boric acid and others. Chitosan possesses groups such as carbonyl and amine groups (NH) which are the areas where antimicrobial activity takes place. Known as a weak base with pKa 6.5, possesses amino groups which are primary and easily get protons in an acidic medium, enhances the solubility of water at different pH levels.

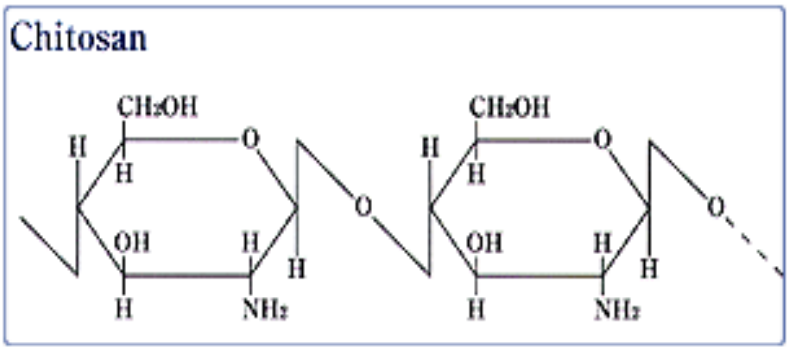

Figure 1: structure of chitosan

1.4 Poly (vinyl alcohol)

PVA is a synthetic, hydrophilic and soluble polymer which is obtained by the dissolution of polyvinyl acetate in an alcohol and later on treated with sodium hydroxide (8). PVA is mostly used in blending other natural materials due to the fact that, it is non toxic, very polar, soluble in water, non carcinogenic, biocompatible with a lot of biopolymers, biodegradable and can as well form fibers (8– 10). Due to these features, PVA possesses many biomedical applications such as replacement material for the skin and cartilage replacement material and others (7). PVA as well possesses satisfactory physical properties such as its elasticity, its high degree of swelling in solutions which are aqueous, its adhesivity, its emulsifying nature and most importantly its good film foaming capacity. PVA is mostly blended with chitosan due to its elasticity and good tensile strength (7). It is associated to boric acid during paper adhesives fabrications and to formaldehyde and butyraldehyde during resin formation.

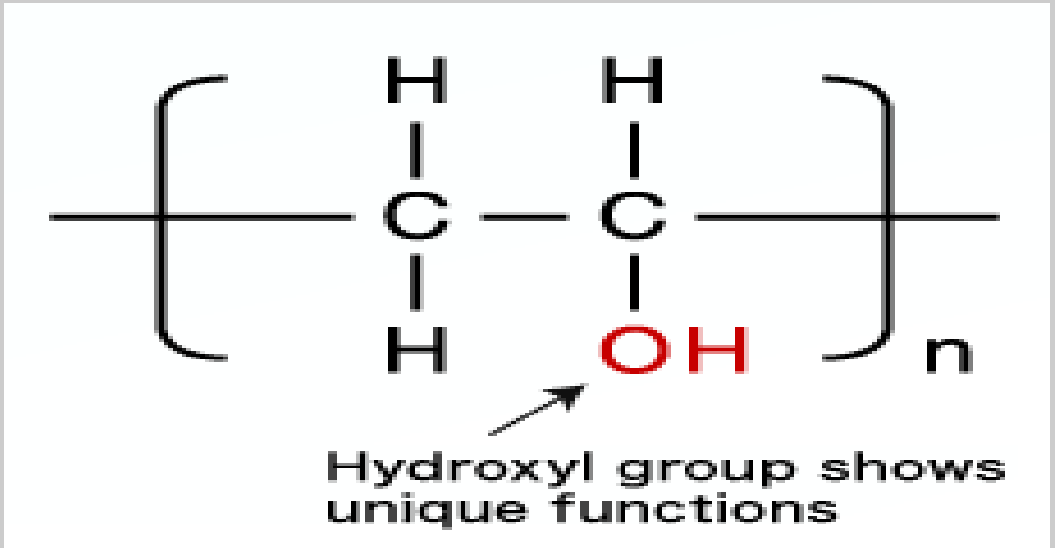

Figure 2: structure of Poly (vinyl alcohol)

1.5 Zinc oxide nanoparticles

Nanoparticles are generally defined as particles of sizes between 1-100nm and their small size makes it easier for them to penetrate the samples or hydrogel and play its role either biomedical or else wise reason for most researchers' interest in them. According to some researchers, we have 3 types of nanoparticles; metal nanoparticles, polymer nanoparticles and metal oxide nanoparticles. Zinc oxide nanoparticles on its own are used in areas such as; cosmetics, solar cells, chemical sensors, biosensors, gas sensors and drug delivery (11–15). Researchers who worked using ZnO nanoparticles found out that its antibacterial activity range is very wide especially towards gram negative andpositive ones meaning that they easily destroy the growth of these bacterial. This nanoparticle possesses reacting oxygen specie(ROS). This ROS stimulates the destruction of bacterial cell wall, enhancing the permeability of the membrane and favoring the easy diffusion of the nanoparticle. During this process, the proton motive force is lost and the dissolved toxic zinc ions are assimilated leading to the inability of the mitochondria to properly assume its functions, outflow of the intracellular contents and the release of the gene expression oxidative stress causing the inhibition of cell growth and consequently its death (16– 18).

1.6 Boric acid cross- linker

Cross linking is defined as the formation of covalent bonds between the cross linked molecules. A lot of cross-linkers have been used such as formaldehyde, glutaraldehyde, Quinone and various others but boric acid was chosen in this case. Boric acid is chosen because it is known to be the perfect cross-linker for PVA since crosslinking them slows the PVA molecular action leading to the increase in the

viscosity of the solution and giving rise to a viscoelastic gel (28). Boric acid is said to possess antibacterial properties than PVA and helps to harden PVA gel beads.

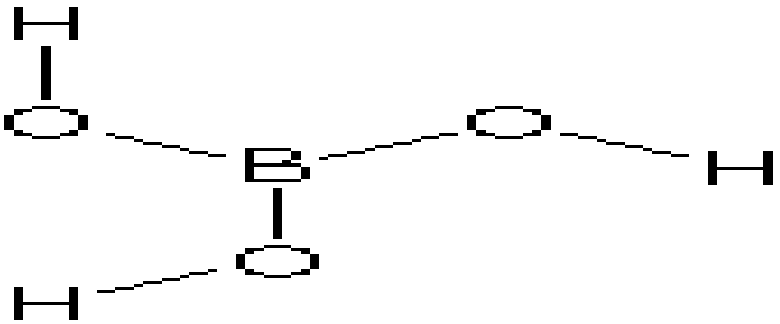

Figure 3: structure of boric acid

1.7 Bacteria

Bacteria are micro organisms possessing only 1 cell and without nucleus. *E.coli* is known as a gram negative, rod shaped, facultative anaerobic bacteria which stays in the digestive tracts of animals as well as humans. Many types of harmless *E.coli* exist but the harmfull ones are few and they can cause infections such as: severe anemia, bloody diarrhea, kidney failure, food poisoning, urinary tract infections and others (24). The harmless ones forms part of the normal floral gut and helps their hosts in the production of vitamin K_2 thereby preventing their intestines to be colonised with pathogenic bacteria (25-28). This bacterial infection is gotten by being in contact with the stool of humans or animals either by drinking or eating contaminated foodstuffs. This can be prevented by constantly keeping our environment clean and this infection can be treated by using antibiotics such as fluoroquinolones, azithromycin or rifaximin (29).

Chapter 2

EXPERIMENTAL

2.1 Materials

- Sodium hydroxide(pellets, 97+%, A.C.S.reagent, Aldrich, cas no 1310-73-2, made in Germany)
- Zinc sulphate(AnalaR*),
- Chitosan of high molecular weight(600000g/mol, Fluka and made in Switzerland)
- PVA(99+%) hydrolysed, Aldrich with molecular weight between 85000-146000g/mol)
- Boric acid(cas no 10043-35-3, molecular weight 61.83g/mol, Sigma-Aldrich,USA)
- Acetic acid 100%(cas no 64-19-7, Riedel-deHaen, made in Germany)
- Tryptone (Sigma Aldrich, cas no 91079-40-2)
- Yeast extract (Sigma Aldrich , cas no 8013-01-2)
- Sodium chloride (Sigma Aldrich , cas no 7647-14-5)
- Agar (Sigma Aldrich , 9002-18-0)
- Distilled water

2.2 Methods of preparation

2.2.1 Preparation of Zinc Oxide nanoparticles

3g of sodium hydroxide (NaoH)was dillutedin 250ml of distilled water and 6.5g of zinc sulphate (ZnS04)was dilluted in 200ml of distilled water as well.The dissolved $ZnS0_4$ solution containing a magnetic stirrer is placed on a stirring balance and NaoH was gradually added in to the solution while it stirs continuously at 150^{o}C for a period of 12hrs and the substance obtained is rinsed with distilled water numerous times till its pH gets neutral. It was then filteredwith the use of filter paper, dried inside an oven,analyzed using scanning electron microscope (SEM) and then used.

2.2.2 Preparation of Chitosan/PVA hydrogel /Boric acid

PVA(5% w/v) solution was made by dilluting5g of it in distilled water at 85^{o}C for a period of 6hrs while stirring continuously with a magnetic stirrer and Chitosan polymer (2% w/v) on the other hand was made by dissolving 2g of Chitosan in acetic acid (1%v/v) because it doesn't dissolve in water. They were then mixed at varying ratios and stirred overnight, then boric acid (10%w/v) was dillutedin distilled water and added to the various samples and stirred and the various prepared samples were then soaked in sodium hydroxide (10%w/v) which was dilluted in pure water and kept overnight for the membranes of the hydrogels to be removed. These prepared samples were then washed, casted in to Petri dishes and freeze/thawed at −20^{o}C for 6hrs and at +37^{o}C for 2hr respectively for 5times in order for 5 cycles to be obtained. The freeze- thawed samples are then dried in an oven at 80^{o}C for 48hrs and the swelling and gelation properties of the various samples analyzed (20).

Table 1: Composition of samples 1, 2 and 3

Sample number	Amount of PVA/g	Amount of Chitosan/g	Boric acid/g	Sodium hydroxide/g
S1	1.5	1.5	1	1
S2	1	2	1	1
S3	2	1	1	1

2.2.3 Preparation of ZnO nanoparticles/Boric acid/Chitosan/PVA hydrogel

Different amounts of chitosan and PVA were diluted and mixed as mentioned above, then equal amount of ZnO nanoparticles (5% w/v) was diluted in water and then added in to the chitosan/ PVA mixture and then blended as well before the addition of boric acid and sodium hydroxide and finally washed, freeze/ thawed and dried. The nanoparticle was first of all diluted in distilled water before its addition in order for the blending to be effective.

Table 2: Compositions of samples 4, 5 and 6

Sample no	PVA/g	Chitosan/g	Boric acid/g	Sodium hydroxide/g	ZnO nanoparticles/g
S4	1.5	1.5	1	1	0.1
S5	1	2	1	1	0.1
S6	2	1	1	1	0.1

2.2.4 Preparation of LB medium and LB agar

For the LB medium, 1g of tryptone was added to 0.5g of yeast extract and 1g of sodium chloride and was well stirred with 100ml of distilled water then sterilised by autoclave for 1hour at 115^{o}c. The solution was cooled down and 50µl of the stock solution of E.coli was inoculated and incubated in a rotator shaker at 120rpm at 37^{0}c for 16hrs and a homogenous mixture with a slight color change indicating the bacterial growth was obtained. We always use bacteria with their logarithmic phase.

For the LB agar, 2g of tryptone was added to 1g of yeast extract, 2g of sodium chloride and to 3g of agar. This mixture was stirred with 200ml of distilled water and autoclaved for 1hour at 115^{0}c, cooled and then 12.5ml of the mixture was poured in Petri dishes under a lamina flow cabinet which is a sterilized environment and allowed to cool before closing them in order to avoid vapor formation on top of the dishes.

2.3 Swelling and Gelation percentages of the hydrogels

2.3.1 Swelling percentage (%)

A known amount of each of the dried samples of the hydrogels without nanoparticles were cut and recorded as W_2 and then soaked in distilled water at different time intervals (2hrs, 4hrs, 6hrs, 8hrs, 10hrs and 24hrs), removed from distilled water, wiped using filter paper,reweighed and recorded as W_1 for the first step. The second step was done by measuring again the dried weights of a known amount of each of the samples (W_2) and then they were soaked this time in different buffer pH solutions (2, 6 ,7 ,10 ,12) for 24hrs and then reweighed (W_1). The formula used for the calculation is stated below:

$$\text{Swelling \%} = \frac{W1 - W_2}{W_2} \times 100$$

2.3.2 Gelation percentage (%)

This was done in order to know the total amount of gel formed after being dried by each of the prepared samples that is to know if the total quantity of materials used at the beginning during their various preparations all formed gels or not . This was then obtained by making use of the following formula;

$$\frac{\text{Weight of the dried hydrogel}}{\text{Initial quantity of hydrogel used}} \times 100$$

2.4 Hydrogels samples analysis

Each of the above prepared samples were analyzed using techniques known as scanning electron microscope and fourier transform infrared in order to determine the surfaces of each hydrogel sample and the various functional groups present in the samples respectively.

2.5 Hydrogels Antibacterial Activities

E.coli ATCC^{R} 25922TM strain was used in analyzing the antibacterial activities of the samples.100µl of the prepared LB E.colimedium was introduce ın 6 of the LB agar prepared petri dishes and spread evenly then each of the hydrogels with and without nanoparticles were placed in the petri dishes, labelled and incubated at 37^{0}C for 24hrs and their various inhibition zones measured.The antibacterial activities of each of the samples were later analyzed.

Chapter 3

RESULTS AND DISCUSSION

3.1 Characterization

3.1.1 Scanning electron microscope (SEM) analysis

Various structures of the hydrogel samples were analyzed and it could be observed from their various figures below that all the hydrogel samples contain pores on their surfaces. It is seen that the surface appearances of all the samples are similar, but their pore sizes and distribution varies. Their porosity distribution became denser and consequently very uniform as the amount of chitosan was increased (figure 5) and the porosity distribution became even more and more denser and uniform when the nanoparticles was added as seen in figure 8. So the uniformity and density of the pore distribution increases with increase amount of chitosan and with nanoparticles. The pore sizes decreases with the addition of nanoparticles (23).

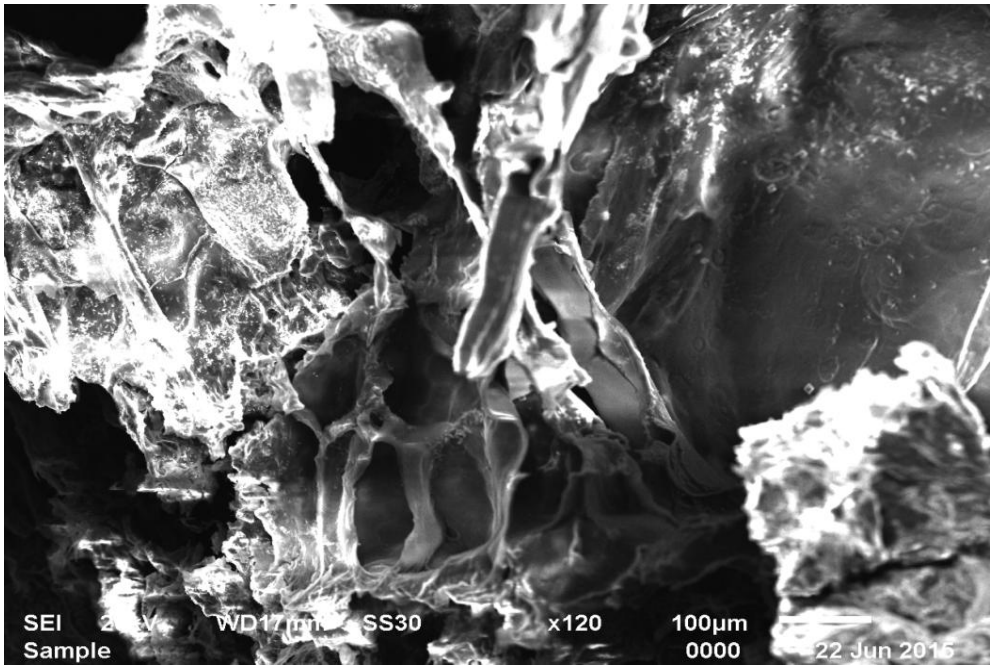

Figure 4: SEM of sample 1 (chitosan-PVA equal amounts)

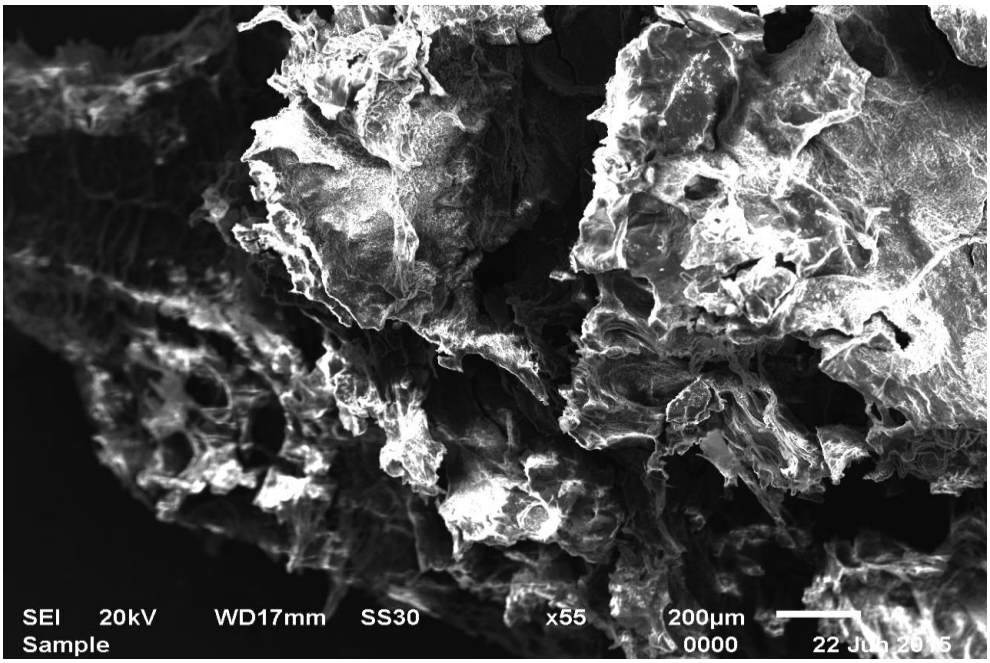

Figure 5: SEM of sample 2 (chitosan high, PVA low)

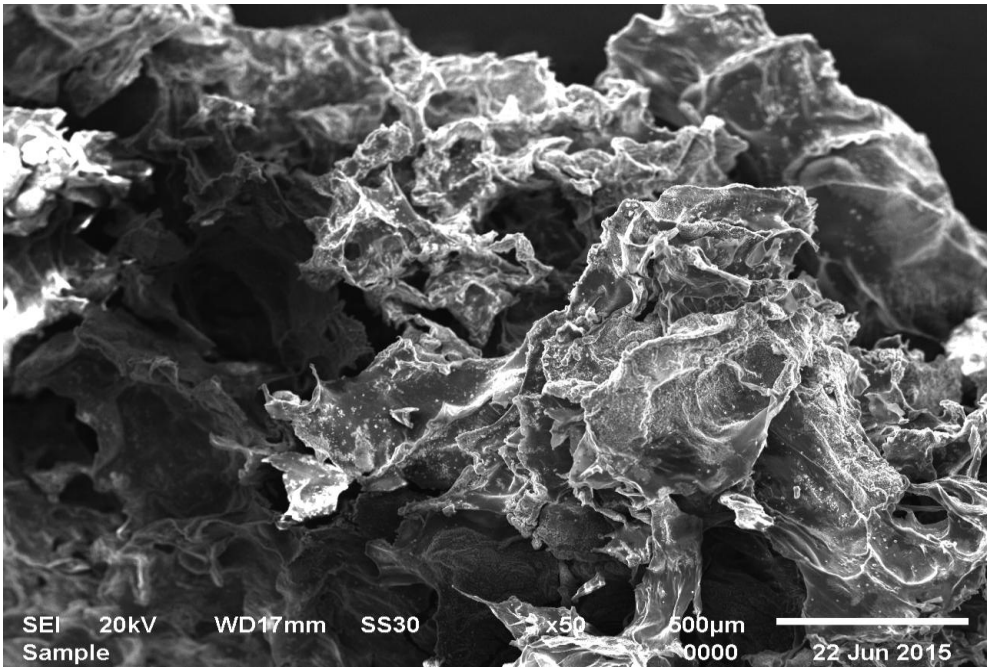

Figure 6: SEM of sample 3 (chitosan low, PVA high)

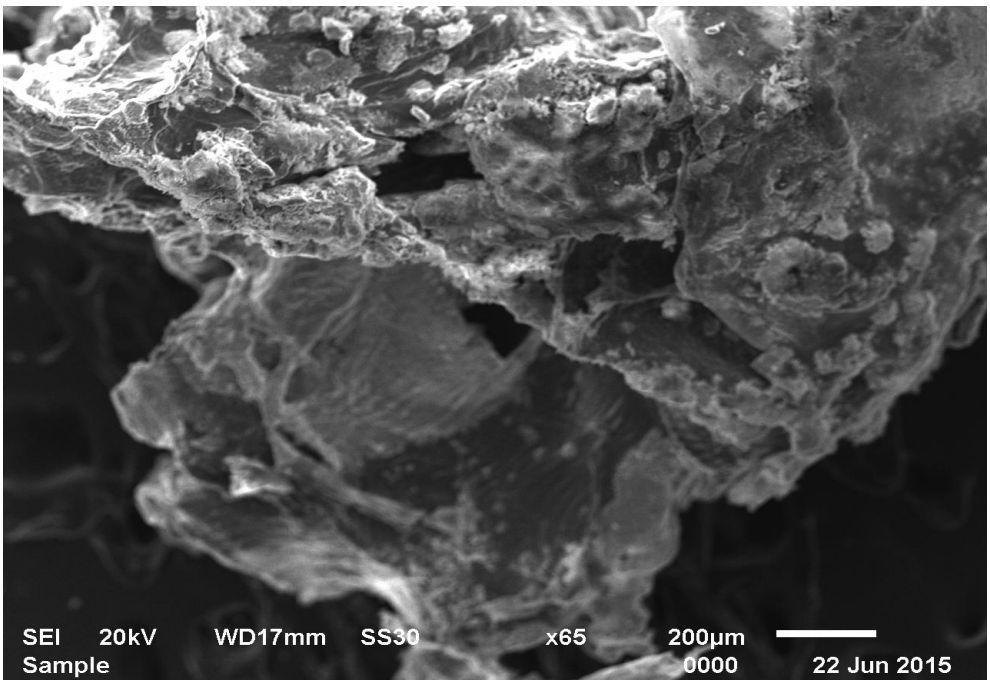

Figure 7: SEM of sample 4 (chitosan-PVA equal amounts + ZnO)

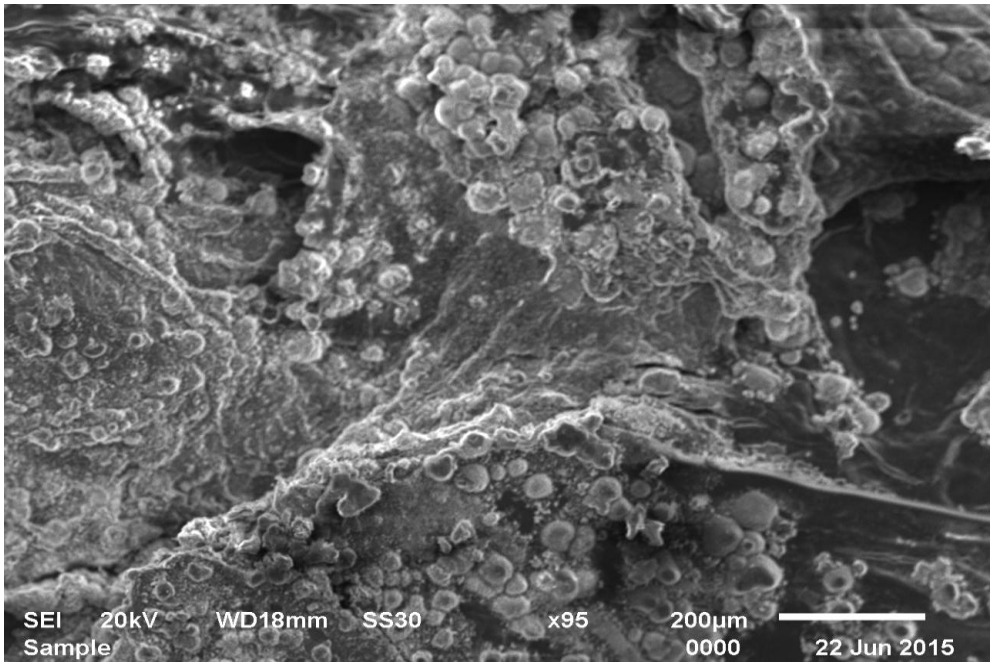

Figure 8: SEM image of sample 5 (chitosan high, PVA low + ZnO)

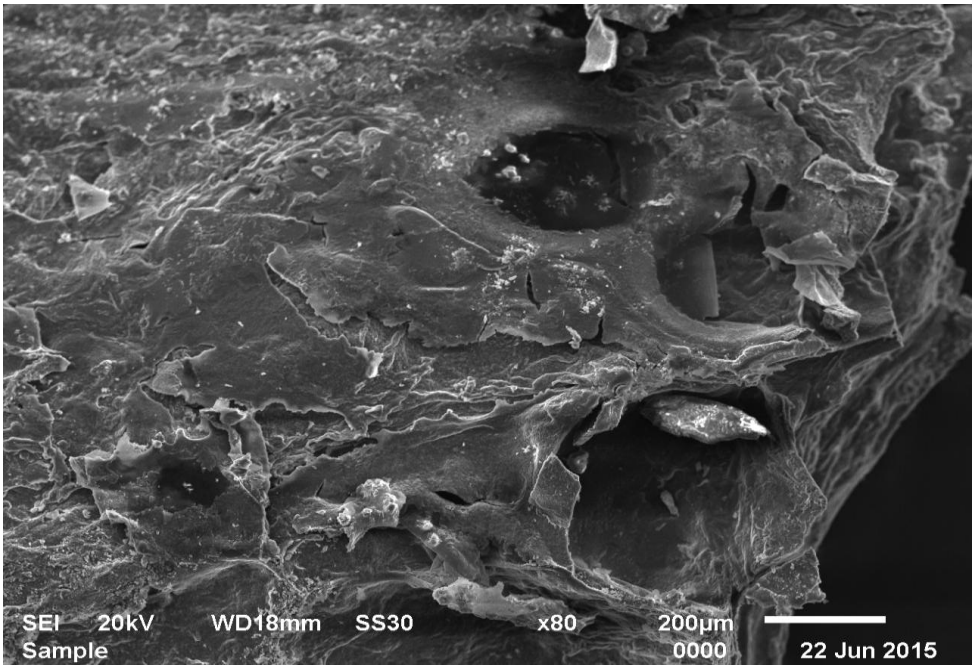

Figure 9: SEM images of sample 6 (PVA high, chitosan low + ZnO)

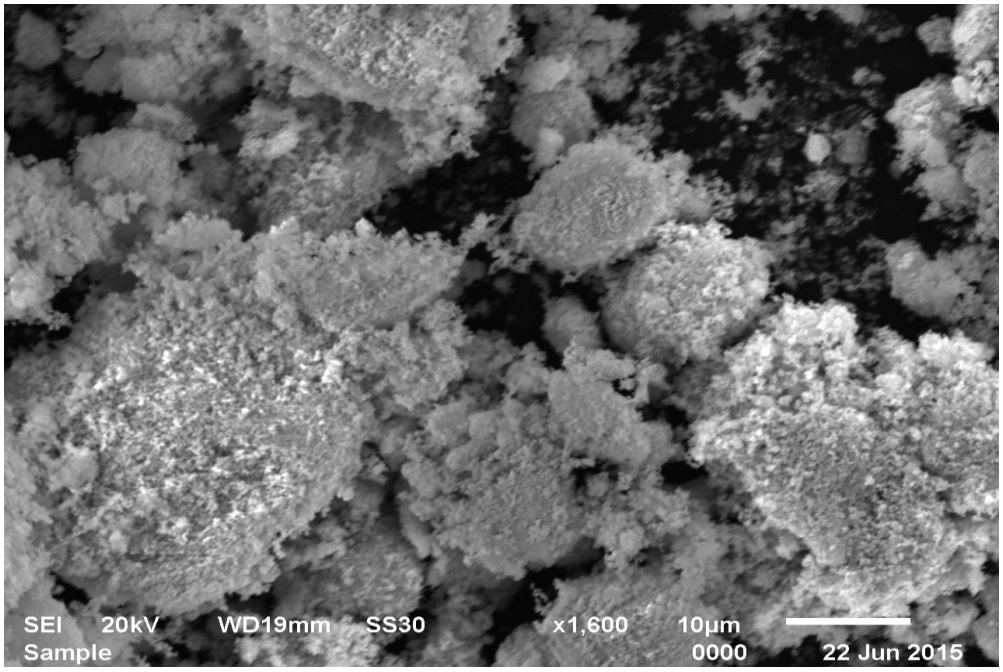

Figure 10: SEM of ZnO nanoparticles

3.1.2 Fourier transform infrared analysis

Four important pics are observed from chitosan as seen in figure 11. The pics are observed at 3421, 2954, 1633, 1585cm^{-1} representing hydroxyl groups, alkane group, amide 1 and amide 11 groups respectively. The presence of amides groups indicates that the deacetylation degree of chitosan is high. It is seen that the various absorption bands broadens as we move to the PVA / CH / BA sample due to the physical cross linking involved between the molecules and the overlapping of the groups in the chitosan. A peak is observed at 3291cm^{-1} and this simply indicates that there are hydrogen bonds inbetween chitosan and PVA causing the extension of hydroxyl and amine groups. Some other peaks are observed at 1706, 1615 and 1403cm^{-1} due to the stretching of the carbonyl group, $NHCOCH_3$ (amide) absorption and the presence of C-H bonds respectively in the chitosan-PVA polymer chains. Carbonyl group is still present in the hydrogel crosslinked with boric acid and this is due to the fact that hydroxyl moieties from PVA are still free due to incomplete crosslinking with the

crosslinker. The free hydroxyl groups serve as a good binding site for the zinc ions and this can be viewed from the fact that the addition of the nanoparticle in to the hydrogel enhances the expansion of the hydrogels backbone thereby leading to the increase in the hydrogels flexibility.

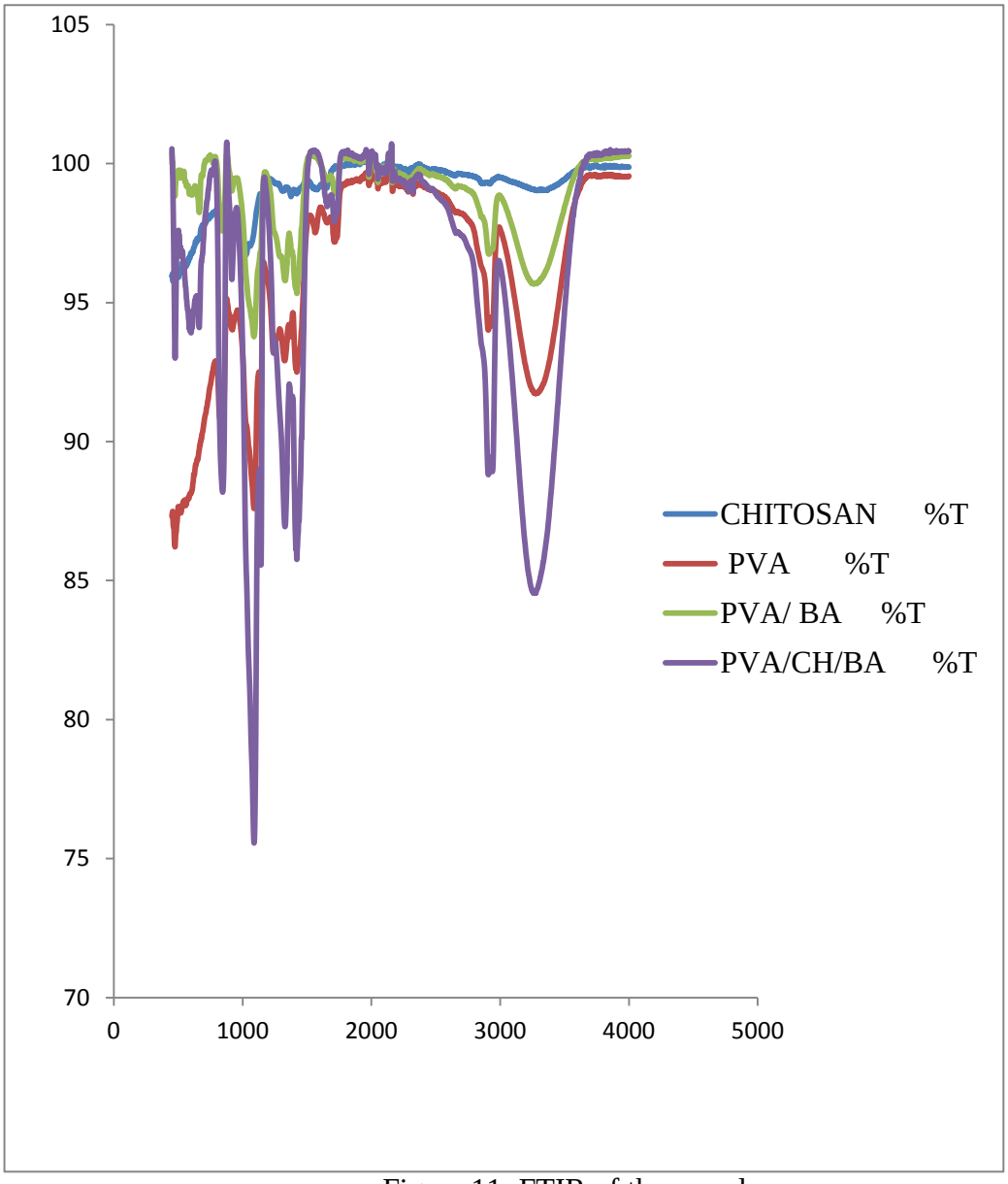

Figure 11: FTIR of the samples

3.2 Swelling behavior

3.2.1 Swelling of the samples in distilled water

Only the swelling properties of the samples without ZnO nanoparticles were done that is samples 1, 2 and 3. These samples were soaked in distilled water for variable number of hours and the results showed from their various graphs that, the sample containing the highest amount of PVA (S3) increases with increase in time and this is due to the fact that PVA is soluble in water and its degree of swelling is elevated in solutions due to its free hydroxyl groups (OH) groups.PVA has a tendency of forming hydrogen bonds with water. The sample (S2) with the highest amount of chitosan decreases with increase time simply because chitosan is insoluble in water as well as in some organic solvents but soluble inacetic solutions which are diluted and the one with equal amounts of chitosan and PVA increases slightly with increase time (S1).

Table 3: Swelling percentages of samples1, 2, 3 in distilled water at varying times

Samples	Swelling % in water /2hrs	Swelling % in water /4hrs	Swelling % in water /6hrs	Swelling % in water /8hrs	Swelling % in water /10hrs
1	405.6	527.8	572.2	572.2	427.8
2	623.3	579.2	541.5	554.1	522.6
3	82.6	716.5	849.4	969.6	950.6

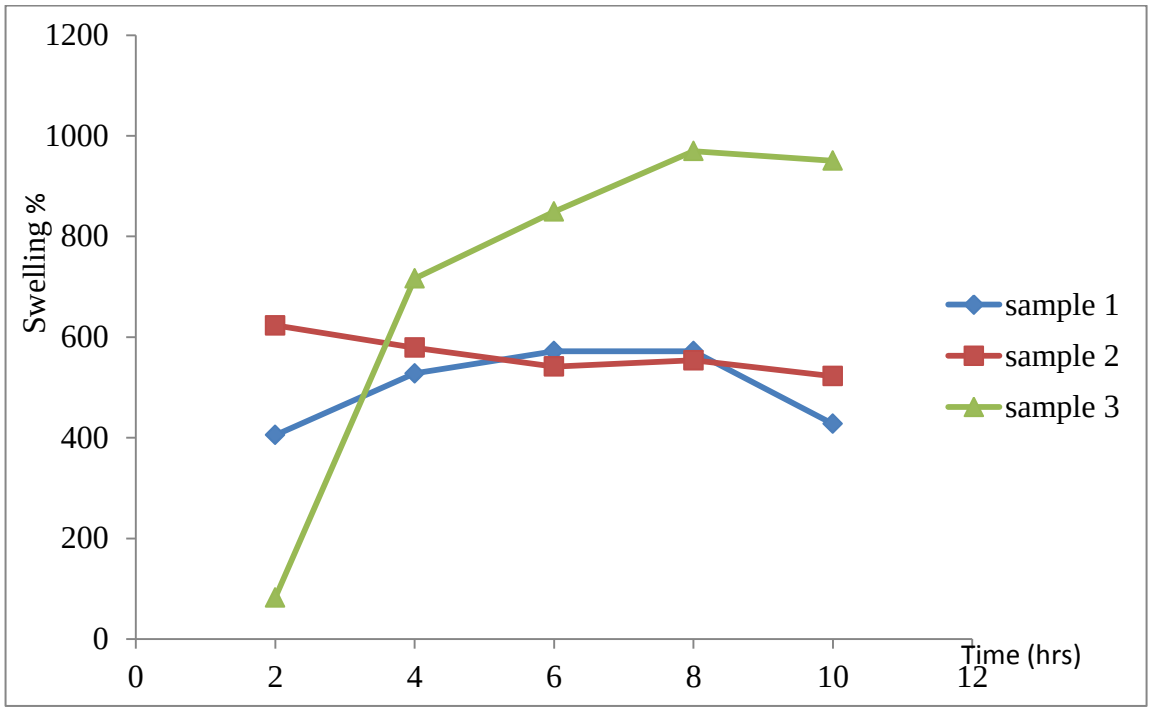

Figure 12: Graph of the swelling percentages of samples 1, 2 and 3 in distilled water

3.2.2 Swelling of the hydrogels in buffer pH solutions

Samples without nanoparticles were soaked in to buffer solutions of different pH and from their graphs it was noticed that, the sample with a high amount of chitosan(S2) increases highest with an increase in pH due to the fact that chitosan is soluble in buffer solutions than in water. The sample with more of PVA (S3) increases slightly whereas the one with equal amounts (S1) increases the least. It is seen that at pH 2 and 6, S2 increases higher than the others because chitosan contains amine groups (NH_2) which favors the formation of polycations (NH_3^+). Chitosan dissolves well in aqueous acidic solution.

Table 4: Swelling percentages of samples 1, 2, 3 in different buffer solutions

Samples	Swelling % / pH2	Swelling % /pH6	Swelling % / pH7	Swelling % / pH10	Swelling % / pH12
1	325.9	383.3	372.7	200	316.7
2	409.1	509.1	705.3	266.7	497.5
3	316.7	460	707.7	191.9	532.9

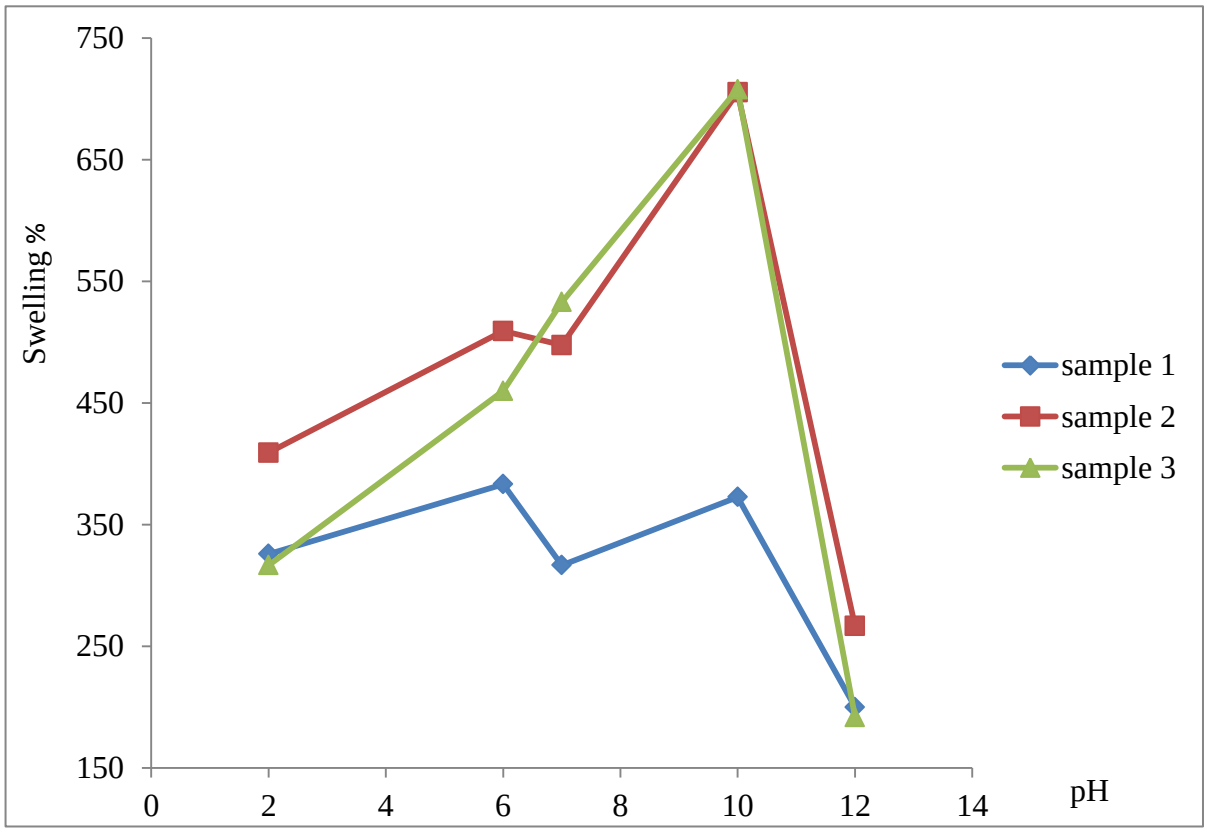

Figure 13: Graph of the swelling percentages of samples 1, 2 and 3 in different buffer solutions

3.3 Gelation behavior

3.3.1Gelation of the hydrogels

It was calculated and it was discovered that, S3 with high amount of PVA has the highest because PVA forms good covalent bonds with boric acid the cross linker than the way chitosan does. Cross linking PVA and boric acid increases the viscosity of the gel.

Table 5: Gelation percentages of samples 1, 2, 3

Samples	Gelation %
1	22.8
2	22.0
3	63.7

3.4Antibacterial evaluation of the hydrogels

The antibacterial activities of all the prepared hydrogels were gotten by measuring their various inhibition zones as showed in table 6. It is seen that the higher the quantity of chitosan in a hydrogel, the higher its inhibition zone and the higher it's antibacterial activity. This can firstly be associated to the fact that; the positively charged molecules of chitosan interact with the negative microbial charges present on the surfaces of the bacteria cell membranes causing repulsion between them. This reaction is favored by the formation of forces which are electrostatic between chitosans protonated amine groups (NH_3^+) and the negative ones present on the surfaces of the microbes. This reaction leads to: (i) internal osmotic imbalances, cell

wall permeability disruption and consequently bacteria growth inhibition; and (ii) peptidoglycan hydrolysis in the bacteria cell wall, causing intracellular electrolytes and low molecular weight protein leakages (21). Chitosan can as well inhibit bacterial growth by simply binding to the DNA thereby inhibiting the mRNA and synthesis of protein (22). The sample with Zn0 increases highest because metal chelation suppresses the elements present in spores thereby preventing the binding of nutrients which are essential for bacterial growth (23).

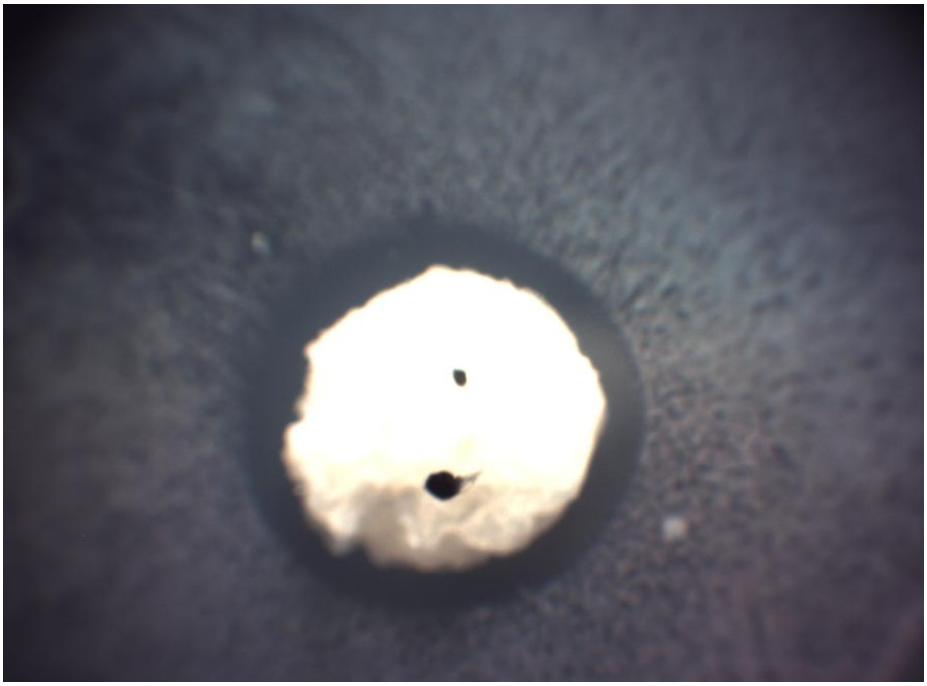

Figure 14: Inhibition zone of sample 1(chitosan-PVA equal amounts)

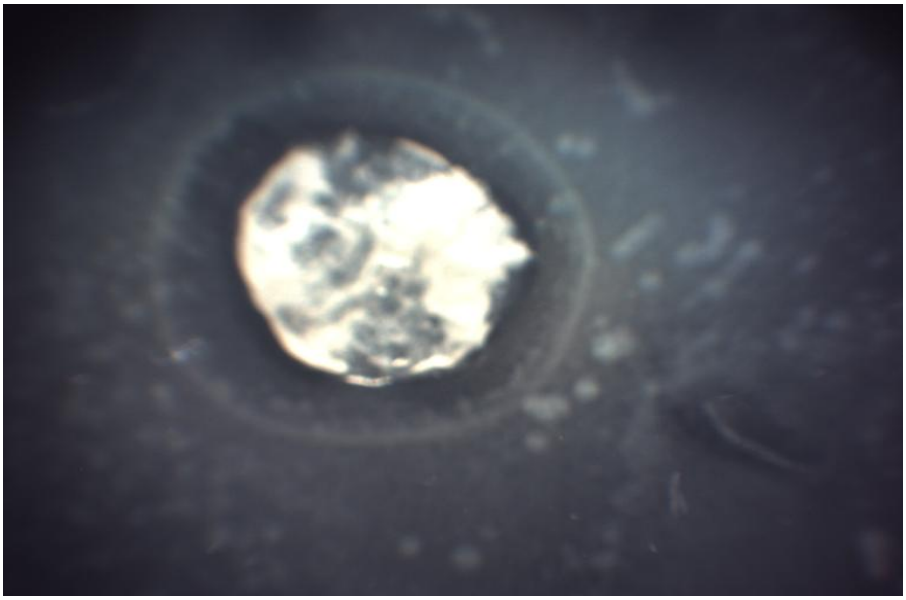

Figure 15: Inhibition zone of sample 2 (chitosan high, PVA low)

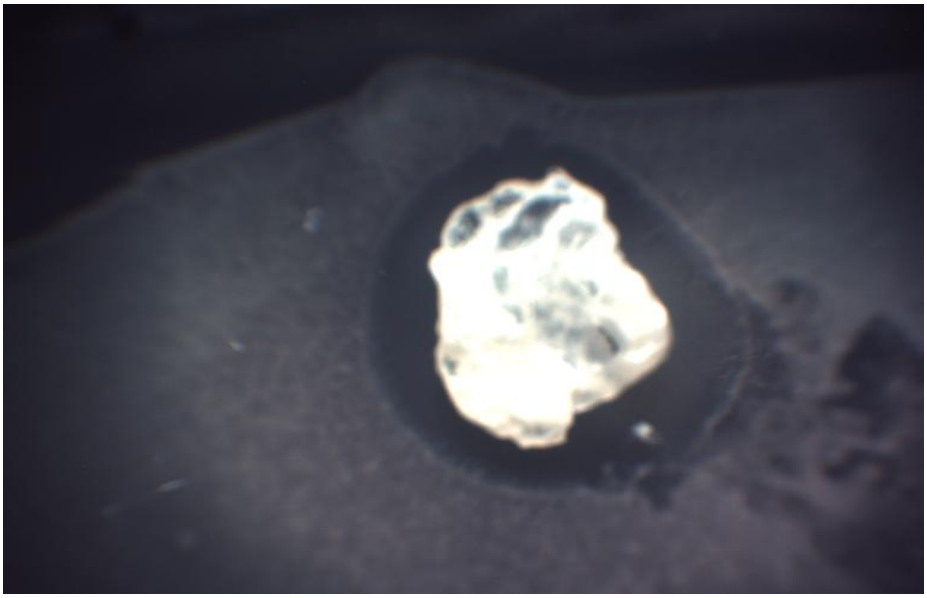

Figure 16: Inhibition zone of sample 3 (PVA low, chitosan high)

Figure 17: Inhibition zone of sample 4 (chitosan-PVA equal + ZnO)

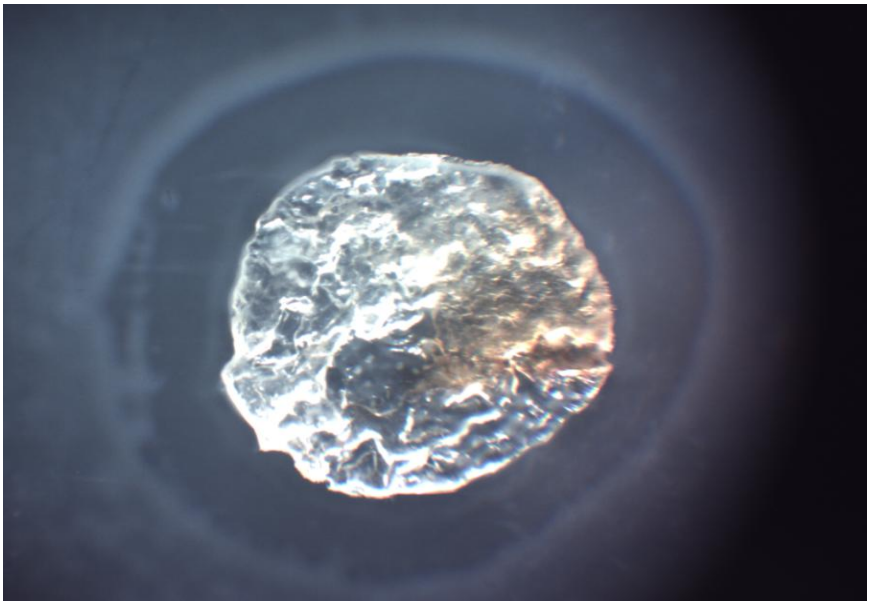

Figure 18: Inhibition zone of sample 5(chitosan high, PVA low + ZnO)

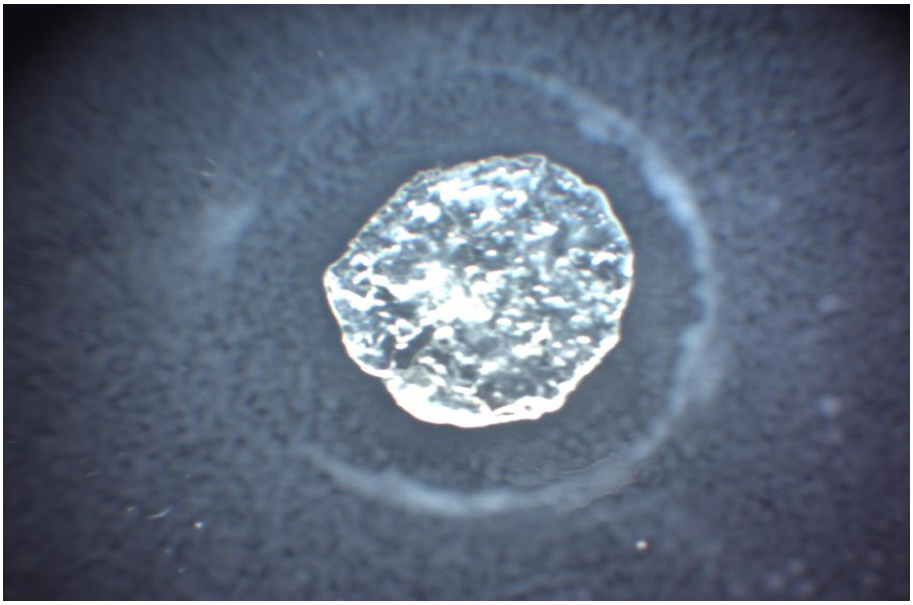

Figure 19: Inhibition zone of sample 6 (PVA high, chitosan low + ZnO)

The various measured zones of inhibition of each of the samples are as follows:

Table 6: Inhibition zones of the various hydrogel samples

Hydrogel samples	Chitosan (g)	PVA (g)	ZnO (g)	Inhibition zone of E.coli (mm)
Sample 1	1.5	1.5	0	10
Sample 2	2	1	0	19
Sample 3	1	2	0	14
Sample 4	1.5	1.5	0.1	13
Sample 5	2	1	0.1	26
Sample 6	1	2	0.1	20

From the values obtained above we see that S2 has the highest inhibition zone as compared to S1 and S3. This is because sample 2 contains more chitosan than PVA and is followed by S3 which contains more of PVA than of chitosan and the last is S1 which contains equal amounts of PVA and chitosan. So we conclude for this first observation that chitosan has a better antibactericidal activity as compared to that of PVA. An increase in the antibactericidal activities of samples 4,5 and 6 was observed after the addition of ZnO nanoparticle. It is clearly seen that sample 5 is the best firstly because it contains high amounts of chitosan and secondly because of the presence of the nanoparticles.

Chapter 4

CONCLUSION

Various hydrogel samples were gotten by varying chitosan and PVA amounts in order to determine firstly which amongst them greatly affects bacterial growth and secondly by determining the effect of the addition of ZnO nanoparticles on the bacterial growth. From the results obtained, we conclude that increasing the amounts of chitosan in a hydrogel increases antibactericidal effect and Vis versa since it is proved by other researchers that chitosan possesses a broad range of antibacterial spectrum because of its free amino groups which stimulates bacterials destruction at a quicker rate. The addition of the nanoparticles ZnO increased the rate of destruction of the bacterial since this nanoparticles is known as well to possess a wide antibacterial activity range. So therefore an increase in the amounts of chitosan and of the nanoparticles greatly favors bacterial death.

4.1 Future research proposals

- We can do the antıbacterıal analysıs of gram posıtıve and negatıve bacterıals and compare theır actıons toward the hydrogels and even fungı.
- Vary the quantity of cross linker and see the effect
- Doıng the swellıng properties of the hydrogels contaınıng the nanopartıcles and observıng ıf the presence of nanopartıcles reduces or ıncreases the kınetıcs
- Changıng the types of nanopartıcles and concludıng whıch has the best antıbacterıal range
- Work on antıfungal analysıs
- We can use different methods to measure antibacterial effects

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