Synthesis and Characterisation of Chemically Crosslinked Chitosan Citrate Gels

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ABSTRACT

Chitosan was modified using citric anhydride to produce film membranes via solvent evaporation. The films dissolved in acidic media which showed that crosslinking was not achieved, but it showed an appreciable amount of swelling (896%) as a result of the modification with maximum % swelling obtained by 1.0 g citric acid (CA):0.5g chitosan at pH 11 as compared with chitosan films with maximum swelling % of 314% at the same pH.

N-protected chitosan was chemically crosslinked with a non-toxic crosslinker citric anhydride to obtain citrate esters and incorporate the carboxylic group onto chitosan. Products obtained did not dissolve in acidic media which confirms crosslinking and there was also a high % of swelling observed with the maximum %swelling of 875% by 1.0 g CA: 0.5g at pH 4.

Characterisation was done using FTIR and C-13 NMR. Free amine content was determined by titration and the kinetic study revealed that both modified and citrate crosslinked chitosan samples obeyed pseudo 2nd order. The effect of time on crosslinking showed that as time increases crosslinking also increased.

In this study, we were able to develop a pH sensitive gel that could find a wide range of applications.

Keywords: Chemical Crosslinking, Citrate ester, Crosslinking Density

ÖZ

Kitosan sitrik anhidrit ile reaksiyona sokularak modifiye edilmiştir. Elde edilen ürünün çapraz bağlanmaya uğramadığı dolayısıyla asitli ortamda cözündüğü ve bu çözeltilerden filmler elde edilebildiği gösterilmiştir. Ağırlıkça 1.:0:0.5 sitrik asit:kitosan oranında reaksiyona sokularak elde edilen üründen oluşturulan filmlerin çözünmeden önce pH 11 çözeliti içerisinde %896 şişme derecesine ulaştıkları bu değerin kitosan filmler için %314 olduğu saptanmıştır.

Amin grupları benzaldehit ile korunarak elde edilen kitosan örneği sitrik anhidrit ile reaksiyona sokularak çapraz bağlanmayla birlikte kitosanın sitrat esterleri elde edilmeye çalışılmıştır. Elde edilen ürünlerin asitli ortamda çözünmemesi çapraz bağlanmaya uğratıldıklarını düşündürmüştür. Ağırlıkça 1.:0:0.5 sitrik asit:korunmuş kitosan oranında reaksiyona sokularak elde edilen ürünün pH 4 çözeltide %875 oranında şiştiği gözlemlenmiştir.

Ürünler FTIR ve C-13 NMR yöntemleri ile karakterize edilmişlerdir. Serbest amin miktarları asit-baz titrasyonu yöntemi ile bulunmuştur. Örneklerin şişme kinetiğinin ikinci dereceden olduğu ve çapraz bağlanma oranının reaksiyon zamanı ile arttığı saptanmıştır.

Bu çalışmada, pH'a duyarlı, çeşitli uygulama alanları olabilecek poliamfolit ürünler elde edilmiştir.

Anahtar Kelimeler: çapraz bağlanma, sitrat ester, kitosan

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If no be God.......

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Chapter 1

INTRODUCTION

Chitosan is a linear polysaccharide obtained by deacetylation of a naturally occurring polymer chitin (which is present in the outer skeletons of marine animals). It consists of 2- amino – deoxy – (1-4)-β –D- glucopyranose residues (D- glucosamine units) and has small amount of N-acetyl D glucosamine units ($NHCOCH_3$) depending on the degree of deacetylation. It is polyfunctional (containing both amine and hydroxyl groups) making it easy to carry out various modifications and giving it a wide range of applications.

Chitosan degrades in acidic medium and this usually limits its application. To prevent this effect, chitosan is usually crosslinked by physical or chemical means. Physical gels formed are not as strong or stable as chemically crosslinked gels and dissolve easily while most chemical crosslinkers used are toxic, hence the need to develop new methods to form chemical crosslinking with crosslinkers that are not toxic.

This study focuses on the esterification reaction of chitosan with citric anhydride gotten from the treatment of citric acid in the presence of sodium hypophosphite with heat to act as a source of crosslinking for the chitosan via the hydroxyl groups. In this study, amine groups of chitosan were protected with Benzaldehyde according to the method in literature by Sabarudin et al., (2005) [1]. The N protected chitosan will

be chemically crosslinked with the use of citric acid (nontoxic) according to the method used by Salam et al., (2011) [2] to crosslink hemicellulose before deprotection of the amine group was carried out. This should produce a polyampholyte gel with superior hydrophobicity. The effect of reaction time and amount of citric acid will be investigated and used to determine optimum conditions for the esterified chitosan. Swelling properties will be investigated and related to the crosslinking density of the product. The gel will possess both amine and carboxylic acid group which will make it useful for environmental applications and medical purposes. Research method applied will be quantitative.

1.1 Chitosan, Properties and Applications

Chitosan is produced from chitin (which after cellulose is the 2nd most abundant polysaccharide found in nature). This is achieved by treating chitin with concentrated NaOH (40 -50%) solution at 60 –120 °C to obtain a 95% deacetylated product. Several applications of chitosan in medicine include drug, protein, gene delivery and transfection, tissue repair and to aid/promote wound healing [3, 4].

Chitosan is biocompatible, biodegradable, bio-adhesive, muco-adhesive, polyfunctional, nontoxic and abundant. All these properties make chitosan to receive a lot of interest for both medical and pharmaceutical applications [5].

Chitosan as compared with chitin can be easily modified because it is less crystalline and more soluble [6].

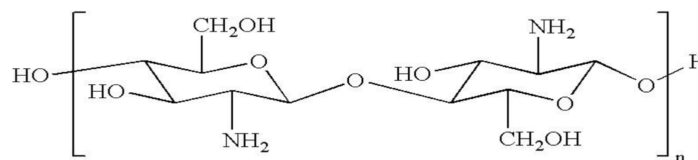


Figure1: Structure of chitosan

Unlike other polysaccharides, chitosan is cationic in nature (polycation) when in solution giving it good complex forming and ion adsorbing properties. Also, as compared with other polysaccharides that are mostly neutral or acidic, chitosan and chitin are the only basic ones with chitosan pKa value of 6.5 [3].

For chitosan to be useful in drug delivery, it requires crosslinking so as to increase the time and how consistent the drug is being delivered [7].

1.2 Chemical Modification of Chitosan

Due to the polyfunctionality of chitosan, several types of modification reactions can be carried out on it to obtain different materials with different characteristics. The advantages of modifying chitosan are that modification improves the properties or gives it new properties, the backbone of chitosan is not affected and chitosan retains its properties after modification [6].

Modification of chitosan via the amine groups makes chitosan to lose one of its most interesting properties/functions which is its biological and cationic property in solutions hence the modification via the hydroxyl groups is favoured [8,9]. The presence of two OH groups on the backbone of chitosan makes it suitable for it to undergo reactions common to alcohols e.g. etherification, esterification.

Esterification reaction is a reaction between the OH group of alcohols and carboxylic acids, or acid anhydrides and alcohols to form an ester (RCOOR). This research

work focuses on forming a chitosan citrate using citric anhydride and the OH group present in chitosan.

Several modification reactions that are carried out on chitosan include; grafting, N-phthaloylation, alkylation, Schiff base formation, silylation, carboxymethylation, oligomerization, tosylation etc.

Graft copolymerization is one of the most attractive methods of expanding the applications of chitosan and to add various functional groups unto chitosan. Several methods used for grafting unto chitosan include radiation, cationic, free radical, microwave assisted and enzymatic polymerisation [10, 11]. A good example of the effects of grafting is the grafting of Polyaniline on the amine groups of chitosan which produced conductive polymers [12]. Also, Chitosan-g-polycaprolactone copolymer showed lower crystallinity, was more soluble in organic solvents and resulted in production of hydrophobic side chains onto the backbone of chitosan [9].

N-phthaloylation of chitosan reduces the hydrogen bonds present and makes chitosan more soluble in organic solvents. It is also used to protect the amine groups of chitosan thereby making it easy to carry out region-selective reactions [8, 12].

Oligomerization involves the depolymerisation of the chitosan chain to form low molecular weight chitosan called chitooligomers. These oligomers formed have different properties and applications from that of chitosan. For example, they are more soluble and still retain the physiological properties and are used in medicine, food and also for cosmetics. Oligomers can be formed via chemical (use of strong acids), physical or enzymatic (enzymes such as lysozymes, chitonases etc.) methods

with the enzymatic means preferred because the oligomers formed and the reaction can be controlled by means of pH, temperature and reaction time [6].

1.3 Chitosan Hydrogels

Hydrogels are 3D network that swell in water and biological fluids and are able to retain up to 1000 times their dry mass of these fluids. They are also referred to as "intelligent or smart materials" because of their responses to changes in whatever media they find themselves in e.g. pH [13]. They are divided into 2 based on the nature of the linkages formed and are; chemical and physical hydrogels.

Chemical hydrogels are formed as a result of covalent bonds which are irreversible in nature while physical hydrogels are formed as a result of ionic interactions [5]. Chitosan forms gels as a result of intermolecular links through hydrophobic interaction between residual acetyl groups. Forming stronger intermolecular links can be achieved by chemically crosslinking chitosan which is used to increase the chemical stability, mechanical strength, reduce its swelling in aqueous media and control its solubility [6].

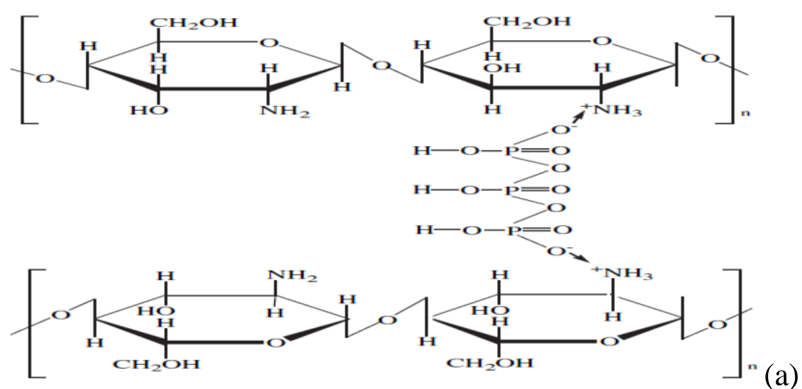
Physical gels (e.g. with TPP, citric acid) and chemical gels (crosslinkers e.g. EDGE, PEG) formed are used to make films, fibers, sponges, microspheres, beads etc. for various applications.

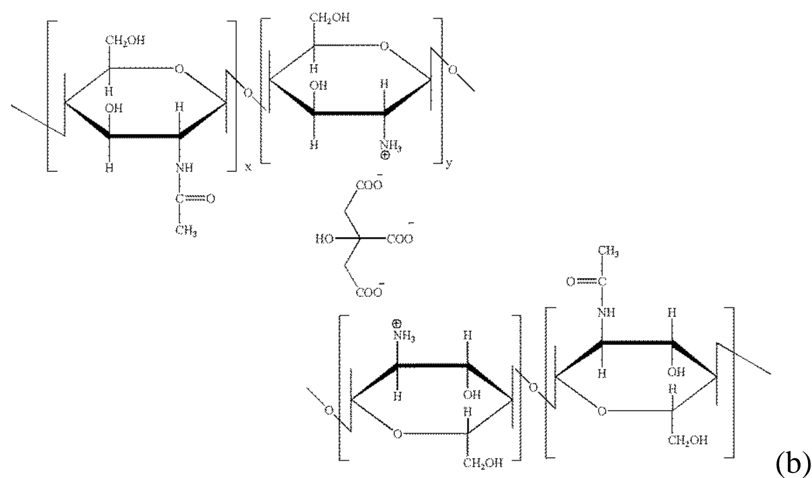
1.3.1 Physical Gels

Chitosan as well known is a polycation and can interact with negatively charged ions to form a kind of bridge between the chitosan polymer chains and the anions via electrostatic attraction. Hydrogels that are formed via physical crosslinking are used in drug delivery because no chemical reagent or solvents are used that can be

harmful to the body after drug loading and release [4]. Physically crosslinked gels are one of the simplest and safest methods to crosslink chitosan but they lack mechanical stability and dissolve easily and this limits their applications.

Several well-known anions used are citric acid, sodium tripolyphosphate (TPP), sodium citrate, sodium sulphate etc. the interaction observed between chitosan and the various crosslinkers is shown in Figure 2 below. It should be noted that all ionic interactions are between the protonated amines and the crosslinkers and pH greater than 6 would affect the crosslinking since less of the amines will be protonated.





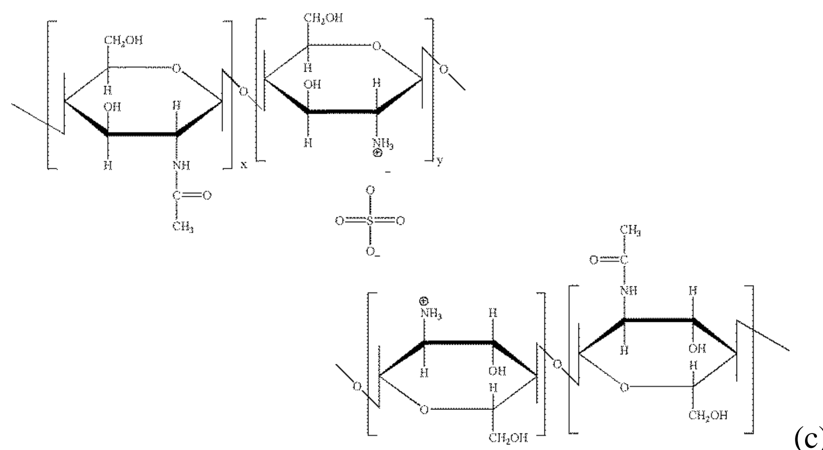


Figure 2: (a) Chitosan TPP, (b) Chitosan citrate, (c) Chitosan sulphate ion

Chitosan TPP finds various applications in different fields. They can be used for metal adsorption and waste water treatment, in agriculture for oral delivery of genes, drug loading and delivery [14-17].

A lot of study has been done on chitosan citrates and their applications especially in drug delivery [18-22]. This is due to the fact that they produce non-toxic materials upon degradation, swell considerably in aqueous media and are pH sensitive but they all involve physical crosslinking which limits their applications while this study involves chemical crosslinking using the same citrate.

Another good example of a physically crosslinked gel is the formation of polyelectrolyte complexes (PEC). PEC's are formed by two polymers of opposite charges e.g. xanthan gum and chitosan. The availability of the glucuronic acid and pyruvate on the backbone of xanthan gum makes it form an anionic polyelectrolyte which interacts with the polycation chitosan to form a PEC. This xanthan-chitosan PEC can also be used for oral drug delivery and release because it produces non-toxic materials on degradation and has a pH dependent Swelling property [23].

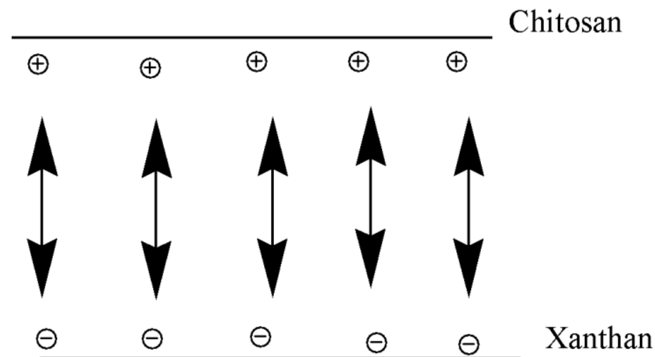


Figure 3: Xanthan Chitosan PEC

1.3.2 Chemical Gels

Chemical gels are formed via crosslinking reactions to produce covalent bonds. Several well-known crosslinkers used are the dialdehydes (glutaraldehyde, glyoxal), formaldehyde, ethylene glycol diglycidyl ether EDGE, epichorohydrin, but their applications especially for pharmaceutical use is limited as a result of the toxicity caused by these crosslinkers (14). PEG and Genipin are also used as chemical crosslinkers and are known not to be toxic in nature.

There are 3 types of chemically crosslinked hydrogels according to their structure and they are;

a) Hybrid polymer network: are formed when the crosslinking is between chitosan polymeric chains and no other polymer is involved. The crosslinking can occur between 2 different polymer chains or on the same chain.
b) Semi interpenetrating network: is formed when another polymer is added to chitosan before crosslinking takes place. The polymer is not involved in the crosslinking but is trapped inside the crosslinked chitosan network. The mechanical strength and biocompatibility of hydrogels are improved upon and the polymers involved are known to retain their individual properties

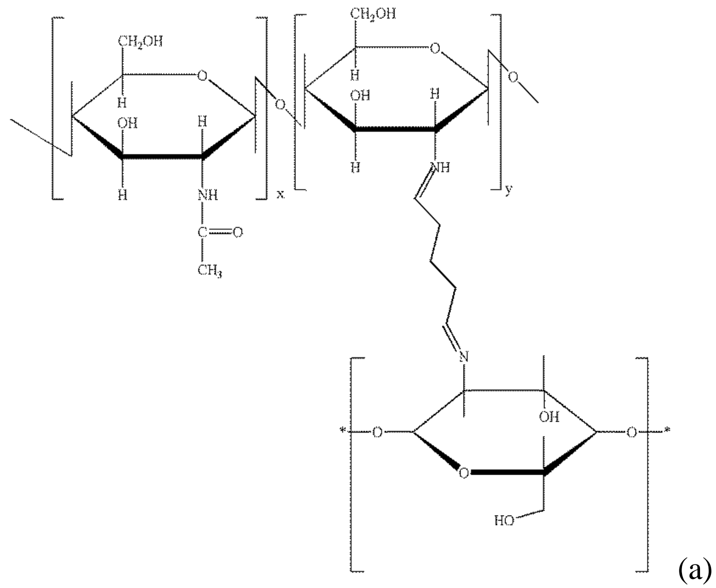
[24]. Semi IPNs also possess porosity which aids the diffusion of solutes particles in and out of the network structure [25].

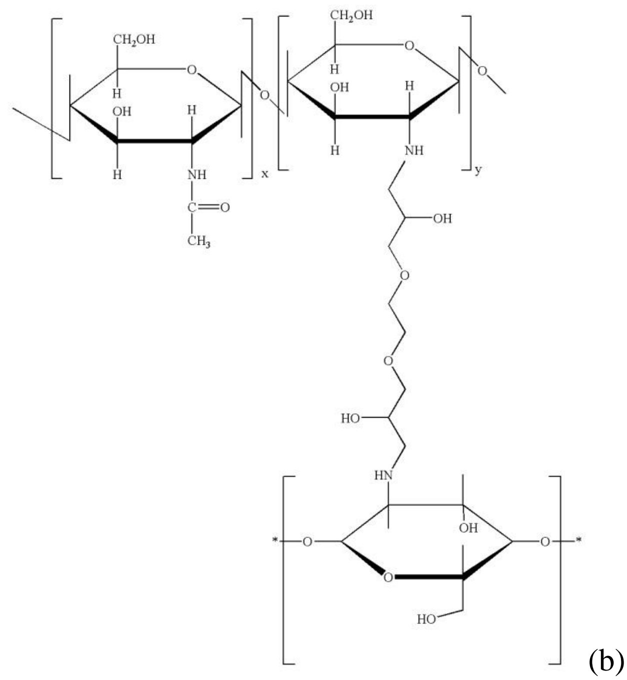
c) Full Interpenetrating network: is similar to the semi IPN but the difference is that the polymer added to chitosan is already crosslinked. Their properties and structures are found to be different from those of the semi IPNs.

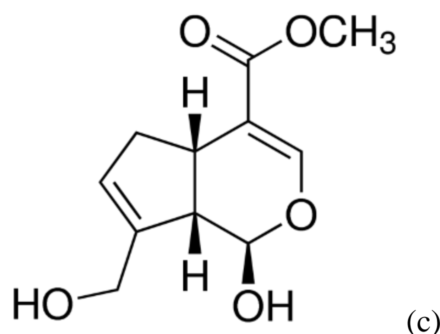
Chitosan crosslinked by glutaraldehyde has several advantages. Chitosan glutaraldehyde can be used for enzyme immobilization and metal adsorption [12]. Another study revealed the use of chitosan crosslinked with glutaraldehyde for antibacterial growth. Ordinary chitosan could not inhibit the growth of the bacteria *Burkholderia cepacia* but the crosslinked chitosan was able to hinder bacteria growth [26]. Papain which is an enzyme that possesses both anti-bacterial and anti-inflammatory property was loaded via absorption to an already crosslinked chitosan microparticle using glutaraldehyde and TPP as crosslinkers. A controlled release of the papain was observed confirming that glutaldehyde can be useful as crosslinker for controlled drug release [27].

Genipin is another crosslinker reportedly used to crosslink chitosan. It is less toxic than glutaraldehyde, degrades slower and can be used for clinical purposes. The mechanism of crosslinking between genipin and chitosan is not really clear or well explained [5, 28]. Yuan et al (2007) used genipin to crosslink chitosan microspheres to determine its effect on protein/drug release and the swelling of the chitosan microspheres. The degree of crosslinking was shown to increase with an increase in time and genipin concentration which led to a decrease in the swelling ratio. The release rate was also slower than the uncrosslinked microsphere which makes it useful as a proponent for increasing the time for drug delivery [29]. Figure 4 below

shows the structures of the chemical crosslinking of chitosan with glutaraldehyde and EDGE and genipin.







(c)

Figure 4: Structure of a) chi glutaraldehyde b) chi EDGE c) Genipin

1.4 Citric Acid

Citric acid is a white crystalline powder at room temperature with molecular formula $C_6H_8O_7$. For any compound to serve as a suitable crosslinker, it must have more than one functional group to form a network between two polymer units and must have a lower molecular weight than that of the polymer chains crosslinked together [5]. Citric acid has this property with 3 carboxylic acid groups (COOH). It is found in fruits giving them their characteristic sour flavour and used as a natural preservative.

Citric acid finds various applications such as additives to food, to soften water, anti-coagulant and anti-viral tissues. Citric acid when used to crosslink provides the ester bond crosslink, helps to balance the Hydrophilicity of the polymer, provides hydrogen bonding and adds a new functional group to the material [22].

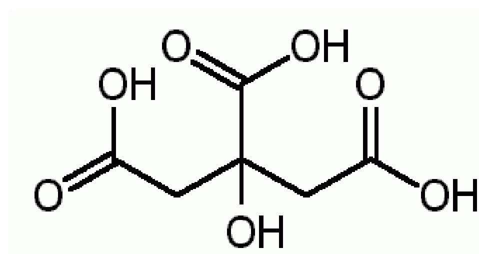


Figure 5: Citric Acid

1.5 Aim

The aim of this research work is to synthesize the citrate ester of chitosan via esterification of N- protected chitosan by citric anhydride to give a chemically cross-linked network which is expected to form a polyampholyte with superior hydrophobicity. Citric acid will be grafted on the backbone of both N- protected and unprotected chitosan to determine the effect of protecting the amino group and type of gel formed. The effect of reaction time will also be observed and used to determine the optimum conditions where we can obtain the best yield and crosslinking for our grafted chitosan. Research method applied will be quantitative.

Chapter 2

EXPERIMENTAL

2.1 Materials

All chemicals used in this thesis work are depicited in Table 1.

Table1: Materials and their Manufacturing Company

Material	**Manufacturer**
Chitosan medium molecular weight	Aldrich
Acetic acid	Reidel-deHaen
Sodium Hydroxide	Aldrich
Citric Acid	BDH
Sodium Hypo Phosphite	Aldrich, Switzerland
Poly ethylene glycol 8000	Aldrich
Hydrochloric acid	BDH
DMSO	Sigma Aldrich
Potassium Chloride	Sigma Aldrich
Potassium Hydrogen Phthalate	Aldrich
Sodium biCarbonate	BDH

All other manufacturing companies are in Germany except BDH (British Drug Houses Ltd.) which is in England. Distilled water was used to prepare all solutions.

2.2 Solution Preparation

2.2.1 Acetic Acid Solution

A 1% (v/v) acetic acid solution was prepared by diluting 5mL of 99.9% acetic acid solution in a 500mL volumetric flask and made up to mark with distilled water.

2.2.2 Sodium Hydroxide Solution

Various concentrations of sodium hydroxide solution were prepared for the removal of the modified chitosan films. They include 4%, 6%, 8% and 14% NaOH solutions and were prepared by dissolving 4 g, 6 g, 8 g and 14 g of NaOH in 100mL of distilled water.

2.2.3 Buffer Solutions

All buffer solutions prepared were used for the swelling experiments. A pH meter was used to confirm the pH values of the prepared buffers before use.

For buffer solution of pH 1.2, 125mL of 0.2M KCl and 212.5mL of 0.2M HCl was mixed in a 500mL volumetric flask and made up to mark with distilled water.

For pH 4.0, in a 1L volumetric flask, 5.1g of potassium hydrogen phthalate was dissolved in 10mL of 0.01M HCl and made up to mark with distilled water.

For pH 7.0, 122mL of 0.1M HCl and 378mL of 0.1M sodium hydrogen phosphate was mixed in a 500mL volumetric flask and made up to mark with distilled water.

For pH 11.0, 1.05g of sodium bi carbonate was dissolved in 113.5mL of 0.1M NaOH in a 500mL volumetric flask and made up to mark with distilled water.

2.2.4 Preparation of 0.5M HCl

Stock solution of hydrochloric acid was found to be 10.18 M. 49.12mL of the HCl was measured (using a measuring cylinder) into a 1 L volumetric flask and made up to mark with distilled water.

2.2.5 Preparation of Citric Anhydride Solution

Procedure used in the formation of citric anhydride was obtained from Salam et al (2011) as shown in Figure 6 below. Several masses of citric acid (0.5, 1.0, 1.5, 2.5g) were weighed in a 100mL beaker. 0.1g of sodium hypophosphite (SHP) was added to each one of them with varying amount of distilled water. Heat was applied to increase the solubility of both salts and aid the formation of citric anhydride.

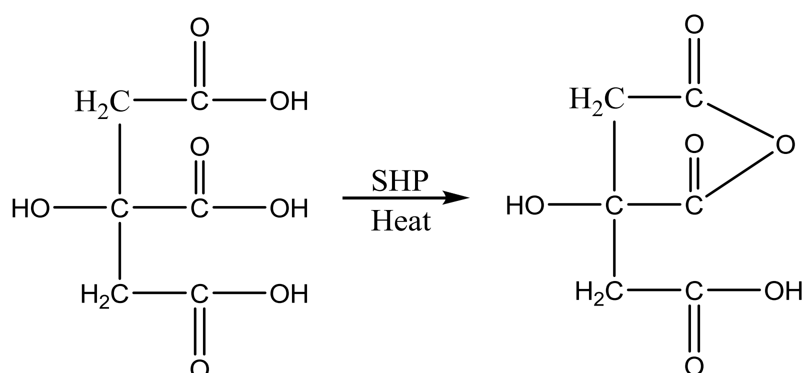


Figure 6: Formation of Citric Anhydride

2.3 Modification of Unprotected Chitosan; Formation of Citrate Ester of Chitosan

Modification of pure chitosan was carried out using citric anhydride. A sample of chitosan weighing 0.5g was dissolved in 100mL of 1% acetic acid solution to form a 0.5% chitosan solution. 0.0625g of PEG was added to act as a plasticizer. To the prepared chitosan PEG solution, varying amounts of the citric anhydride was added to check the effect of increasing the amount of the citric anhydride. The reaction was

carried out at 110°C with constant stirring of 250 rpm for 3hr. Modified chitosan (MC) was precipitated out of the solution using ethanol and then filtered. The precipitate was washed several times with distilled water to remove the unreacted species and dried at room temperature to determine the yield.

$$\% \, yield = \frac{W_2}{W_1} \times 100 \qquad (1)$$

W_2= weight of filtrate- initial weight of chitosan

W_1= weight of chitosan

2.4 Film Preparation

To form the modified chitosan films, 25mL of the modified chitosan solution was transferred into a Petri-dish and put in the oven for 48hours at 50°C. The NaOH solution prepared was used to remove the dried films from the Petri dish via solvent evaporation method. The film formed was washed with 90% ethanol solution and distilled water respectively before it was dried at room temperature.

2.5 Protection of Chitosan using Benzaldehyde

The procedure used was reported by Sabarudin et al (2005). 10g of chitosan was suspended in 100mL of ethanol in a 500mL beaker. 38.3mL (40g) of Benzaldehyde was added and stirred at room temperature for 12hours to protect the amino groups of chitosan by formation of Schiff base. The resulting product was filtered and washed several times with ethanol and water before it was dried at room temperature.

2.6 Crosslinking of Benzaldehyde Protected Chitosan

For the crosslinking, 0.5g protected chitosan was weighed into a 3 neck round bottom flask, 50mL of DMSO and the citric anhydride solution of varying amounts in ratio of 1:1, 1:2, 1:3 and 1:5) were added. The reaction conditions were 110°C, 3 hours and at constant stirring of 250 rpm. At the completion of the reaction, the cross

linked chitosan was filtered using a filter paper, washed several times with ethanol and water to remove traces of unreacted species and DMSO. The filtrate was dried at room temperature to determine the yield.

2.6.1 Effect of Time on Crosslinking of Chitosan

Effect of time on crosslinking was determined. Crosslinked chitosan with the highest % swelling was used for this experiment. The same procedure used in section 2.8 was used with only the time for crosslinking varied as 3, 5 and 7 hours respectively.

2.7 Deprotection of Crosslinked Chitosan

The filtered chitosan from above was transferred to a beaker, 200mL of 0.5M HCl was added, stirred at ambient temperature for 12 hours to remove the Schiff base i.e. deprotect the chitosan. The product was filtered out and the procedure was repeated for another 12 hours. Product was dried at room temperature.

2.8 Characterization

2.8.1 Fourier Transform Infrared Analysis (FTIR)

This was done with the aid of a Perkin Elmer spectrum 65 FT-IR spectrometer. It was used to determine the chemical structures, have an idea of the functional groups and thereby show the interactions between the chitosan, its modified form, protected and crosslinked chitosan product. Film membranes produced were analyzed by placing them directly on the FTIR instrument cell while other samples were ground with KBr powder (1:10) and made into pellets.

2.8.2 Carbon 13 NMR

For the C-13 NMR analysis, 0.5g of Benzaldehyde chitosan, crosslinked protected chitosan and crosslinked deprotected chitosan was sent for BCDMAS analysis and this was carried out in ODTU Merkez laboratory using a Braker Super Conductivity FTNMR Spectrometer Avance TM300MHz WB instrument.

2.8.3 Free Amine Content

The free amine content was determined by titration. 0.1-0.2g of the samples; chitosan, Benzaldehyde chitosan and the cross linked chitosan. The samples were dissolved in 20mL of 0.1N hydrochloric acid solution and left for 24hours at constant stirring so as to dissolve properly. The resulting solution was filtered and then titrated using 0.1N NaOH and phenolphthalein as an indicator. A blank experiment was also performed. Amine content was determined using the equation below;

$$\% NH_2 = \frac{(v_1 - v_2) \times c \times E}{M \times 1000} \times 100 \qquad (2)$$

V_1= Vol of Sodium Hydroxide used for blank titrant

V_2= Vol of Sodium Hydroxide used for sample titrant

C= concentration of NaOH

E= mol weight of amine group

M= mass of sample

2.8.4 %Weight Loss

A known mass of the sample was immersed in pH buffers 1.2, 4, 7 and 11 (25mL) for 24 hours. After that the film/crosslinked chitosan was carefully removed and left to dry completely at room temperature.

$$\% weightloss = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3)$$

W_1= Initial mass of film / crosslinked chitosan

W_2= Final mass after drying

2.8.5 Swelling Test

Swelling test was done by weighing a small piece of the modified chitosan film on an electronic balance. The weighed film was immersed in 25mL of the prepared buffer solutions with different pH values. To determine the weight after specific time

intervals (1, 3, 5, 7 and 24hrs), each film was carefully removed from the buffers, placed on a filter paper to remove the surface moisture and weighed.

$$\% swelling = \frac{W_2}{W_1} \times 100 \qquad (4)$$

Where;

W_1 is the initial mass of film

W_2 represents the difference in mass at a time t from the initial mass of film.

2.9 Swelling Kinetics

The data from the swelling experiment was used to determine the kinetics of the swelling whether it was first-order kinetics or second-order kinetics. This was determined using the sample that showed maximum amount of swelling. The equations used are shown below;

First order kinetics
$$In\left(\frac{W_{max}}{W_{max} - W_t}\right) = k_1 t \qquad (5)$$

Second order kinetics
$$\frac{t}{W_t} = \frac{1}{(k_2 W_{max}^2)} + \frac{t}{W_{max}} \qquad (6)$$

Where;

W_{max}= weight at maximum water uptake

W_t= weight of sample at time t

t represents time in mins

K_1 and K_2 are the rate constants

2.10 Crosslinking Density

The crosslinking density will be related to the degree of swelling of the samples. It was determined by the equation below;

$$crosslinking\ density = \frac{moles\ of\ crosslinking\ agent}{moles\ of\ polymer\ repeat\ unit} \qquad (7)$$

Chapter 3

RESULTS AND DISCUSSION

3.1 Modification of Unprotected Chitosan; Formation of Citrate Ester of Chitosan

Chitosan was modified as mentioned in section 2.3. The mass of PEG and SHP were kept constant at 0.1g and 0.0625g respectively. The percentage yield was determined by Equation 1. As seen from Table 2 below, the % yield increased as we added more citrate into the chitosan solution. This is probably due to the fact that the reaction was carried out under homogenous conditions as compared with that of the Benzaldehyde protected chitosan so there was favourable interaction between the reacting species. The resulting %yield is shown in Table 2.

Table2: % yield of Modified Chitosan (MC)

Chitosan(g)	**Citric acid(g)**	**Ratio of chitosan citric acid**	**Yield(g) MC**	**% Yield MC**
0.5	0.5	1:1	0.651	30
0.5	1.0	1:2	0.667	33
0.5	1.5	1:3	0.696	39
0.5	2.5	1:5	0.780	56
0.5	5.0	1:10	0.938	88

Chitosan and Chitosan +PEG films were easily removed via solvent extraction with 4% (1M) NaOH solution due to the presence of a lot of protonated amines in it. Chitosan films prepared without the addition of PEG were brittle hence PEG was added to act as a plasticizer. Films prepared using PEG was found to be flexible and less brittle. The only difference observed in the physical appearance of the films was that adding PEG to the 0.5% chitosan solution reduced the transparency of the chitosan films. This may be due to incomplete miscibility of the chitosan and PEG under the given conditions.

Sodium hydroxide was used for the removal of the films formed via solvent evaporation technique. This is due to the ions of the protonated amines and the OH^- ions interacting together (electrostatic interaction). As observed in this study, the increase in the amount of citric acid used required a more concentrated sodium hydroxide solution to be used for the film removal.

For the 1:1 chitosan citric anhydride solution, a 4% NaOH solution was used but as the ratio of chitosan to citric anhydride increased; the concentration of NaOH used was also increased. This shows that the amount of citrate increased and they were less protonated amines for the OH ions to interact with. For the 2.5g citric acid (1:5), a 14% NaOH solution was used for film removal. Hence the film forming ability of chitosan reduced as we increased the amount of citric acid.

In the case of the crosslinked chitosan, no film could be formed because the crosslinked product was not completely soluble in acetic acid.

The proposed reaction between unprotected chitosan and citric anhydride is shown in Figure 7 below. Since the amide group of chitosan is more reactive than the hydroxyl group, more of the citrate is expected to be grafted on the backbone of the chitosan via the amide group. It is also presumed that some esterification reactions (OH and citrate) might also take place on the OH groups of chitosan; however we don't have any direct evidence of that.

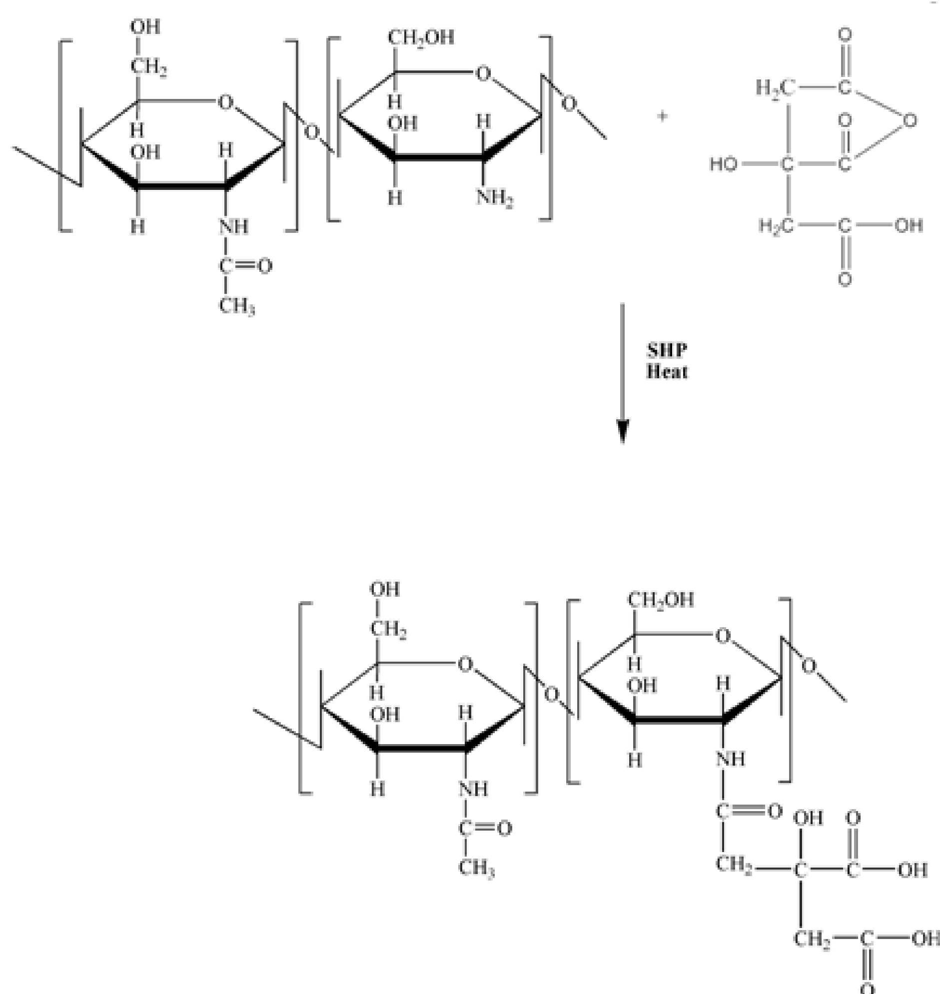


Figure 7: Reaction of Unprotected chitosan with citric anhydride

3.2 Synthesis of N-Protected Chitosan and Formation of Citrate Crosslinked Chitosan

Figure 8 below shows the overall reaction scheme carried out in this study. The first step involves the protection of the chitosan using benzaldehyde. As can be observed, the amine groups of chitosan are protected via formation of a Schiff Base N=C. Step 2 is the crosslinking of the N–protected chitosan via the hydroxyl group using citric anhydride obtained from section 2.2.5. The red part observed in the reaction scheme, shows the citrate crosslinking of the N-protected chitosan and the incorporation of a new functional group COOH into the crosslinked chitosan. The final step is the deprotection of the protected chitosan to recover the amine groups of chitosan back into the crosslinked structure.

Reaction Pathway

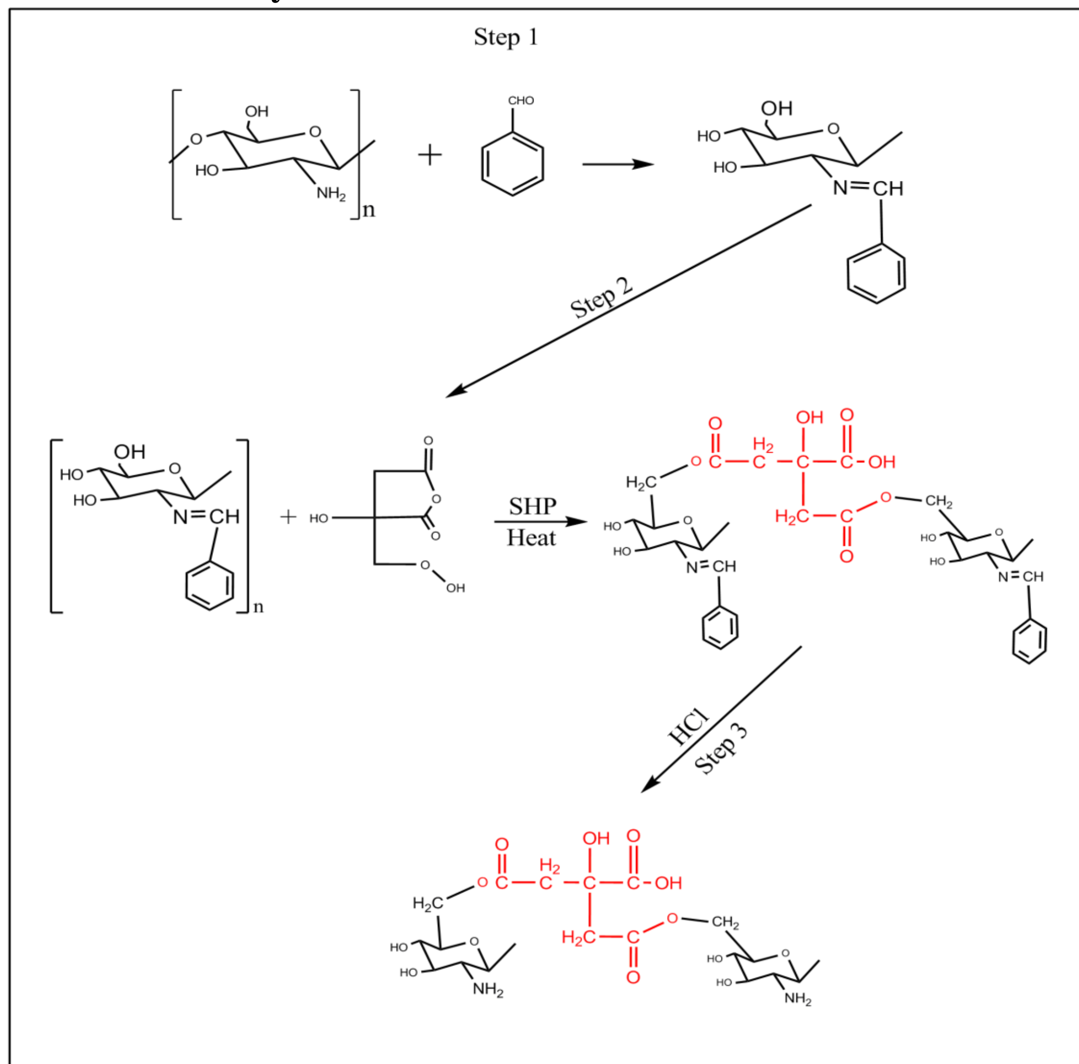


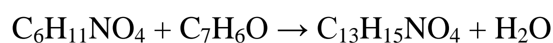
Figure 8: Scheme of Reaction

3.3 Amine Content

Amine content of chitosan, N protected chitosan and crosslinked chitosan was determined. Amine content of chitosan was determined to be 10.34% by mass while that of protected chitosan was 0.21% by mass indicating that almost 98% of free amine groups were protected. In the case of the crosslinked chitosan which was deprotected, the amine content was found to be 4.83% by mass showing that we were able to recover some of the amine groups back into the crosslinked chitosan which is what we are trying to achieve in this study.

3.3.1 N-Protected Chitosan

Amine groups of chitosan were protected by the method stated in section 2.5. The protection was confirmed by the use of FTIR and amine content determination. The limiting reactant was chitosan and the % yield was determined.



$$C_6H_{11}NO_4 + C_7H_6O \rightarrow C_{13}H_{15}NO_4 + H_2O$$

Mass of empty Petri dish – 40.8302g

Mass of Petri dish and protected chitosan – 54.6106g

Mass of Benzaldehyde protected chitosan – 13.7804g

The percentage yield was determined to be 88.9 %.

Table 3: %weight loss in Crosslinked Chitosan (CC)

Benzaldehyde Protected Chitosan(g)	Citric acid(g)	Ratio of chitosan citric acid	Yield(g) CC	% weight loss CC
0.5	0.5	1:1	0.490	2
0.5	1.0	1:2	0.469	6
0.5	1.5	1:3	0.408	18
0.5	2.5	1:5	0.328	34

Benzaldehyde protected chitosan was not soluble in DMSO hence the reaction was carried out under heterogeneous conditions whereby the more citric acid is added, the

more the solution became viscous and the yield reduced as also observed from the Table 3.

3.4 Instrumental Analysis

3.4.1 FTIR Analysis

3.4.1.1 FTIR Analysis of Chitosan

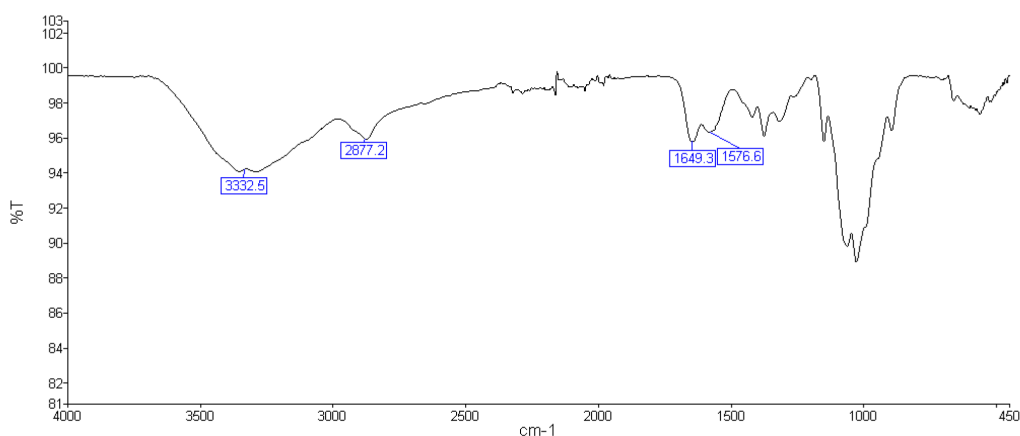


Figure 9: FTIR spectrum of Chitosan

The FTIR spectrum of chitosan is shown in Figure 9. In chitosan, we observe the peaks at 3332 cm^{-1} and 2877 cm^{-1} which represents the O-H, N-H stretch and C-H stretch. Bands at 1649 and 1577 cm^{-1} represent the amide I and II of chitosan. The C-O-C stretching peak is at 1152 cm^{-1} while the peak at 1060 cm^{-1} represents the C-O skeletal vibration of the primary alcohol at C6.

3.4.1.2 FTIR Analysis of Citrate Modified Chitosan (Unprotected)

The FTIR spectrum of the modified chitosan is shown in Figure 10. In the case of the modified chitosan, a very broad band is seen at 3384 cm^{-1} as a result of the addition of the OH groups present in citrate i.e. overlapping of OH. The peak at 1656 cm^{-1} represent the C=O stretching in amide group formed as a result of the interaction between amine of chitosan and citric anhydride. Also, a stretch is observed and a new peak is formed at 1590 cm^{-1} which represents the amide II band. The peaks at

1377 cm^{-1} and 1418 cm^{-1} which are also present in chitosan became very sharp which could be the C-O symmetric vibration in COO^- ions and the C-O anti-symmetric vibration of COO^- ions [13].This confirms that citrate is incorporated in the backbone of chitosan.

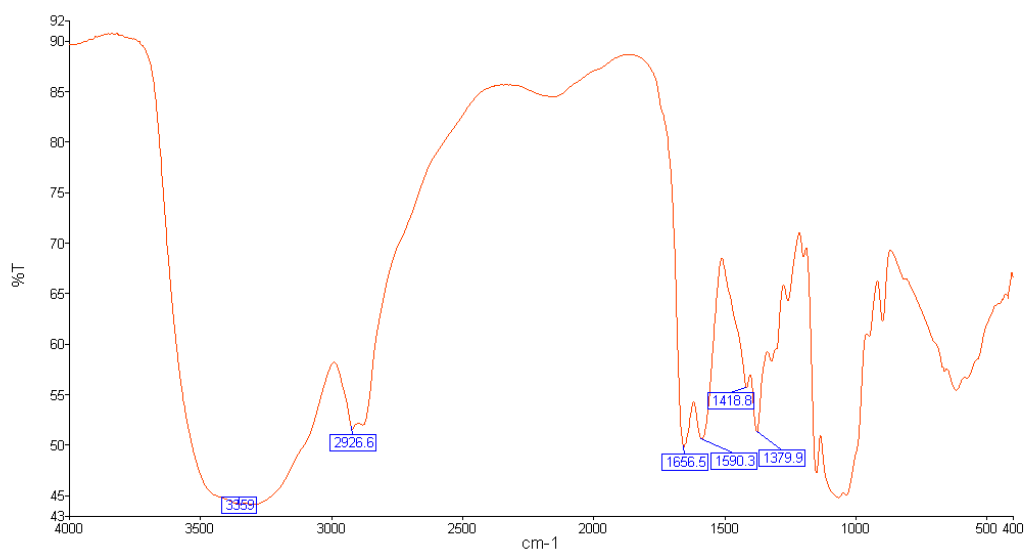


Figure 10: FTIR spectrum of Citrate Modified Chitosan

3.4.1.3 FTIR Analysis of Benzaldehyde Protected Chitosan

Protection was carried out using benzaldehyde as shown by the FTIR spectrum in Figure 11. A broad peak was observed at 3359 cm^{-1} which represents the O-H stretch. A new peak was observed at 1637 cm^{-1} which represents the N=C (Schiff base) [30]. A sharp peak obtained at 1581 cm^{-1} represents the aromatic ring stretch. Also new peaks were observed at 895, 757 and 689 cm^{-1} representing C-H aromatic bending shows the incorporation of aromatic structure to the chitosan backbone.

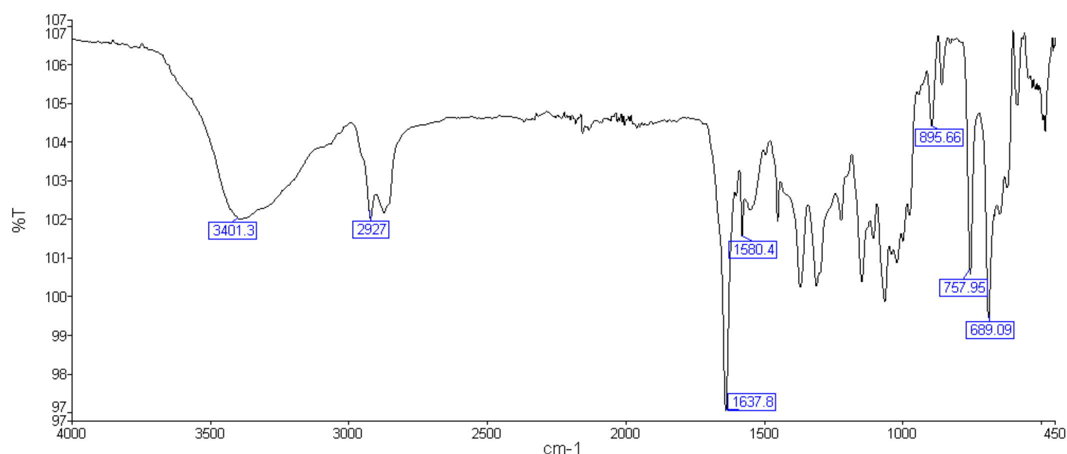


Figure 11: FTIR spectrum of Benzaldehyde Protected Chitosan

3.4.1.4 FTIR Analysis of Protected Crosslinked Chitosan

The FTIR spectrum of the N-protected and crosslinked chitosan is illustrated in Figure 12. It showed the emergence of a new peak at 1710 cm^{-1} which represents the C=O formed as a result of ester formation. The OH peak reduced and became broader as compared with the protected chitosan, this proves that there was esterification i.e. crosslinking / grafting on the OH group. The peak for the Schiff base at 1637 cm^{-1} still remained while the aromatic ring stretch is observed at 1530 cm^{-1}.

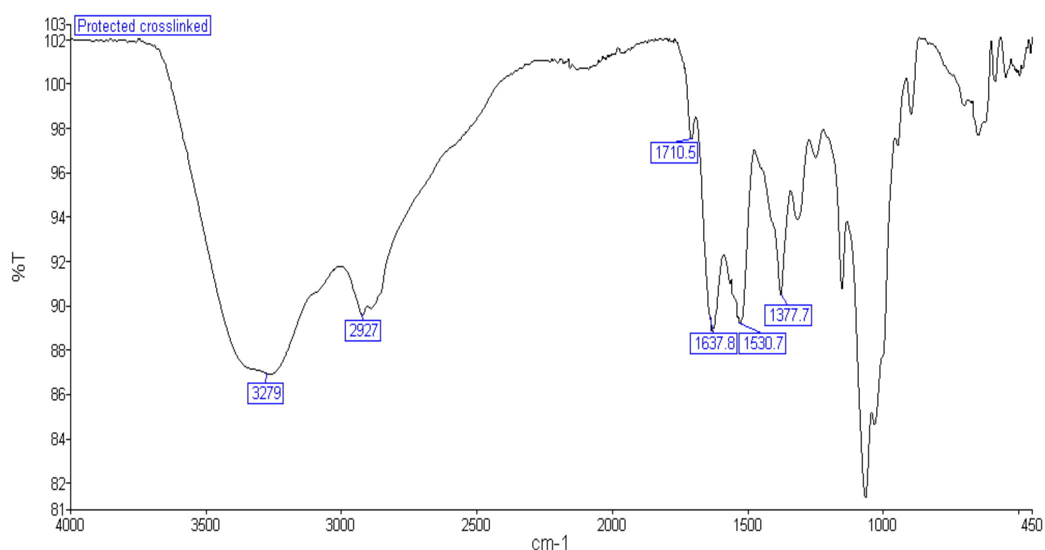


Figure 12: FTIR spectrum of Protected Crosslinked Chitosan

3.4.1.5 FTIR Analysis of Deprotected Crosslinked Chitosan

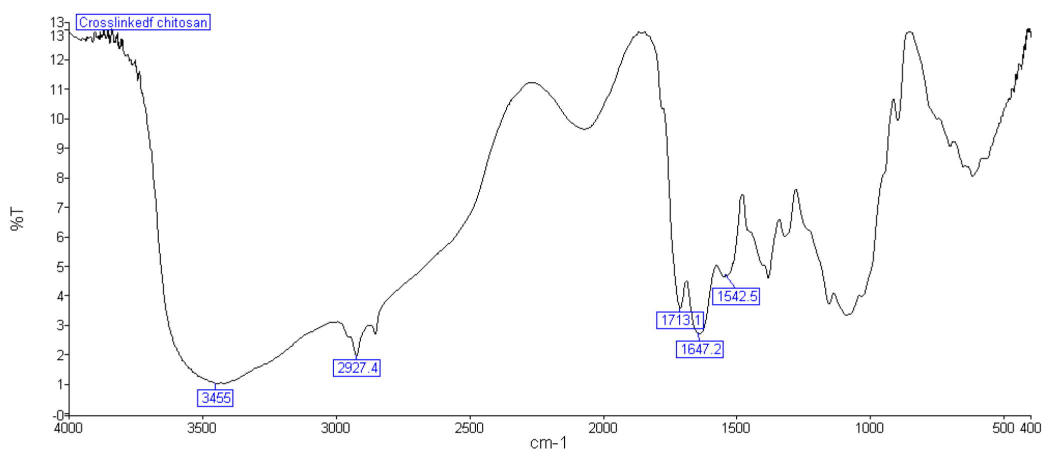


Figure 13: FTIR spectrum of Crosslinked Chitosan

The FTIR spectrum of crosslinked chitosan is shown in Figure 13. The C=O peak remained at 1713 cm^{-1} and the Schiff base protection peak at 1637 cm^{-1} disappeared and we got peaks at 1647 and 1542 cm^{-1} representing our amides. A stretch is observed on the peak 1378 cm^{-1} showing the C-O symmetric vibration in COO^- ions. Hence, the proposed modification was achieved according to the FTIR analysis.

3.5 C-13 NMR Analysis

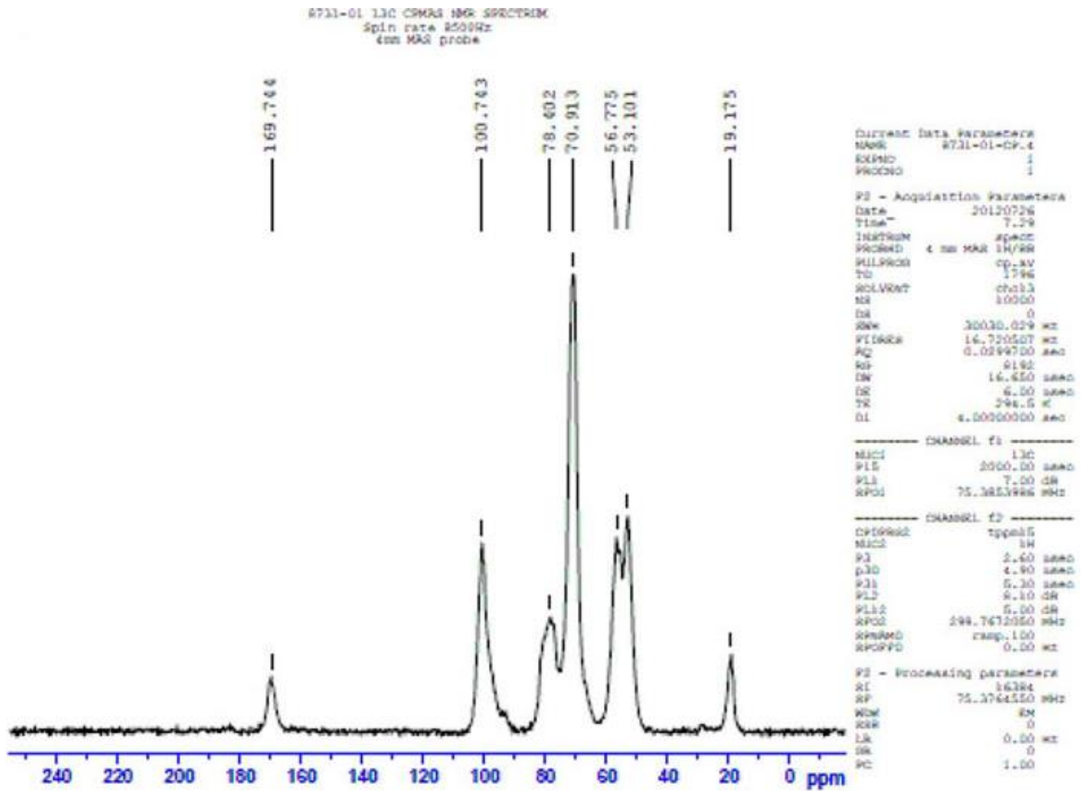


Figure 14: ^{13}C NMR of Chitosan

Source [34]

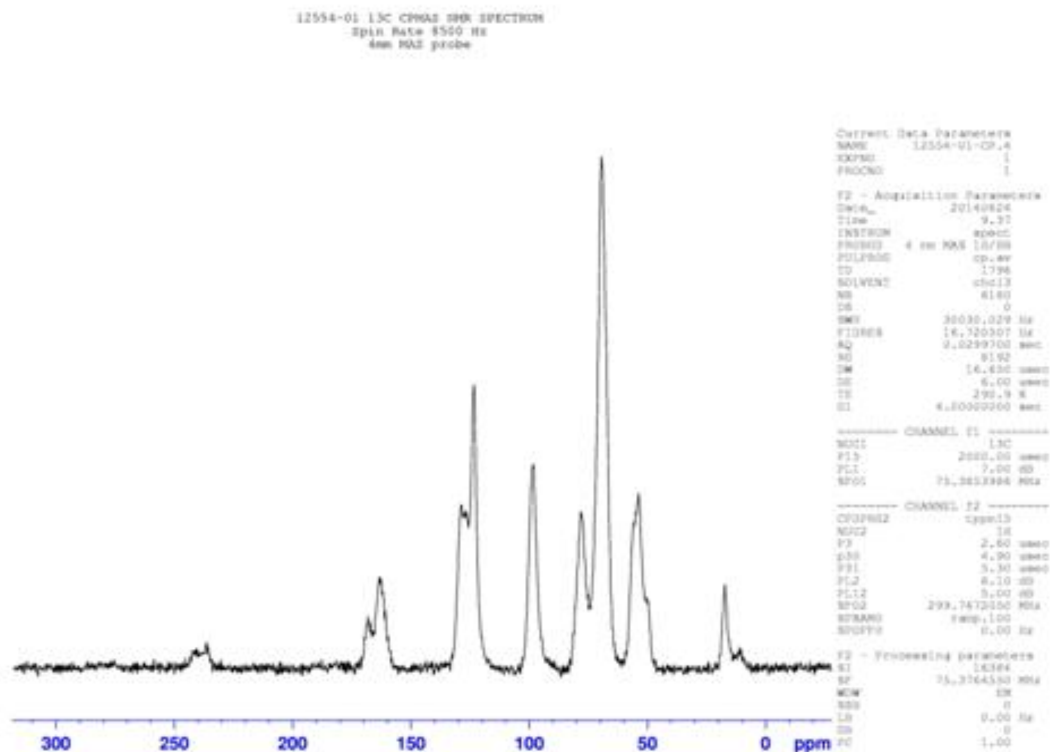


Figure 15: ^{13}C NMR of Benzaldehyde Protected Chitosan

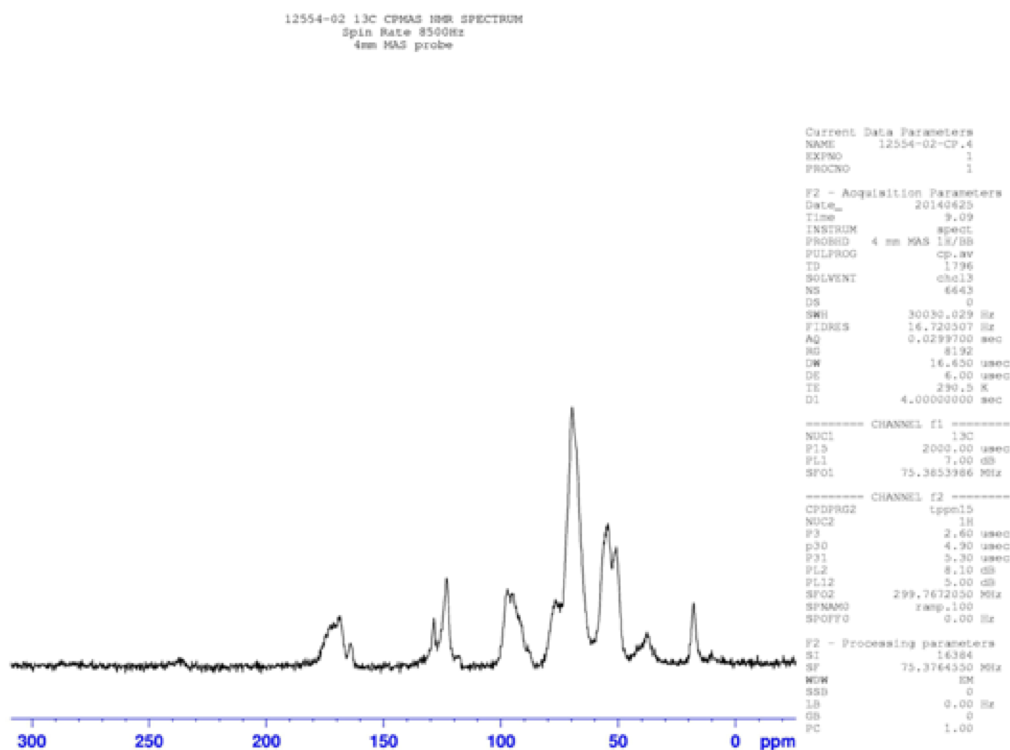


Figure 16: ^{13}C NMR of Protected Crosslinked Chitosan

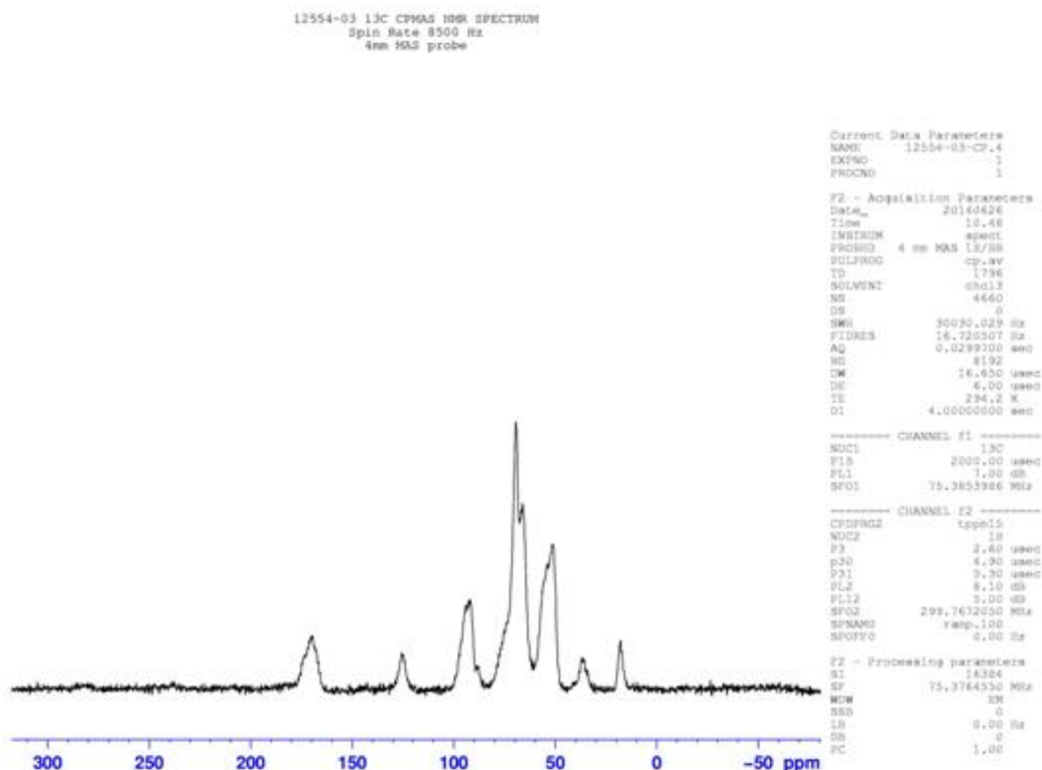


Figure 17: ^{13}C NMR of Deprotected Crosslinked Chitosan

Carbon13 NMR of chitosan was obtained and explained by Yalinca et al (2013). The signals at 171 and 19 ppm represented the C=O (carbonyl carbon) and CH_3 present in the acetamide group of chitosan. C1, C4, C3,5, C6 and C2 are represented by signals at 100.7, 78.4, 70.9, 56.8 and 53.1 ppm respectively [34]. NMR for Benzaldehyde is shown in Figure 15. As expected from the scheme of reaction in Figure 8, new signals were obtained at 125 and 129 ppm which is as a result of the C=C double bonds present in aromatic compounds. Another new signal is at 165 ppm which is due to the N=C i.e. Schiff base formation. There were also some slight chemical shifts in the C1-C6 of chitosan. A new signal at 38 ppm observed in the Benzaldehyde protected crosslinked chitosan represents the CH_2 (carbon-hydrogen bond) group attached to the carbonyl group in the citrate crosslinking which pushed it further downfield. Intensity of other signals reduced and this could be as a result of

the new environment around the chitosan [34] as seen in Figure 16. Figure 17 shows the C NMR of the deprotected crosslinked sample. The signal at 170 ppm is for the carbonyl group of an ester (RCOOR). The small signal at 125ppm probably shows that there was not 100% deprotection of the crosslinked product. This same trend where there was incomplete deprotection was also observed by Liu et al (2005) when chitosan was protected and deprotected using phthalic anhydride and hydrazine monohydrate [9]. This also confirms the proposed modification of chitosan using citric anhydride.

3.6 Swelling Results

3.6.1 Swelling results for Chitosan and Modified Chitosan films

The results for swelling experiments for chitosan, chitosan+ PEG, modified chitosan film is shown in Tables 4 and 5 below. The swelling experiments were carried out using 4 different pH values (1.2, 4.0, 7.0, and 11.0) in the acidic, neutral and basic medium. The effect of adding citrate to chitosan on the swelling capacity was studied with respect to time and pH.

Table 4: Swelling % of chitosan, chitosan + peg and modified chitosan at pH7

Time (hours)	Swelling %					
	Chitosan	Chi +PEG	0.5g CA	1.0g CA	1.5g CA	2.5g CA
1	168	260	390	489	318	205
3	289	401	455	465	283	169
5	239	459	428	437	259	96
7	227	346	403	443	276	86
24	208	253	323	470	208	52

Table 5: Swelling % of chitosan, chitosan + PEG and modified chitosan at pH11

Time (hours)	Swelling %					
	Chitosan	Chi +PEG	0.5g CA	1.0g CA	1.5g CA	2.5g CA
1	314	290	514	896	794	778
3	237	520	454	830	731	689
5	189	416	419	808	672	612
7	176	301	412	830	659	553
24	153	287	408	846	629	457

The swelling behaviour of chitosan and chitosan+PEG films at pH7 and 11 is given in Figure 18 and 19 respectively.

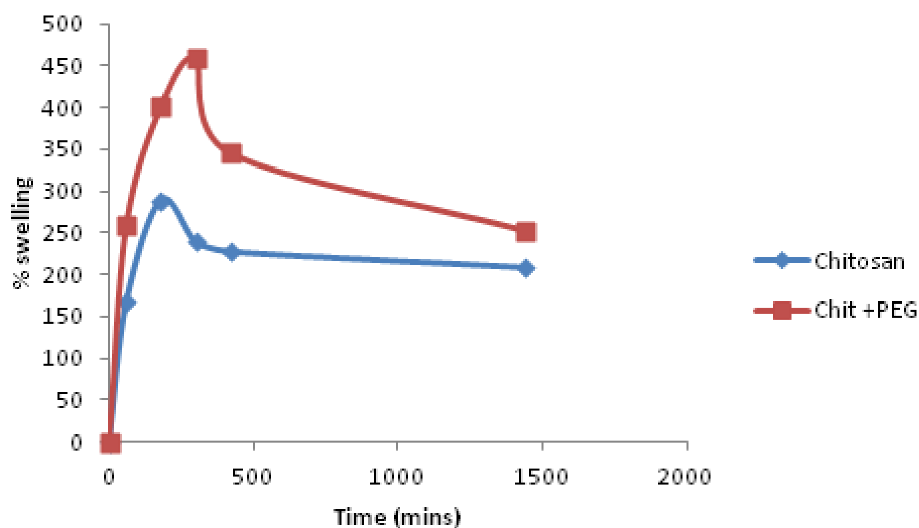


Figure 18: Swelling percentage for Chitosan and chitosan + PEG at pH7

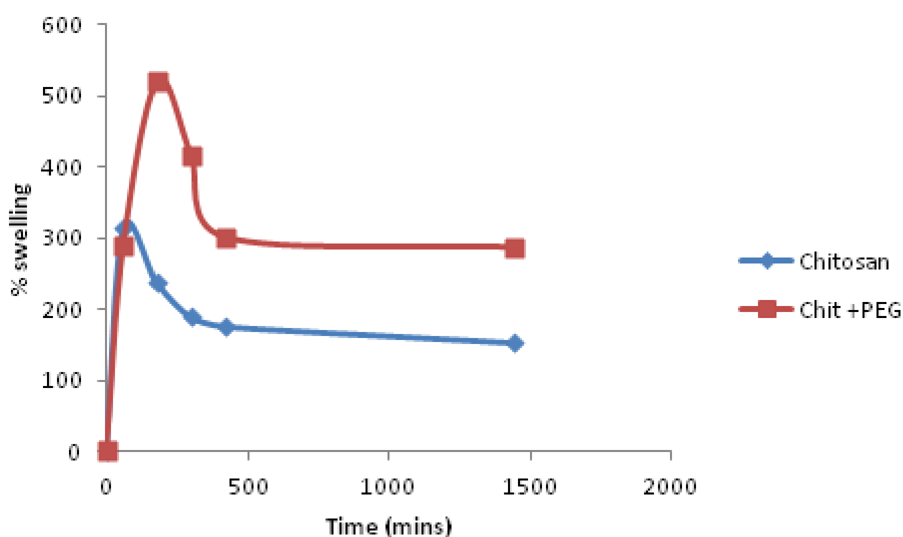


Figure 19: Swelling percentage of chitosan and chitosan + PEG at pH11

All samples dissolved in the acidic medium (pH 1.2 and 4) as a result of the protonation of chitosan amine groups to NH_3^{+}. All chitosan membranes are known to exhibit very little resistance in acidic medium and they dissolved [13]. The only observable difference is that adding PEG and citrate increased the time taken for

dissolution by a few minutes. Hence, it can be concluded that modification of unprotected chitosan by reacting with citric anhydride does not give rise to a crosslinked product.

The swelling of membranes is usually affected by the hydrophilicity, degree of crosslinking, pH and ionic strength [13].

The PEG added to chitosan increased the maximum % swelling from 288% in pH 7 to 458% and from 314% to 520% in pH 11. PEG acted as a plasticizer and this increase in swelling was due to the fact that PEG is also hydrophilic [31].

It is also observed that it took 1 hour more for chitosan PEG to reach maximum swelling% as compared to chitosan in both pH media. Adding PEG to chitosan affected the inherent crystalline structure of chitosan [31] and this would result in the formation of some amorphous regions in chitosan making it take longer for the water molecules to fuse into both the amorphous and crystalline regions as compared to chitosan which only had crystalline regions.

The swelling behaviour of citrate modified chitosan is shown in Figures 20 and 21. The amine group (NH_2) in chitosan is responsible for its hydrophilicity and even though modifying it resulted in the loss in some of the amine groups, citrate is hydrophilic too hence an increase was also observed in the % swelling as compared to just chitosan and chitosan PEG with the maximum swelling of modified chitosan membrane at 1.0g citrate at 1hr 489% and 895% respectively in pH 7 and 11. As observed from Tables 4 and 5, there was rapid swelling at the initial stage which is due to the presence of enough negative surfaces (COO-) present on the surface of the

modified chitosan films. Swelling in pH 11 was more than that at pH7 because at pH 7, there exists mostly NH_2 and COOH which have hydrogen bonding and this would result in lesser swelling [33]. At pH 11 (basic buffer) more ionisable groups are left on the surface of the sample and this made the material to swell due to enhanced electrostatic repulsion which resulted in higher hydration of the membrane.

After about 300mins, decreasing trend was observed and this may be attributed to the saturation of the polymer chains and reduction in repulsive forces on the surface of the material leading to aggregation of the polymer chain. As the chains aggregate, water molecules are extruded hence a decrease volume is observed. This trend is also confirmed by the %weight loss after 24hours as seen from Table 6. The decrease in swelling could also be due to the screening effect of the COO- groups by the Na+ ion used in preparing the buffer. Chitosan and chitosan PEG as compared to the modified films had a 20.6%, 2.3% and 45.7%, 9.52% increase in weight at pH 7 and 11 respectively.

All samples showed a high percentage of weight loss as compared to the chitosan, chitosan PEG samples which showed a considerable weight gain as a result of the entrapment of water molecules in the network of the films.

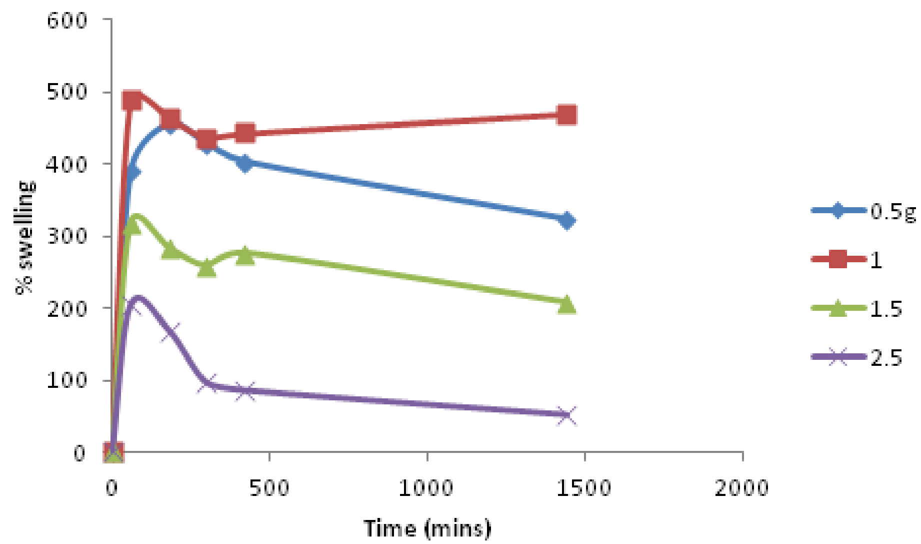


Figure 20: Swelling % of modified chitosan at pH 7

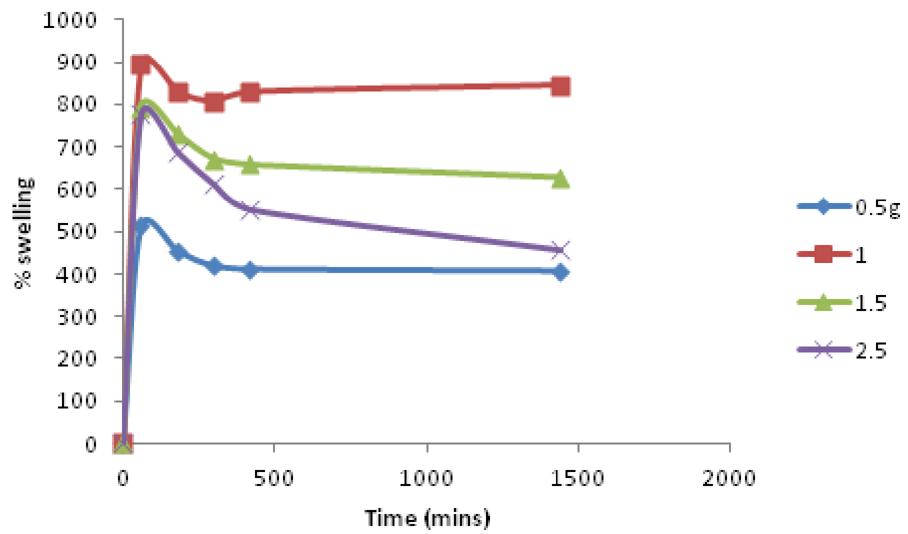


Figure 21: Swelling % of modified chitosan at pH 11

Table 6: Weight loss modified chitosan film membranes

Sample	%Weight Loss	
	pH7	pH11
0.5g CA	40.6	28.8
1.0g CA	50.7	45.8
1.5g CA	57.3	80.9
2.5g CA	64	85

3.6.2 Swelling Behaviour of Citrate Crosslinked Chitosan

The crosslinked chitosan has 2 functional groups, the NH_2 and COOH. The pKa of chitosan is 6.5 while that of COOH is approximately 4.7 (33). Swelling in the acidic medium could be attributed to the protonation of the amine groups of chitosan as seen in pH 1.2 and 4 due to the repulsion of the protonated amines.

The equilibrium % swelling values for citrate crosslinked chitosan with 0.5 g, 1.0 g, 1.5 g and 2.5 g citrate are tabulated in Table 7, 8, 9 and 10 respectively. Similarly, the swelling trend for each sample is shown in Figure 22-25.

Taking the swelling trend of 0.5g citrate crosslinked as an example, at pH 1.2, the amines were protonated and the sample experienced a repulsion ($NH_3^+NH_3^+$) which led to the swelling of the product after the first hour. As time went on, a considerable decrease was observed in the swelling percentage due to favourable interaction of the

Cl^- (used to prepare the buffer) and the protonated amines which caused a screening effect on the NH^{3+} and reduced the swelling.

At pH 4 there was an observable increase in the swelling as compared to pH 1.2 (from 498 to 521%) due to reduced screening of the protonated amines since the screening effect of counter ions is high in very acidic pH (<3).

At pH greater than 4 up till pH 7, both functional groups exist in the form NH_2 and COOH, NH_3^+ and COO-. This causes the reduction in swelling as compared to pH 4 (from 521 to 396%) and this is due to the favourable ionic interactions between COO- and NH_3^+ and the hydrogen bonding present.

At pH 11, the deprotonated COO- is responsible for the swelling which results also due to repulsion of charges. This causes the increase in swelling we experienced from pH 7 to 11(maximum swelling at pH11 is 519 as compared to 396%). The trend at pH 11 is not the same as the other pH since maximum swelling did not occur in the first hour. In the case of the 0.5 and 1.0g, the % swelling reduced after the 1st hour probably due to the screening effect of Na^+ while it later increased which could be due to the hydrophilic NH_2 also present at this pH. For 1.5g citrate, the % swelling increased as time increased which is probably due to the presence of more COOH in the sample as compared to 0.5 and 1.0g. This is also confirmed when we look at the swelling % at pH 1.2 and 4 which is considerably lower than that at 0.5g and 1.0g citrate (408 as compared to 498% and 570% at pH 1.2 and 377 as compared to 521 and 875% at pH 4) showing that we have less of the amines and more of the citrate incorporated into it.

Crosslinking reduced in 2.5g citrate due to the viscosity of the medium which reduced (hinder) the amount of citric anhydride that was able to interact and crosslink the chitosan. This resulted in the dissolution of the sample in pH 1.2 and 11. The slight interaction between the NH_3^+ and COO- must have being the reason why it did not completely dissolve at pH 4 and 7 (but had a high % weight loss of 71 and 75.6% respectively) as compared to when it is only NH_3^+ and COO- in pH 1.2 and 11 which had a high electrostatic repulsion and disintegrated completely.

Table 7: % swelling for 0.5g CA sample

pH/time(hours)	**1.2**	**4**	**7**	**11**
1	498	521	396	494
3	457	428	340	438
5	404	411	266	509
7	364	387	266	509
24	355	281	283	519

Table 8: % swelling for 1.0g CA sample

pH/Time(hours)	**1.2**	**4**	**7**	**11**
1	570	875	756	736
3	461	496	325	472
5	495	409	325	574
7	463	368	282	511
24	456	340	256	547

Table 9: % swelling for 1.5g CA sample

Time(hours)	**pH**			
	1.2	**4**	**7**	**11**
1	408	377	327	258
3	396	385	303	316
5	405	329	200	410
7	406	294	194	457
24	405	242	228	595

Table 10: % swelling for 2.5g CA sample

pH/Time(hours)	**1.2**	**4**	**7**	**11**
1	316	178	18	247
3	-62	36	18	204
5	Dissolve	-22	60	4
7	Dissolve	-58	64	-62
24	Dissolve	-58	44	Dissolve

*negative values show weight loss

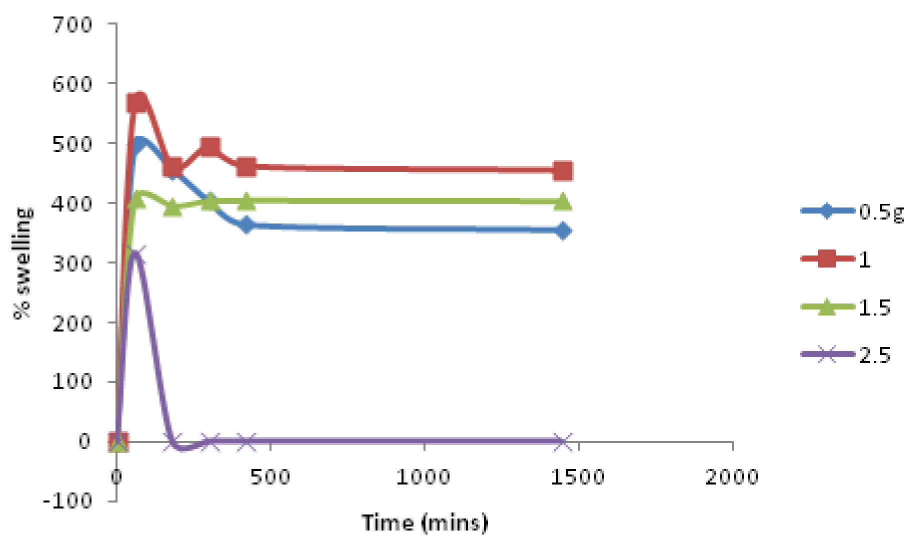


Figure 22: swelling % of crosslinked chitosan at pH 1.2

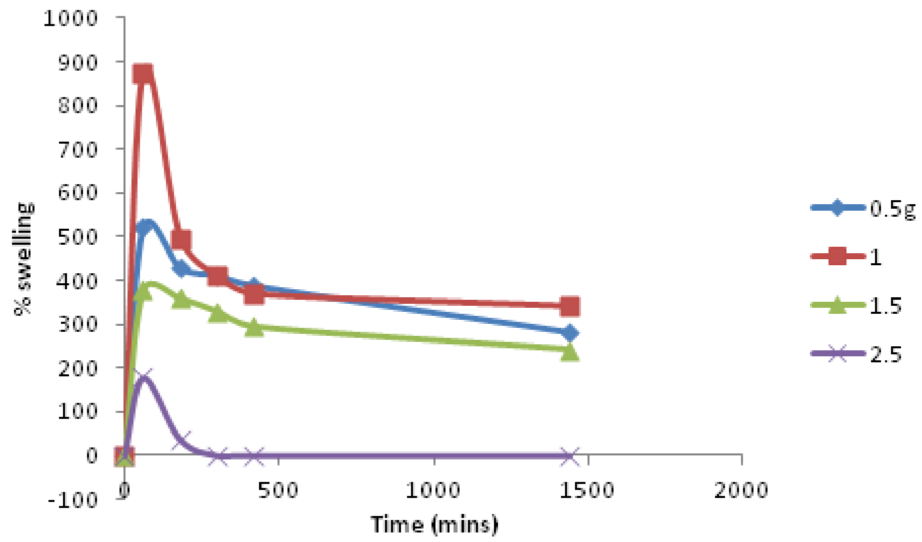


Figure 23: Swelling % of crosslinked chitosan at pH 4

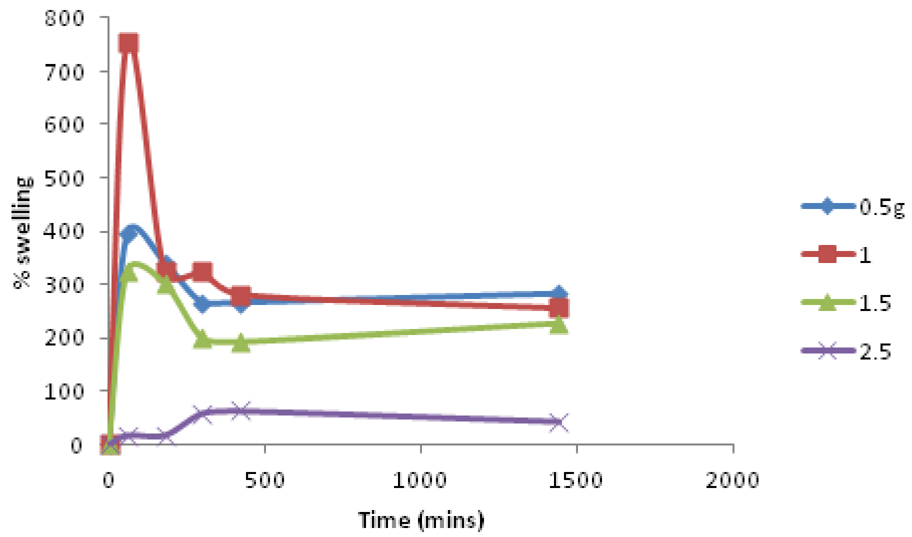


Figure 24: Swelling % of crosslinked chitosan at pH 7

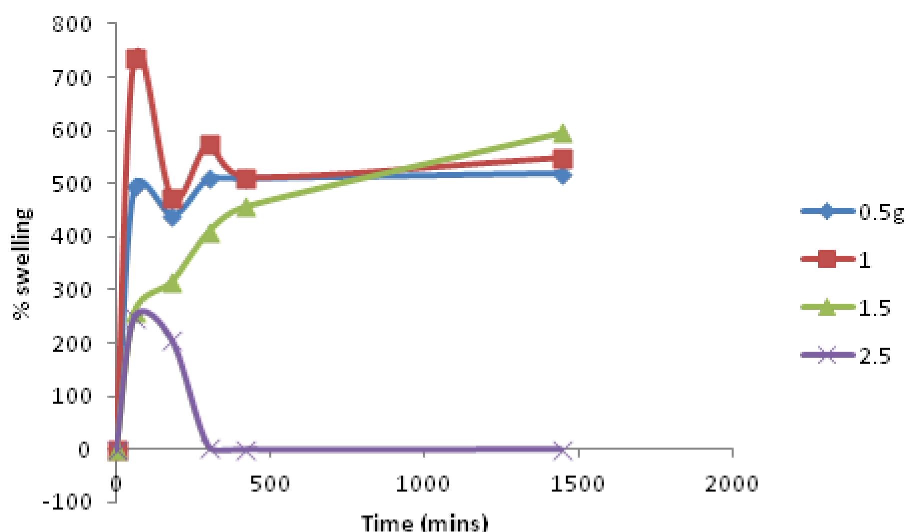


Figure 25: Swelling % for crosslinked chitosan at pH 11

Comparing the crosslinked chitosan with the modified one, it was observed that there was little dissolution of the crosslinked chitosan in acidic pH which proves that there was chemical crosslinking involved since crosslinking reduces the ability of a hydrophilic hydrogel to dissolve in aqueous solutions. All crosslinked samples showed very little %weight loss with only the 2.5g sample dissolving at pH 1.2 and 7 and having a very high % weight loss.

3.7 Swelling Kinetics

3.7.1 Modified Chitosan

A swelling kinetics analysis was undertaken in order to understand the swelling mechanism. The film with the maximum swelling ratio i.e. 1.0g citrate modified film was used for this analysis. The first order and second order kinetic equations were used to plot graphs as given in Figure 26 and 27 respectively. The plot should give a straight line if the swelling behaviour obeys the corresponding equation (32). It was observed that the swelling did not follow the first order as seen from the R^2 values and the theoretical weight at a specific time (60mins) when calculated was 0.0003g which does not agree with that gotten experimentally as 0.0495 and 0.0478g. The

swelling followed second order kinetics with R^2 values of 0.9996 and 0.9999 at pH7 and 11 with the theoretical value W_{max} determined to be 0.0481g and 0.0455g as compared to the experimental which were 0.0495 and 0.0478 respectively. All plots of the modified samples using second order gave straight line graphs with the R^2 values ranging from 0.9979-1.

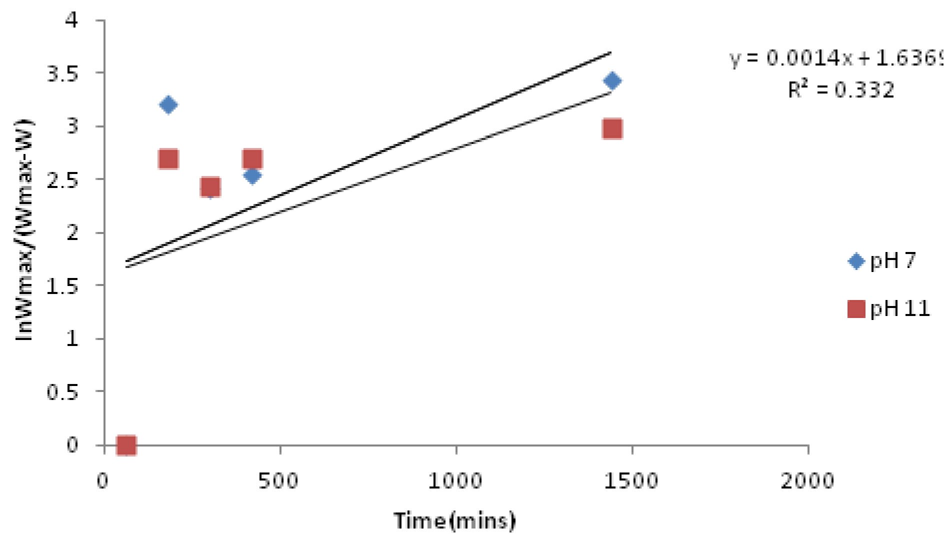


Figure 26: Pseudo 1st order kinetic of modified chitosan

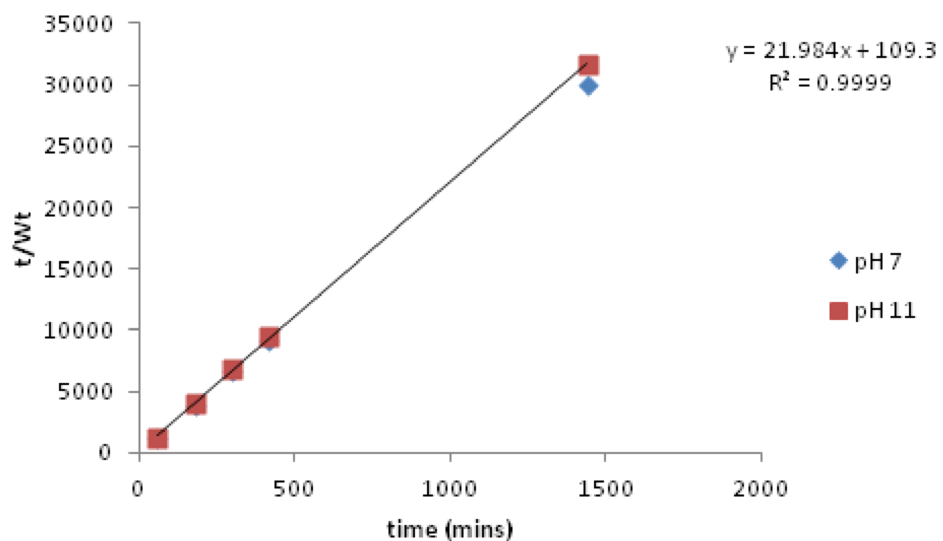


Figure 27: Pseudo 2nd order kinetics

3.7.2 Crosslinked Chitosan

1.0g crosslinked chitosan was used to plot the first order and second order kinetic graphs. As seen from the graph below in Figure 29, the R^2 value for 2nd order (0.9988) was also closer to 1 as compared to the 1st order plot as seen in Figure 28 (0.1156) and a linear graph was obtained showing that the swelling also followed second order kinetics which is similar to that of the modified chitosan also obtained in Figure 26 above.

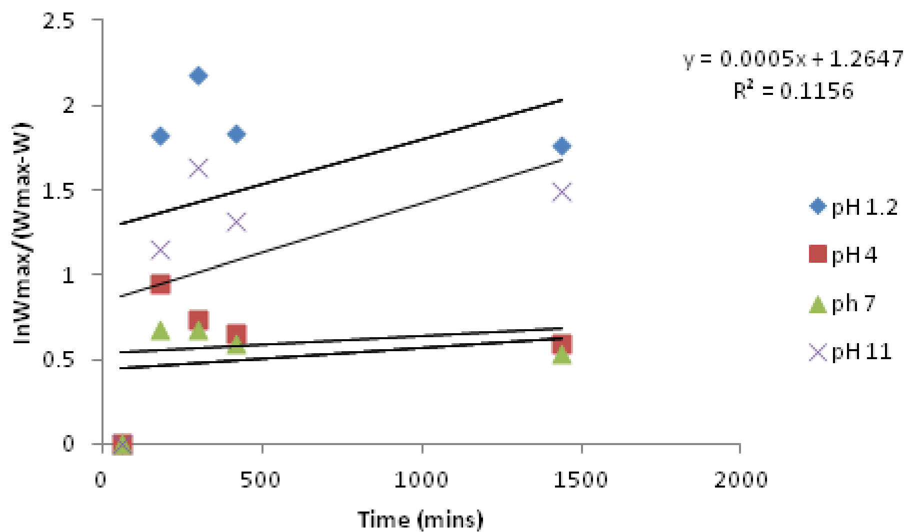


Figure 28: Pseudo 1st order kinetics for crosslinked chitosan

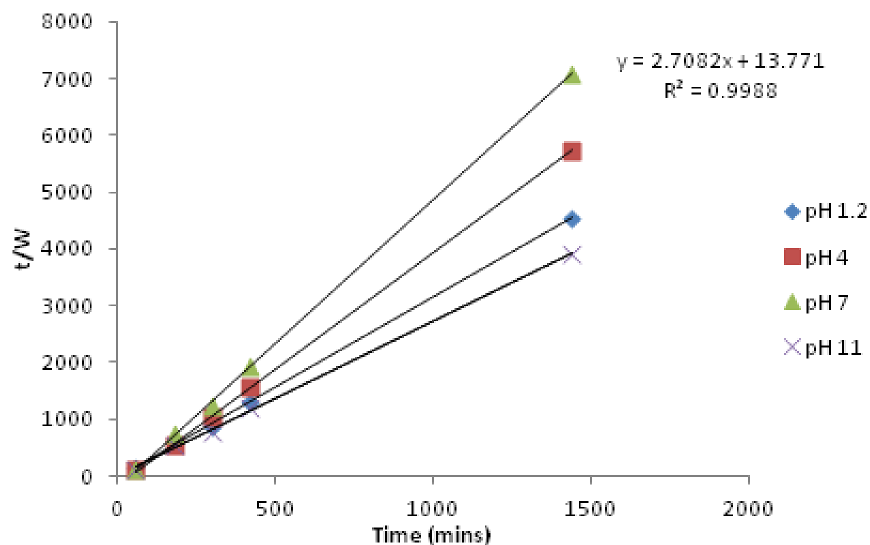


Figure 29: Pseudo 2nd order kinetics for crosslinked chitosan

3.8 Crosslinking Density

The maximum swelling% of each crosslinked sample at different pH was plotted against the crosslinking density at several masses of crosslinker. The results are shown in Table 11 and 12 and in Figure 30. The general expected trend is that as crosslinking increases, the % swelling is supposed to increase up to a point. This is due to the increase in the number of functional groups (NH_2 and COOH) present in the crosslinked chitosan but as the amount of crosslinker increases, the network connecting the chitosan and the crosslinker increases hence we experienced lesser swelling due to more interaction between chitosan and the crosslinker. Crosslinking density of modified chitosan was determined according to equation 7 and shown below:

Table 11: crosslinking density

Chitosan(g)	**Citric acid(g)**	**Crosslinking density**
0.5	0.5	0.84
0.5	1.0	1.68
0.5	1.5	2.52
0.5	2.5	4.19

A plot of the maximum swelling at each pH values of each crosslinked chitosan is shown below. As can be observed, at pH 1.2, the % swelling increased from 0.5g crosslinker (498%) to 1.0g crosslinker (571%) before we observe a downward trend

in the % swelling. The same is true for all pH values we carried out the swelling of crosslinked chitosan in as observed from the graph in Figure 30 below.

Table 12: maximum swelling % at different pH values

Crosslinked chitosan	**Maximum % swelling**			
	pH 1.2	**pH 4**	**pH 7**	**pH 11**
0.5	498	522	396	519
1.0	571	875	755	737
1.5	408	377	327	595
2.5	314	179	64	250

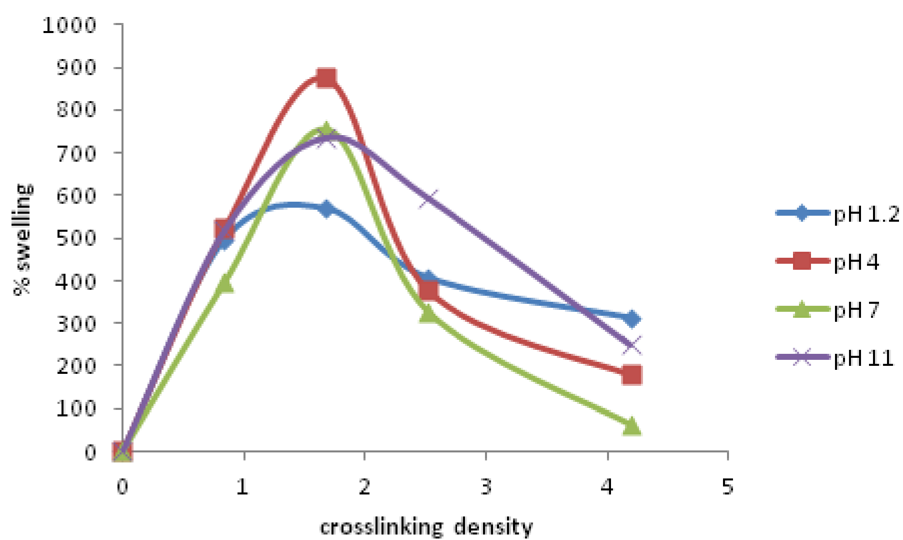


Figure 30: crosslinking density against % swelling

3.9 Effect of Time on Crosslinking

As we increase the time of the reaction, the crosslinking is expected to increase due to increased interaction between chitosan and the crosslinker to form a more compact structure that does not swell easily. This effect was confirmed after varying the time of reaction by 3, 5 and 7 hours. Crosslinked chitosan with the highest amount of swelling was used as a reference i.e. 1.0g citric acid.

Table 13: Swelling % at 5hours

Time(hrs)	**pH**			
	1.2	**4**	**7**	**11**
1	292	294	208	300
3	308	277	149	246
5	306	259	127	288
7	308	246	124	346
24	290	246	122	462

Table 14: swelling % at 7 hours

Time (hrs)	**pH**			
	1.2	**4**	**7**	**11**
1	364	306	219	211
3	364	260	186	234
5	328	193	186	260
7	321	183	200	268
24	321	190	270	361

As can be observed from Table 13 and 14 above which shows the swelling % at 5hr and 7hr reaction time, the swelling % reduced as we increased time. At 3hr (Table 8), maximum swelling % for pH 1.2, 4, 7 and 11 was 570, 875, 756 and 736% respectively while that for 5hr and 7hr reaction time were 308, 294, 208, 462% and 364, 306, 219 and 361%. The decrease in maximum swelling % at 7hr in pH 11 (where COOH is the functional group causing swelling) as compared to 5hr pH 11 is due to the reduced amount of free COOH available as free sites for swelling because more of it has being incorporated into the crosslinked structure. This trend of the swelling behaviour is shown in the Figures 31-34.

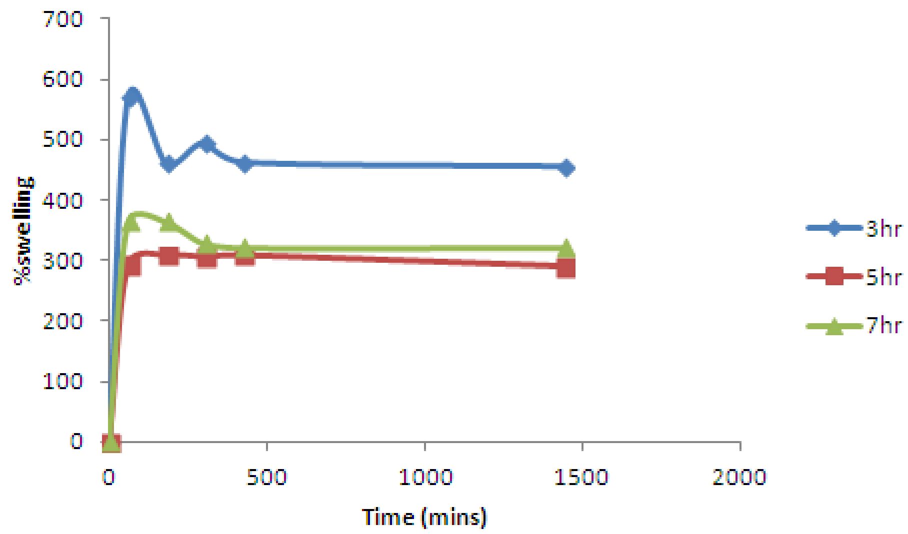


Figure 31: Swelling % of 1.0 g CA at 3, 5 and 7 hrs at pH 1.2

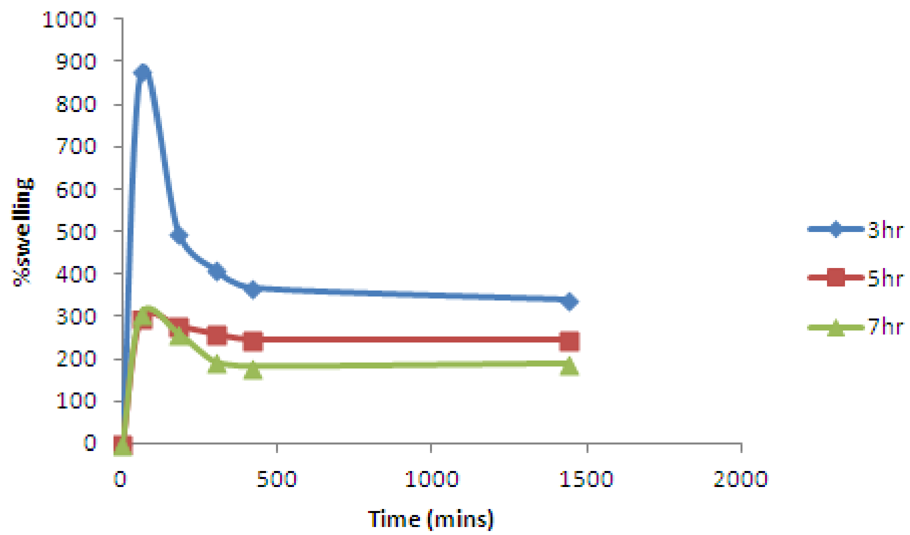


Figure 32: Swelling % of 1.0 g CA at 3, 5 and 7 hrs at pH 4

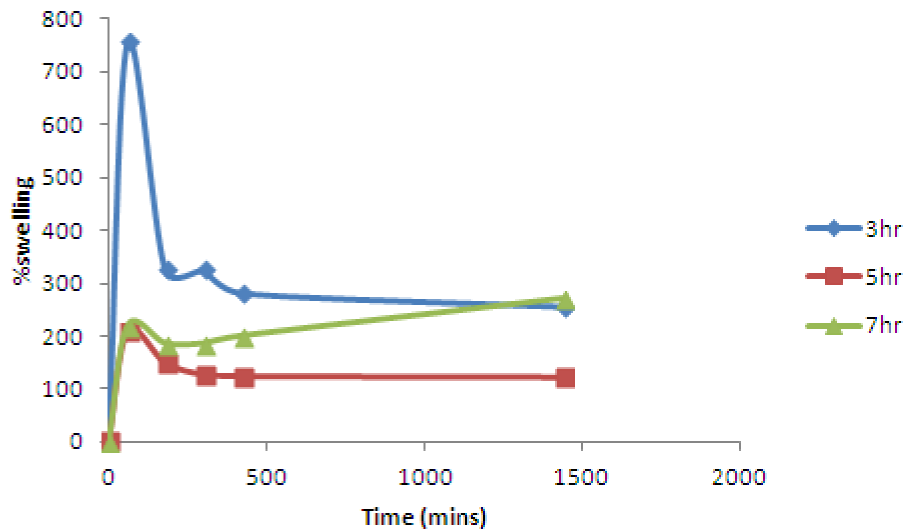


Figure 33: Swelling % of 1.0 g CA at 3, 5 and 7 hrs at pH 7

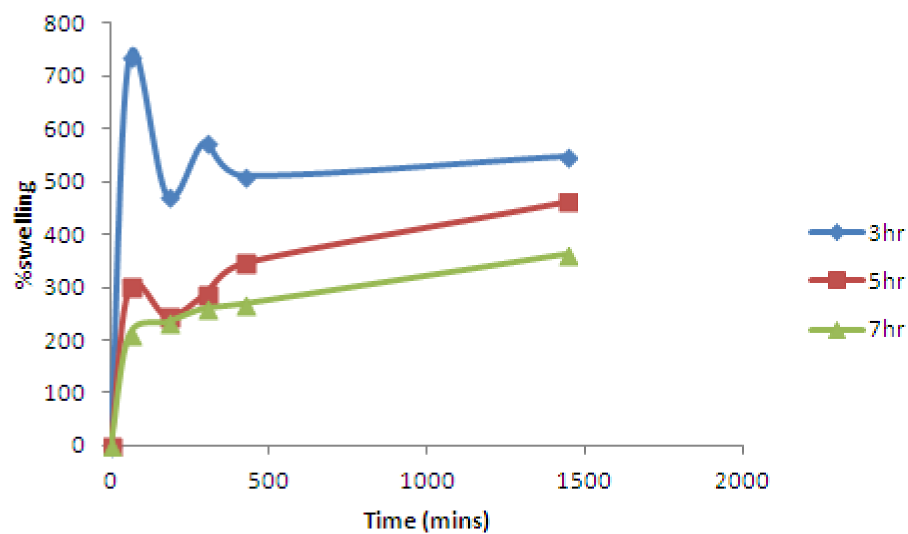


Figure 34: Swelling % of 1.0g CA at 3, 5 and 7 hrs at pH 11

Table 15 shows the %weight of both samples at 5 hours and 7 hours respectively. Weight loss in the sample at 7 hrs was lower as compared to that at 5hours showing that it was more compact and crosslinked. Hence, it can be concluded that increasing the time of crosslinking resulted in more crosslinked products, same trend was observed by Moura et al (2007) [28].

Table 15: % weight loss at 5hours and 7 hours reaction time

Time	**pH**			
	1.2	**4**	**7**	**11**
5 hours	27	22	24	23
7 hours	2	10	9	7

Chapter 4

CONCLUSION

Chitosan was modified using citric anhydride to form film membranes. PEG was added to act as a plasticizer. The incorporation of citrate into the backbone of chitosan was successful as shown by FTIR spectra and swelling experiments. Adding citrate to chitosan increased the %swelling of chitosan but the films were found to dissolve in acidic solution and as amount of citrate increased (2.5g). Swelling kinetics followed pseudo second order kinetics.

Amine groups of Chitosan were protected using Benzaldehyde. FTIR spectra and amino content was used to confirm the N protection of chitosan and the crosslinked chitosan.

Protected chitosan was crosslinked with the use of citric anhydride to incorporate the COOH functional group into chitosan. The presence of a peak at 1713cm^{-1} showed the incorporation of the carbonyl group into chitosan. Swelling also followed pseudo second order and with maximum swelling at 1.0g citric acid and at pH 4.

The effect of time on crosslinking showed that as time increased crosslinking increased which resulted in lesser swelling of the sample in aqueous media and formation of a more compact crosslinked product.

The grafting of chitosan with citric anhydride formed a gel that can be used for environmental purposes in removing metals, dyes, pesticides from aqueous solutions. It could also find biomedical applications as a result of the amino groups attached to the polymer which can make it useful for specific drug delivery systems.

Further research could be done by preparing a blend (semi IPN) with another polymer.

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