Preparation and Characterization of Phosphorylated Chitosan Films via Graft Copolymerization

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ABSTRACT

Graft copolymerization of bis (2-methacryloyl oxy ethyl) acid phosphate (BAP) on to chitosan initiated by potassium per sulphate (KPS) under N_2 atmosphere has been studied. The effect of polymer concentration, monomer concentration, polymerization temperature, initiator concentration and polymerization time on the grafting yield have been investigated.

The copolymers were characterized by FTIR, SEM, DSC, and contact angle measurement. Swelling and dissolution behaviour of grafted polymer was followed in different buffer solutions (pH = 3, 7, and 11).

Keywords: Chitosan, Phosphorylated Chitosan, Contact Angle, BAP

ÖZ

Azot atmosferi altında potasyum persülfat (KPS) redoks başlatıcısı kullanılarak farklı konsantrasyonlardaki kitosan sulu çözeltilerinin bis(2-metakriloil oksi) asit fosfat (BAP) ile kopolimerizasyonu incelenmiştir. Polimer konsantrasyonunun, monomer konsantrasyonu, polimerizasyon sıcaklığı, başlatıcı konsantrasyon ve polimerizasyon süresinin % aşılama etkisi çalışılmıştır.

Aşılı kopolimerler FTIR, SEM, DSC ile karakterize edilmiş ve temas açısı ile hidrofilik özelliği incelenmiştir. Sentezlenen yeni fosforile kitosan filmlerin morfolojileri ile termal davranışları SEM ve DSC analizleri ile test edilmiştir. Aşılı polimerin şişme davranışı farklı pH' lardaki tampon çözeltilerde (pH = 3, 7 ve 11) test incelenmiştir.

Anahtar Kelimeler: Kitosan, Fosforile Kitosan, Temas Açısı, BAP

To …..

My honourable father Mohammed Taher; he was a significant driving force in the continuation of my education.

My dear mother, who offered me unconditional love, support and encouragement throughout the years.

My respectable brothers, Sabir and Ameer; your support always keeps me persistent and provides me perseverance in everything I do.

My sisters.

My wife, who has put up with me for reasons not always obvious.

My lovely daughter (Asia)

My all loves in my village (Argosh)

Unknown candle bear who toil hard to serve humanity, peace and rights of the persecuted peoples.

Best Regards

Zirar Argoshy

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LIST OF ABBREVIATIONS

a) BAP : bis(2-methacryloyl oxyethyl)acid phosphate

b) CS : Chitosan solution

c) DMF : Dimethylformamide

d) DMSO : Dimethyl sulfoxide

e) DS : Degree of substitution

f) DSC : Differential scanning calorimetry

g) EDC : 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

h) FT-IR : Fourier transform infrared

i) KPS : Potassium persulfate

j) M : Molar

k) NMPC : N-methylene phosphonic chitosan

l) rpm : Rounds per minute

m) SEM : Scanning electron microscope

n) SGF : Simulated gastric fluid

o) SIF : Simulated intestinal fluid

p) TPP : Tripolyphosphate

Chapter 1

INTRODUCTION

Preparation of phosphorylated chitin and chitosan have drawn attention since they form a class of polymers with many useful properties such as antibacterial activity and metal chelating ability. They also have potential applications in tissue regeneration, drug delivery and in the food industry. Phosphorylated chitosan shows electrical conductivity and have been tested as proton conducting membranes in fuel cells. Several synthesis methods have been proposed for the synthesis of phosphorylated chitins and chitosans. The most widely studied methods are (i) the reaction of chitin or chitosan with phosphoric acid in the presence of urea at high temperatures, (ii) reaction with P_2O_5 in methane sulfonic acid. Even though these attempts gave successful results, they involve harsh reaction conditions. Modification under mild conditions can be achieved by graft copolymerization which allows improvements in physical characteristics such as better film forming ability, improved mechanical and thermal properties due to incorporation of another polymer into the system.

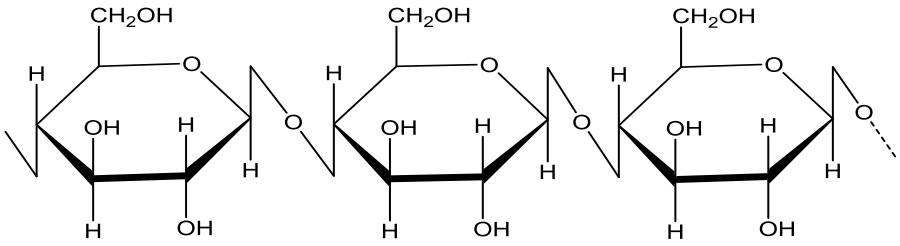
In this study, graft copolymerization of bis(2-methacryloyl oxyethyl)acid phosphate (BAP) onto chitosan will be investigated. There is only one article in the literature describing grafting of mono(2-methacryloyl oxyethyl)acid phosphate monomer onto chitosan by ceric ion initiation (Jung, Kim, Choi, Lee, & Kim, 1999). In this article, the effect of monomer concentration on the grafting percentage and grafting

efficiency was reported; the grafting conditions were not optimized. This thesis aims at finding the optimum grafting conditions of BAP onto chitosan. Optimum grafting conditions was determined by changing BAP concentration, temperature, time and initiator concentration. The grafting percentage was determined by gravimetric analysis. Solubility of the products was tested in aqueous media. Swelling properties of the cross-linked gels were also determined. Swelling and dissolution properties were determined in acidic, neutral and basic media. The effect of grafting percentage on the physical properties of the products was investigated. Morphology of the films was investigated by SEM analysis. Thermal stability of prepared films was determined by DSC. Contact angle of the films was also investigated.

1.1 Cellulose, Chitin and Chitosan

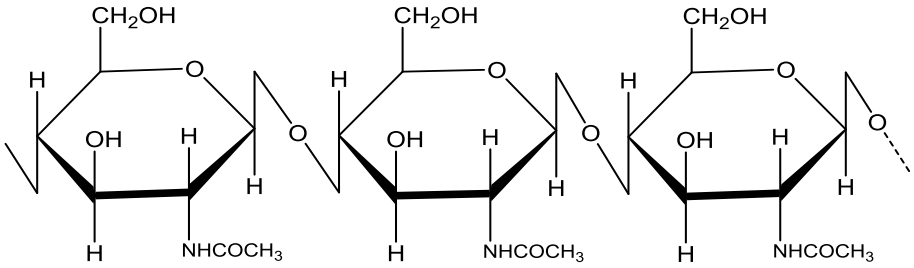
The most common polysaccharide is cellulose (Scheme 1) (Huber et al., 2012; Kono & Zakimi, 2013). The second most abundant polysaccharide after cellulose is chitin which was first extracted from the mushrooms (Dutta, Dutta, & Tripathi, 2004; Liu, Zhou, Wang, Xu, & Sun, 2013; Synowiecki & Al-Khateeb, 2003). Chitin resembles cellulose in chemical structure, the only difference being the existence of the acetamido group at position C-2 in chitin which replaces the hydroxyl group in cellulose. Exoskeletons of crustaceans and insects are the common sources of chitin as well as the cell walls of fungi. Chitin is made up of the glucose derivatives, N-acetyl-D-Glucosamine, (1-4) bonded 2-Acetamido-2-Deoxy-β-D-Glucan, (Scheme 2) (Kumar, Ramya, Jayakumar, Nair, & Lakshmanan, 2013; Mohammed, Williams, & Tverezovskaya, 2013; Park & Kim, 2010; Yang, 2011). Chitosan is a cationic polysaccharide that is derived from chitin by deacetylation (Nunthanid et al., 2004; Sankararamakrishnan & Sanghi, 2006). The chemical structure of chitosan is shown

in Scheme 3. β (1-4) linkage the glycosidic bond forms between glucosamine and N-acetyl glucosamine to produce chitosan (Honarkar & Barikani, 2009; Rashid, Rahman, Kabir, Shamsuddin, & Khan, 2012).



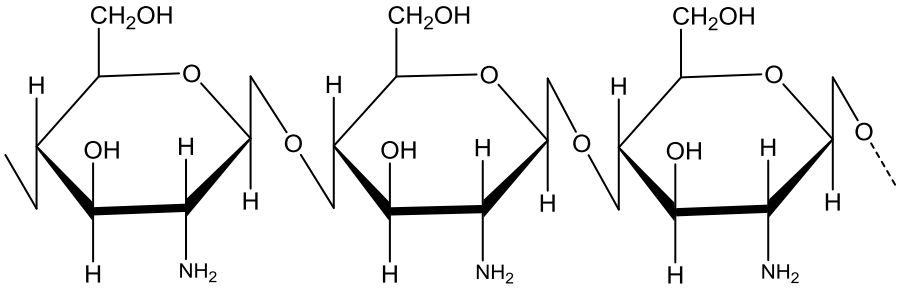
Cellulose

Scheme 1. Chemical Structure of Cellulose



Chitin

Scheme 2. Chemical Structure of Chitin

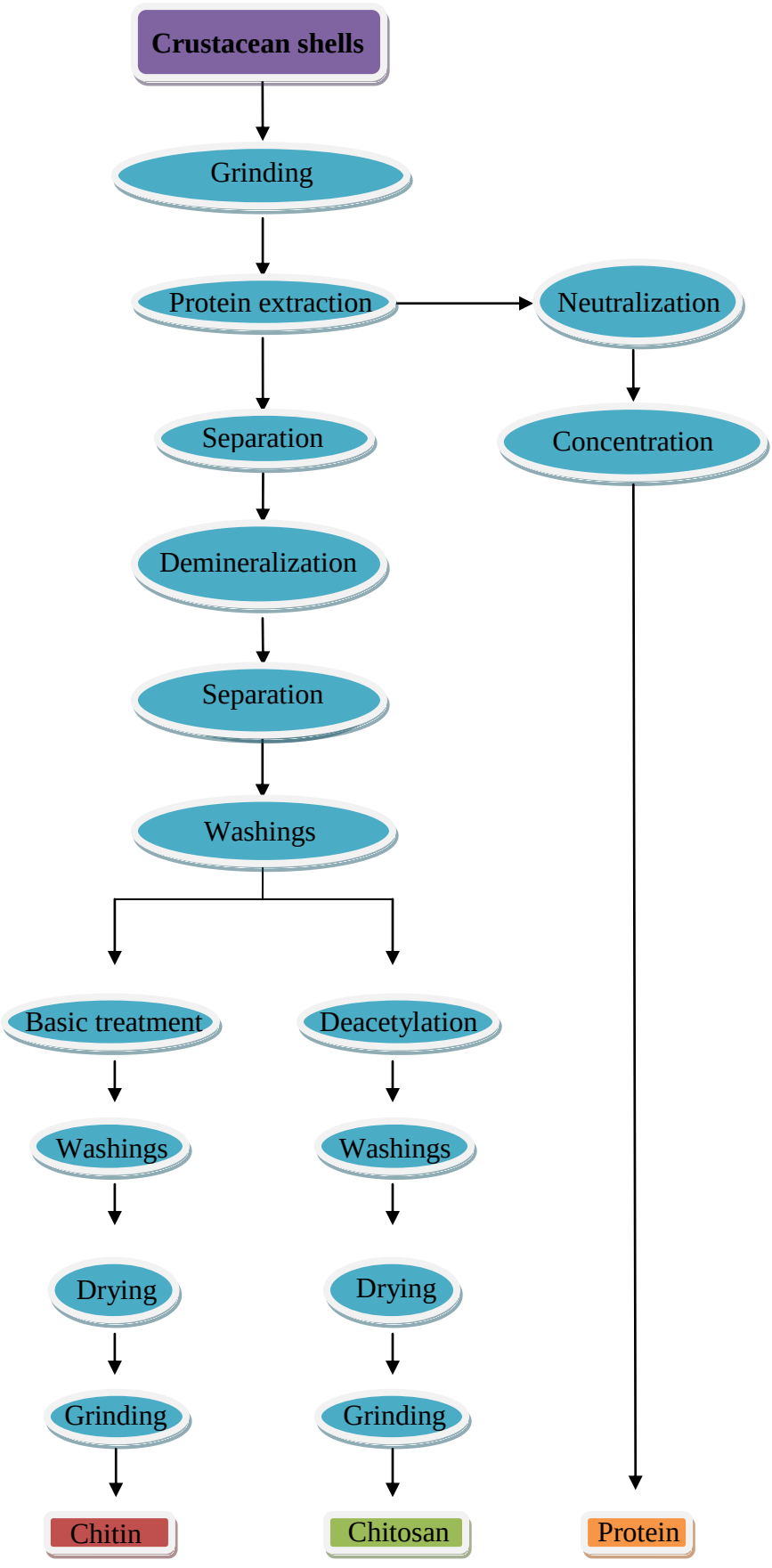


Chitosan

Scheme 3. Chemical Structure of Chitosan

Commercial chitin can be isolated from crustacean wastes of the fishing industry and then it can chemically be converted to chitosan. The major chitin sources are the shells of shrimp, crab, lobster, prawn and krill. The percentages of chitin present in these crustacean wastes varied between (20 – 30%), protein (30 – 40%), calcium carbonate and phosphate (inorganic salts) (30 – 50%) and lipids (0 –14%). These percentages vary with species and season.

Consequently, the techniques of the isolation are very wide-ranging, since they depend significantly on the compositions of the source. The majority of the techniques developed depend on chemical processes of hydrolysis of the protein and the elimination of the inorganic material. A workable sequence of isolation steps is schematically represented in Scheme 4 (Belgacem& Gandini, 2008). Extraction processes for chitin generally can be implemented via the following consecutive steps: raw material conditioning, protein extraction (deproteinization), removing of inorganic components (demineralization) and decolouration. This sequence is preferred when the extracted protein is to be used as food additive for livestock feeding. Otherwise, removing of mineral can be carried out first (Beaney, Lizardi-Mendoza, & Healy, 2005; Belgacem& Gandini, 2008).



Scheme 4. Isolation Procedures for Preparation of Chitin and Chitosan (Belgacem & Gandini, 2008)

1.2 Chemical, Physical and Biological Properties of Chitosan and Its Applications

Cationic nature of chitosan is very important, because it accounts for its many unique properties. The positive charge originates from the protonation of amino group under acidic conditions. Even though chitosan's solubility properties are improved in comparison to those of chitin, still the applications of chitosan are limited due to its insolubility in most common organic solvents (Elsabee & Abdou, 2013; Jayakumar, Selvamurugan, Nair, Tokura, & Tamura, 2008; Zhang et al., 2010). Because of the large molecular weight, polyelectrolyte nature, presence of active functional groups, gel-forming, and adsorption-abilities, chitosan is the most important derivative of chitin (Anaya, Cardenas, Lavayen, Garcia, & O'Dwyer, 2013). Furthermore, modification of chitosan can occur chemically or enzymatically, leading to products with biodegradability and biocompatibility (Sashiwa & Aiba, 2004; Zhang, et al., 2010). Both degree of N- acetylation and molecular weight play an important role in most of the applications. These two parameters affect the physiochemical as well as biological properties such as solubility, immunological activity and biocompatibility (Synowiecki & Al-Khateeb, 2003).

Chitosan, has multipurpose biological features like biocompatibility, hydrophilicity, biodegradability, antibacterial property, ability for chelating lipids, and a strong affinity for many proteins (Hasiploglu, Yilmaz, Yilmaz, & Caner, 2005). Degradation products of chitin and chitosan can be applied as wound dressings in gene, drug and vaccine delivery and in food industries (Yang, 2011). On the other hand, chitosan and its modified forms are used forvarious cosmetic purposes, in tooth-paste formulation and in some face and hand creams. Furthermore, chitosan

derivatives are used for hair-care treatment (Dutta, et al., 2004; Synowiecki & Al-Khateeb, 2003).

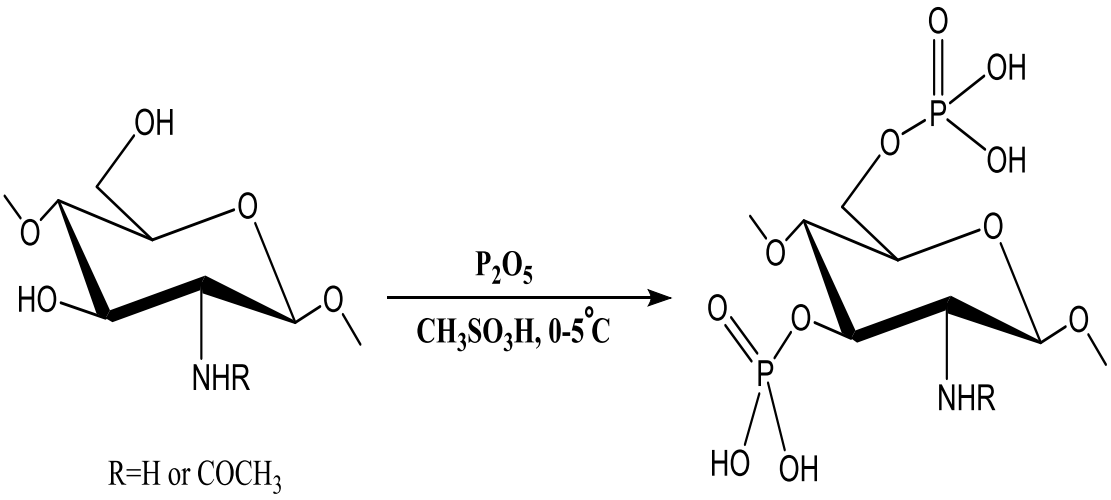
1.3 Phosphorylated Chitosan

One of the water soluble derivatives of chitosan is phosphorylated chitosan,which has significant importance for drug delivery. Both phosphorylated chitin and phosphorylated chitosan have the ability to form polyelectrolyte hydrogels and to make complexes with metals.They have anti-inflammatory property, and blood compatibility(Amaral, Granja, Melo, Saramago, & Barbosa, 2006; Jayakumar, Reis, & Mano, 2006).

1.3.1 Preparation Methods of Chitin and Chitosan Phosphorylation

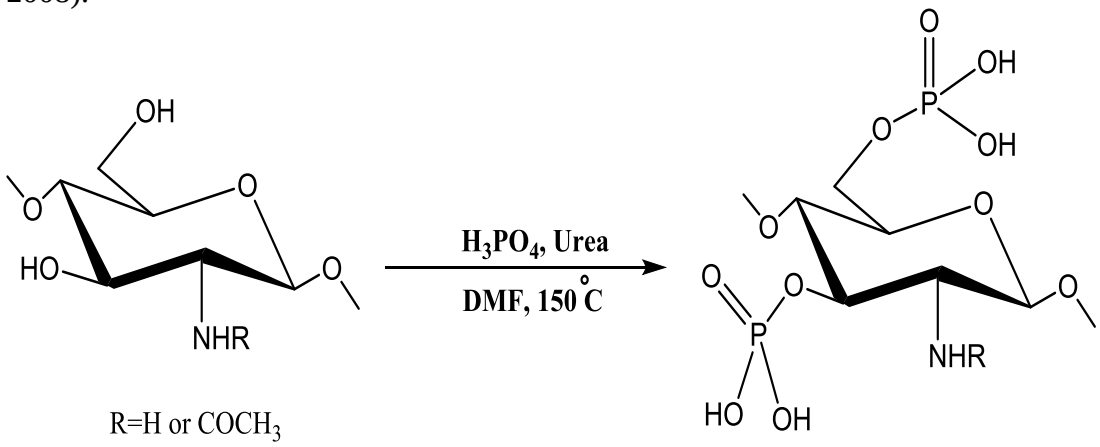
Recently, phosphorylated derivativesof chitin and chitosan were prepared using several different methods (Jayakumar, et al., 2008; Li, Huang, Wang, Ma, & Xie, 2011). The presence of amine group and hydroxyl groups in chitosan is quite advantageous to conduct modification reactions (Li, et al., 2011). The structure and the reaction pathway of products of phosphorylated chitosan depend on the nature of the phosphorylating agents, ratio of reactants, and reaction conditions (Matevosyan, Yukha, & Zavlin, 2003). There are several different methods to form phosphorylated chitin and chitosan, using different catalysts and different reaction conditions.

Phosphorous pentoxide in methanesulphonic acid can be used to carry out phosphorylation in chitin and chitosan at low temperature. Water soluble products with high degree of substitution (DS) were obtained. Methanesulphonic acid acts both as a perfect solvent for chitin and chitosan, and as a catalyst (Scheme 5) (Jayakumar, et al., 2006; Jayakumar, et al., 2008; Prabaharan, 2008).



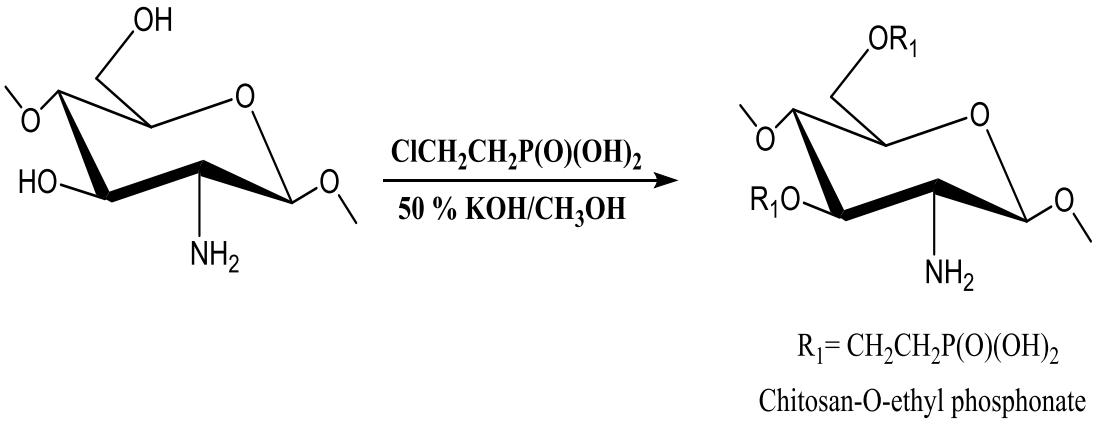
Scheme 5. Chitin and Chitosan Phosphorylation UsingP_2O_5/CH_3SO_3H

Phosphorylated chitin and chitosan could be prepared by heating (150 °C) chitin or chitosan with orthophosphoric acid and urea in (DMF), urea acts as reaction promoter (Scheme 6) (Jayakumar, et al., 2006; Jayakumar, et al., 2008; Prabaharan, 2008).



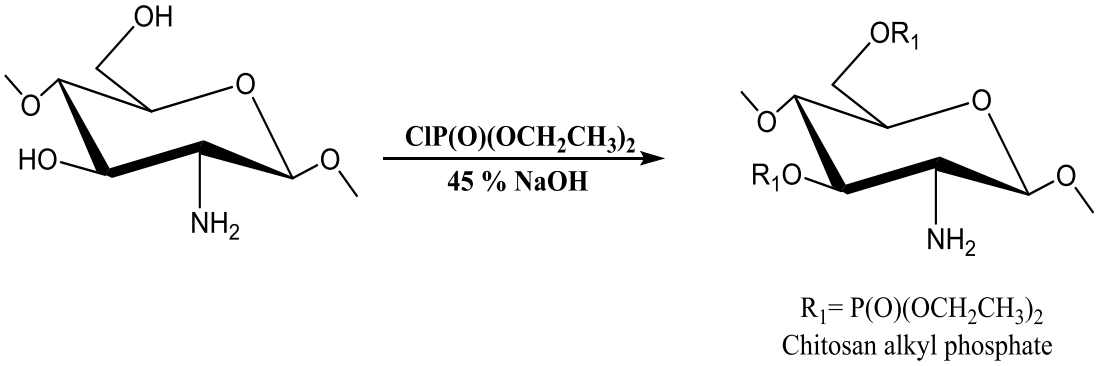
Scheme 6. Chitin and Chitosan Phosphorylation Using H_3PO_4/Urea/DMF

Chitosan-O-ethyl phosphonate can be prepared by using KOH/CH_3OH and $ClCH_2CH_2P(O)(OH)_2$ under moderate conditions (Scheme 7) (Jayakumar, et al., 2008).



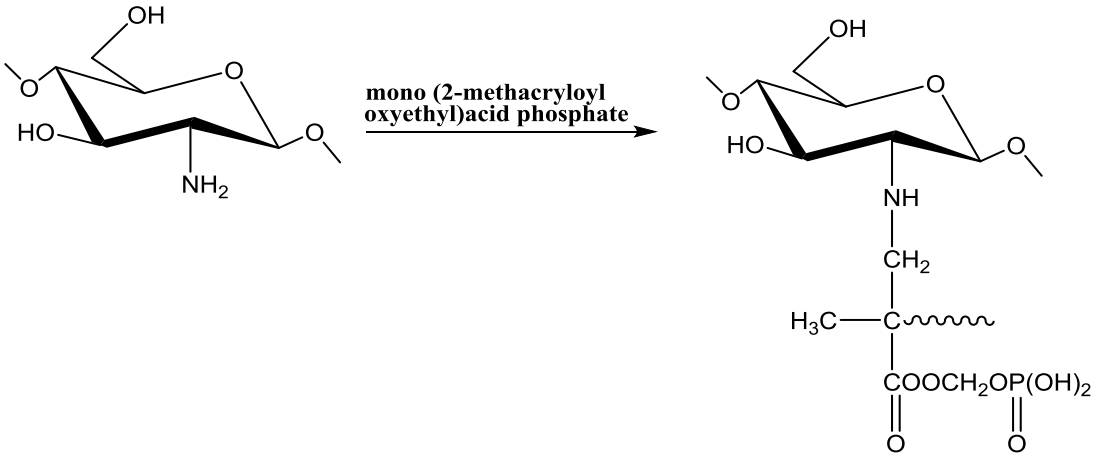
Scheme 7. Preparation of Chitosan-O-ethyl Phosphonate

Alkali chitosan has been used for preparing the chitosan alkyl phosphate/chitosan-O-ethyl phosphonate, with a view to make hydroxyl groups more active and to allow the coupling reaction with diethyl chlorophosphate/2-chloro ethyl phosphonic acid (Scheme 8) (Jayakumar, et al., 2008).



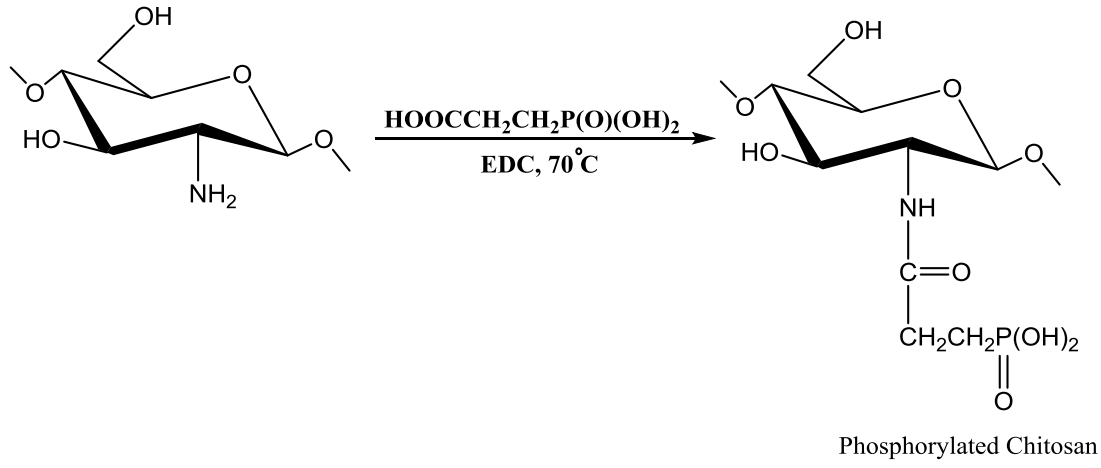
Scheme 8. Preparation of Chitosan Alkyl Phosphate

The phosphorylated chitosan was also prepared by graft copolymerization method, using mono (2-methacryloyl oxyethyl) acid phosphate. The phosphorylated chitosan was observed to have zwitter ionic character and to have improved antimicrobial properties (Scheme 9) (Jayakumar, et al., 2006; Jayakumar, et al., 2008).



Scheme 9. Preparation of Phosphorylated Chitosan Using Grafting Method

Phosphorylated chitosan could be prepared by graft copolymerization technique using 2-carboxethylphosphonic acid, chitosan with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as a catalyst at (70°C) (Scheme 10) (Jayakumar, et al., 2006; Jayakumar, et al., 2008).

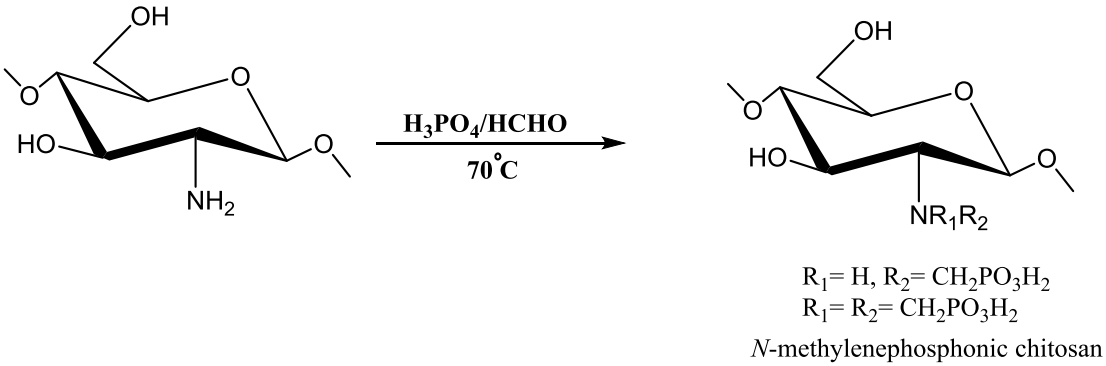


Phosphorylated Chitosan

Scheme 10. Preparation of Phosphorylated Chitosan Grafting Method

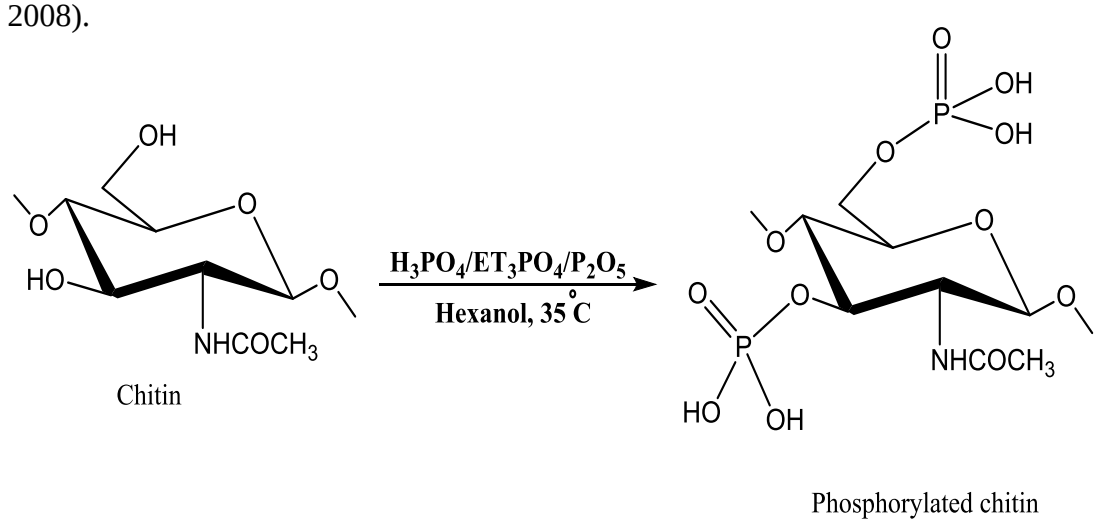
A novel N-methylene phosphonic chitosan can be synthesized by using chitosan, H_3PO_4 and HCHO. The combination of methylene phosphonic groups into chitosan allows solubility in water under mild conditions while keeping its filmogenic

properties (Scheme 11) (Jayakumar, et al., 2006; Jayakumar, et al., 2008; Ramos, Rodriguez, Rodriguez, Heras, & Agullo, 2003).



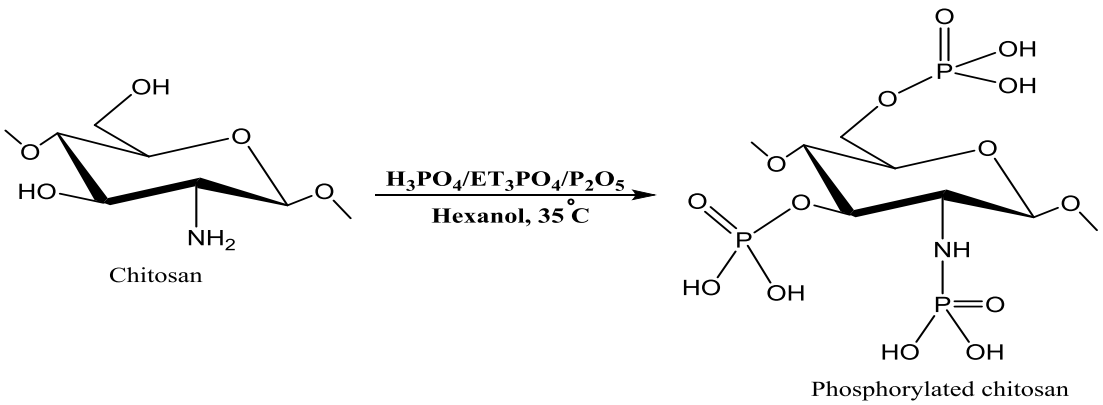
Scheme 11. Preparation of N-Methylene phosphonic Chitosan

Phosphorylated chitin also could be prepared by using chitin with $H_3PO_4/Et_3PO_4/P_2O_5$/hexanol (Scheme 12) (Jayakumar, et al., 2006; Jayakumar, et al., 2008).



Scheme 12. Preparation of Phosphorylated Chitin Using $H_3PO_4/Et_3PO_4/P_2O_5$

Chitosan phosphorylation, using $H_3PO_4/Et_3PO_4/P_2O_5$, phosphorylated chitosan can also be prepared by chitosan with $H_3PO_4/Et_3PO_4/P_2O_5$/hexanol (Scheme 13) (Jayakumar, et al., 2006; Jayakumar, et al., 2008).



Scheme 13. Preparation of Phosphorylated Chitosan Using $H_3PO_4/Et_3PO_4/P_2O_5$ Method (Surface Phosphorylation)

1.3.2 Phosphorylated Chitin and Chitosan Applications

Phosphorylated chitosans have drawn attention since they form a class of polymers with many useful properties such as water solubility, and metal chelating ability. They also have potential applications in tissue regeneration, drug delivery, fuel cells and in the food industry (Jayakumar, et al., 2006).

1.3.2.1 Adsorption of Metal Ions

The phosphorylated chitin and chitosan has capability to bind metals (Li et al., 2013). Especially their adsorption ability for uranium is higher than of the other heavy metal ions. Modified chitin and chitosan by phosphorus, have the ability to complex with alkali metals. After converting chitin to phosphorylated chitin by chemical modification the ability of binding to all metal ions would be increased. However, transition metals excluding Mn^{2+} could be adsorbed strongly to phosphorylated chitosan than phosphorylated chitin. Both insoluble phosphorylated chitin (less than 5% deacetylated) and insoluble phosphorylated chitosan (97% deacetylated) showed less performance than insoluble phosphorylated chitosan (45% deacetylated) for binding to Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+}. Hence DD is an important characteristic in addition to phosphorylation in determining metal binding capacity. Insoluble

phosphorylated chitin and chitosan were superior in their metal-binding ability to chitin or chitosan. Ability of Ca^{2+} adsorbing of insoluble phosphorylated chitin and phosphorylated chitosan were much greater than their starting materials in an ample pH range. This gives evidence on the large contribution of the phosphoryl group to the Ca^{2+} adsorption. It also indicates that insoluble phosphorylated chitosan (45% deacetylated) display the highest adsorption ability in the studied pH (Jayakumar, et al., 2006).

1.3.2.2 Food Applications

N-lauryl-N-methylene phosphonic chitosan and N-methylene phosphonic chitosan have found applications in the food industry. These biobased polymers show a wide range of applications including biodegradable film formation, enzyme immobilization, and protection of food from microbial spoilage, (as fruits deacidification and color stabilization). Phosphorylation provided chitosan with water solubility and emulsifying abilityfor food applications (Jayakumar, et al., 2006; Ramos et al., 2003).

1.3.2.3 Applications for Fuel Cell

The membranes of phosphorylated chitosan in their dry states are non conductive, while ionic conductive properties have been shown by hydrated membranes of phosphorylated chitosan. Phosphorylated chitosan displays ionic conductivity one order of magnitude higher than membranes of chitosan which are not modified. On the other hand, crystallinity of modified chitosan films and the corresponding swelling behaviour changed considerably, while they almost kept their thermal stability and strength when compared to unmodified chitosan films. According to these results, membranes of phosphorylated chitosan could have a chance to be

applied in alkaline polymer electrolyte fuel cells. Anhydrous proton-conducting membranes were prepared by Yamada and Honma using a composite of phosphorylated chitin imidazole (Yamada & Honma, 2004). The utilization of a biopolymer such as phosphorylated chitin for polymer electrolyte membrane fuel cell technologies is novel, challenging, inexpensive, and environmentally friendly (Jayakumar, et al., 2006).

1.3.2.4 Drug Delivery Applications

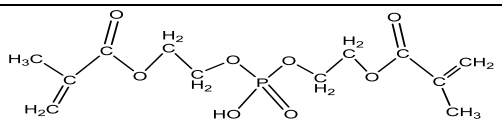
Gel beads could be prepared from phosphorylated chitosan by using TPP to improve the controlled release system in a gastrointestinal fluid (Win, Shin-ya, Hong, & Kajiuchi, 2003). Ibuprofen as a model drug had been used in one work which included the *in vitro* drug release profiles monitored at different pH media at (37°C). Released amounts of ibuprofen from gel beads of phosphorylated chitosan were found to decrease with decreasing pH of the dissolution medium. In this way, it was shown that pH is one of the factors that affect drug release, as well as the electrostatic difference between negative ion of phosphate groups in phosphorylated chitosan and carboxyl groups of ibuprofen. For example rate of release in simulated gastric fluid (pH = 1.4) is lower than that, in simulated intestinal fluid (pH = 7.4), enabling the drug delivery or release to take place preferentially in the intestine with preventing smultaneously the drug discharge in the stomach. All of these beneficial characteristics evidenced that gel beads of modified chitosan could be used as drug carrier for controlled drug delivery in oral administration. To minimize the enzymatic degradability and to enhance the sustained release property, polyelectrolyte complex microspheres based on phosphorylated chitosan by using tripolyphosphate (TPP) were developed and characterized. The ibuprofen released from phosphorylated chitosan microspheres sustained more effectively than that from CS microspheres in

the medium of proteolytic enzymes such as pepsin and trypsin, respectively. These phosphorylated chitosan microspheres could serve as a good candidate for oral drug-delivery systems with sustained release properties due to their higher stability in SGF and SIF containing hydrolytic enzymes (Jayakumar, et al., 2006; Prabaharan, 2008).

1.4 Bis(2-Methacryloyl Oxyethyl)Acid Phosphate

In this thesis, graft copolymerization of bis(2-methacryloyl oxyethyl)acid phosphate (BAP) onto chitosan was performed in aqueous medium by redox initiation using methods reported earlier (Adali & Yilmaz, 2009; Caner, Yilmaz, & Yimaz, 2007; Yilmaz, Adali, Yilmaz, & Bengisu, 2007), novel chitosan-graft-BAP films were obtained. There is very limited information about BAP in the literature except that its boiling point is 221^{o}C, and density which is 1.28 g/mL.

Table 1. Chemical Properties & Physical Properties of Monomer

Bis(2-methacryloyl oxyethyl)acid phosphate)	
General formula	— $C_{12}H_{19}O_8P$
— Chemical structure	 Bis(2-methacryloyl oxyethyl) acid phosphate
— Boiling point	221 ^{o}C
— Density	1.28g/mL
— Refractive index	n20/D 1.47(lit.)
— Flash Point	>230 °F
— Melting point, pH, solubility	No data available
— Storage temperature	—2-8 °C
— Formula Weight	—322.25g/mole

Initially, the chemical and physical properties of BAP were investigated. Optimum grafting conditions were determined by changing the monomer concentration, temperature, time and the initiator concentration. Products either in the form of film or powder were obtained depending on the chitosan:BAP ratio. Solubility and swelling properties of the products were determined in aqueous media. The products were characterized by FTIR, SEM and DSC analysis.

Chapter 2

EXPERIMENTAL

2.1 Materials

The chemicals used are listed below. All materials were used as received.

No	Chemicals	Manufacturers
1	Chitosan (medium molecular weight)	Aldrich-Germany
2	Sodium Hydroxide Pellets	Aldrich-Germany
3	Sodium Hydrogen Carbonate	AnalaR-UK
4	Potassium Per Sulfate	Aldrich-Germany
5	Potassium Hydrogen Phthalate	AnalaR-UK
6	Ammonium Molybdate	AnalaR-UK
7	Tween 80	Fluka-UK
8	Bis(2-methacryloyl oxyethyl)Acid Phosphate	Aldrich-Japan
9	Acetic Acid	Riedel-deHaen-Germany
10	Sulfuric Acid (95-96%)	Riedel-deHaen-Germany
11	Hydrochloric Acid	AnalaR-UK
12	Toluene	Sigma-Aldrich-Germany
13	Food Grade Ethanol	Sema Ltd.-Turkey
14	Acetone	Kemiteks Kimyevi Maddeler Tic.Ltd.Sti.-Turkey
15	Ascorbic Acid	Biochemical &BDH-UK
16	Potassium Antimony Tartarate	AnalaR-UK
17	Dimethyl Sulfoxide	Merck-Germany
18	Dimethyl Formamide	Analar-UK
19	Hexane	Emplura-Germany
20	Chloroform	Sigma-Aldrich-Germany

2.2Methods

2.2.1 Preparation of Bis (2-Methacryloyl Oxyethyl) Acid Phosphate (BAP) Copolymerized Chitosan Films

Chitosan solution with 2% (w/v), 1% (w/v), 0.75% (w/v) and 0.5% (w/v) were prepared by dissolving enough amount of chitosan in 1% (v/v) acetic acid solution. Then, 50 mL of chitosan solution at a given concentration was taken and stirred at 1400 rpm, at (40, 50, 60 and 70°C) under N2 atmosphere for 30 minutes. Tween 80 of volume 0.5 mL and the initiator, KPS of required amount were added in the mixture. Then, the required amount of monomer, BAP dissolved in 5mL of toluene was added into the solution. The reaction was carried out for a predetermined period of time (1, 2, 3 and 6 hours). The obtained product was washed with acetone and poured into a glass petri dish. It was dried at 45°C in the oven. Then, the films cast were removed with 25 mL of 0.1 M NaOH solution and washed with double distilled water and then soaked in ethanol for several minutes for the removal of the homo polymer. The preparations of all products are summarized in Table 2.

Table 2. Preparation Conditions of Phosphorylated Chitosan Films and Grafting Percentages

Sample no.	Sample ID	Chitosan (w/v%)	BAP (mL)	KPS (g)	Time (h)	Temperature (°C)	% Grafting
1	C2BAP0.5 (0.2 × KPS)	2	0.5	0.0136	2	60	12
2	C1BAP1 (0.2 × KPS)	1	1	0.0136	2	60	95
3	C1BAP0.5 (0.2 × KPS)	1	0.5	0.0136	2	60	74
4	C1BAP0.25 (0.2 × KPS)	1	0.25	0.0136	2	60	46
5	C0.75BAP1 (0.2 × KPS)	0.75	1	0.0136	2	60	156
6	C0.75BAP0.5 (0.2 × KPS)	0.75	0.5	0.0136	2	60	105
7	C0.75BAP0.25 (0.2 × KPS)	0.75	0.25	0.0136	2	60	127
8	C0.5BAP1 (0.2 × KPS)	0.5	1	0.0136	2	60	231
9	C0.75BAP0.5 (0.2 × KPS)	0.75	0.5	0.0136	1	60	61
10	C0.75BAP0.5 (0.2 × KPS)	0.75	0.5	0.0136	3	60	100
11	C0.75BAP0.5 (0.2 × KPS)	0.75	0.5	0.0136	6	60	78
12	C0.75BAP0.5 (0.04 × KPS)	0.75	0.5	0.00272	2	40	68
13	C0.75BAP0.5 (0.04 × KPS)	0.75	0.5	0.00272	2	50	86

14	C0.75BAP0.5 (1 × KPS)	0.75	0.5	0.068	2	60	261(powder)
15	C0.75BAP0.5 (0.4 × KPS)	0.75	0.5	0.0272	2	60	181
16	C0.75BAP0.5 (0.04 × KPS)	0.75	0.5	0.00272	2	60	120
17	C0.75BAP0.5 (0.022 × KPS)	0.75	0.5	0.0015	2	60	79
18	C0.75BAP0.5 (0.04 × KPS)	0.75	0.5	0.00272	2	70	76
19	C0.75BAP0.5 (0.04 × KPS)	0.75	0.5	0.00272	2	90	269(powder)

2.2.2 FTIR Analysis

The FT-IR spectra of the products in film or powder form were recorded on a Perkin Elmer Spectrum Two^{TM} FT-IR spectrometer.

2.2.3 Gravimetric Analysis

Percent yield of grafting was calculated by the following equation (1)

$$\%\text{Grafting} = \frac{(m_{film} - m_{chitosan})}{m_{chitosan}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, m_{film} is the mass of grafted film and $m_{chitosan}$ is the mass of chitosan.

2.2.4 Dissolution Characterisics of Monomer

Solubility of the monomer was determined in double distilled water, acetic acid, ethanol, toluene, dimethyl formamide, dimethyl sulfoxide, hexane, chloroform and acetone. A sample 0.50 mL monomer was mixed with 5.0 mL of solvent at room temperature, and the results were observed.

2.2.5 Swelling Experiments

The swelling behaviour of the prepared films was qualitatively measured in different pH buffer solutions at room temperature at 20 °C. All weights were measured using sartorius handy, H 110 analytical balance of ±0.001 accuracy.

pH=3 buffer solution was prepared by dissolving 10.21g potassium hydrogen phthalate in 223mL of 0.10M HCl, then diluted it to 1000mL by adding double distilled water. A buffer solution of pH=7 was prepared by dissolving 6.81g potassium dihydrogen phosphate in 291mL of 0.10M NaOH, then diluted to 1000mL by adding double distilled water. A buffer solution of pH=11 was prepared by

dissolving 2.10g sodium bicarbonate in 227mL of 0.10M NaOH, followed by completion to 1000mL by adding double distilled water.

Films 50.0 mg were kept in the beaker with 50mL of solution and stirring at 50 rpm at 37°C. Excess water was removed from the surface of the membranes carefully, using filter paper. Then,they were weighted immediately, using an electronic analyticalbalance. The swelling ratios of the films were calculated using equation (2)

$$\%\text{Swelling} = \frac{(m_{film2} - m_{film1})}{m_{film1}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, m_{film1} and m_{film2} are the weights of the films in dry and swollen states, respectively.

2.2.6 Film Thickness

The measured average thicknesses of the prepared films were recorded by using a micrometer *(MOORE & WRIGHT, England*). Different measurements of thickness were made for each film at different positions on each specimen and the average value was reported as film thickness.

2.2.7 Contact Angle Measurements

Drop method technique was used for measurement of water contact angle for the prepared pure chitosan film and phosphorylated chitosan films at room temperature, using an optical contact angle meter (KSV/ Attention Theta, Finland) forexamination of wettability of the prepared films surfacely. Glass microscope slides were used for proceeding the experiments (76.2 mm × 25.4 mm, 1 mm thick). The slides were sterilized by soaking them in ethanol for 2 hours before use. Contact angle

measurements were carried out in Merkez Laboratuvar – Middle East Technical University Ankara.

2.2.8 Differential Scanning Calorimeter (DSC) Analysis

Perkin Elmer Diamond differential scanning calorimeter was used to perform differential scanning calorimeter (DSC) measurements for chitosan film and phosphorylated chitosan film samples. The process was done by Merkez Laboratuvar –Middle East Technical University in Ankara, under a nitrogen atmosphere at a constant heating rate of 10^{o}C/min.

Chapter 3

RESULTS AND DISCUSSION

3.1 Solubility Characteristics of Monomer (BAP)

Solubility test was done for Bis(2-methacryloyl oxyethyl)acid phosphate), using following organic solvents.

Table 3. Solubility Test for (BAP)

Solvents	Results	Observations
Water	+/-	Immiscible
Toluene	+	Miscible
DMF	+	Miscible
DMSO	+	Miscible
1% (V/V)Acetic acid	+/-	Immiscible
Ethanol	+	Miscible
Hexane	+/-	Immiscible, jelly like
Acetone	+	Miscible
Chloroform	+	Miscible

Note: (+) means dissolved completely and (+/-) means immiscible.

3.2 Optimization of Grafting Conditions

Optical pictures of phosphorylated chitosan films and powders are shown in Figures 1 and 2. As can be observed from the pictures, homogeneous transparent films were obtained.

(a)

(b)

Figure 1. (a) Phosphorylated Chitosan Films (C0.75BAP0.5-2h60°C (0.2 × KPS) Left and C1BAP0.5-2h60°C (0.2 × KPS) Right) and (b) Phosphorylated Chitosan Film (C1BAP0.25-2h60°C (0.2 × KPS))

The products in the powder form were coarse particles which included some small film-like parts as well.

(a)

(b)

Figure 2. (a) Phosphorylated Chitosan Powder (0.068 g) Initiator Used at 60°C and (b) Phosphorylated Chitosan Powder (0.00272 g) Initiator Used at 90°C

3.2.1 Effect of Monomer Concentration

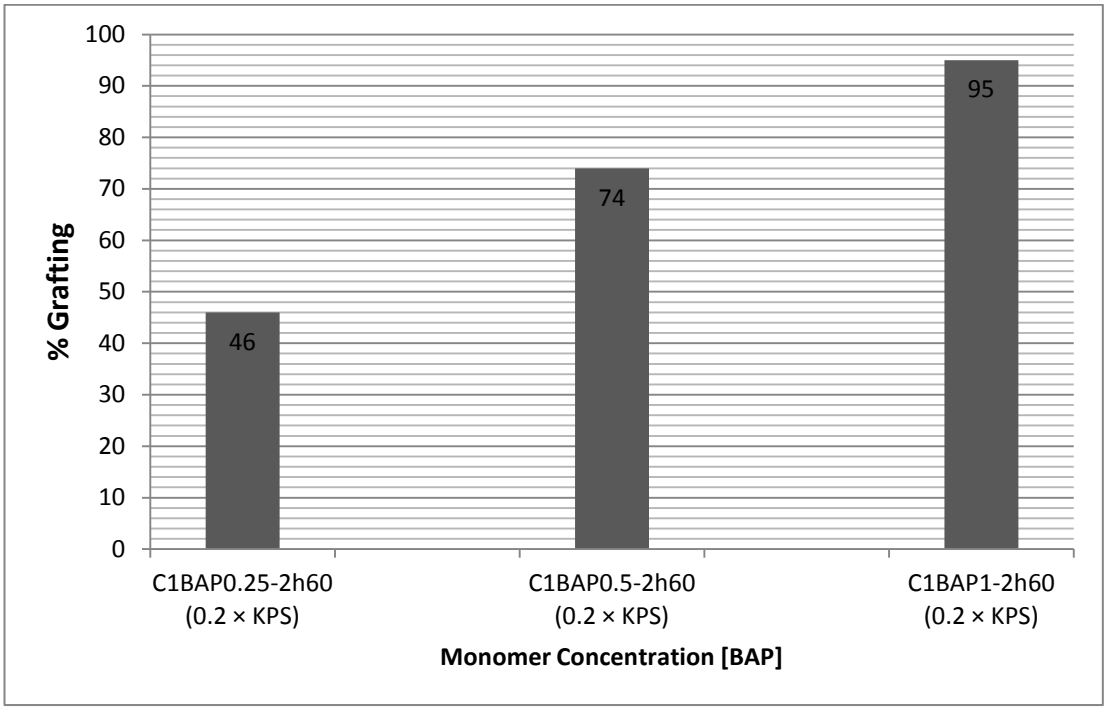


Figure 3. Effect of Monomer (BAP) Concentration on Grafting Percentage

BAP was grafted onto chitosan in aqueous medium using KPS as the redox initiator. The increase in mass of monomer (BAP) provided evidence of successful grafting reactions. There is a steady increase in grafting as shown in Figure 3. After copolymerization of BAP onto chitosan is initiated, increasing amount of monomer results in more monomer grafted on the polymer due to availability of active sites.

Table 4. Effect of Monomer Concentration on Grafting of BAP onto Chitosan (Reaction Condition: Chitosan = 1% g (w/v 1% Acetic acid), 0.0136 g KPS, Time = 2h and Temp. = 60°C in 50 mL Solution)

Sample ID	**[BAP]×10^{-4} (mole/L)**	**% Grafting**
C1BAP0.25-2h60 (0.2 × KPS)	178	46
C1BAP0.5-2h60 (0.2 × KPS)	355	74
C1BAP1-2h60 (0.2 × KPS)	703	95

3.2.2 Effect of Chitosan Concentration

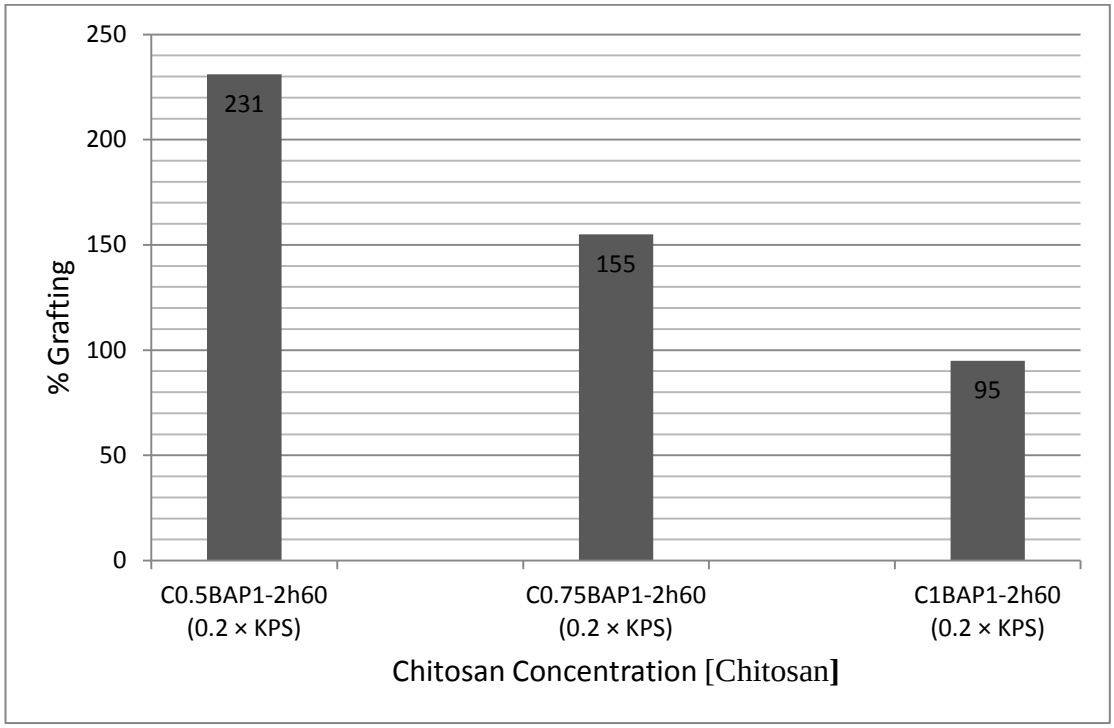


Figure 4. Effect of Chitosan Concentration on Grafting Percentage

As the chitosan concentration is increased, the grafting yield decreases. The viscosity of the solution will be increasing with increase chitosan concentration, results in restricted mobility of the molecules. Therefore, the probability of collisions between chitosan molecules and monomer and initiator molecules decreases. In this way, % grafting yield of phosphorylated chitosan decreases with increasing chitosan concentration as shown in Figure 4.

Table 5. Effect of Chitosan Concentration on Grafting Percentage

Sample ID	%Chitosan (w/v)	% Grafting
C0.5BAP1-2h60(0.2×KPS)	0.5	231
C0.75BAP1-2h60(0.2×KPS)	0.75	155
C1BAP1-2h60(0.2×KPS)	1	95

3.2.3 Effect of Reaction Time

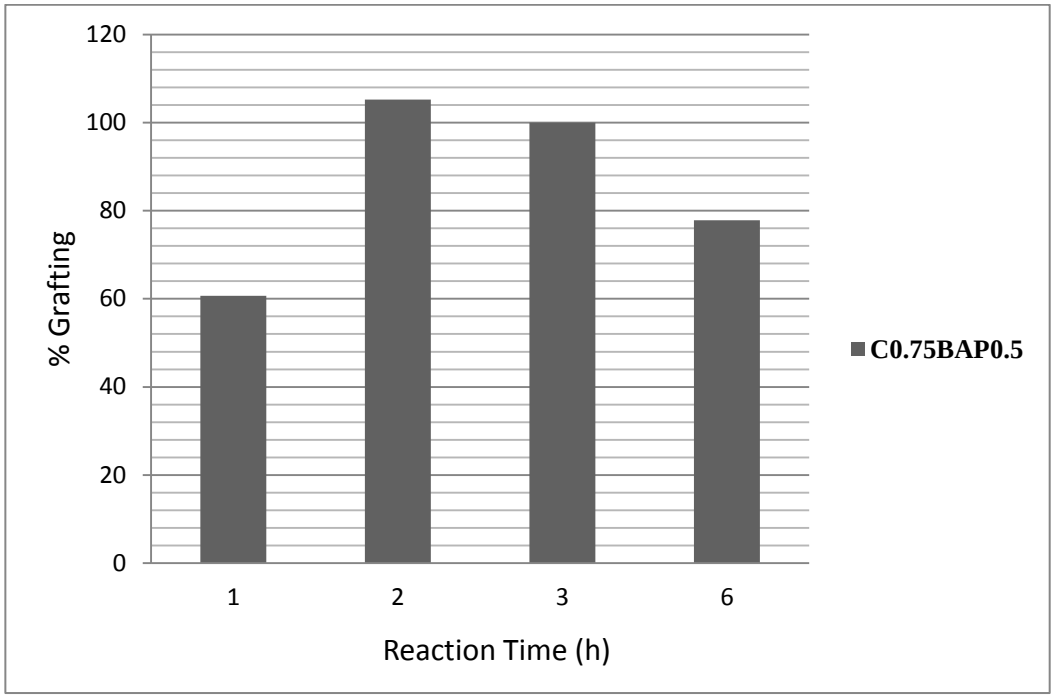


Figure 5. Effect of Reaction Time on Grafting Percentage

The effect of reaction time on the % grafting was examined and the results are shown in the Figure 5. The maximum grafting was obtained at 2 hours reaction time. At longer reaction times the grafting yield decreases. This may be due to oxidative degradation of chitosan with time, as well as decreasing number of the grafting sites with further increase in time due to increased probability of termination reactions.

Table 6. Effectof Reaction Time on Grafting Percentage

Sample ID	Time (h)	% Grafting
C0.75BAP0.5-1h60 (0.2 × KPS)	1	61
C0.75BAP0.5-2h60 (0.2 × KPS)	2	105
C0.75BAP0.5-3h60 (0.2 × KPS)	3	100
C0.75BAP0.5-6h60 (0.2 × KPS)	6	78

3.2.4 Effect of Reaction Temperature

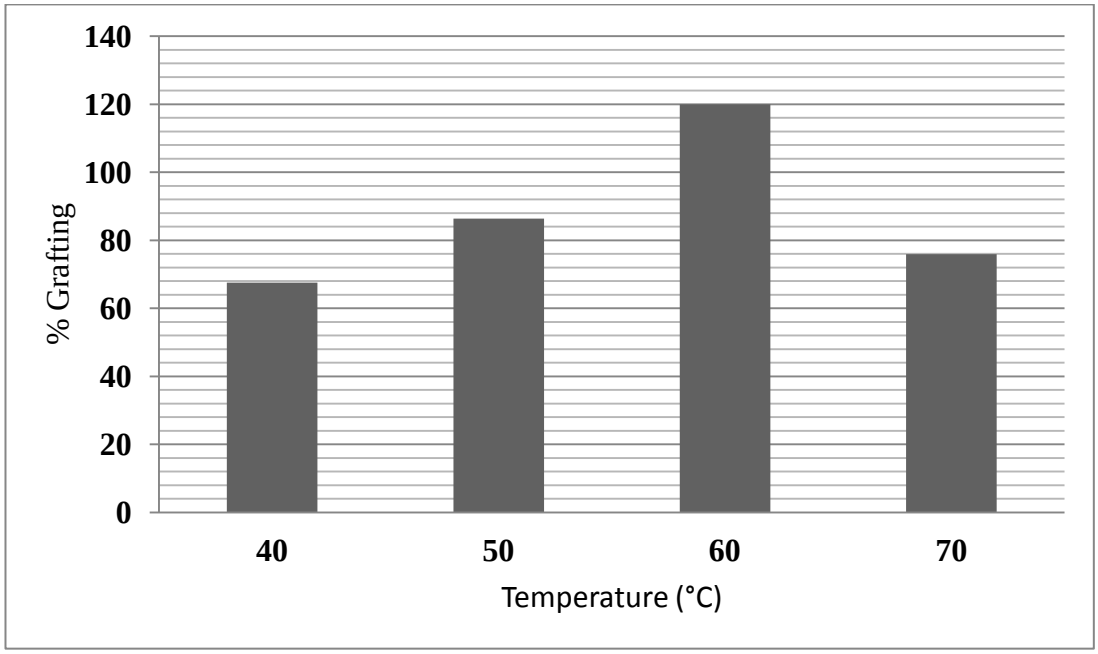


Figure 6. Effect of Reaction Temperature on the Grafting Percentage (C0.75BAP0.5-2h (0.04 × KPS))

The temperature range studied was 40-90°C. The effect of reaction temperature on the grafting percentage is given in Figure 6 and Table 6 for the phosphorylated chitosan films. The % grafting yield of phosphorylated chitosan films increased gradually with increasing temperature up to 60°C. The maximum value of % grafting is observed at 60°C and then decreases at 70°C. A further increase in temperature may favor homopolymerization reactions. Another factor is that chain transfer reactions with higher activation energy will increase leading to termination reactions. Furthermore, oxidation rate of polymers may increase as temperature increases. All of these factors may affect the temperature dependency of grafting reactions.

It is interesting to observe that further increase in temperature to 90°C gives rise to a powdery product instead of a film with a % grafting value of 269. This product was

found to have a considerably higher swelling ratio (~3000%) when compared to the films as discussed in section (3.4). therefore, it can be interpreted that the reaction conditions of chitosan concentration 0.75%, C0.75BAP0.5-2h60 (1×KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) results in cross linked products.

Table 7. Effect of Reaction Temperature on the Grafting Percentage

Sample ID	**Temperature (**°C)	**% Grafting**
C0.75BAP0.5-2h-40(0.04 × KPS)	40	68
C0.75BAP0.5-2h-50 (0.04 × KPS)	50	86
C0.75BAP0.5-2h-60 (0.04 × KPS)	60	120
C0.75BAP0.5-2h-70 (0.04 × KPS)	70	76
C0.75BAP0.5-2h90 (0.04 × KPS)	90	269

3.2.5 Effect of the Amount of Initiator

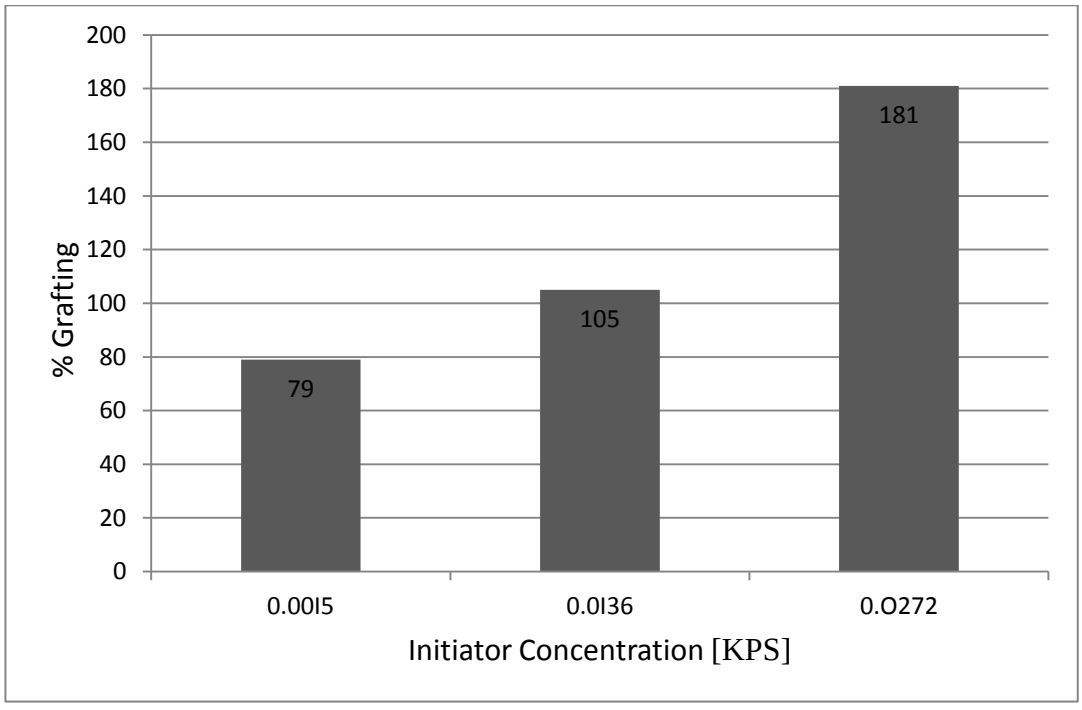


Figure 7. Effect of the Amount of Initiator on the Grafting Percentage

The effect of the initiator on % grafting has been examined and the results are shown in Figure 7 and Table 7. Increase in initiator concentration resulted in an increase in % grafting yield. It was observed that the maximum concentration of KPS ($18\times\mathbf{10^{-4}}$ M) gave the maximum grafting yield due to increasing active sites on the backbone.

Table 8. Effect of the Amount of Initiator on the Grafting Percentage

Sample ID	KPS amount (g)	[KPS]×10^{-4} (mole/L)	% Grafting
C0.75BAP0.5-2h60 (0.022 × KPS)	0.0015	1	79
C0.75BAP0.5-2h60 (0.2 × KPS)	0.0136	9	105
C0.75BAP0.5-2h60 (0.4× KPS)	0.0272	18	181

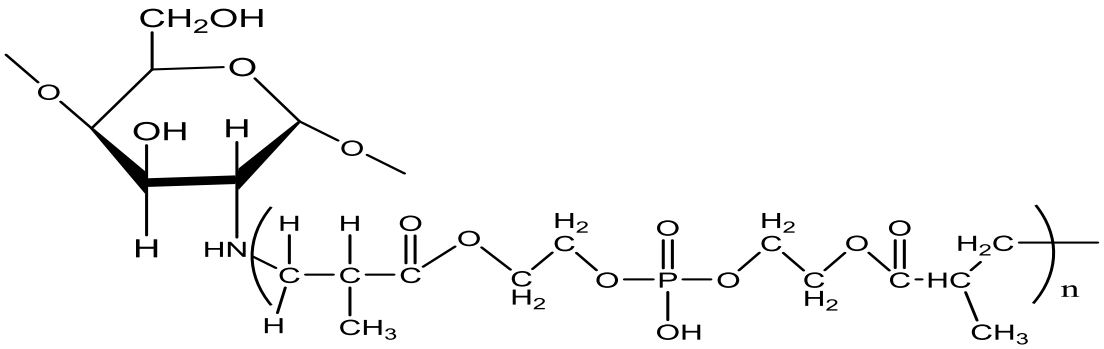
3.3 FT-IR Analysis

Some of the possible chemical structures of phosphorylated chitosan and cross linked chitosan are shown in Scheme 14 (a), (b), (c,)(d), (e) and (f). FTIR spectra of the samples are shown in Figure 8 and 9. In figure 8(a) the spectrum of chitosan film is shown. The spectrum of BAP and phosphorylated chitosan film is shown in Figure 8 (b) and (c) respectively. Amide I and amide II bands are observed at 1665 cm^{-1} and 1571 cm^{-1} as appeared in FT-IR spectrum of the chitosan film.

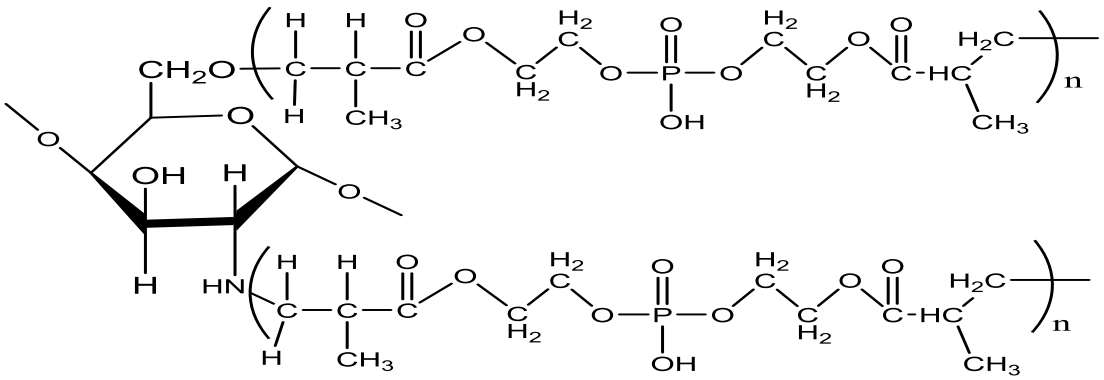
The presence of the P=O stretching at 1176 cm^{-1}, C=O stretching in ester bond in the 1725 and C-CH_2 bending 700-796 cm^{-1} range was taken as evidence of successful copolymerization of BAP with chitosan as shown in Scheme 14.

The spectra of chitosan, BAP and the cross linked product are compared to each other in Figure 9 (a), (b) and (c) respectively. In the cross linked product due to high

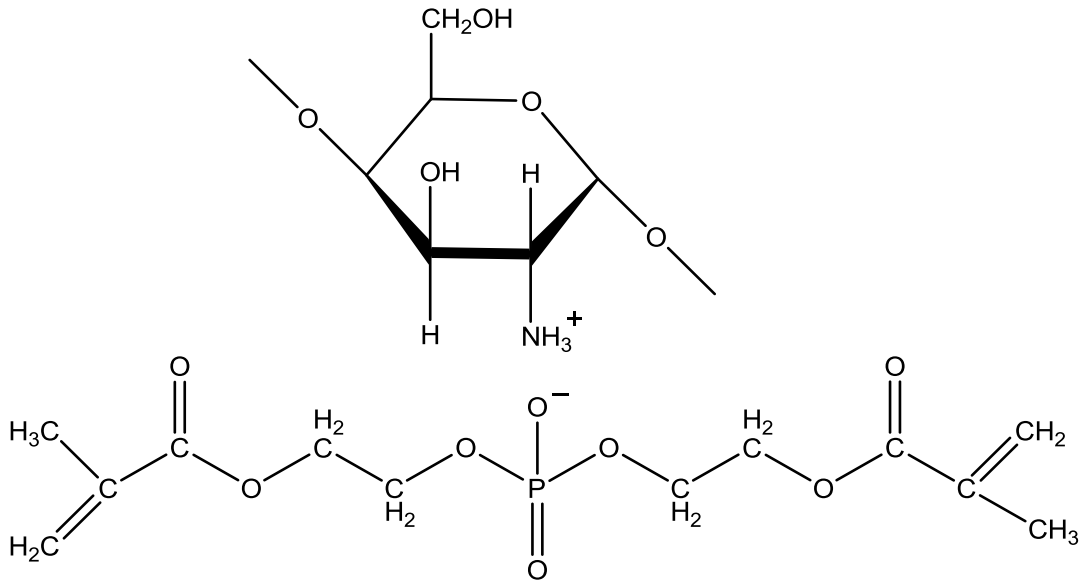
percentage of grafting the –CH_2– stretching bands are clearly observable at 2857 and 2930cm^{-1}. The characteristic bands of the monomer and chitosan are also available.



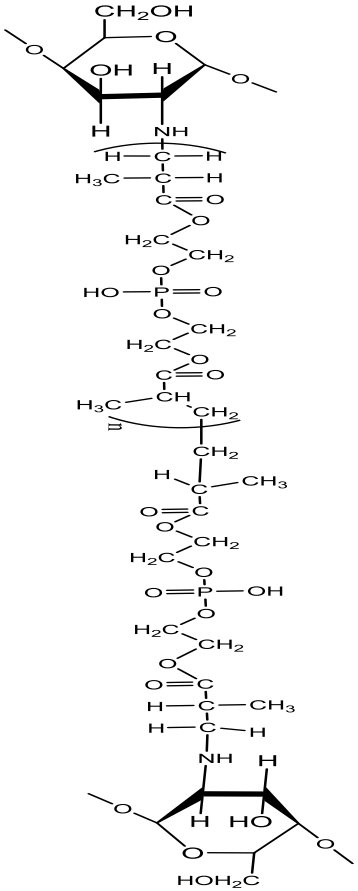
(a)



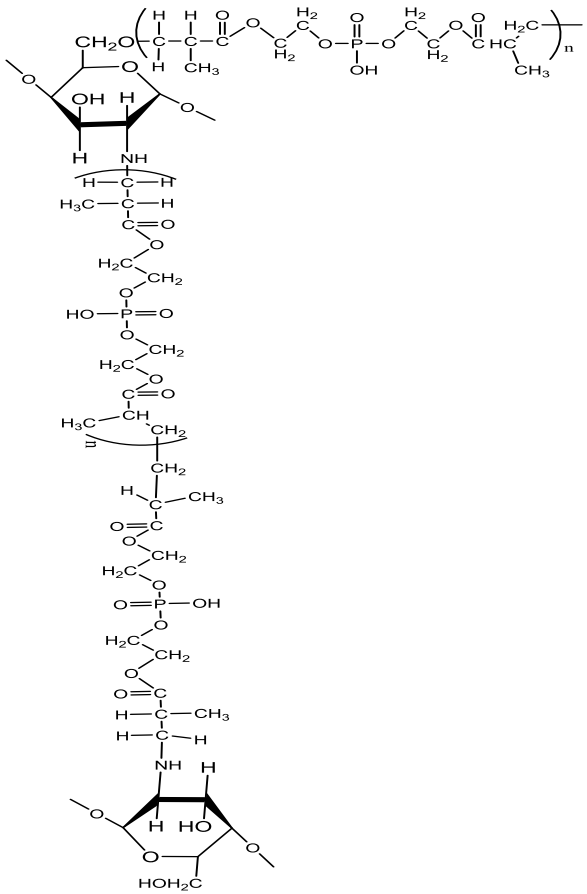
(b)



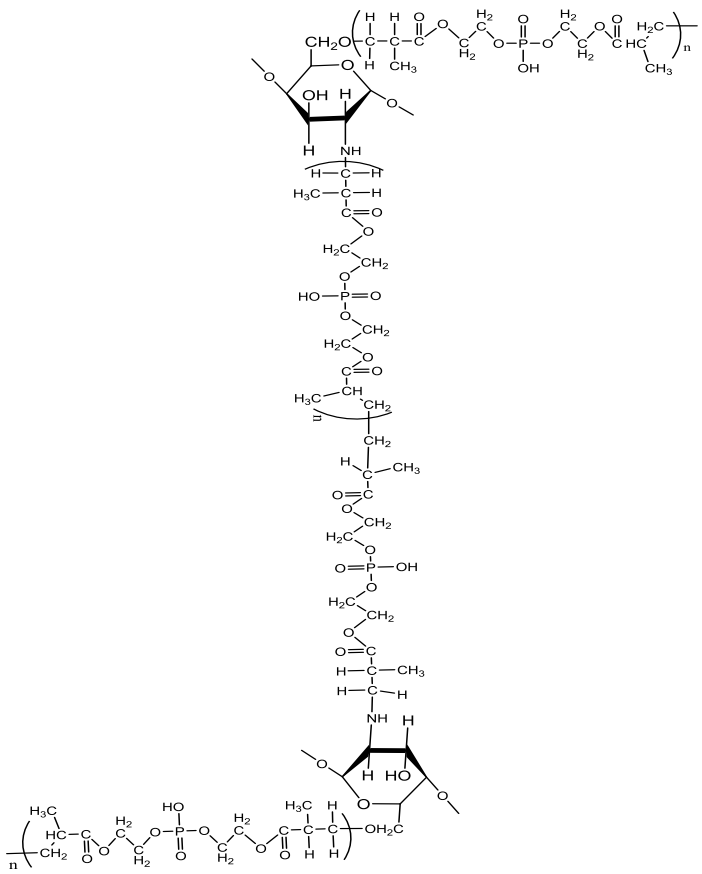
(c)



(d)



(e)



(f)

Scheme 14. (a) N-Phosphorylated Chitosan, (b) N,O- Phosphorylated Chitosan, (c) Phosphorylated Chitosan Formation via Ionic Crosslinking, (d) Crosslinked Chitosan, and (e) and (f) N,O-Phosphorylated and Cross linked Chitosan

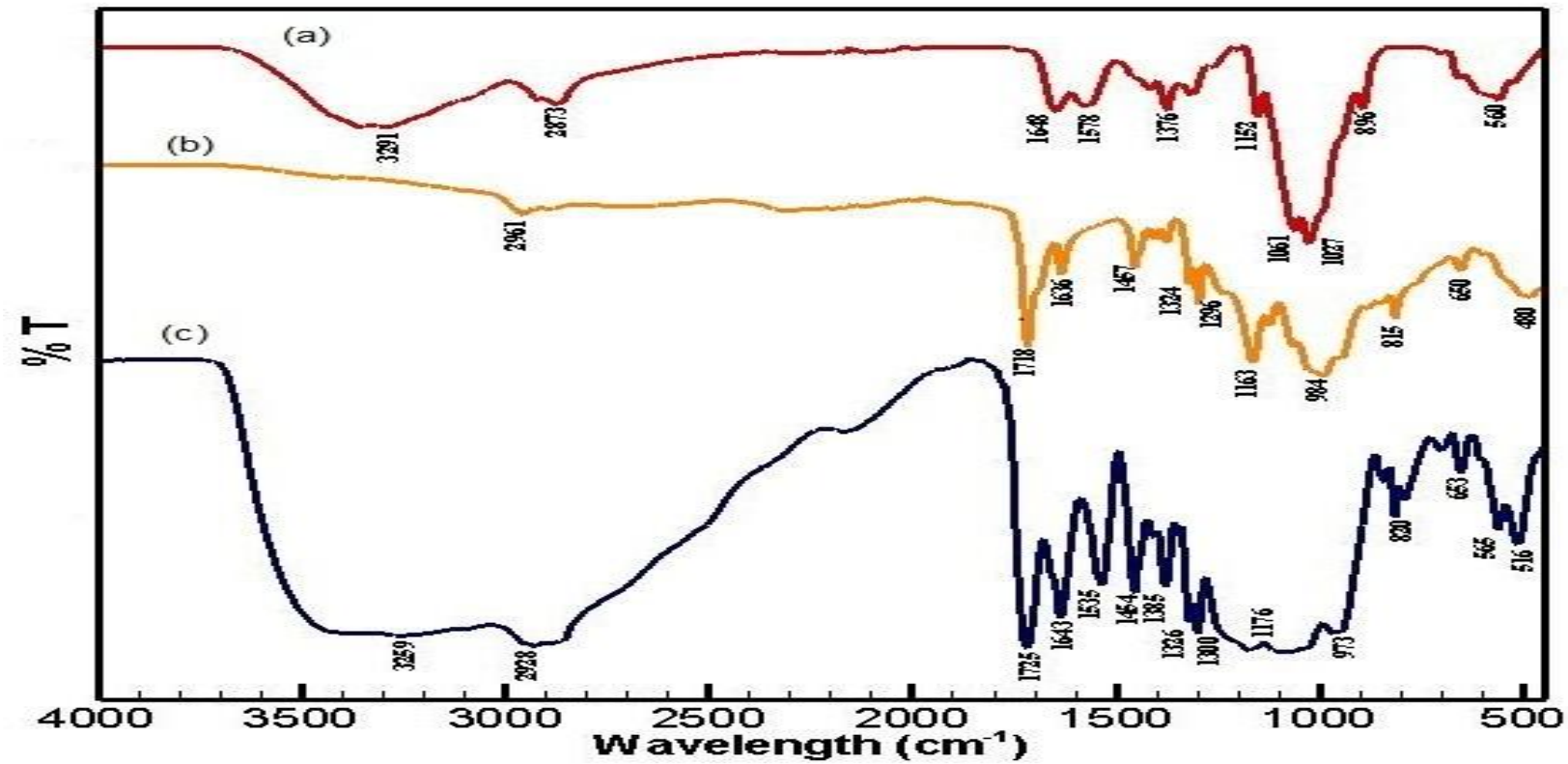


Figure 8. (a) Pure Chitosan Film, (b) Pure BAP, and (c) Phosphorylated Chitosan Film (C0.75BAP1 (0.2 × KPS))

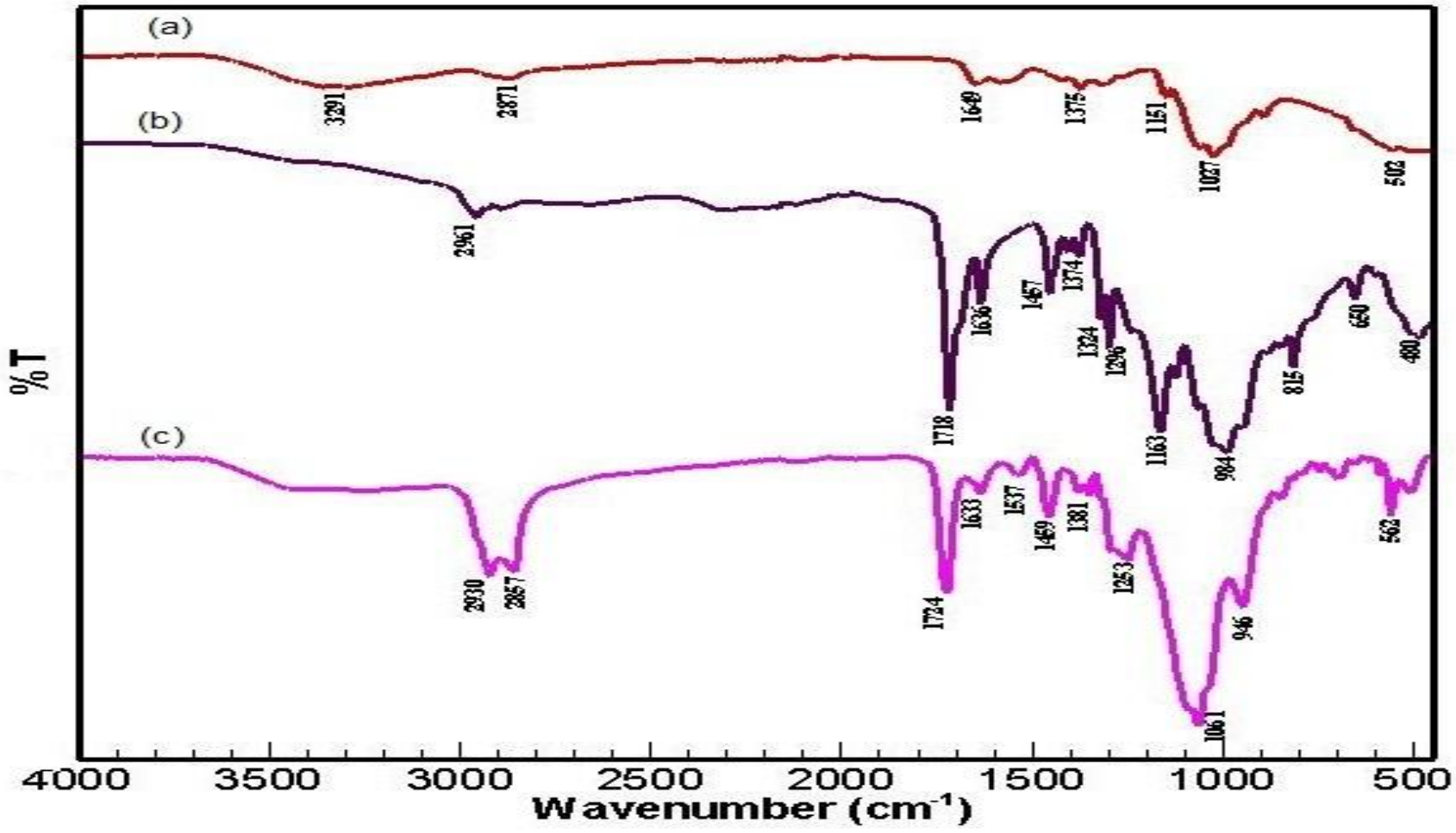


Figure 9. (a) Pure Chitosan Powder, (b) Pure BAP, and (c) Phosphorylated Chitosan Powder (C0.75BAP0.5 (1×KPS))

3.4 Swelling Ratio of Prepared Films

At pH=3 all samples dissolve. The swelling percentages of a chitosan film and some of the Phosphorylated chitosan films at pH=7 are shown in Table 9.

Table 9. % Swelling of 0.75% Chitosan Film, C0.75BAP0.5-1h60°C (0.2 × KPS), C0.75BAP0.5-3h60°C (0.2 × KPS) and C0.75BAP0.5-6h60°C (0.2 × KPS) in Buffer Solution pH=7 (Film Products)

Time (min.)	0.75% Chitosan film	C0.75BAP0.5-1h60°C(0.2×KP) (%61G)	C0.75BAP0.5-3h60°C(0.2×KPS) (%100G)	C0.75BAP0.5-6h60°C(0.2×KPS) (%78G)
30	205	376	189	293
60	207	330	201	284
90	206	368	183	269
120	206	280	203	277
150	206	355	175	252
180	206	309	180	255
210	206	334	195	252
240	206	320	195	291
270	206	313	221	276
300	206	332	196	265
330	206	322	188	255
360	206	292	181	248

As can be observed in Figure 10 and Table 9 the swelling degrees of all samples are comparable to each other. However, there is a tendency in % swelling to decrease with increasing % grafting. The sample with the highest % grafting value (100%) has the % swelling value as chitosan film itself. At pH=7 amine groups of chitosan are

neutralized, and ionization of the phosphate groups are neutralized. The factor which is predominant of the swelling is the ease of diffusion of H_2O molecules into the film structure. The film properties which will affect this behavior could be the crystallinity, the film thickness and the porosity of the membrane. A combination of all these factors may be operating to result in the observed behaviors.

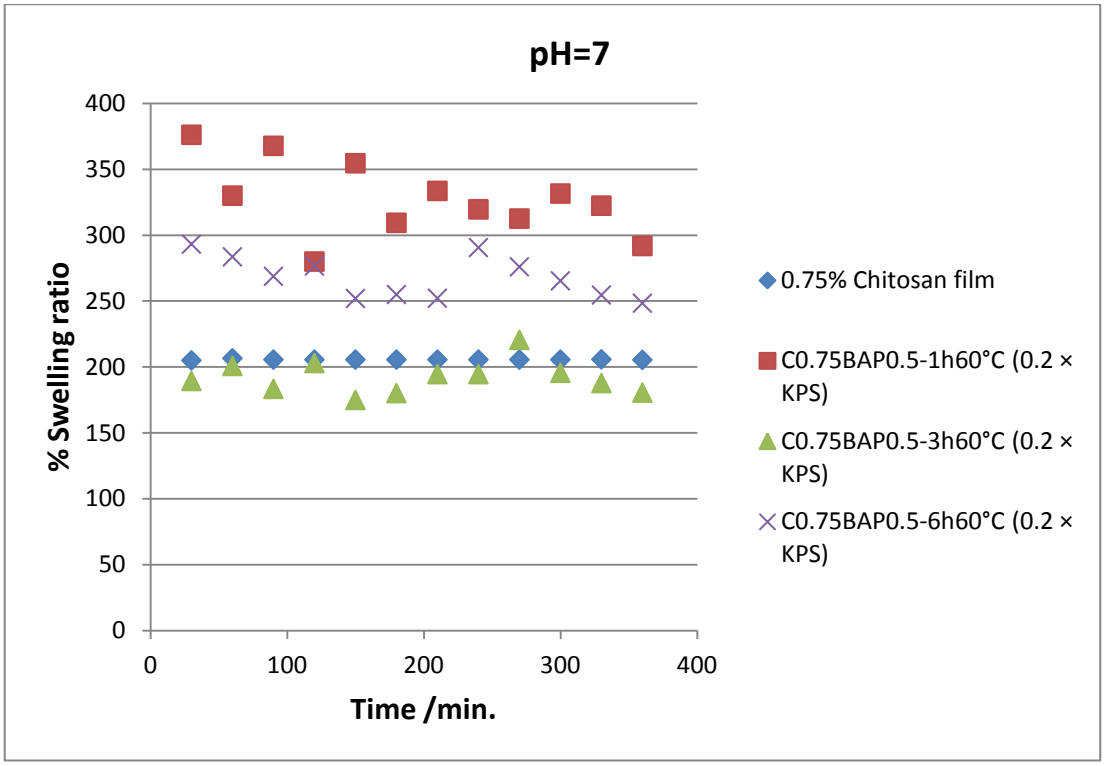


Figure 10. Effect of Reaction Time Duration of Phosphorylated Chitosan Films and Pure Chitosan Film on the Swelling Behavior, Using (pH = 7) Buffer Solution

Table 10. % Swelling of 0.75% Chitosan Film, C0.75BAP0.5-1h60°C (0.2 × KPS), C0.75BAP0.5-3h60°C (0.2 × KPS) and C0.75BAP0.5-6h60°C (0.2 × KPS) in Buffer Solution pH=11 (Film Products)

Time (min.)	0.75% Chitosan film	C0.75BAP0.5-1h60°C(0.2×KPS) (%61G)	C0.75BAP0.5-3h60°C(0.2×KPS) (%100G)	C0.75BAP0.5-6h60°C(0.2×KPS) (%78G)
30	225	381	465	389
60	220	383	516	350
90	222	438	631	273
120	210	284	565	381

150	206	445	493	440
180	201	332	427	446
210	197	498	617	374
240	195	505	596	525
270	193	415	474	395
300	198	323	346	389
330	205	400	609	333
360	195	259	339	232

At pH=11 as the % grafting increases % swelling increases due to a higher phosphate content and more ionized species at this pH as shown in Figure 11 and Table 10.

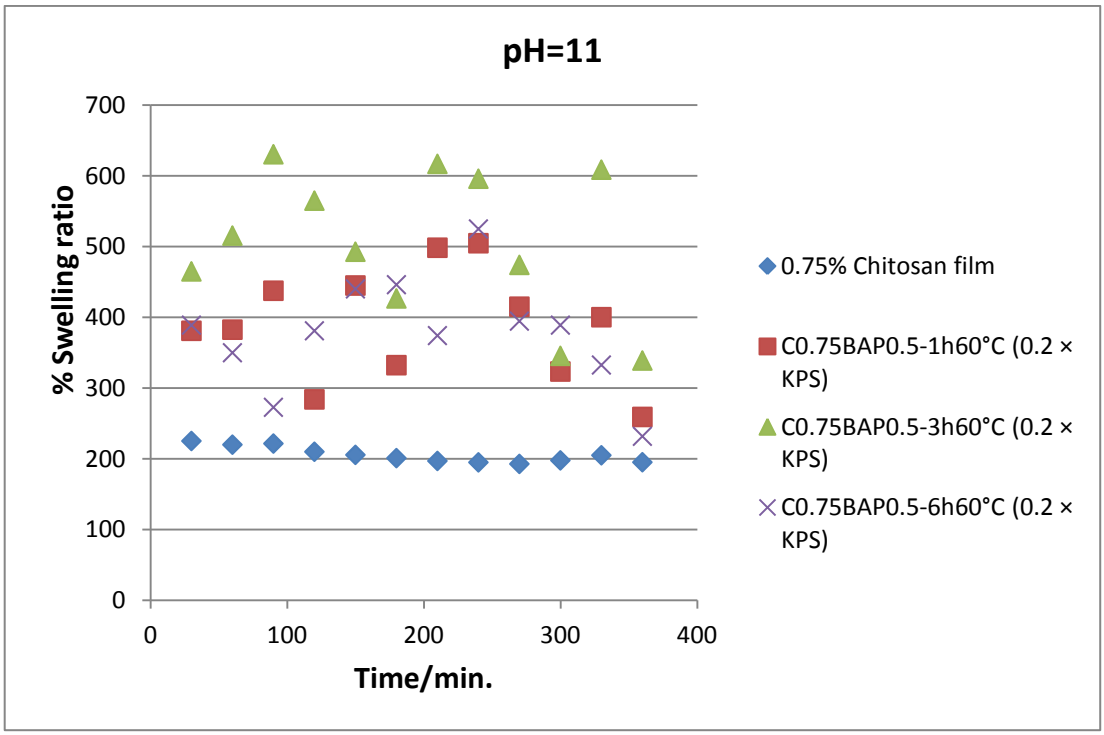


Figure 11. Effect of Reaction Time Duration of Phosphorylated Chitosan Films and Pure Chitosan Film on the Swelling Behavior, Using (pH = 11) Buffer Solution

The swelling behavior of the cross linked products (powders) are shown in Figure 12, 13, and 14, and Table 11, 12 and 13.

Table 11. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) andC0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=3

Time/h	C0.75BAP0.5-2h60°C (1 × KPS)	C0.75BAP0.5-2h90°C (0.04 × KPS)
1	3870	3664
2	3650	3578
3	3896	3573
4	3696	3537
5	3698	3537
6	3700	3539
24	3421	2616
48	2704	2044
72	2710	2473

Contrary to the film samples the powder samples do not dissolve at pH=3 due to their cross linked structure. They show super absorbent character with swelling percentage up to 3700%.

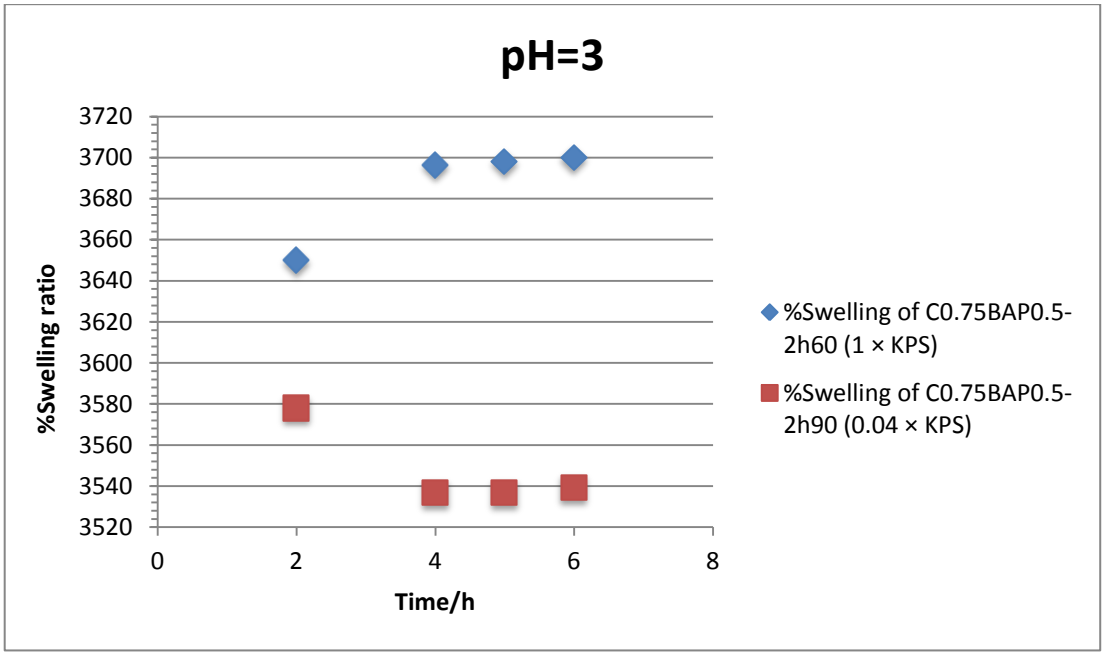


Figure 12. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=3

The samples have similar grafting percentage of the order of 260%, and hence shown similar swelling degrees of the order of 3500% and 3700%. In acidic media cross linked powders will be protonated and repulsion forces between protonated amine groups will be predominant. Therefore the increased swelling was observed.

In the neutral pH both powders showed similar swelling tendency and they reached equilibrium at the end of fourth hour. There are less ionic interactions resulting in lowered swelling, since the copolymer carries minimum electric charge.

In the alkaline medium the % swelling ratio increased in comparison to neutral pH, and acidic media due to the ionization of the phosphate groups.

Table 12. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=7

Time/h	**%Swelling of C0.75BAP0.5-2h60°C (1 × KPS)**	**%Swelling of C0.75BAP0.5-2h90°C (0.04 × KPS)**
1	3884	3614
2	3900	3614
3	3115	3580
4	3184	3240
5	3218	3404
6	3220	3418
24	2822	2819
48	2384	2213
72	2300	2732

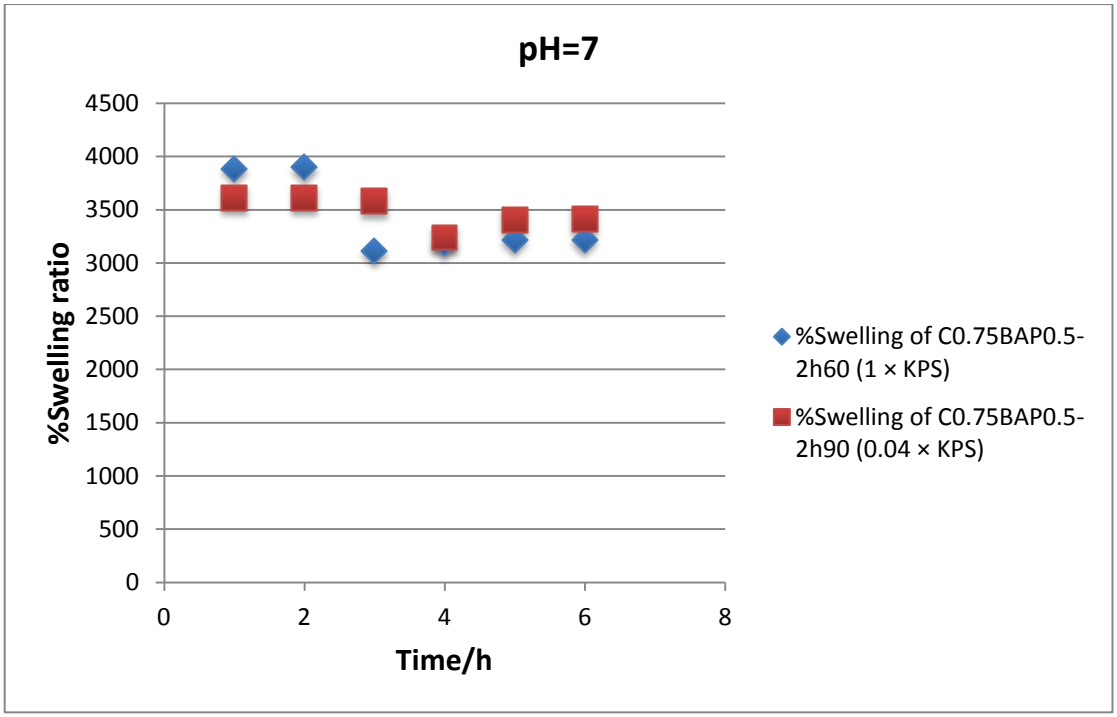


Figure 13. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=7

Table 13. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=11

Time/h	%Swelling of C0.75BAP0.5-2h60°C (1 × KPS)	%Swelling of C0.75BAP0.5-2h90°C (0.04 × KPS)
1	3738	4235
2	3648	4030
3	3579	4071
4	3420	3726
5	3466	3745
6	3484	3735
24	2888	3104
48	2840	2684
72	2633	2437

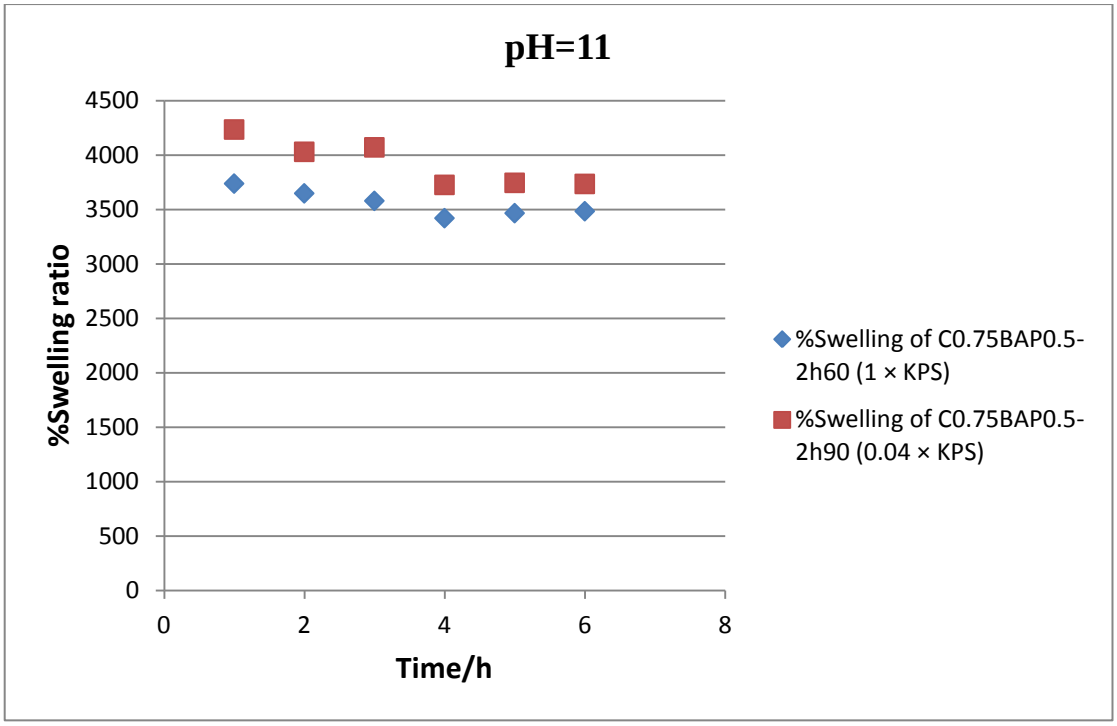


Figure 14. % Swelling of C0.75BAP0.5-2h60 (1 × KPS) and C0.75BAP0.5-2h90 (0.04 × KPS) in Buffer Solution pH=11

Table 14. % Swelling of Phosphorylated Chitosan Films in pH=3 Buffer Solution

Time (min.)	C1BAP1/2h60 (0.2 × KPS)	C1BAP0.5/2h60 (0.2 × KPS)	C1BAP0.25/2h60(0.2 × KPS)	C0.75BAP0.5/1h60 (0.2 × KPS)	C0.75BAP0.5/2h60 (0.2 × KPS)	C0.75BAP0.5/3h60 (0.2 × KPS)	C0.75BAP0.5/6h60 (0.2 × KPS)	C0.75BAP0.5/2h60 (0.4 × KPS)
30	Readily soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	113
60	Readily soluble	Soluble	Soluble	Readily soluble	Soluble	Readily soluble	Soluble	94
90	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	91
120	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	94
150	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	125
180	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	110
210	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	130

240	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	105
270	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	112
300	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	90
330	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	86
360	Readily soluble	Soluble	Soluble	Readily soluble	Readily soluble	Readily soluble	Soluble	86

3.5 Thicknesses of Prepared Films

3.5.1 The Effect of Temperature on the Thickness

Table 14. Effect of Temperature on the Prepared Film Thicknesses

Sample ID	Temperature/ °C	Average Thickness/mm
C0.75BAP0.5-2h(0.04 × KPS)	40	0.01025
C0.75BAP0.5-2h(0.04 × KPS)	50	0.01275
C0.75BAP0.5-2h(0.04 × KPS)	60	0.02900
C0.75BAP0.5-2h(0.04 × KPS)	70	0.01025

It can be noted that thickness of films reached a maximum value at 60°C (%105) then decreased steadily as temperature increases; at 90°C powdered form was formed. At constant amount of chitosan, BAP and initiator concentration, increasing temperature lowered the thickness. The optimum temperature was obtained at 60°C which leads to highest grafting yield.

3.5.2The Effect of Amount of Initiator on the Thickness

Table 15. Effect of Initiator Amount on the Prepared Film Thicknesses

Sample ID	Initiator amount/g	Average Thickness/mm
C0.75BAP0.5-2h-60 (0.022 × KPS)	0.00150	0.0095
C0.75BAP0.5-2h-60 (0.04 × KPS)	0.00272	0.0290
C0.75BAP0.5-2h-60 (0.2 × KPS)	0.01360	0.0315

It was observed that increasing amount of initiator in the reaction system leads to increase in thickness of phosphorylated chitosan film as shown in Table 16. At

constant concentration of chitosan, BAP, temperature and reaction duration, increased in initiator amount leads to increase the thickness. On the other hand, % grafting was increased with increasing the amount of initiator.

3.5.3 The Effect of Chitosan Concentration on the Thickness

The polymer concentration effected on the thicknesses of films prepared proportionally as shown in Table 17, by decreasing chitosan concentration, the thickness were decreased.

Table 17. Effect of Chitosan Concentration on the Prepared Film Thicknesses

Chitosan film alone (w/v%)	Average Thickness/mm
2	0.05125
1	0.02575
0.75	0.02550
0.5	0.01275

3.5.4 The Effect of Monomer (BAP) Concentration on the Thickness

For 1% chitosan solution with increasing BAP amount the thicknesses of the films increased, and proportionally of the grafting yield also increased. For 0.75% chitosan solution the measured thicknesses were increasing with increasing BAP amount, but the high yield of grafting percentage was observed when (1, 0.25 and 0.5mL) of BAP were used respectively as shown in Table 2 and 18.

Table 16. Effect of (BAP) Concentration on the Prepared Film Thicknesses

Sample ID	(BAP) Volume/mL	Average Thickness/mm
C1BAP1-2h60 (0.2 × KPS)	1	0.02250
C1BAP0.5-2h60 (0.2 × KPS)	0.5	0.00650
C1BAP0.25-2h60 (0.2 × KPS)	0.25	0.00525
C0.75BAP1-2h60 (0.2 × KPS)	1	0.04000
C0.75BAP0.5-2h60 (0.2 × KPS)	0.5	0.03150
C0.75BAP0.25-2h60 (0.2 × KPS)	0.25	0.00875

3.5.5 The Effect of the Duration (Reaction Time) on the Film Thicknesses

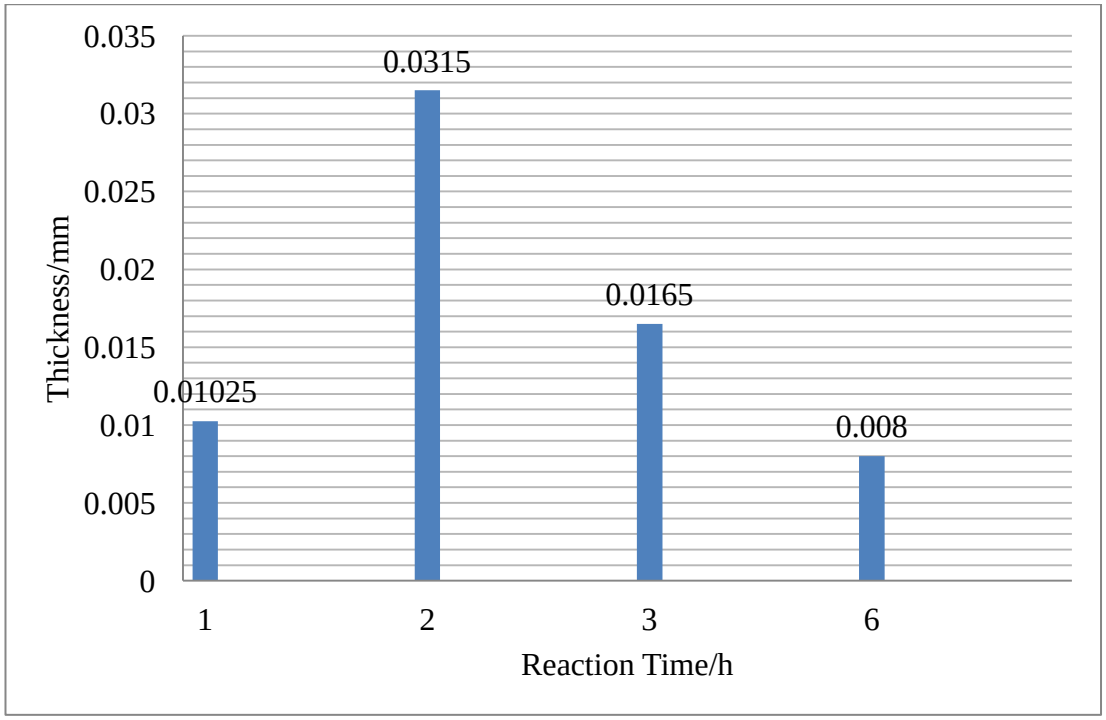


Figure 15. Effect of Reaction Time on the Prepared Film Thicknesses

With increasing time grafting yield increases reaching a maximum of 105% in 2 hours. This trend is observed in the film thicknesses of the products as well. The product with the highest grafting percentage (105%) is the thickest film among all with a thickness of 0.03150 mm.

Table 179. Effect of Reaction Time on the Prepared Film Thicknesses

Sample ID	Time/hour	Average Thickness/mm
C0.75BAP0.5 (0.2 × KPS)	1	0.01025
C0.75BAP0.5 (0.2 × KPS)	2	0.03150
C0.75BAP0.5 (0.2 × KPS)	3	0.01650
C0.75BAP0.5 (0.2 × KPS)	6	0.00800

3.6 Contact Angle Measurements

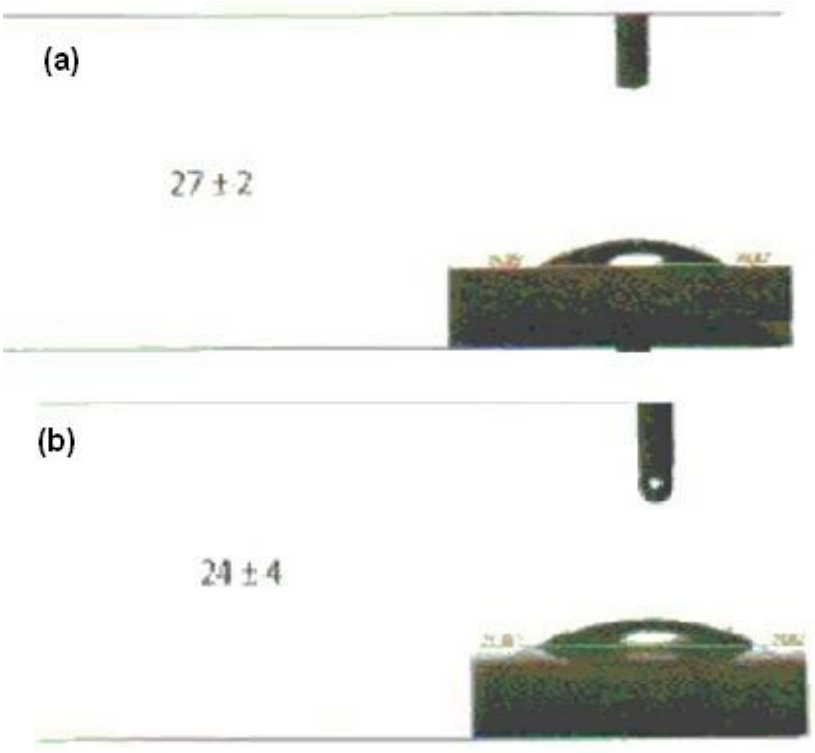


Figure 16. Contact Angles of (a) Pure Chitosan Film and (b) Phosphorylated Chitosan Film

Hydrophilicity of pure chitosan film and Phosphorylated chitosan film can be detected by contact angle measurements of water. Usually contact angle of water is increase when surface hydrophilicity of the film is lower. Phosphorylated chitosan

film posses smaller contact angle than that of chitosan due to increased hydrophilicity with copolymerization as shown in Figure 16.

Table 20. Average Thicknesses and Contact Angles of the Films

Sample ID	Thickness Average/mm	Contact Angle
C0.75 (Chitosan film)	0.02550	27±2
C0.75BAP0.5-2h60(0.2 × KPS)	0.03150	24 ± 4

3.7 Scanning Electron Microscope (SEM) Analysis

Scanning electron microscope (SEM) pictures (X500 & X2000) of chitosan and phosphorylated chitosan films are shown in (Figure 17 & 18) respectively. Chitosan film cast from acetic acid solution has a surface that contains nanosized particles which adhere onto the film surface. This must be due to the inhomogeneities in the solution at the molecular level, or inconsistent drying conditions. During solvent evaporation some polymer molecules which phase separate from solution may adhere onto the surface of the film being formed. When the surface of the phosphorylated chitosan film is examined it can be observed that there is a homogenous film surface containing adhered particles similar to that of chitosan film.

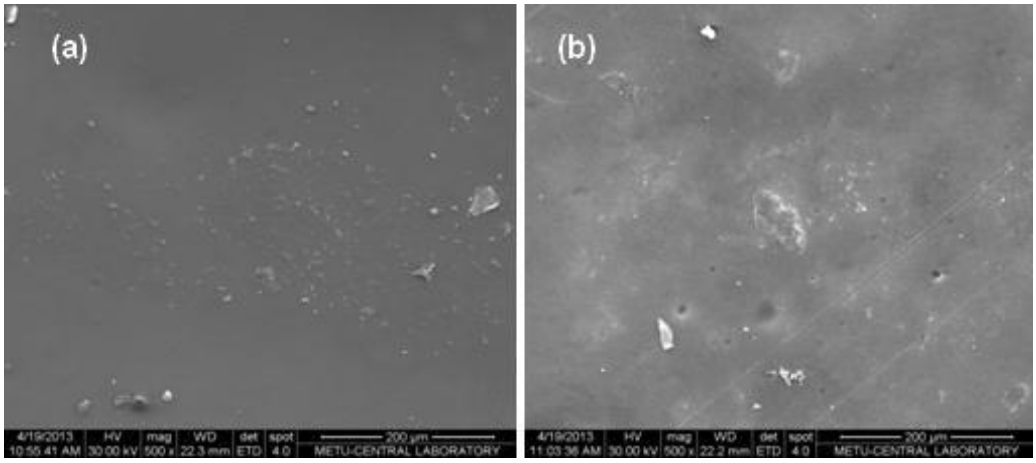


Figure 17. Scanning Electron Microscope (SEM) Pictures (X 500) of (a) Pure Chitosan Film and (b) Phosphorylated Chitosan Film

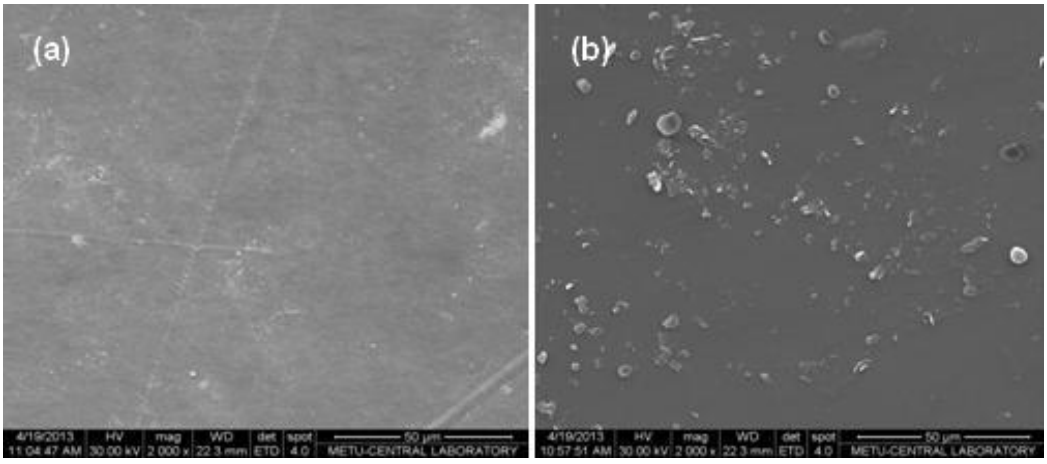


Figure 18. Scanning Electron Microscope (SEM) Pictures (X 2000) of (a) Pure Chitosan Film and (b) Phosphorylated Chitosan Film

3.8 Differential Scanning Calorimetry (DSC) Analysis

DSC thermograms of pure chitosan film and phosphorylated chitosan films with % grafting values of 127% (C0.75BAP0.25) and 105% (C0.75BAP0.5) are shown in (Figure 19, 20 & 21) respectively. Samples were first heated up to 200°C to remove any moisture and possible impurities. Chitosan lost water at 46.01°C, whereas phosphorylated chitosan films with % grafting values of 127% (C0.75BAP0.25) and 105% (C0.75BAP0.5) show water loss peaks at 47.99°C and 64.18°C. After cooling, the second runs were taken up to 400°C. It can be observed that pure chitosan film

decomposes with an endothermic decomposition peak at 309°C. This behaviour is contradictory to what is observed with chitosan powders without any preheating (Hasiploglu, et al., 2005). This should be due to the conformational changes and chain rearrangement during preheating and cooling. Phosphorylation via grafting results in a decrease in the decomposition temperature, hence, phosphorylated products are thermally less stable. Phosphorylated chitosan film with % grafting value of 127% (C0.75BAP0.25) has a decompose at 270.02°C whereas phosphorylated chitosan film with % grafting value of 105% (C0.75BAP0.5) has a decomposition temperature of 283.54°C. The reason is the disruption of H-bonding as a result of grafting.

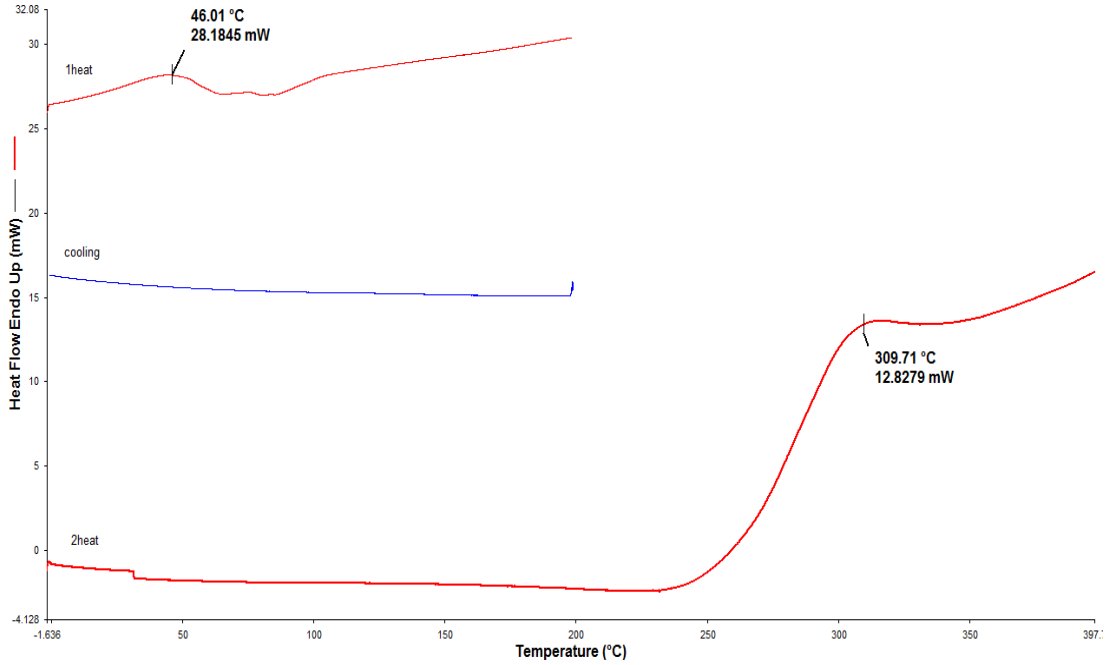


Figure 19. DSC Runs of Pure Chitosan Film

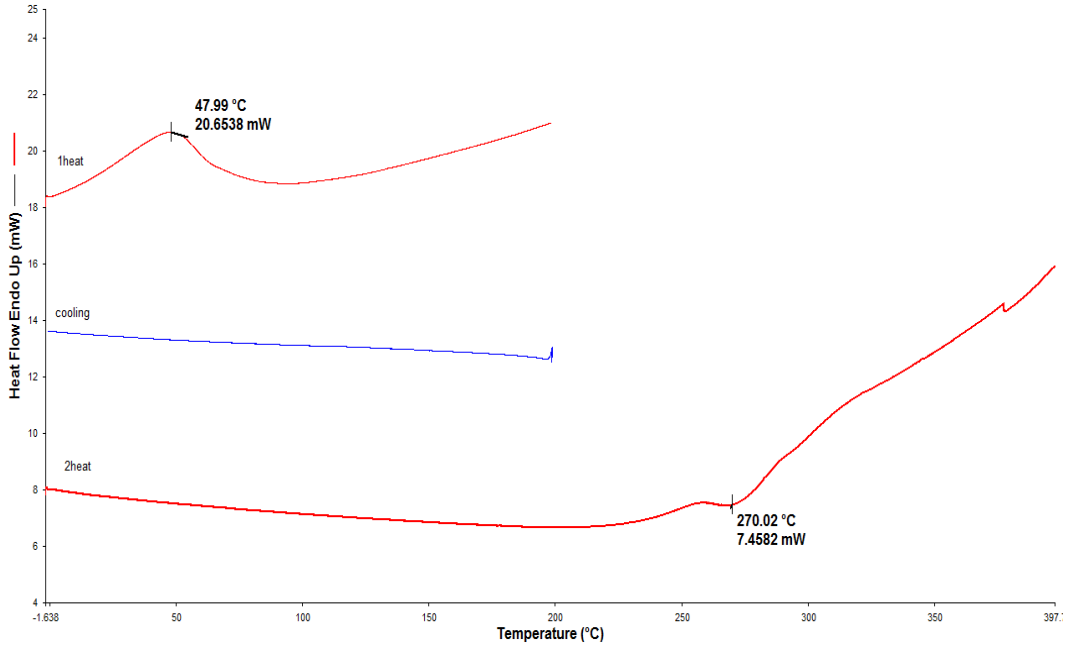


Figure 20. DSC Thermogram of C0.75BAP0.25-2h60°C (0.2 × KPS) Phosphorylated Chitosan Film

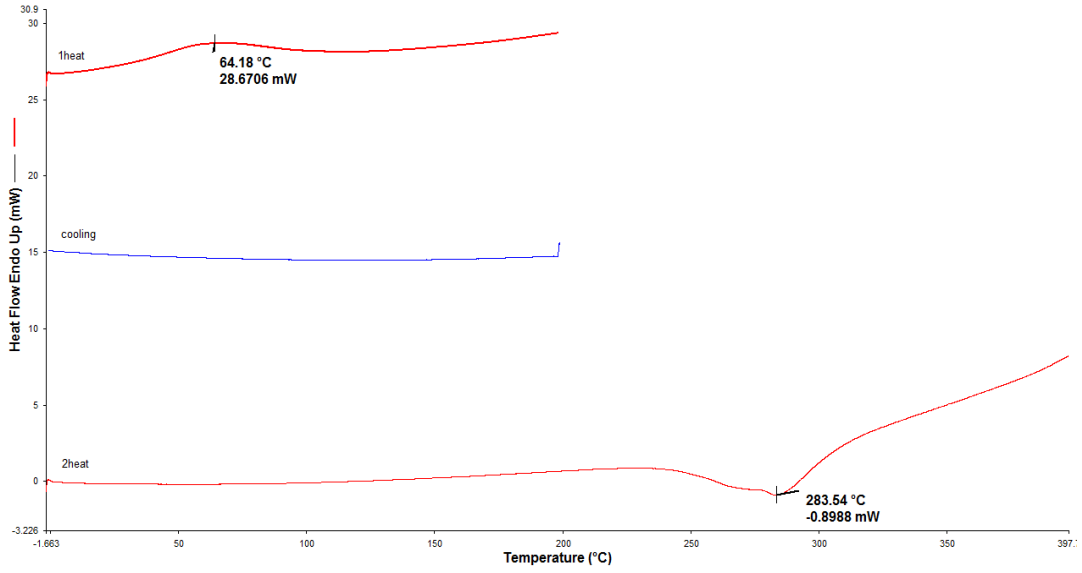


Figure 21. DSC Thermogram of C0.75BAP0.5-2h60°C (0.2 × KPS) Phosphorylated Chitosan Film

Chapter 4

CONCLUSIONS

BAP was grafted onto chitosan in aqueous solution by using KPS as the redox initiator. Grafting yield was affected by reaction temperature, reaction time, concentration of polymer and monomer. The effect of reaction time on the grafting percent yield was more pronounced than the effect of monomer concentration, initiator concentration or temperature. The maximum grafting percentage (231%) was obtained at (C0.5BAP1-2h-60°C (0.2×KPS)) for the products in the film form and (269%) at (C0.75BAP0.5-2h-90°C (0.04×KPS)) for the powders.

The presence of P=O stretching at 1176 cm^{-1}, C=O stretching in ester bond at 1725 cm^{-1} and C-CH2 bending in the 700-796 cm^{-1} range in the FTIR spectra represent successful grafting of BAP onto chitosan.

Solubility characteristics and % grafting were observed in a correlation. Depending on the ratio of degree of ionization of the amine and phosphate groups present in the synthesized copolymer, the copolymer is capable of various swelling degree in acidic, neutral or basic media.

SEM analysis showed homogeneous film surface formed upon copolymerization of BAP.

All grafted films showed lower thermal stability than chitosan film itself. This is explained by the loss of H- bonding during grafting.

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