DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PREPARATION AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)-BORATE/CHITOSAN FILMS

by
Refika YILDIZ

June, 2013
İZMİR

PREPARATION AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)-BORATE/CHITOSAN FILMS

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by
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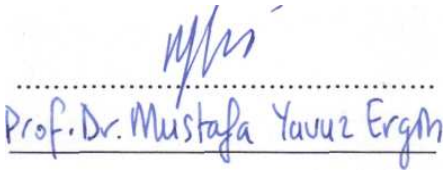
M. Sc. THESIS EXAMINATION RESULT FORM

We have read the entitled **PREPARATION AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)-BORATE/CHITOSAN FILMS** completed by **REFİKA YILDIZ** under supervision of **PROF. DR. KADİR YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

Prof. Dr. Kadir YURDAKOÇ

Supervisor



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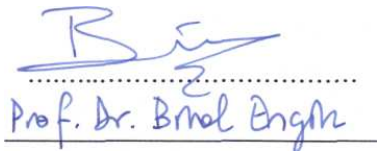

Jury Member

Prof.Dr. Ayşe OKUR

Director

Graduate School of Natural and Applied Science

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PREPARATION AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)-BORATE/CHITOSAN FILMS

ABSTRACT

In this study, poly(vinyl alcohol) (PVA)/Borate/Chitosan films were prepared by using boric acid as a crosslinker. Synthesized films were characterized by FTIR, AFM, XRD and SEM methods. On the otherhand, thermal properties of the films were determined by TG/DTG analysis. At the same time, swelling properties of the films were investigated at two different temperatures (25 and 37 centigrade) in distilled water, KCl/HCl buffer (pH 1.2) and phosphate buffer (pH 7.4) solutions. For the enhancement of the physical and chemical properties of the films, PVA, CS and boric acid amounts used the film production was changed and the results were compared with each other.

In the comparison of the FTIR spectra of the prepared films with different ratios, there were not observed great differences. On the other hand, spherical structures observed in SEM images were increased with the increasing amounts of chitosan. Furthermore, it was observed in the TG/DTG analysis that some films were degradated in three steps while others were in four steps degradation. In the swelling experiments, all of the films were swollen more in KCl/HCl buffer than less swollen in phosphate buffer solutions.

Keywords: Chitosan, chitosan/PVA, borate, PVA/borate gel,

POLİVİNİL ALKOL/BORAT/KİTOSAN FİLMLERİN HAZIRLANMASI VE KARAKTERİZASYONU

ÖZ

Bu çalısmada, poli(vinil alkol) (PVA), kitosan (CS) ve çapraz bağlayıcı olarak borik asitin kullanıldığı filmler elde edilmiştir. Sentezlenen filmler FTIR, AFM, XRD ve SEM yöntemleriyle karakterize edilmiştir. Filmlerin termal özellikleri ise TGA/DTG analizleri ile belirlenmiştir. Aynı zamanda filmlerin saf su, potasyum klorür/hidroklorik asit tamponu (pH 1.2) ve fosfat tamponu (pH 7.4) içerisindeki şişme özellikleri iki farklı sıcaklıkta (25 ve 37 santigrat derecede) incelenmiştir. Elde edilen filmlerin, fiziksel ve kimyasal özelliklerinin iyileştirilmesi adına hazırlanan filmlerde kullanılan PVA, CS ve borik asit oranları değiştirilerek sonuçlar karşılaştırılmıştır.

Farklı oranlarda hazırlanan filmlerin karakterizasyonu amacıyla yapılan FTIR ölçümlerinin karşılaştırılmasında çok büyük farklılıklar gözlenmezken; SEM görüntülerindeki küresel yapıların artan kitosan miktarıyla arttığı gözlemlenmiştir. TGA sonuçlarında tüm filmlerde ortak ve belirgin olarak gözlemlenen iki pik vardır. Bunun yanı sıra, TG/DTG analizlerinde bazı filmler üç basamakta bozunurken; bazılarının dört basamakta bozunduğu tespit edilmiştir. Yapılan şişme deneylerinde tüm filmler en fazla KCl/HCl tamponunda şişerken; en az fosfat tamponunda şişmiştir.

Anahtar sözcükler: Kitosan, kitosan/PVA, borat, PVA/borat jel,

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CHAPTER ONE
INTRODUCTION

1.1 Chitosan

1.1.1 The Properties of Chitosan

Chitosan, is a linear copolymer polysaccharide consisting of D-glucosamine (β-(1–4)-linked 2-amino-2-deoxy- D-glucose) and *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) units which is an *N*-deacetylated derivative of chitin (Vieira, Cestari, Airoldi & Loh, 2008) (Figure 1.1) (Figure 1.2 and Figure 1.3). Chitin is the second most plenty polysaccharide in nature, after cellulose (Rathke & Hudson, 1994). Chitin is associated with other polysaccharides in the fungal cell walls, while in animal forms chitin is associated with proteins (Muzzarelli, 1977) (Figure 1.2 and Figure 1.3).

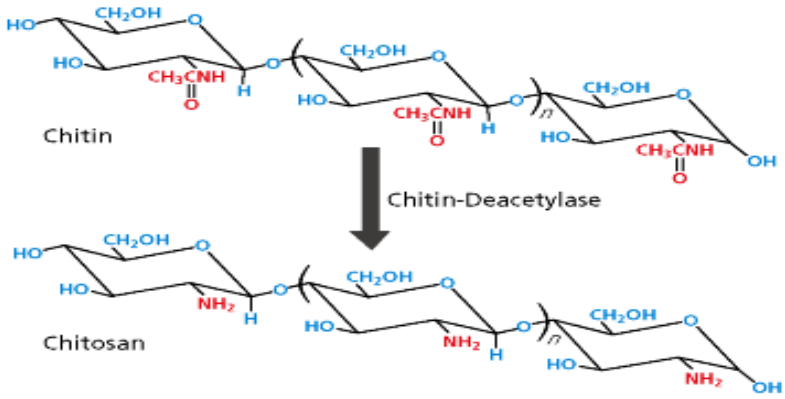


Figure1.1 Structures of chitin and chitosan

Chitosan contains three functional groups. These are two hydroxyl groups (–OH) and one amino group ($–NH_2$), per glucosamine unit (Crini, 2006). However, pure chitosan materials have some obvious disadvantages such as poor chemical resistance, low mechanical strength and difficult recovery (L. Wang & A. Wang, 2008). In addition, the adsorption capacity for crosslinked chitosan was lower when compared with free chitosan, because of functional group ($–NH_2$) being crosslinked (Shawky, 2009).

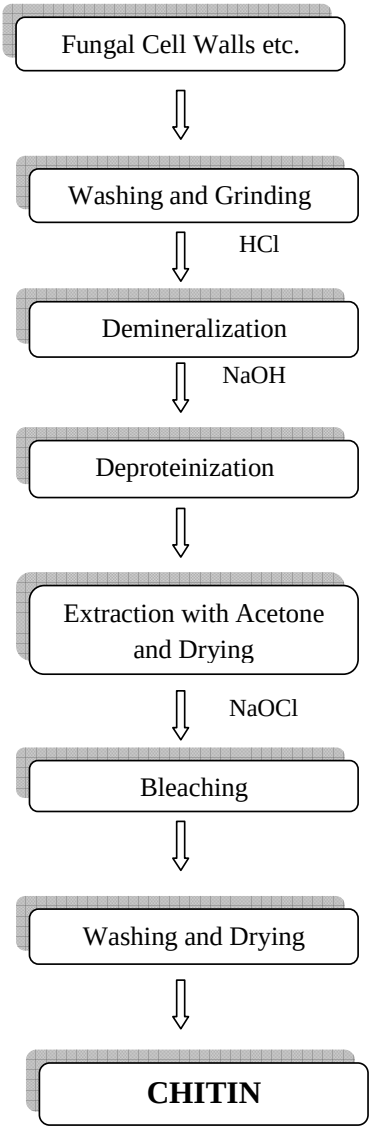


Figure 1.2 Synthesis of Chitin

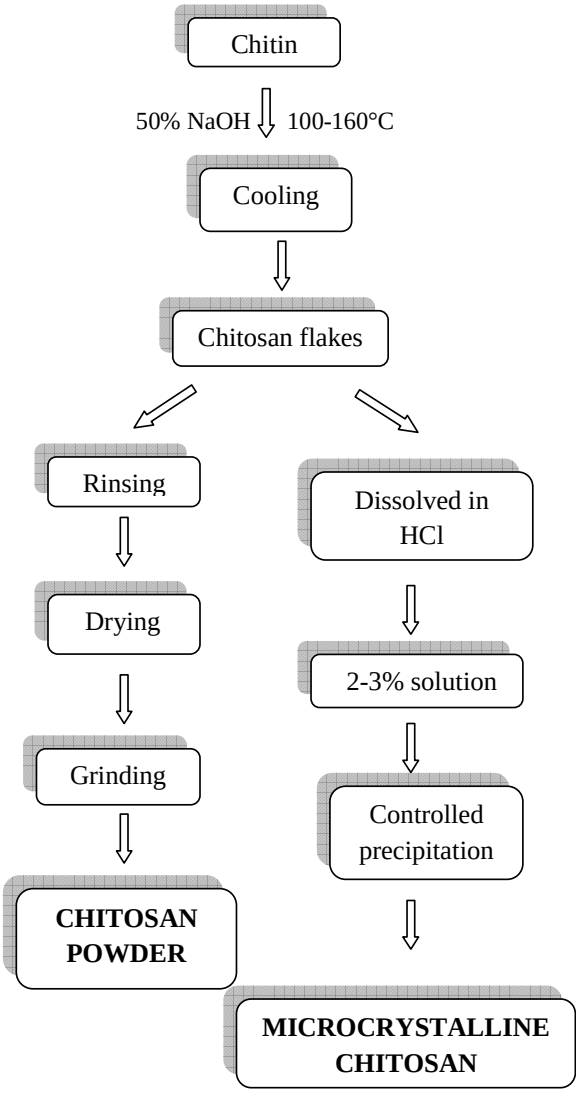


Figure 1.3 Synthesis of Chitosan (Aranaz et al. 2009)

Chitosan is only soluble in aqueous solutions of some acidic and some selective N-alkylidinations (Muzarelli, 1997; Hirano, 1997) and N-acylation (Li, Dunn, Grandmaison & Goosen,1997; Sashiwa & Shigemasa, 1999) have also been attempted. Although several water soluble (C. Zhang, Ping, H. Zhang & Shen, 2003) or highly swelling (Hudson & Smith, 1998) derivatives are obtained, it is difficult to develop the solubility in common organic solvents by these methods. Modification of the chemical structure of chitin and chitosan to improve the solubility in conventional organic solvents has been reviewed by many authors (Kim, Lee & Cho, 1995; Kurita, Tomita, Ishii, Nishimura & Shimoda, 1993)

Chitosan are naturally occurring biopolymers, (De & Robinson, 2003; Koga, 1998) has been used to obtain biocompatible and biodegradable with low toxicity (Chen, Wu, Mi, Lin, Yu & Sung, 2004; Rasool, Yasin & Akhter, 2008). Chitosan is used as a gel, film, and fiber-forming material (Wang, Qiang, Fang, Hu, Cui & Fu, 2000). These properties make it an important material, and it has many applications in tissue engineering, pharmaceutical industry, biotechnology, and delivery of drugs, nonviral genes, enzymes, and so on (Islam, Yasin, Bano & Riaz, 2012).

Chitosan is generally prepared by deacetylation of α-chitin using 40-50% aqueous alkali solution at 100-160°C for a few hours. The resulting chitosan has a degree of deacetylation (DA) up to 0.95. According to US Department of Commerce's research; when chitin is deacetilated in 40% NaOH at 120°C for 1-3 h, obtained with a degree of deacetylation of chitosan is 70%. (P. Dutta, J. Dutta & Tripathi, 2004)

Chitosan hydrogels are very fragile and can be improved through the incorporation of other monomers and polymers and/or by crosslinking. (Jin, Teixeira, Dijkstra, Karperien, VanBlitterswijk & Zhong, 2009; Tang, Du, Li, Wang & Hu, 2009) Certain reagents have been used for cross linking chitosan such as glutaraldehyde, tripolyphosphate, ethylene glycol, diglycidyl ether and diisocyanate (Nishi, Nakajima, & Ikada, 1995; Speer, Chvapil, Eskelson, & Ulreich, 1980).

1.1.2 Applications of Chitosan

1.1.2.1 Water Filtration

Chitosan can also be used in water processing engineering as a part of a filtration process. Chitosan causes the fine sediment particles to bind together, and is subsequently removed with the sediment during sand filtration. It also removes phosphorus, heavy minerals, and oils from the water. Chitosan is an important additive in the filtration process. Sand filtration apparently can remove up to 50% of the turbidity alone, while the chitosan with sand filtration removes up to 99% turbidity (Woodmansey, 2002). Chitosan has been used to precipitate caseins from bovine milk and cheese making (Ausar, Passalacqua, Castagna, Bianco & Beltramo, 2002).

Chitosan is also useful in other filtration situations, where one may need to remove suspended particles from a liquid. In combination with bentonite, gelatin, silica gel, isinglass, or other fining agents, it is used to clarify wine, mead, and beer. Added late in the brewing process, chitosan improves flocculation, and removes yeast cells, fruit particles, and other detritus that cause hazy wine. Chitosan combined with colloidal silica is becoming a popular fining agent for white wines, because chitosan does not require acidic tannins (found primarily in red wines) with which to flocculate (Rayner, 2006).

1.1.2.2 Antioxidant

Chitosan has an antioxidant properties. Two types of fungal chitosan, B or C, have been prepared by alkaline N-deacetylation of crude chitin B or C for different durations of 60, 90, and 120 min (Yen, Tseng, Li, & Mau, 2007). Results show that chitosan has antioxidant activities of 61.6-82.4% at 1 mg/cm^{-3} and shows reducing powers of 0.42-0.57 at 10 mg/cm^{-3}. Also, no significant difference in antioxidant properties between chitosan B and C has been observed.

1.2 Poly(vinyl alcohol) PVA

1.2.1 The Properties of PVA

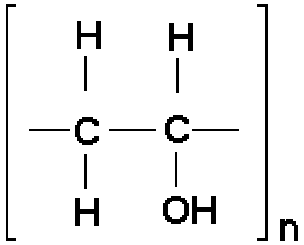


Figure 1.4 Poly(vinyl alcohol)

Attention has been focused on poly(vinyl alcohol) (PVA) for more than 30 years because of its unique chemical and physical properties as well as its industrial applications (Sakurada, 1985). Some of the unique properties come from the large number of hydroxy groups which react chemically with many kinds of functional groups. The hydrogen bond related to the hydroxy group also plays an important role in the physical properties of PVA, e.g. high water solubility, wide range of crystallinity and high crystal modulus (Shibayama, Sato, Kimura, Fujiwara & Nomura, 1988).

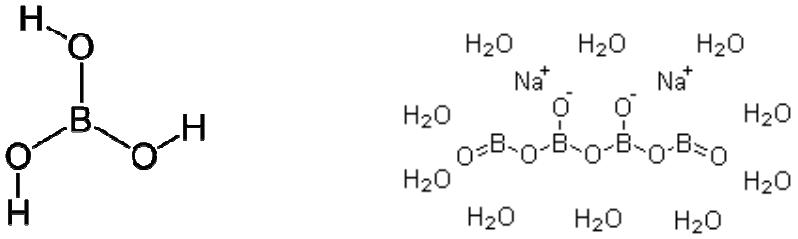
PVA is a water-soluble material containing large amounts of hydroxyl groups. PVA has been widely applied because it has many advantages such as low cost, non-toxicity, biocompatibility, high durability and chemical stability (Khoo & Ting, 2001; Kao, Wu, C. Chang & J. Chang, 2009).

PVA is highly hydrophilic, nontoxic and biocompatible with excellent film forming property. PVA films have good mechanical strength, long-term thermal and pH stability. These properties of PVA have led their use in areas of medical and pharmaceutical applications. Cross linked PVA membranes show good swelling property and are useful in sustaining drug release too (Gholap, Jog & Badiger, 2004).

PVA can be prepared by hydrolysis of a variety of poly(vinyl esters) and poly(vinyl ethers). Most commercially available PVA is produced from poly(viny1 acetate) (PVAc) (Tanigami, Shirai, Yamaura & Matsuzawa, 1994; Tanigami, Yano, Yamaura & Matsuzawa, 1995). The structure of crystalline PVA was shown to be monoclinic (Hodge, Bastow, Edward, Simon & Hill, 1996a; Hodge, Bastow, Edward & Simon, 1996b). The high crystallizability of PVA compared to PVAc should be attributed to the fact that the hydroxyl groups are of sufficiently small size to allow the chains to adopt a planar zig-zag conformation. PVA has found many applications in pharmaceuticals, cosmetics and in the paper and food industries, either alone or in blends with other polymers (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto & Aiba, 1997).

1.3 Borate

1.3.1 General Definitions



(a) (b)

Figure 1.5 a) $B(OH)_3$ b) $Na_2B_4O_7.10H_2O$

Boron is an element which is commonly found in hot spring water and seawater. Boron is widely distributed in nature, mainly in the forms of boric acid ($B(OH)_3$) or borax ($Na_2B_4O_7.10H_2O$). (Figure 1.5) Industrial applications of the boric compounds include glass manufacturing, soaps and detergents, flame retardants, doping in the semiconductor industries, and neutron absorption in nuclear power plants (Shimada, Iizuka, Shiojiri, Yamasaki & Yanagisawa, 2006). Boron compounds have a wide range of applications ranging from medicines such as eye drops and gargles to glost

and metallic plating in various industries (Harada, Takagi, Kataoka, Yamamoto & Endo, 2011).

1.3.2 Adsorption Mechanism of Borate on PVA

Crosslinking increases mechanical properties and resistance of PVA. Crosslinking of PVA can be easily attained by adding alkaline reagent. Boric (Deuel & Neukom, 1949; Lorand & Edwards, 1959), cupric (Saito & Okuyama, 1954), and titanic (Crisp, 1946) reagents also were crosslinked with PVA. There are at least two kinds of proposed structure of PVA and boric acid reaction. These are monodiol type and didiol type (Figure 1.6). The didiol type complex was more dominant than mono type complex (Ochiai, Shimizu, Tadokoro & Murakami, 1981).

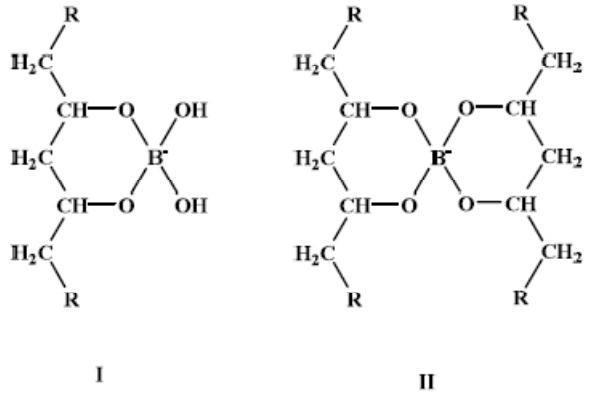


Figure 1.6 Chemical structure of monodiol complex (I) and didiol complex (II)

1.4 Aim of The Study

In this study, poly(vinyl alcohol) and chitosan will be crosslinked with boric acid to form film type structures. For this purpose, PVA and chitosan will be mixed at different ratios to obtain the correct stoichiometry. Then, the synthesized film will be characterized by using different methods such as FTIR, TG/DTG, XRD, SEM and AFM. At the same time, swelling properties will also be investigated at two different pH values.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials

Chitosan (CS) (highly viscous) was purchased from Fluka (degree of deacetylation: 75-85%, average molecular weight: 500000-700000 g mol^{-1}) as a flaked material. PVA was purchased from Fluka (average molecular weight 72 000). Boric acid was purchased from Sigma-Aldrich 11607-puriss. (Meets analytical specification of Ph. Eur., BP, NF, 99.5-100.5%) powder.

2.2 Preparation of Films and Solutions

2.2.1 Chitosan Solution and PVA-Borate gel Preparation

Six different concentration of chitosan solution were prepared. 0.2 g Chitosan was dissolved in 10 mL of distilled water with 1% of CH_3COOH at room temperature about 24 hours for first solution. 0.16 g Chitosan was dissolved under the same conditions for second solution. Third and fourth solutions were included 0.12 g Chitosan. 0.08 g Chitosan was dissolved for fifth solution and 0.02 g Chitosan for sixth solution.

PVA solutions were prepared at two different concentrations. First solution was prepared by fully dissolving 0.115 g polymer powder without further purification in 5 mL of distilled water, under heat about 1 hours and solution was left to cool down to room temperature. Second PVA solution was prepared with 0.1 g PVA under the same conditions like first solution.

Boric acid solutions were prepared at two different concentrations too. 0.04 g Boric Acid was dissolved in 2 mL of distilled water for first solution and 0.08 g Boric acid was dissolved for second solution.

Then PVA and Boric Acid solutions of different concentrations were stirred at room temperature for 15 min. Table 2.1 shows ratio of between PVA and Boric Acid.

Table 2.1. Rate of PVA-borax gel

	Distilled Water (mL), PVA (g)		Distilled Water (mL), Boric Acid (g)
1.	5 mL, 0.1 g	+	2 mL, 0.04 g
2.	5 mL, 0.115 g	+	2 mL, 0.04 g
3.	5 mL, 0.115 g	+	2 mL, 0.08 g

2.2 Preparation of Chitosan/PVA-Borate Films

Chitosan solutions which were different concentrations were dried on Petri dish at room temperature for 2 days. Then prepared PVA-Borax Gels have been added on the dried chitosan and left to dry in room temperature for 2 days. The ratio of prepared films was shown at Table 2.2.

Table 2.2. Ratio of between chiotsan-PVA-boric acid

Sample Code	Chitosan(g)	PVA(g)	Boric Acid(g)
C10PV100B1	0.2	0.1	0.04
C8PV115B1	0.16	0.115	0.04
C6PV115B1	0.12	0.115	0.04
C6PV115B2	0.12	0.115	0.08
C4PV115B2	0.08	0.115	0.08
C1PV115B2	0.02	0.115	0.08

2.3 Characterization

2.3.1 Fourier Transform Infrared (FTIR) Spectra of the Samples

The hydrogels were characterized by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. ATR-FTIR was used to investigate the formation of crosslinked networks from the blends with borate.

ATR-FTIR spectra were measured on a Pelkin-Elmer FTIR spectrophotometer Spectrum BX-II in the range 4000-700 cm^{-1} with the sum of 50 scans at a resolution of 4 cm^{-1}.

2.3.2 Thermal Analysis (TGA)

The thermal properties of the prepared films were analyzed by thermogravimetric analysis (TGA). TGA was performed from 30°C to 600°C at a rate of 10°C/min with a Perkin Elmer Diamond TG/DTA instrument in N_2 atmosphere and Al sample pans.

2.3.3 Scanning Electron Microscopy (SEM)

The surface morphology of the films obtained was assessed by scanning electron microscopy (SEM). The images were obtained using an accelerating voltage of 10 kV. SEM photographs were taken at different magnifications (in the range of 1000x and 10000x) by using FEI Quanta 250 FEG in the Laboratories of Materials Research Center of İzmir Institute of Technology.

2.3.4 Crystallinity by X-Ray Diffraction (XRD)

X-ray diffraction (XRD) patterns of the hydrogels were recorded on a Philips X" Pert Pro X-Ray diffractometer using Cu K_α radiation at 45 kV and 40 mA. The

scanning scope of 2θ was range from 5 to 40º. These analysis were carried out at the Laboratories of Materials Research Center of İzmir Institute of Technology.

2.3.5 Atomic Force Microscopy (AFM)

The surface friction characteristics of synthesized films were analyzed by atomic force microscope (AFM) using MMSPM Nanoscope IV in the Laboratories of Materials Research Center of İzmir Institute of Technology.

It was equipped with an E scanner which possesses the maximum xy range of 15.0 µm. The topographies were obtained at the scan size of 2.0 µm and the scan frequency of 1.2 Hz by a silicon nitride cantilever.

2.3.6 Swelling Tests

Swelling experiments were conducted with films in distilled water at 25°C and in phosphate buffer (pH=7.4) at 37°C also in KCl/HCl buffer (pH=1.2) at 37°C during which the polymer attained equilibrium swelling. The amount of water kept in the films was determined with the following equation:

$$S\% = \frac{Ms - Md}{Ms} \times 100 \qquad (2.1)$$

where M_s and M_d symbolize the weight of swollen and dried samples.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared (FTIR) Spectra of the Samples

ATR-FTIR spectroscopy technique was used to characterization of the crosslinked hydrogels with borate. Figure 3.1 shows the FTIR spectra of CS/PVA/Borate.

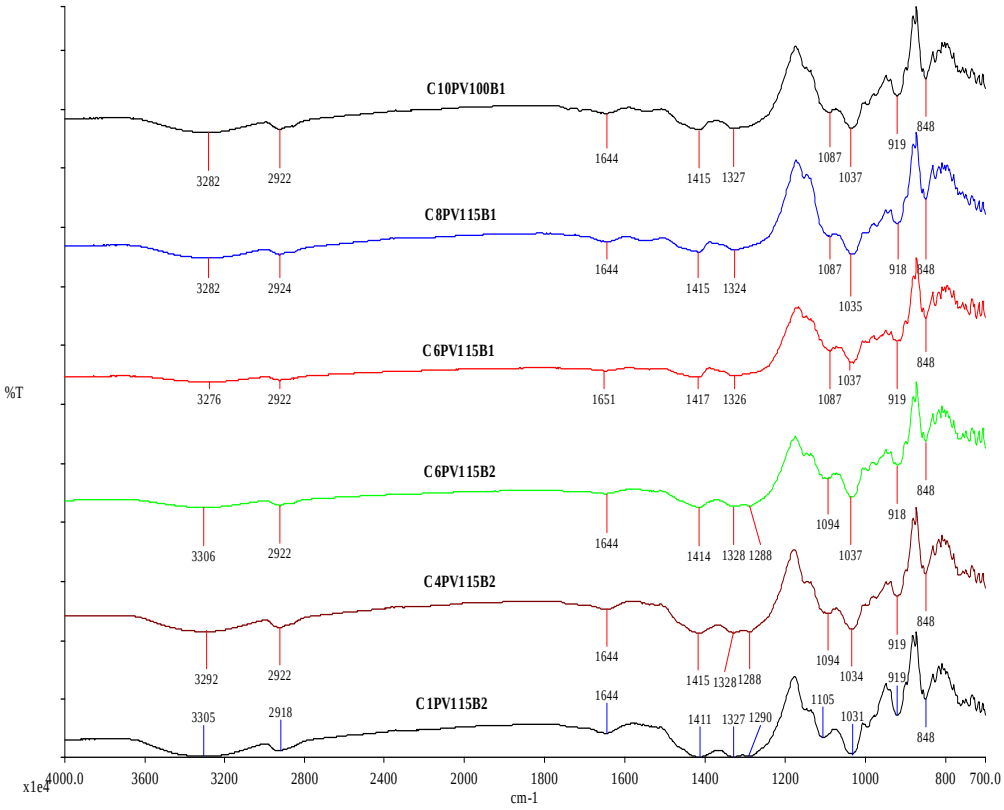


Figure 3.1 FTIR spectra of CS/PVA/Borate hydrogel films

The spectrum of chitosan shows the characteristic absorption bands at 1650 cm^{-1} (amide I) 1556 cm^{-1} (amide II), and 1410 cm^{-1} (-CH_2 bending). The absorption bands at 1140 cm^{-1} (asymetric stretching of the C-O-C bridge), 910 and 1056 cm^{-1} (C-O stretching) are characteristics of its saccharine structure (Marchessault, Ravenelle & Zhu, 2006). The extensive band at 3286 cm^{-1} is caused by the overlapped stretching

of hydrogen bond bounded –OH and $–NH_2$. The stretching of its $–CH_2$ appears at 2921 cm^{-1} (Liang, Liu, Huang & Yam, 2009)

PVA has characteristic bands at 1648 cm^{-1} (C=C vibrations), 3500-3000 cm^{-1} (-OH stretching), 1084 cm^{-1} (C-O-C vibrations), 893 cm^{-1} (C-O symetric stretching) (Abdelrazek, Elashmawi & Labeeb, 2010)

According to FTIR spectra, the shapes and positions of most bands are exhibited similarly. While FTIR spectrum of films were compared with chitosan film spectrum, intensity of the amide I band at 1650 cm^{-1} and the amide II band significantly decreased. The center of the peak located at 3286 cm^{-1} for samples is corresponding to the overlapped stretching of hydrogen bond bounded –OH and $–NH_2$. The stretching of its $–CH_2$ appears at 2921 cm^{-1}. Unexpectedly, both location and density of these two absorbance bands show no obvious changes even though there is a cross-linking reaction between hydroxyl groups in PVA chains and borate molecules.

3.2 Thermogravimetric Analysis (TGA)

Table 3.1 Results of thermogravimetric analysis

Sample	First Stage		Second Stage		Third Stage		Forth Stage	
	T (^oC)	Mass Loss%	T (^oC)	Mass Loss%	T (^oC)	Mass Loss%	T (^oC)	Mass Loss %
C10P100B1	46-102	8.1	158-220	27.6	254-292	17.3	383-419	13.5
C8P115B1	52-102	5.3	170-238	45.5	264-287	10.6	385-407	13.5
C6P115B1	-	-	169-244	30.9	257-309	28.8	385-405	16.4
C6P115B2	63-104	7.9	175-228	23.8	257-285	15.2	383-405	23.3
C4P115B2	37-96	5	158-235	37	-	-	380-398	12
C1P115B2	-	-	154-216	37.9	-	-	387-405	48.7

TG and DTA analyzes were performed to determine the thermal stability of synthesized films. Figure 3.2 and Figure 3.3 show the TGA and DTG curves of

films. According to TGA curves, we can see that the degradation of films are different.

Two degradation stages was observed in the measurements taken in several films, some of the degradation on the three steps and others on the four steps is completed. The first step of decomposition of all of the films was relative to physically adsorbed water. The decomposition of C10P100B1 was in the range 46-102, 158-220, 254-292 and 383-419°C with a mass losses of 8.1%, 27.6%, 17.3% and 13.5% respectively for the first, second, third and fourth stages. C8P115B1 decomposed in four steps too. The first and second stage decomposition was in the range 52-102, and 170-238°C with a mass losses of 5.3% and 45.5% and the third and fourth stage decomposition was in the range 264-287, and 385-407°C with a mass losses of 10.6% and 13.5%, respectively. C6P115B1 decomposed in three steps. The first decomposition was in the range 169-244°C with a mass losses of 30.9% , the second decomposition was in the range 257-309°C with a mass losses of 28.8% and the third decomposition was in the range 285-405°C with a mass losses of 16.4%. The first, second, third and fourth decomposition of C6P115B2 was in the range 63-104, 175-228, 257-285 and 383-405°C with a mass losses of 7.9%, 23.8%, 15.2% and 23.3%. The decomposition of C4P115B2 was in the range 37-96, 158-235, and 380-398°C with a mass losses of 5%, 37%, and 12% respectively for the first, second and third stages. C1P115B2 decomposed in two steps unlike others. The first and second decomposition was in the range 154-216°C and 387-405°C with a mass losses of 37.9% and 48.7%. All these are summarized in the Table 3.1.

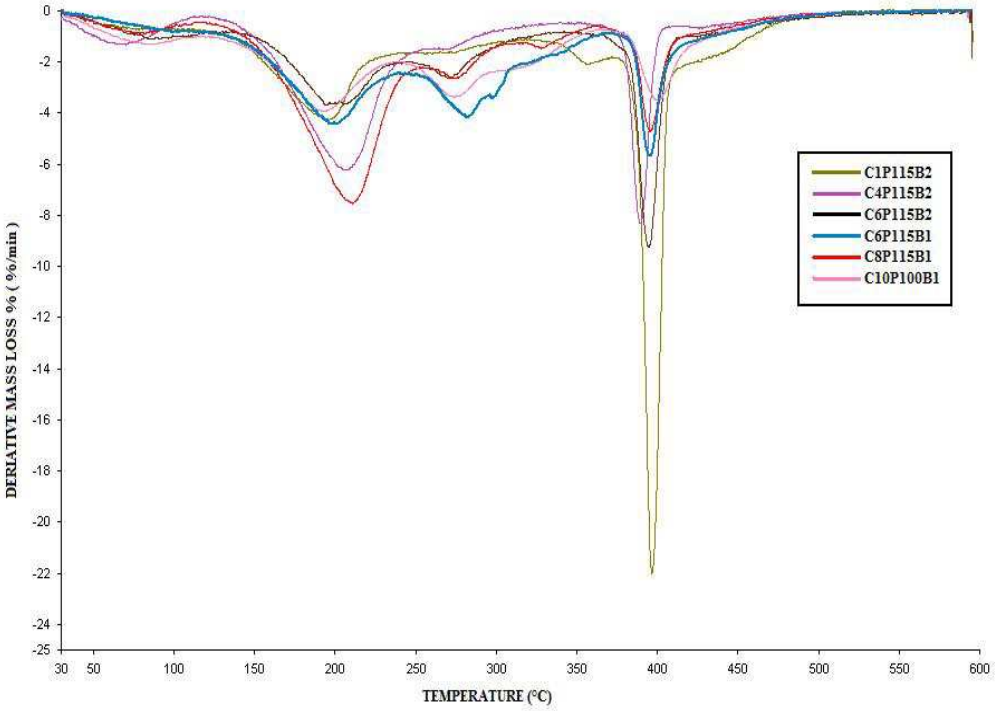


Figure 3.2 DTG curves of films

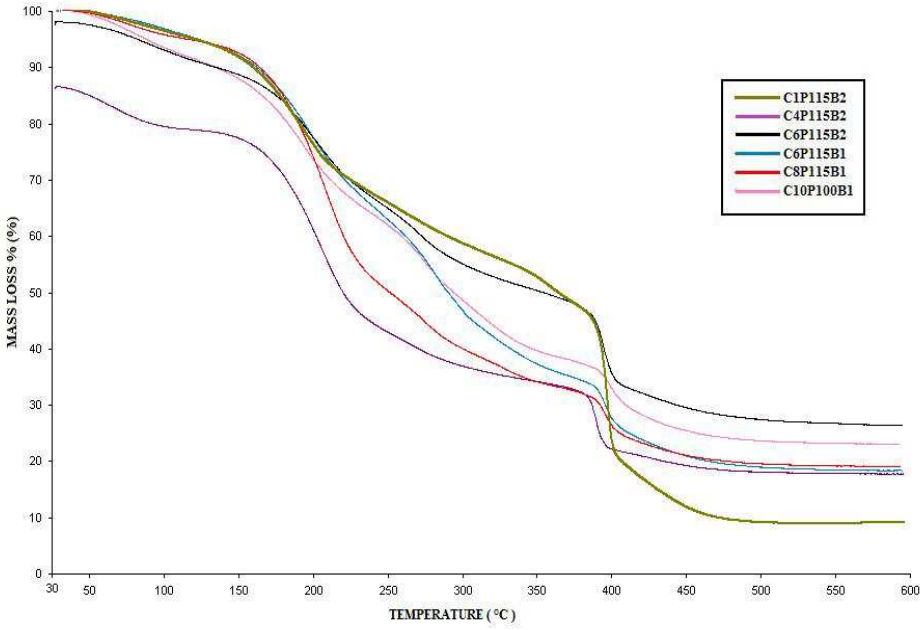
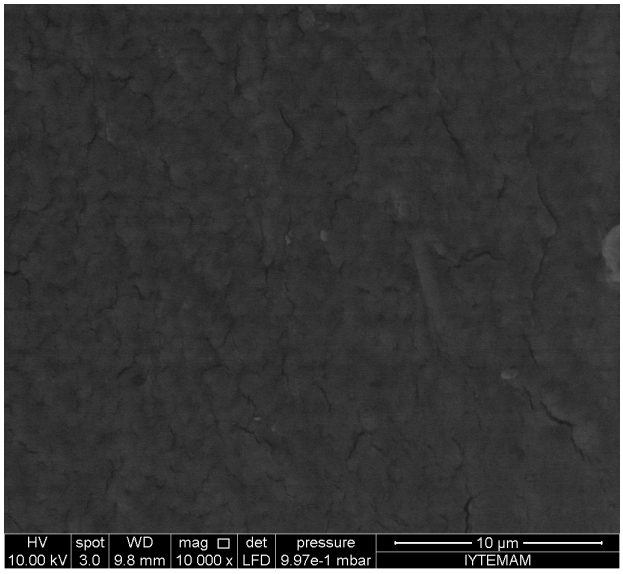


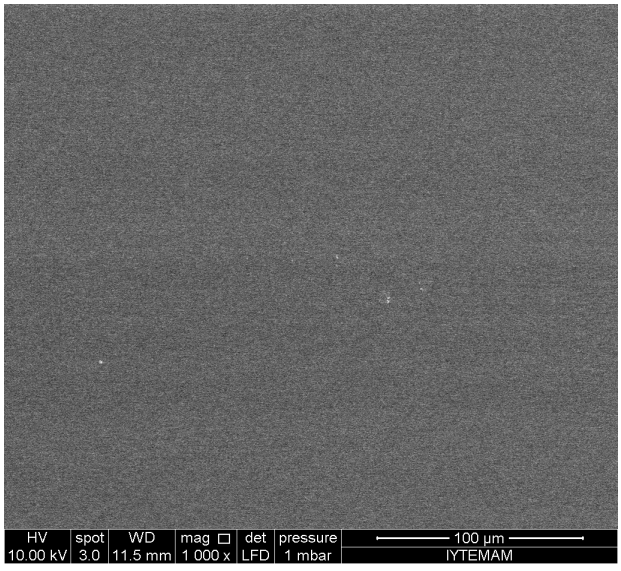
Figure 3.3 Thermogravimetric curves of films

3.3 Scanning Electron Microscopy Analysis (SEM)

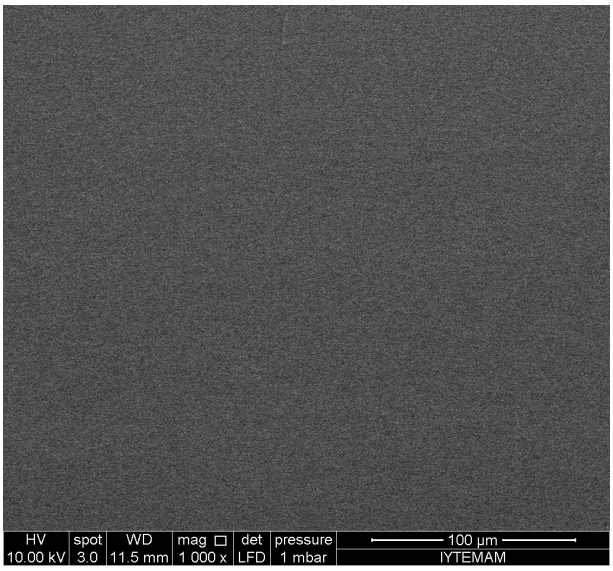
The surface morphologies of hydrogels were viewed with scanning electron microscopy (SEM). SEM micrographs were represented in Figure 3.4 and Figure 3.5. Surfaces of hydrogels are homogeneous as shown Figure 3.4. Although all the surface appearance of hydrogels are homogeneous; one of the hydrogel's surface which is the lowest ratio of chitosan, is observed cracks (Figure 3.4.a).



(a)



(b)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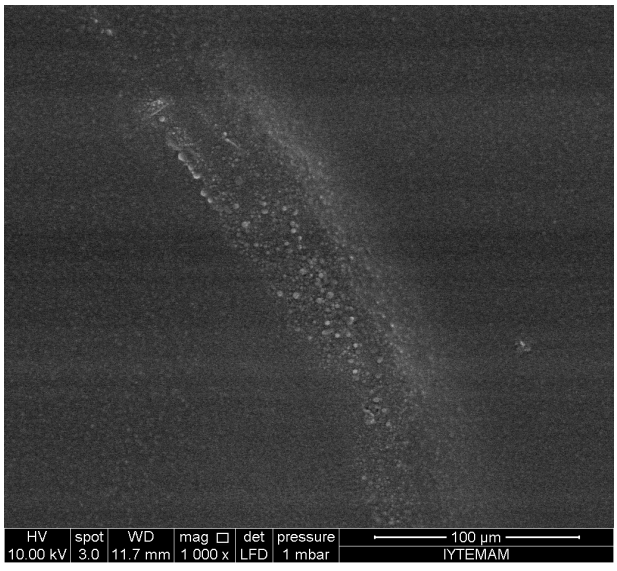
100 µm
IYTEMAM

(c)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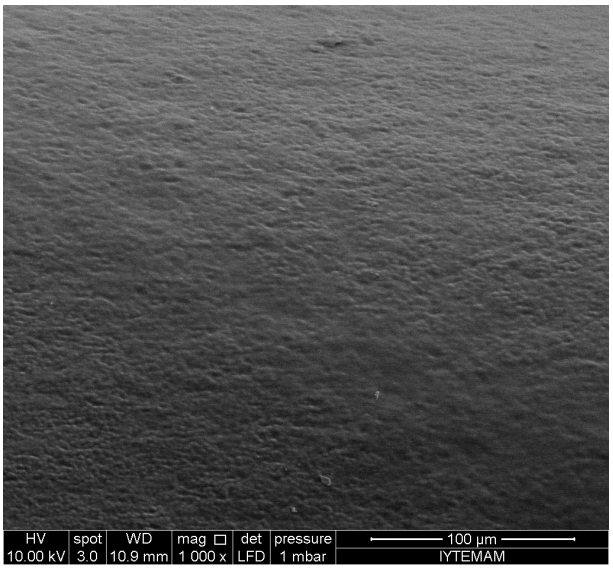
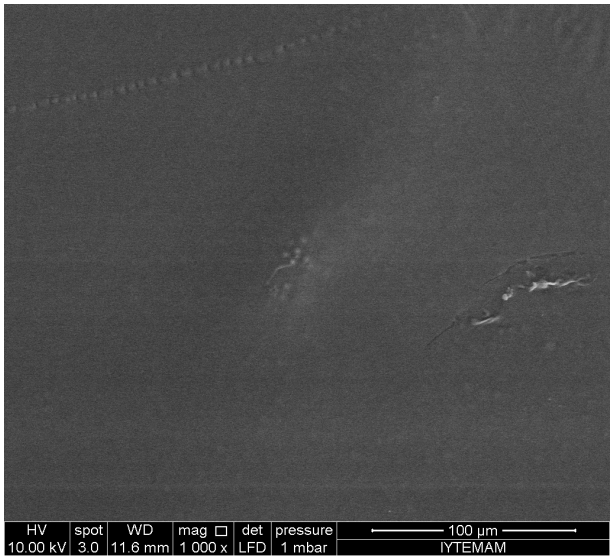
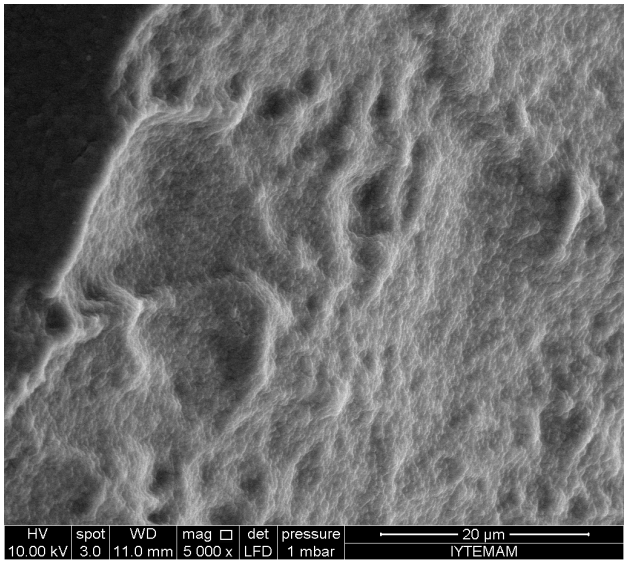


(e)



(f)

Figure 3.4 SEM images of surface of hydrogels; a) C1PV115B2, b) C4PV115B2, c) C6PV115B2, d) C6PV115B1, e) C8PV115B1, f) C10PV110B1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1 mbar
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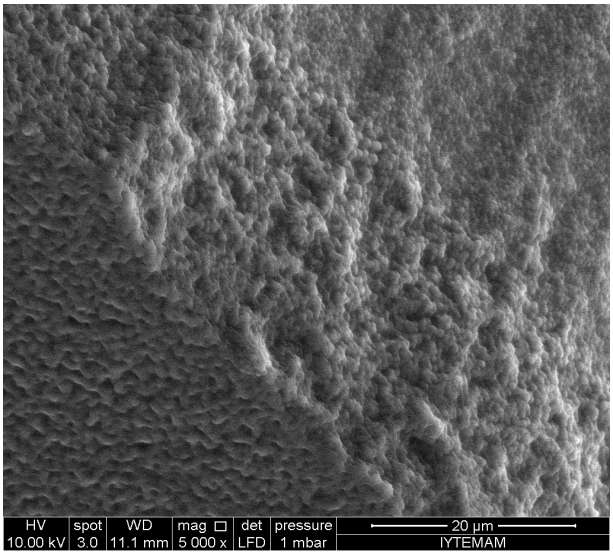
(a)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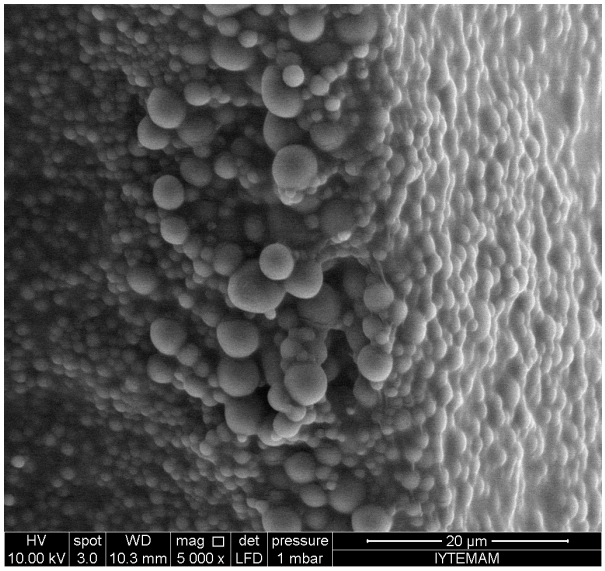
(b)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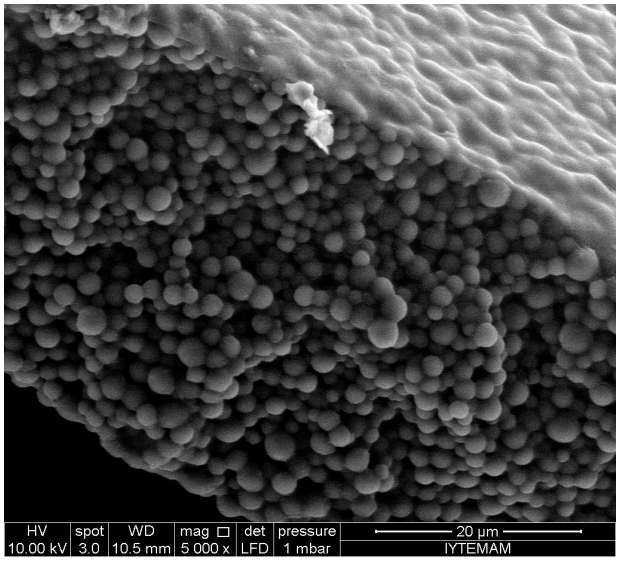
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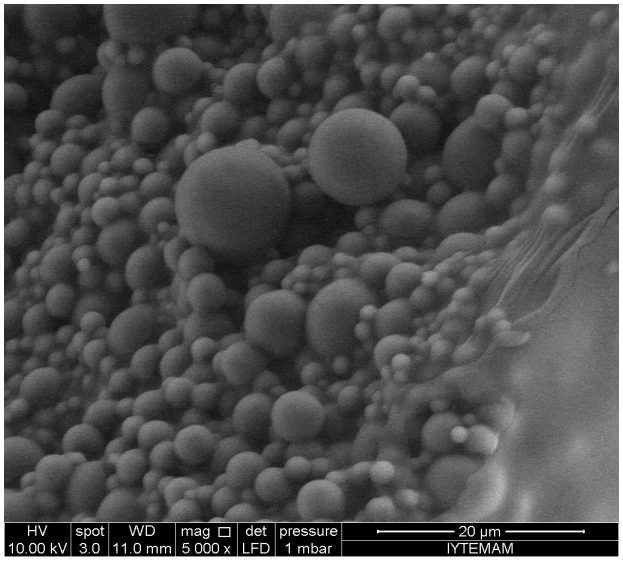
(d)



(e)

Figure 3.5 Cross-sectional SEM images of hydrogels; a)C4PV115B2, b)C6PV115B2, c)C6PV115B1, d)C8PV115B1, e)C10PV110B1

Spherical structures are observed on cross-sectional images of the hydrogels (Figure 3.5). The significance and size of these structures are increasing as the rate of chitosan. C6PV115B1, C8PV115B1 and C10PV110B1 (Figure 3.5.c,d,e) have also very clear and distinct spherical structures, while C4PV115B2 and C6PV115B2 (Figure 3.5.a,b) have less and not obvious spherical structures. The amount of chitosan is same in C6PV115B2 and C6PV115B1 (Figure 3.5.b,c), while the amount of borate is different. Although the lower amount of borate in the C6PV115B1 (Figure 3.5.c), spherical structures are observed more pronounced.

3.4 X-Ray Diffraction (XRD) Analysis

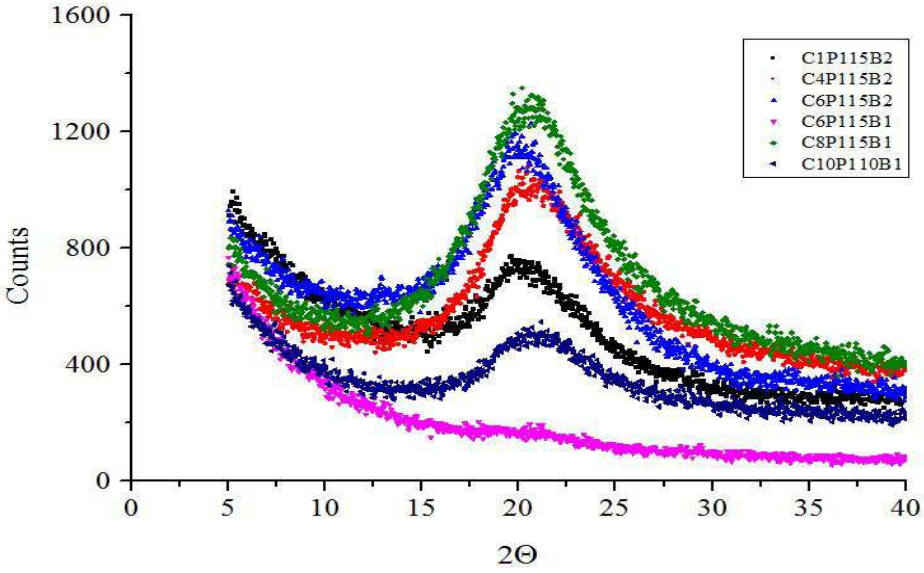


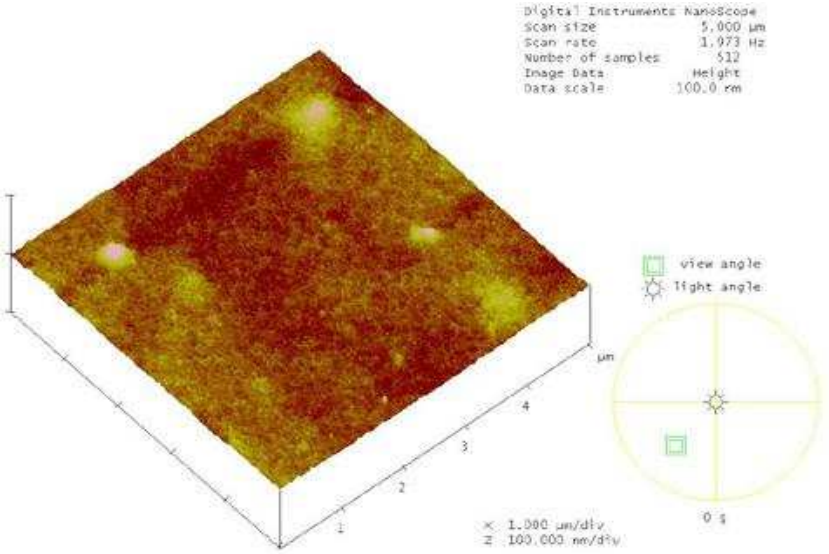
Figure 3.6 XRD patterns of gels

The crystallographic structure of Chitosan and hydrogel films were determined by XRD and shown in Figure 3.6. It can be said that all of the films had amorphous structure. The broad peak at 2θ=20° reflects the structure of chitosan. Diffraction patterns of PVA were analyzed based on the monoclinic unit cell (Bunn, 1948) and patterns of chitosan on the orthorhombic and monoclinic unit cell (Clark & Smith, 1936; Mazeau, Winter, & Chanzy, 1994; Ogawa, Yui, & Okuyama, 2004). Chitosan sample shows a characteristic peak at 2θ=20° (Wang & Hon, 2005). In generally, for the Chitosan diblock copolymer, the peak associated with Chitosan at about 20° decreased. This result indicated that crystalline structure has been disrupted to a great extent by the chemical bond between Chitosan and other polymer structure in the diblock copolymer (Kong et al., 2010).

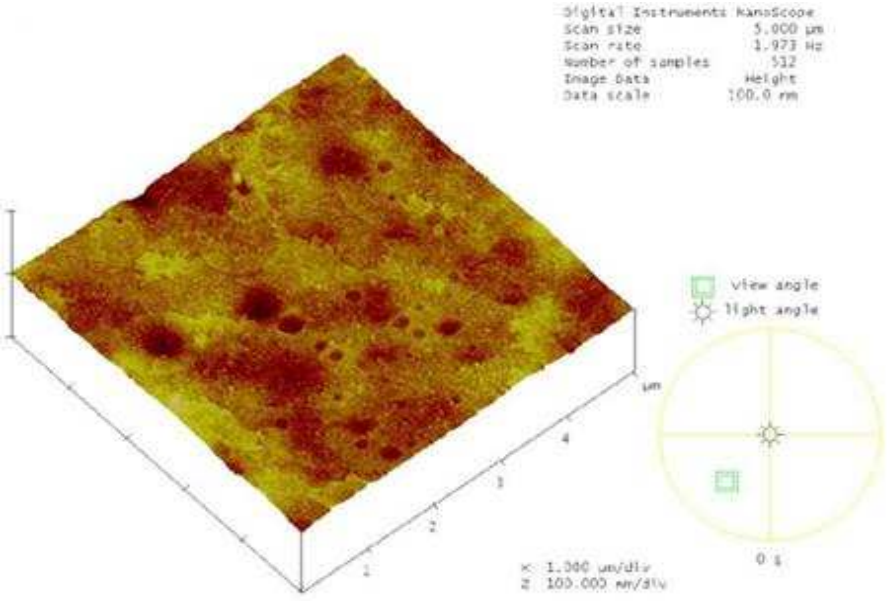
Figure 3.6 shows the diffractogram of films. According to diffractogram; the intensity of the characteristic peak of Chitosan at 20° was increased, except C6P115B1. Chitosan and crosslinked Chitosan yield similar XRD patterns but the cross linked Chitosan shows higher and sharper crystalline peaks. Chitosan is

crosslinked with PVA and boric acid there is a change in the crystalline structure. The crystallinty of Chitosan hydrogel film increased with crosslinking reaction.

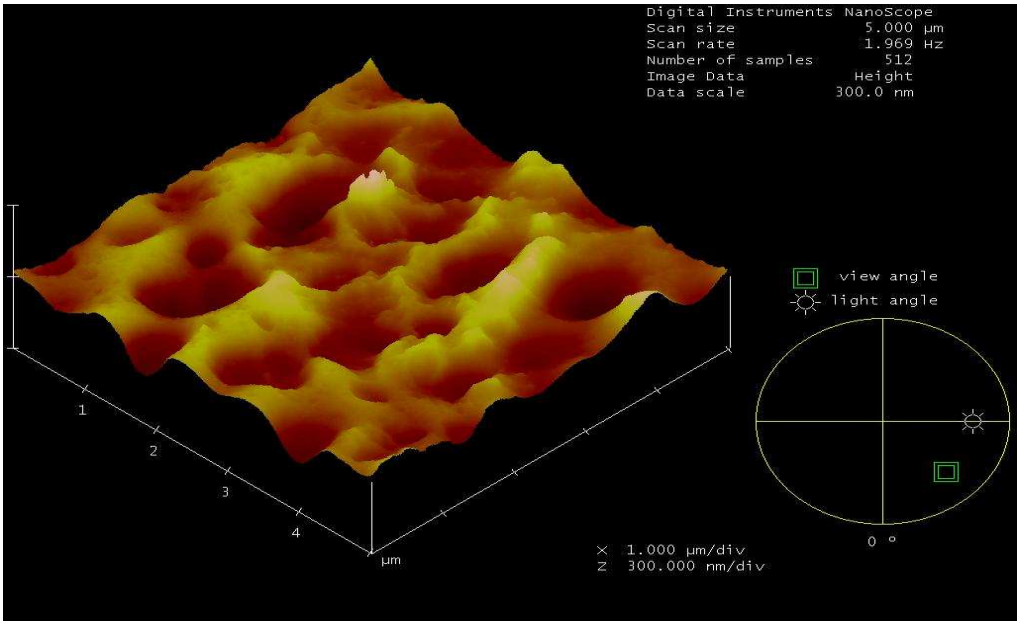
3.5 Atomic Force Microscopy (AFM) Analysis



(a)



(b)



(c)

Figure 3.7 AFM images a) Pure chitosan b) Pure PVA c) C10PV100B1

The AFM images of pure chitosan, pure PVA and their blends (C10PV100B1) are shown in Figure 3.7. As can be seen, the AFM images show differences in surface properties of homopolymer films and their blends. The chitosan and PVA films have relatively smooth and flat surfaces, whereas their blends show two separated phases. This observation agrees very well with the results of thermal analysis. The values of the *Rms* surface roughness is 38.112 nm.

3.6 Swelling Test Results

Swelling behavior of the gels was measured at different temperatures and different pH values (pH=1.2 and pH=7.4 at 37°C; in distilled water at 25°C). Then, swelling (S%) was calculated with equation 2.1. these results showed us, gels swollen up to at pH=1.2 most less than at pH=7.4.

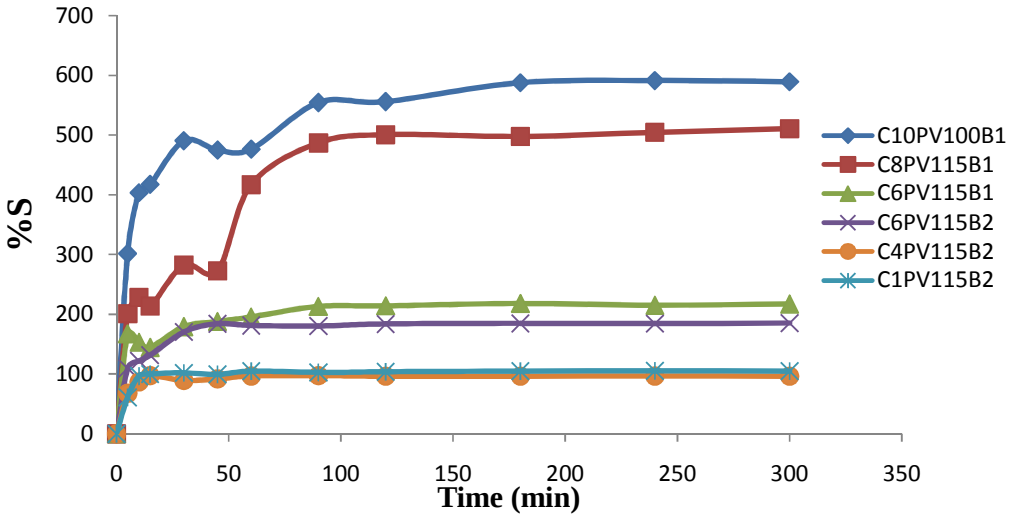


Figure 3.8 Swelling degree of gels in distilled water at 25°C

Figure 3.7 shows that swelling of gels in distilled water at 25°C. Swelling values of C1PV115B2 and C4PV115B2 are too close to each other, from about 60% to 100%. Likewise, swelling volume of C6PV115B2 and C6PV115B1 are close to each other, from about 109% to 213%. But; C8PV115B1, C10PV100B1 are different from them and quite high. Maximum swelling value is 500% for C8PV115B1 while maximum swelling value is 587% for C10PV100B1.

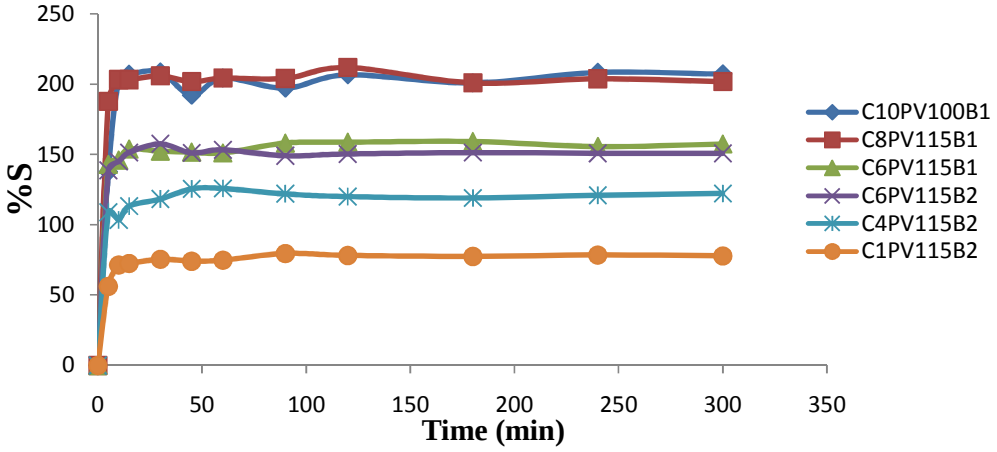


Figure 3.9 Swelling degree of gels in phosphate buffer (pH=7.4) at 37°C

We can see swelling behaviour of gels in Figure 3.8. According to this maximum swelling value of C8PV115B1 and C10PV100B1 are the same and 211%. Swelling

volume of C6PV115B2 and C6PV115B1 are too close to each other, as in the other solutions, from 138% to 159%. Swelling value of C4PV115B2 is slightly lower than them. It's swelling value from 108% to 120%. Finally, C1PV115B2 has a value of the lowest swelling in the phosphate buffer, from 56% to 77%.

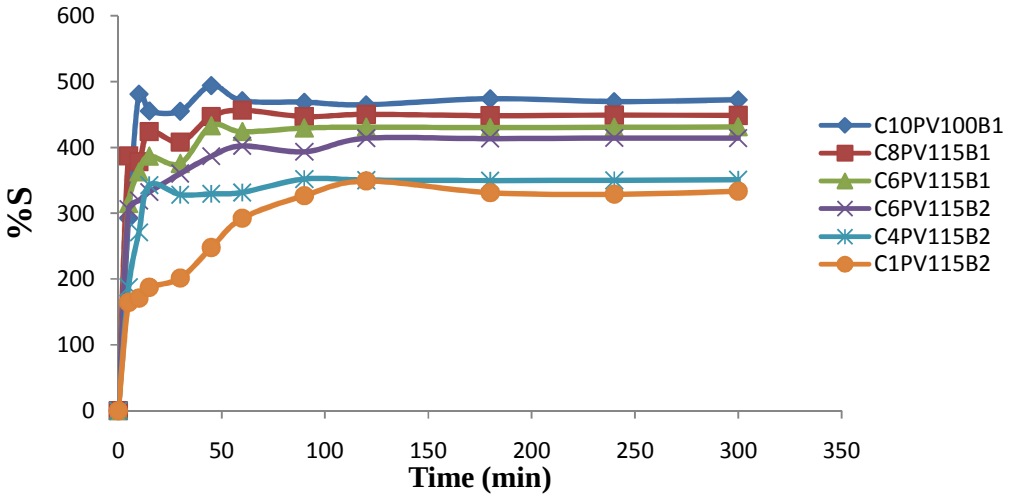


Figure 3.10 Swelling degree of gels in KCl/HCl buffer (pH=1.2) at 37°C

All gels have very high swelling values in KCl/HCl buffer. Even all gels, except C10PV100B1 and C8PV115B1 were reached to the high swelling values. Swelling value of C10PV100B1 is from 306% to 473%, C8PV115B1 is from 386% to 448%, C6PV115B1 is from 306% to 430%, C6PV115B2 is from 306% to 413%, C4PV115B2 is from 187% to 349% and C1PV115B2 is from 187% to 331%.

CHAPTER FOUR
CONCLUSION

Cross-linked hydrogels, the subject of many studies that are used in various biomedical applications as drug delivery. Hydrogels are flexible, powerful, not easy-degradable and biocompatible materials. These properties make them quite convenient structures for these applications.

In this study, cross-linked hydrogels synthesized with Chitosan/PVA/Boric acid, for the optimization of gels, ingredients used at different rates. Also, they were characterized by FTIR, SEM, XRD, AFM and thermal methods (TGA/DTG).

The FTIR spectra of samples were shown in Figure 3.1. As shown in the spectra of all the samples had similar bands.

Thermal stability of gels measured by TG/DTA and the results are shown in Figure 3.2 and 3.3. Pure chitosan is degraded in two steps but some synthesized films were degraded in three steps and some of them were degraded in four steps. According to the thermograms, thermal stabilities of gels were increased by the crosslinking of chitosan.

The surface morphology of the samples was investigated by SEM analysis. The SEM images were shown in Fıgure 3.4 and 3.5. The surface morphologies of all samples were homogeneous and the spherical structures were observed on sections of them. Spherical structures was found more specific and clear as increase amount of chitosan on cross section images.

XRD results were shown in Figure 3.6. Pure chitosan had a characteristic peak at $2\theta=20°$. The same peak was also observed for the synthesized gels, but the intensity of the peak was much more smaller due to the crosslinking. A broad peak centered at $2\theta=20°$ was dominant and indicated amorphous structure.

The Swelling behavior of gels were shown in Figure 3.8, 3.9 and 3.10. Swelling ratio was increased with increasing chitosan amount. In addition, swelling ratio in KCl/HCl was found higher than in distilled water and in PBS.

The synthesized chitosan/borate/PVA films may be used as network structures, support or medium for a drug delivery systems due to their swelling properties.

REFERENCES

Abdelrazek, E. M., Elashmawi, I. S., & Labeeb, S., (2010). Chitosan filler effects on the experimental characterization, spectroscopic investigation and thermal studies of PVA/PVP blend films. *Physica B, 405,* 2021–2027.

Aranaz, I., Mengibar, M., Harris, R., Panos, I., Miralles, B., Acosta, N., Galed, G. & Heras, A., (2009). Functional characterization of chitin and chitosan. *Current Chemical Biology, 3,* 203-230.

Arvanitoyannis, I., Kolokuris, I., Nakayama, A., Yamamoto, N., & Aiba, S. (1997). Physico-chemical studies of chitosan-poly(viny1 alcohol) blends plasticized with sorbitol and sucrose. *Carbohydrate Polymers, 34,* 9-19.

Ausar, S. F., Passalacqua, N., Castagna, L. F., Bianco, I., & Beltramo, D. M. (2002). Growth of milk fermentative bacteria in the presence of chitosan for potential use in cheese making. *International Dairy Journal, 12,* 899–906.

Bunn, C. W. (1948). Crystal structure of polyvinyl alcohol. *Nature, 161,* 929–930.

Chen, S. C., Wu, Y. C., Mi, F. L., Lin, Y. H., Yu, L. C., & Sung. H. W. (2004). A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. *Journal of Controlled Release, 96,* 285-300.

Clark, G. L., & Smith, A. F. (1936). X-ray diffraction studies of chitin, chitosan and derivatives. *The Journal Physical Chemistry A, 40,* 863–879.

Crini, G. (2006). Non-conventional low-cost adsorbents for dye removal: a review. *Bioresource Technology. 97,* 1061–1085.

Crisp, J. D. (1946). *US Patent 258, 193.*

De, S. J., & Robinson, D. J. (2003). Polymer relationships during preparation of chitosan-alginate and poly(L-lysine)-alginate nanospheres. *Journal of Controlled Release, 89,* 101-112.

Deuel, H. & Neukom, H. (1949). Über die reaktion von borsäure und borax mith polysacchariden und anderen hohmolekularen polyoxyverbindungen. *Makromol Chem, 3,* 13-30.

Dutta, P. K., Dutta J., & Tripathi, V. S. (2004). Chitin and chitosan: chemistry, properties and applications. *Journal of Scientific & Industrial Research, 63,* 20-31.

Gholap, S. G., Jog, J. P., & Badiger, M. V. (2004). Synthesis and characterization of hydrophobically modified poly(vinyl alcohol) hydrogel membrane. *Polymer 45(17),* 5863-5873.

Harada, A., Takagi, T., Kataoka, S., Yamamoto, T., & Endo, A. (2011). Boron adsorption mechanism on polyvinyl alcohol. *Adsorption 17,* 171–178.

Hirano, S. (1997). N-acyl, N-arylidine and N-alkylidine chitosans and their hydrogels. *Chitin Handbook,* 71-76.

Hodge, R. M., Bastow, T. J., Edward, G. H., Simon, G. P., & Hill, A. J. (1996a) Free volume and the mechanism of plasticization in water swollen poly(viny1 alcohol). *Macromolecules, 29,* 8137-8143.

Hodge, R. M., Edward, G. H., & Simon, G. P. (1996b) Water absorption and states of semicrystalline poly(viny1 alcohol) films. *Polymer, 37,* 1371-1376.

Hudson, S. M., & Smith, C. (1998). Polysaccharide: chitin and chitosan: chemistry and technology of their use as structural materials. *Biopolymers From Renewable Resources,* 96-118.

Islam, A., Yasin, T., Bano, I., & Riaz, M. (2012). Controlled release of aspirin from ph sensitive chitosan/poly(vinyl alcohol) hydrogel. *Journal of Applied Polymer Science, 124*, 4184–4192.

Jin, R., Teixeira, L. S. M., Dijkstra, P. J., Karperien, M., Van Blitterswijk, C. A., & Zhong, Z. Y., J. Feijen, (2009). Injectable chitosan-based hydrogels for cartilage tissue engineering. *Biomaterials, 30*, 2544-2551.

Kao, W. C., Wu, J. Y., Chang, C. C., & Chang, J. S. (2009). Cadmium biosorption by polyvinyl alcohol immobilized recombinant escherichia coli. *Journal of Hazardous Materials, 169,* 651–658.

Khoo, K. M., & Ting, Y. P. (2001). Biosorption of gold by immobilized fungal biomass. *Biochemical Engineering Journal, 8,* 51–59.

Kim, S. S., Lee, Y. M., & Cho, C. S. (1995). Synthesis and properties of semi-interpenetrating polymer networks composed β-chitin and poly(ethylene glycol) macromer. *Polymer, 36,* 4497-4501.

Koga, D. (1998). In Chitin enzymology – Chitinase. *Advanges in Chitin Science*, *3*, 16–23.

Kong, X. Y., Li, X. Y., Wang, X. H., Liu, T. T., Gu, Y. C., Guo, G., Luo, F., Zhao, X., Wei, Y. Q., & Qian, Z. Y. (2010). Synthesis and characterization of a novel MPEG–Chitosan diblock copolymer and self-assembly of nanoparticles. *Carbohydrate Polymers*, 79, 170–175.

Kurita, K., Tomita, K., Ishii, S., Nishimura, S., & Shimoda, K. (1993). β-chitin as a convenient starting material for acetolysis for efficient preparation of N-acetylchitin to oligosaccharides. *Journal of Polymer Science Part A: Polymer Chemistry., 31,* 2393.

Li, Q., Dunn, E. T., Grandmaison, E. W., & Goosen, M. F. A. (1997). Applications and properties of chitosan. In: Goosen MFA(ed) *Applications of chitin and chitosan*, Lancaster, USA: Technomic Publishing Company, 3-29.

Liang, S., Liu, L., Huang, Q., & Yama, K. L., (2009). Preparation of single or double-network chitosan/poly(vinyl alcohol) gel films through selectively cross-linking method. *Carbohydrate Polymers, 77,* 718–724.

Lorand, J. P. & Edwards, J. O. (1959), Polyol complex and structure of the benzeneboronate ıon. *Journal of Organic Chemistry, 24,* 769-774.

Marchessault, R. H., Ravenelle, F., & Zhu, X. X., (2006). *Polysaccharides for drug delivery and pharmaceutical applications.* Washington: *American Chemical Society, ,* 243-259.

Mazeau, K., Winter, W. T., & Chanzy, H. (1994). Molecular and crystal structure of high-temperature polymorph of chitosan from electron diffraction data. *Macromolecules, 27,* 7606–7612.

Muzarelli, R. A. A. (1997). Some modified chitosans and their niche applications. *Chitosan Handbook,* 47-52.

Muzzarelli, R. A. A. (1977). *Chitin.* Oxford, Pergamon Press.

Nishi, C., Nakajima, N., & Ikada, Y. (1995). In vivo evaluation of cytotoxicity of diepoxy compounds used for biomaterial modification. *Journal of Biomedical Material Research, 29,* 829–834.

Ochiai, H., Shimizu, S., Tadokoro, Y. & Murakami, I. (1981). Complex formation between poly(vinyl alcohol) and borate ion. *Polymer, 22,* 1456-1458.

Ogawa, K., Yui, T., & Okuyama, K. (2004). Three D structures of chitosan. *International Journal of Biological Macromolecules, 34,* 1–8.

Rasool, N., Yasin, T. & Akhter, Z. (2008). Synthesis of carboxymethyl-chitosan/acrylic acid hydrogel using silane crosslinker. *e-Polymers, 142,* 1.

Rathke, T. D., & Hudson, S. M. (1994). Review of chitin and chitosan as fiber and thin formers. *Journal of Macromolecular Science-Macromolecular Chemistry & Physics, 34,* 375-437.

Rayner, T. (2006). *Fining and clarifying agents*. Retrieved July 18, 2006, from http://web.archive.org/web/20060616062535/http://makewine.com/makewine/fining.html.

Ruel-Gariépy E. & Leroux J. C., (2006). Chitosan: A naturel polycation with multiple applications. *Polysaccharides for Drug Delivery and Pharmaceutical Applications*, Washington, American Chemical Society, 243–259

Saito, S. & Okuyama, H. (1954). Die Adsorpsion von Kupfer auf Polyvinylalkohol. *Kolloid Zeitschrift, 139,* 150.

Sakurada, I. (1985). *Polyvinyl alcohol fibers*. NewYork. Marcel Dekker,

Sashiwa, H., & Shigemasa, Y. (1999). Chemical modification of chitin and chitosan 2: preparation and water soluble property of N-acylated or N-alkylated partially deacetylated chitins. *Carbohydrate Polymer, 39,* 127.

Shawky, H. A. (2009). Synthesis of ion-imprinting chitosan/PVA crosslinked membrane for selective removal of Ag(I). *Journal of Applied Polymer Science, 114,* 2608–2615.

Shibayama, M., Sato, M., Kimura, Y., Fujiwara, H. & Nomura, S. (1988). ^{11}B n.m.r. study on the reaction of poly(vinyl alcohol) with boric acid. *Polymer 29,* 336-340.

Speer, D. P., Chvapil, M., Eskelson, C. D., & Ulreich, J. (1980). Biological effects of residual glutaraldehyde in glutaraldehyde-tanned collagen biomaterials. *Journal of Biomedical Material Research, 14,* 753–764.

Tang, Y., Du, Y., Li, Y., Wang, X., & Hu, X. (2009). A thermosensitive chitosan/poly(vinyl alcohol) hydrogel containing hydroxyapalite for protein delivery. *Journal of Biomedical Materials Research Part A, 91,* 953.

Tanigami, T., Shirai, Y., Yamaura, K., & Matsuzawa, S. (1994). Blends between two types of poly(viny1 alcohol)s with different syndiotacticities. *Polymer 35,* 1970-1976.

Tanigami, T., Yano, K., Yamaura, K., & Matsuzawa, S. (1995). Anomalous swelling of poly(viny1 alcohol) film in mixed solvents of dimethylsulfoxide and water. *Polymer 36,* 2941-2946.

Vieira, E. F. S., Cestari, A. R., Airoldi, C., & Loh, W. (2008). Polysaccharide based hydrogels: preparation, characterization, and drug ınteraction behaviour. *Biomacromolecules, 9,* 1195–1199.

Wang, J. W., & Hon, M. H. (2005). Preparation of poly(ethylene glycol)/chitosan membranes by a glucose-mediating process and ın vitro drug release. *Journal of Applied Polymer Science,* 96, 1083–1094.

Wang, L., & Wang, A. (2008). Adsorption properties of Congo Red from aqueous solution onto N,O-carboxymethyl-chitosan. *Bioresource Technology, 99,* 1403–1408.

Wang, M., Qiang, J., Fang, Y., Hu, D., Cui, Y. & Fu, X. (2000). Preperation and properties of chitosan-poly(N-Isopropylacrylamide)semi-IPN hydrogels. *Journal of Polymer Science Part A: Polymer Chemistry*, *38*, 474-481.

Woodmansey, A. (2006). *Chitosan treatment of sediment laden water,* Washington State I-90 Issaquah Project. Federal Highway Administration.

Yen, M. T., Tseng, Y. H., Li, R. C., & Mau, J. L. (2007). Antioxidant properties of fungal chitosan from shiitake stipes. *LWT-Food Science and Technology, 40,* 255–261.

Zhang, C., Ping, Q., Zhang, H., & Shen, J. (2003). Synthesis and characterization of water-soluble o-succinyl-chitosan. *European Polymer Journal*, *39*, 1629-1634.