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Characterization and Performance Improvement of Chitosan Films As Affected by Preparation Method, Synthetic Polymers, and Blend Ratios

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Jiajie Li
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Abstract

Chitosan films prepared with addition of other polymers have been widely studied for their modified properties. In this dissertation, poly (ethylene oxide) (PEO) and poly (N-vinyl-2-pyrrolidone) (PVP) were blended with chitosan. The objectives of the study were (1) to investigate the the effects of film thickness, blend ratios, and preparation methods on the physical, and mechanical properties and functional performance of chitosan/PEO films, and (2) to compare characteristics and functional properties of chitosan/PVP and chitosan/PEO films.

The results demonstrated that regular cast chitosan/PEO films have altered properties than films produced from either polymer alone. Regardless of molecular weight, chitosan decreased the tendency to spherulitic crystallization of PEO. Production of ultra-thin chitosan and chitosan/PEO films with thickness below 80 nm was possible by spin-coating on silicon wafers. The increase of PEO content did not affect thickness of the films but the surface of corresponding films became rougher probably due to formation of PEO crystallites.

Comparing the functional properties of thick, thin and ultra-thin chitosan/PEO films, the latter showed a significantly higher chromium binding capacity compared to the regular cast films. However, ultra-thin chitosan/PEO films did not show significant antibacterial properties due to their extremely low weight. A decreased film-forming time, especially in the spin-coating method, greatly reduced extent of film crystallization.

Incorporation of PVP or PEO into chitosan films reduced the yellowish color and made films easier to puncture and tear. Although chitosan/PEO blend films showed lower water vapor permeability (WVP) values than chitosan/PVP films, blending chitosan with hydrophilic polymers was not an effective way to significantly improve the WVP. Replacing even 50% of chitosan with PVP or PEO in chitosan films did not significantly decrease the metal-binding and antibacterial properties of the films. Since synthetic polymers are less expensive than biopolymer chitosan, blending chitosan and synthetic polymers could reduce the amount of chitosan and lower the production cost with no effect on functionality of the films. Chitosan/PVP and chitosan/PEO blend

films have the potential to be used in the food industry as active packaging materials to inhibit food borne pathogens and as absorbent to bind heavy metal from the environment.

Table of Contents

CHAPTER 1. INTRODUCTION 1
References 5

CHAPTER 2. LITERATURE REVIEW 9
2.1 Chitosan 10
2.1.1 Sources and Production of Chitosan 10
2.1.2 Physicochemical Properties of Chitosan 11
2.1.3 Antimicrobial and Metal Binding Properties of Chitosan 13
2.1.4 Application of Chitosan in Foods 18
2.2 Chitosan Films 21
2.2.1 General Biopolymer-based Films 21
2.2.2 Physical and Mechanical Properties of Chitosan Films 22
2.2.3 Antimicrobial and Metal Binding Properties of Chitosan Films 28
2.2.4 Application of Chitosan Films in the Food Industry 30
2.2.5 Disadvantages of Chitosan Films 31
2.3 Chitosan Co-polymer Films 32
2.3.1 Advantages of Chitosan Co-polymer Films 32
2.3.2 Blend Films from Chitosan and Natural Polymers 33
2.3.3 Blend Films from Chitosan and Synthetic Polymers 35
References 42

CHAPTER 3. EFFECT OF MOLECULAR WEIGHT AND BLEND RATIOS ON PHYSICAL AND MECHANICAL PROPERTIES OF THICK CHITOSAN/PEO FILMS 62
3.1 Introduction 64
3.2 Materials and Methods 65
3.2.1 Materials 65
3.2.2 Rheological Characterization of LMW Chitosan/HMW PEO Blend Solutions 65
3.2.3 Preparation of Thick Chitosan/PEO Films 66
3.2.4 Thickness 66
3.2.5 Film Crystallization 66
3.2.6 Mechanical Properties 66
3.2.7 Water Vapor Permeability 67
3.2.8 Fourier Transform Infrared (FTIR) Spectra 69
3.2.9 Statistical Analysis 69
3.3 Results and Discussions 69
3.3.1 Rheological Property of LMW Chitosan/ HMW PEO Blend Solutions 69
3.3.2 Film Appearance and Thickness 71
3.3.3 Film Crystallization 72
3.3.4 Film Mechanical Properties 74
3.3.5 Water Vapor Permeability 76
3.3.6 FTIR Analysis 78
3.4 Conclusions 79
References 81

CHAPTER 4. EFFECT OF BLEND RATIOS ON PHYSICAL, MECHANICAL AND ANTIBACTERIAL PROPERTIES OF THIN CHITOSAN/PEO FILMS 85
4.1 Introduction 87
4.2 Materials and Methods 88

4.2.1 Materials 88
4.2.2 Film Preparation 88
4.2.3 Thickness 89
4.2.4 Film Crystallization 89
4.2.5 Film Mechanical Properties 89
4.2.6 Water Solubility 90
4.2.7 Water Vapor Permeability 90
4.2.8 Fourier Transform Infrared (FTIR) Spectra 91
4.2.9 Antibacterial property in the liquid system 91
4.2.10 Statistical Analysis 91
4.3 Results and Discussions 92
4.3.1 Appearance and thickness 92
4.3.2 Film Crystallization 92
4.3.3 Film Mechanical Properties 95
4.3.4 Water Solubility 96
4.3.5 Water Vapor Permeability 97
4.3.6 FTIR Analysis 100
4.3.7 Antibacterial property in the liquid system 102
4.4 Conclusions 103
References 104

CHAPTER 5. PRODUCTION AND CHARACTERIZATION OF ULTRA-THIN CHITOSAN/PEO FILMS 106
5.1 Introduction 108
5.2 Materials and Methods 110
5.2.1 Materials 110
5.2.2 Preparation of Ultra-thin Chitosan/PEO Films 110
5.2.3 Thickness 110
5.2.4 Atomic Force Microscope (AFM) 111
5.2.5 Scanning Electron Microscope (SEM) 111
5.2.6 Statistical Analysis 111
5.3 Results and Discussions 112
5.3.1 Appearance and Thickness 112
5.3.2 Surface Morphology 113
5.4 Conclusions 116
References 118

CHAPTER 6. COMPARISON OF FUNCTIONAL PROPERTIES OF THICK, THIN AND ULTRA-THIN CHITOSAN/PEO FILMS 121
6.1 Introduction 123
6.2 Materials and Methods 124
6.2.1 Materials 124
6.2.2 Preparation of Chitosan/PEO Films 124
6.2.3 Thickness 125
6.2.4 X-ray Diffractometer 125
6.2.5 Metal-binding Capacity 126
6.2.6 Antibacterial Property in the Liquid System 126
6.2.7 Statistical Analysis 126
6.3 Results and Discussions 127
6.3.1 Appearance and Thickness 127
6.3.2 Film Crystallization 128

6.3.3 Metal-binding Capacity of the Films 130
6.3.4 Antibacterial Property of the Films 134
6.4 Conclusions 136
References 138

CHAPTER 7. CHARACTERIZATION AND COMPARISON OF CHITOSAN/PEO AND CHITOSAN/PVP FILMS 142
7.1 Introduction 144
7.2 Materials and Methods 145
7.2.1 Materials 145
7.2.2 Preparation of Chitosan/PVP and Chitosan/PEO Films 146
7.2.3 Thickness 146
7.2.4 Film Color 146
7.2.5 Film Crystallization 146
7.2.6 Mechanical Properties 147
7.2.7 Water Vapor Permeability 147
7.2.8 Metal-binding Capacity 147
7.2.9 Antibacterial Property in the Liquid System 148
7.2.10 Statistical Analysis 148
7.3 Results and Discussions 148
7.3.1 Film Color and Appearance 148
7.3.2 Film Crystallization 150
7.3.3 Film Mechanical Properties 152
7.3.4 Water Vapor Permeability 154
7.3.5 Metal-binding Capacity of the Films 156
7.3.6 Antibacterial Property of the Films 158
7.4 Conclusions 160
References 161

CHAPTER 8. CONCLUSIONS 164

VITA 167

List of Tables

Table 2.1 Scientific publications reporting the food applications of chitosan 20

Table 2.2 Scientific publications reporting mechanical properties of chitosan, edible and synthetic films. 24

Table 2.3 Scientific publications reporting water vapor permeability (WVP) of chitosan, edible and synthetic films. 26

Table 2.4 Scientific publications reporting blend films from chitosan and natural polymers.....34

Table 2.5 Scientific publications reporting blend films from chitosan and synthetic polymers.....36

Table 3.1 Thickness of chitosan/PEO blend films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100[a]. 72

Table 3.2 Comparison of water vapor permeability of MMW/MMW and LMW/HMW chitosan/PEO films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100 in the environmental chamber at 25°C and 50% RH, calculated by ASTM E96 method and correction method*. 77

Table 4.1 Thickness of chitosan/PEO blend films with weight ratio from 100/0 to 50/50[a]. 92

Table 4.2 Diffraction angle (2θ) of sharp peaks and relative crystallinity percentage (Xc) of the chitosan/PEO blend films with weight ratio from 100/0 to 50/50. 95

Table 4.3 Puncture strength (PS), tensile strength (TS) and elongation at break (%E) of the chitosan/PEO blend films with weight ratio from 100/0 to 50/50*. 96

Table 5.1 Thickness of ultra-thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50 and pure PEO films*. 113

Table 5.2 The RMS roughness parameters for surfaces of ultra-thin chitosan/PEO films with blend ratios of 100/0, 90/10, 80/20, 50/50 and 0/100. 115

Table 6.1 Thickness and mass per area of thick, thin and ultra-thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50*. 127

Table 6.2 Relative crystallinity percentage (Xc) of the thick and thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50. 129

Table 6.3 Thickness of ultra-thin chitosan/PEO films before and after metal-binding tests*.....131

Table 6.4 Comparison of Cr (VI) binding capacities of thick, thin and ultra-thin chitosan/PEO films*. 131

Table 6.5 Inhibitory effects of thick and thin chitosan/PEO films toward *Escherichia.coli* K12 inoculated in sterile phosphate buffer (0.05 M, pH=7.08) and stored for 6h at 25°C*.....135

Table 7.1 Thickness of and mass per area of chiosan/PVP and chitosan/PEO blend films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100*. 149

Table 7.2 Optical properties of chitosan/ PVP and chitosan/PEO films* 150

Table 7.3 Comparison of water vapor permeability of chitosan/PVP films, chitosan/PEO films and other edible, synthetic films* 155

Table 7.4 Inhibitory effects of chitosan/PVP and chitosan/PEO blend films toward *Escherichia.coli* K-12 inoculated in sterile phosphate buffer (0.05 M, pH=7.08) and stored for 6h at 25°C. 159

List of Figures

Figure 2.1 Chemical structures of chitin and chitosan. 10

Figure 2.2 Electrostatic attraction between NH_3^+ groups in chitosan and $Cr_2O_7^{2-}$ anions. 17

Figure 2.3 Chemical structure of poly (ethylene oxide) (PEO).....38

Figure 2.4 Chemical structure of poly (N-vinyl-2-pyrrolidone) (PVP).....40

Figure 3.1 Schematic diagram of water vapor permeability measurement cup indicating locations of water vapor pressure values and air gap. 68

Figure 3.2 Shear viscosity as a function of shear rate at 25°C of LMW chitosan/HMW PEO solutions with different weight ratio: 100/0, 75/25, 50/50, 25/75, 0/100. 70

Figure 3.3 Photographs of LMW chitosan/HMW PEO blend films of various ratios (100/0, 75/25, 50/50, 25/75, 0/100).....71

Figure 3.4 Polarized micrographs of thick films with LMW chitosan/HMW PEO blend ratios of 100/0, 75/25, 50/50, 25/75, 0/100 with 100X magnification within 24 hours after casting. 73

Figure 3.5 Comparison of (A) puncture strength, (B) tensile strength and (C) elongation at break of MMW chitosan/MMW PEO films and LMW chitosan/ HMW PEO films with blend ratio from 100/0 to 0/100. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$. 75

Figure 3.6 A portion (3500-1500 cm-1) of FTIR spectra of LMW chitosan/ HMW PEO films (A), MMW chitosan/ MMW PEO films (B) at blend ratios of 100/0, 75/25, 50/50, 25/75, 0/100. All spectra are plotted in absorbance mode. 80

Figure 4.1 Photographs of LMW chitosan/HMW PEO blend films of various ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50). 93

Figure 4.2 Polarized micrographs of films with chitosan/PEO blend ratios of 100/0, 90/10, 80/20, 70/30, 60/40, 50/50 with 100X magnification within 24 hours after casting. Scale bar: 200 μm. 93

Figure 4.3 2θ-scan Wide-angle X-ray diffraction(WAXD) patterns of films with chitosan/PEO blend ratio of (A) 100/0, (B) 90/10, (C) 80/20, (D) 70/30, (E) 60/40, (F) 50/50. 94

Figure 4.4 Effect of chitosan/PEO blend ratio from 100/0 to 50/50 on water solubility of the corresponding films. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$. 97

Figure 4.5 Effect of measurement period on water vapor permeability of chitosan / PEO blend ratio from 100/0 to 50/50 in the environmental chamber at 25°C and 50% R.H. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$. 99

Figure 4.6 A portion (3600-1500 cm-1) of FTIR spectra of chitosan/PEO blend films with ratio of 0/100, 50/50, 60/40, 70/30, 80/20, 90/10, and 100/0. All spectra are plotted in absorbance mode. 101

Figure 4.7 A portion (4000-1500 cm-1) of FTIR spectra of chitosan /PEO blend films with ratio: (a) 50/50, (b) 60/40, (c) 70/30, (d) 80/20, (e) 90/10, observing on both top and bottom sides on an identical point. 101

Figure 5.1 A photograph of ultra-thin film on one silicon chip used to measure the thickness. O stands for the places where thickness was measured. 111

Figure 5.2 Photographs of ultra-thin chitosan films prepared by placing 0.5 ml chitosan solutions on the silicon chips and spun using a spin coater at spin speed of 2500 rpm for 60 seconds. The size of film with silicon chip was compared to a quarter. 112

Figure 5.3 Tapping mode AFM two-dimensional images (left) and three-dimensional images (right) of 1 μm scans for ultra-thin chitosan/PEO blend films with ratios of (A) 100/0, (B) 90/10, (C) 80/20, (D) 50/50, (E) 0/100. 114

Figure 5.4 SEM images of 1 μm scans for ultra-thin chitosan/PEO blend films with ratios of (A) 100/0, (B) 90/10, (C) 50/50, (D) 0/100. 117

Figure 6.1 2θ-scan Wide-angle X-ray diffraction (WAXD) patterns of films with thick (a) and thin (b) chitosan/PEO blend ratio of (A) 100/0, (B) 90/10, (C) 80/20, (D) 70/30, (E) 60/40, (F) 50/50. 129

Figure 6.2 X-ray Diffractogram of grazing angle pattern on 50/50 ultra-thin chitosan /PEO films. 129

Figure 6.3 Percentage of Cr (VI) bound by thick, thin and ultra-thin chitosan/PEO films with blend ratio from 100/0 to 50/50 in 25 ml 5 mg/l chromium solution for thick and thin films and in 25 ml 1 mg/l chromium solution for ultra-thin films. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p < 0.05$. 131

Figure 7.1 Photographs of LMW chitosan/HMW PVP blend films of various ratios (100/0, 75/25, 50/50, 25/75, 0/100). 149

Figure 7.2 Polarized micrographs of chitosan, PVP and PEO films (top), chitosan/PVP films (middle), and chitosan/PEO films (bottom) with ratios of 75/25, 50/50, 25/75 with 400X magnification within 24 hours after casting. 151

Figure 7.3 WAXD photographs of chitosan/PVP blend films with ratios of 100/0, 75/25, 50/50, 25/75, 0/100 as well as WAXD photograph of 50/50 chitosan/PEO films as comparison... 151

Figure 7.4 Comparison of puncture strength (A), tensile strength (B) and elasticity (C) of chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 0/100. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$. 153

Figure 7.5 Percentage of Cr (VI) bound by chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 25/75 in 25 ml 10 mg/l chromium solution. Error bars represent standard deviation (n=3). 157

Figure 7.6 Weight of Cr (VI) (mg) bounded by 1 g chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 25/75 in 25 ml 10 mg/l chromium solution. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$. 157

CHAPTER 1. INTRODUCTION

In the last few years there has been a growing interest about the development of natural biopolymers partly due to their renewable, sustainable and biodegradable properties. One such biopolymer, chitosan, is a cationic polysaccharide derived from crustacean seafood wastes, e.g. shells of shrimps, crawfishes and crabs, and has been widely studied in the last two decades (Kumar, 2000; Marsano et al., 2004). Due to the presence of reactive amino as well as hydroxyl groups, chitosan exhibits tremendous functionality including antimicrobial activity (Kendra and Hadwiger, 1984; Sudarshan et al., 1992; Fang et al., 1994; Sekiguchi et al., 1994; Chen et al., 1998; Roller and Covill, 1999; Tsai and Su, 1999; No et al., 2002; Tsai et al., 2004) and metal-binding property (Babel and Kurniawan, 2003; Dutta et al., 2004; Nomanbhay and Palanisamy, 2005; Gamage and Shahidi, 2007). Many attempts have been conducted to develop functional materials from chitosan, such as films, sutures, beads, and hydrogels, and to apply them for wound healing (Ueno et al., 2001), drug delivery systems (Uhrich et al., 1999; Gupta and Kumar, 2000; Jin and Song, 2006; Wang et al., 2007), and metal removal from waste water (Deans and Dixon, 1992; Selmer-Olsen et al., 1996; Kaminski and Modrzejewska, 1997).

In the food industry, chitosan films have been studied for their potential as active packaging material to extend shelf life of food products because of their low permeability of oxygen and antimicrobial effects (Butler et al., 1996; Jeon et al., 2002; Zivanovic et al., 2005; Duan et al., 2007; No et al., 2007; Ye et al., 2008). However, their wide application is limited by their yellowish color, inability to regulate the mechanical properties, and relatively poor water vapor barrier characteristics (Butler et al., 1996).

The films formed by blending of two or more polymers usually result in modified physical and mechanical properties compared to films made of the individual components. It has been reported that starch/chitosan blend films exhibit a higher flexibility and enhanced elongation than films produced of single polymers (Mathew et al., 2006). Blending of natural and synthetic polymers is a possibility for development of new materials. For example, the blend of chitosan and quaternized poly (4-vinyl-N-butyl) pyridine showed stronger tensile strength and breaking

elongation than films of pure chitosan (Liu and Xiao, 2004). Blending chitosan with poly (vinyl alcohol) (PVA) (Bahrami et al., 2003), N-methylol nylon 6 (Shieh and Huang, 1998), polycaprolactone (PCL) (Sarasam et al., 2006), have been reported to improve the properties of pure chitosan films. Poly (ethylene oxide) (PEO) and poly (N-vinyl-2-pyrrolidone) (PVP) have been investigated as additives to chitosan films because they are non-toxic, hydrophilic and biocompatible polymers (Herold et al., 1989; Angelova et al., 1995; Yeh et al., 2006). PEO has been widely used in the pharmaceutical industry for the production of hot-melt extruded capsules (Crowley et al., 2002). PVP has been utilized in a broad range of areas including the food industry (Yeh et al., 2006). Alexeev et al. (2000) have examined the mechanical properties of chitosan/PEO blend films and reported that chitosan/PEO films with weight ratio of 10/2 had improved mechanical properties compared to pure chitosan films. Zhao et al. (1995) reported that the compatibility and morphology of chitosan/PEO blends were closely related to their composition based on their results of thermal behavior and morphology of the blends. Chitosan and PVP were miscible in the blend system (Sakurai et al., 2000) and the blends have been investigated as membranes for direct methanol fuel cell applications (Smitha et al., 2006). Incorporation of PEO or PVP in chitosan films may alter physical, mechanical properties and functional performance of the films depending on their blend ratios and polymer structures. In addition, since synthetic polymers are easily obtained and have low production cost, the blending of natural polymer and synthetic polymer may improve the cost-performance ratio of film products (Fried, 1995).

Chitosan has metal-binding and antimicrobial effects because the cationic amino groups ($-NH_3^+$) can attract negatively charged metal ions or interact with cell surfaces (Tsai and Su, 1999; Qian et al., 2000). Although chitosan films also show significant metal binding and antimicrobial effects, these activities are reduced because the chitosan molecules are "entrapped" within the films and possible crystallization among the polymer molecules could reduce the availability of active amino groups. Studies have reported that increasing the surface area to

mass ratios of chitosan films and fibers could significantly improve their functionalities (Sams et al., 2004; Desai et al., 2008). Decreasing the thickness of the films to the nano-scale could greatly increase the surface area to mass ratios. Physical and chemical properties of ultra-thin chitosan films and films prepared of modified chitosan films have been described in literature (Ligler et al., 2001; Nosal et al., 2005a-b; Murray and Dutcher, 2006). The color change of chitosan, cross-linked chitosan and poly (allyl amine) hydrochloride ultra-thin films upon metal binding have also been reported (Schauer et al., 2003; Rajpa et al., 2006). However, there is no data comparing properties of ultra-thin films with regular cast films produced from chitosan and synthetic polymers.

The study was intended (1) to investigate the effects of film thickness, blend ratios, and preparation methods on the physical, mechanical properties and functional performance of chitosan/PEO films, and (2) to compare the characterization of chitosan/PVP and chitosan/PEO films at different blend ratios. In our study, regular cast films with the thickenss in the range of 50-100 μm were considered as "thick" films while films with the thickness in the range of 20-50 μm were considered as "thin" and "ultra-thin" films had thickness of less than 100 nm.

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CHAPTER 2. LITERATURE REVIEW

2.1 Chitosan

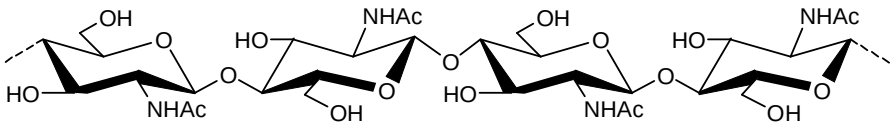
2.1.1 Sources and Production of Chitosan

Chitosan is a natural product derived from chitin, a polysaccharide found in the shells of crustacean, such as shrimp, crab and crawfish, fungal cell walls and insect cuticles (Muzzarelli, 1977; Dutta et al., 2004; No et al., 2007). Chitin is industrially obtained as a by-product from shellfish processing with annual world-wide production of 1.2×10^6 tons (Synowiecki and Ali-Khateeb, 2003). It is the second most abundant natural biopolymer, after cellulose, with estimated annual bioproduction of 10^9-10^{10} tons (Skjak-Break et al., 1989; Peter, 1995; Goosen, 1997).

In the chitin macromolecules, the *N*-acetylglucosamine (GlcNAc) units are combined by β-1, 4 glycosidic linkages and form linear chains (Figure 2.1). The acetamide groups [-NH-($COCH_3$)] at the C-2 positions in chitin molecules could be cleaved to amino (-NH_2) and acetyl groups by concentrated alkali solution to produce chitosan with glucosamine (GlcN) units (Figure 2.1) (Dutta et al., 2004).

Theoretically, chitosan strictly refers to poly (glucosamine); however, since the *N*-deacetylation process is almost never complete, it is also a name for family of partially deacetylated copolymers with different ratios of GlcN and GlcNAc units (Rinaudo et al., 1993).

Chitin



Chitosan

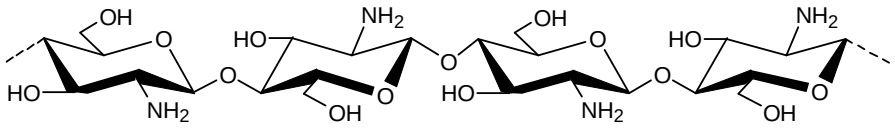


Figure 2.1 Chemical structures of chitin and chitosan.

The degree of deacetylation (DDA), which indicates the content of amino groups in the copolymers (Li et al., 1992), can be used to differentiate chitin and chitosan. In general, chitin with a DDA of above 70% is considered as chitosan (Li et al., 1997a).

Chitosan is conventionally manufactured from crustacean chitin by chemical extraction. The industrial process starts with dried, pulverized crustacean shells and consists of four steps: (1) Demineralization to remove calcium carbonate and calcium phosphate by an acid treatment (0.275-2 M HCl, 0-100°C, 1-48 hours); (2) Deproteinization to remove proteinace materials by an alkali treatment (1 M NaOH, 65-100°C, 1-72 hours); (3) Decoloration to remove pigments by solvent extraction and bleaching (ethanol, acetone, or hydrogen peroxide); and (4) Deacetylation to convert chitin to chitosan by an alkali treatment (40-50% NaOH, 80-150°C, 30 minutes) (Roberts, 1992; No and Meyers, 1997). Chitin and chitosan have been produced commercially in many countries such as India, Poland, Japan, USA, Norway and Australia (Dutta et al., 2004). The market price of chitosan depends of its purity, and the average price in the US is estimated to be $15.43/kg (Babel and Kurniawan, 2003). Estimated worldwide sales of chitin and chitosan for the 1990's were over $ 1.9 billion per year (Knorr, 1991).

2.1.2 Physicochemical Properties of Chitosan

Being from agricultural and marine sources, chitosan is nontoxic, renewable, and ecologically safe (Harish Prashanth and Tharanathan, 2007). Many studies have shown that chitosan is a biodegradable and biocompatible polymer (Hasegawa et al., 1992; Wang et al., 1992; Darmadji and Izumimoto, 1994a; Yin et al., 2006).

The physicochemical properties of chitosan, such as molecular weight, degree of deacetylation, crystallinity and viscosity vary depending on the raw material sources and preparation methods (Sanford, 1989; Li et al., 1992). The temperature and concentration of NaOH solution could affect the removal of acetyl groups from chitin, resulting in the chitosan products with different DDA (Mima et al., 1983; Baxter et al., 1992). Various factors, such as

dissolved oxygen, temperature, and shear stress can cause degradation of chitosan and produce chitosan products with different molecular weight (Li et al., 1992). Chitosan viscosity decreases with an increased time of demineralization (Moorjani et al., 1975). Chitosan prepared from squid pens with parallel chain alignment has weaker intermolecular hydrogen bonding and higher reactivity during deacetylation compared to chitosan prepared from crustacean exoskeletons with antiparallel chain alignment (Shepherd et al., 1997; Tolaimate et al., 2000). Commercial chitosan is generally produced from shrimp and crab shells and has a molecular weight in range between 100 kDa and 1200 kDa (Li et al., 1997a).

Since the only bonds linking GlcNAc and GlcN units are β-1, 4 glycosidic linkages, chitosan has an unbranched structure similar to cellulose. The hydroxyl groups (-OH) and amino groups (-NH_2) easily form intramolecular (O3…O5) and intermolecular (N2…O6) hydrogen bonds resulting in the formation of linear aggregates with crystalline structures (Yui et al., 1994; Harish Prashanth and Tharanathan, 2007). The rigid structure of chitosan molecules and extensive intra and intermolecular hydrogen bonds contribute to the insolubility of chitosan in water at neutral pH. However, in selected aqueous acidic solvents at pH below chitosan's pKa of 6.3 (Sudarshan et al., 1992; Pa and Yu, 2001; Berth and Dautzenberg, 2002), a number of amino groups in the GlcN units can be protonated and dissolve chitosan by forming salt (Muzzarelli, 2002). Organic acids such as acetic, formic and lactic acids are usually used to dissolve chitosan (Nadarajah, 2005). In alkali pH range, chitosan solutions show poor stability due to precipitation and gelation.

Chitosan in aqueous acetic acid solutions generally behaves as typical non-Newtonian shear thinning fluids (Mucha, 1998). Kasaai et al. (2000) have suggested that chitosan (74-80% DDA, 35-2220 kDa) in the 0.25 M acetic acid/0.25 M sodium acetate solution at 25°C behaved as a flexible chain. But the rheological properties of chitosan solutions vary depending on the degree of deacetylation, molecular weight, pH, ionic strength, temperature, and concentration of the solution (Rinaudo et al., 1993; Chen et al., 1994). Pa and Yu (2001) showed that the intrinsic viscosity of chitosan (85% DDA) in aqueous acetic acid solution increased with decreasing pH

value from 3.50 to 1.55 due to the increasing intra-molecular electrostatic repulsion between chitosan molecules with high positive charge density. Matsumoto et al. (1991) reported that 2 wt% of chitosan aqueous solutions with DDA of 62% and 96% were homogeneous whereas that with a DDA of 79% had a certain heterogeneous structure with a long-time relaxation mechanism.

The presence of reactive amino groups and hydroxyl groups enables chitosan to exhibit its functionality. Chitosan has been widely investigated for its binding capacities (Babel and Kuriawan, 2003; Dutta et al., 2004; Nomanbhay and Palanisamy, 2005; Gamage and Shahidi, 2007), and biological activities, such as antimicrobial (Kendra and Hadwiger, 1984; Sekiguchi et al., 1994; Roller and Covill, 1999; No et al., 2002b; Tsai et al., 2004), antitumor (Suzuki et al., 1986; Tokoro et al., 1988), and hypocholesterolemic (Sugano et al., 1992) effect. Due to its solubility in acidic aqueous solutions and high molecular weight, dissolved chitosan can form gels, films, sutures, beads, and fibers to apply these functionalities in the biomedical, food and chemical industries (Hirano et al., 1999; Ghanem and Skonberg, 2002).

2.1.3 Antimicrobial and Metal Binding Properties of Chitosan

2.1.3.1 Antimicrobial Property of Chitosan

The antimicrobial activity of chitosan has been widely researched in recent years. Previous reports have indicated that chitosan inhibits a variety of Gram-positive and Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella* Enteritidis, *Staphylococcus aureus*, *Listeria monocytogenes,* and *Lactobacillus fructivorans* (Papineau et al., 1991; Wang, 1992; Roller and Covill, 2000; Zivanovic et al., 2004), fungi (Allan and Hadwiger, 1979; Stossel and Leuba, 1984; Kendra and Hadwiger, 1984; Hirano and Nigao, 1989; Fang et al., 1994; Roller and Covill, 1999), yeast (Ralston et al 1964; Sudarshan et al., 1992; Roller and Covill, 1999), and viruses (Kochkina et al., 1995; Pospieszny 1997; Chirkov, 2002).

The key factor of chitosan's antimicrobial activity is its positive charge of amino group at C-2 position below its pK_a (pH 6.3). The exact mechanism of the antimicrobial activity of chitosan

is still debatable. Several mechanisms have been proposed to explain the situation when chitosan is directly applied in microbiological media. By one proposed mechanism, polymeric macromolecule chitosan can not pass the outer membrane of bacteria and directly affect the intracellular parts of the cells (Nikaido, 1996). Positive charged chitosan reacts with the negatively charged residues of macromolecules (lipopolysaccharides, proteins) at the cell surface and alters the permeability of the membrane of bacteria, resulting in the leakage of proteinaceous and other intracellular constituents and cell death (Young and Kauss, 1983; Leuba and Stossel, 1986; Fang et al., 1994; Chen et al., 1998; Helander et al., 2001). Several studies have illustrated leakage of glucose, enzymes, and nucleotides from *Escherichia coli* cells during inhibition by chitosan (Tsai and Su, 1999; Helander et al., 2001; Chuang and Chen, 2008). Another suggested mechanism is that oligomeric chitosan could penetrate into the nucleus of a microorganism, binding with DNA and prohibiting mRNA and protein synthesis (Hadwiger et al., 1986). Cuero et al. (1991) proposed that chitosan can selectively bind trace metals and inhibit microbial growth and the production of toxins. Young et al. (1982) suggested that chitosan acted as a water binding agent and inhibited various enzymes. The mechanism most widely accepted relates to damage to the membrane. However, recent reports suggest that the mechanism may be much more complicated because the changes in the hydrophilicity and charge density of the cell surface and changes in the characteristics of chitosan adsorption to the cell wall also need to be considered (Chung et al., 2004).

Antimicrobial activity of chitosan is affected by its molecular weight, DDA, concentration in solution, type and concentration of the acid, other compounds in the system (proteins, lipids, ions), pH of the medium, type of microorganisms, and environment conditions (temperature and relative humidity) (Begin and Calsteren, 1999; Kurita, 2001; Lim and Hudson, 2003; Synowiecki and Al-Khateeb, 2003; Zheng and Zhu, 2003). No et al. (2002b) claimed that chitosan in aqueous solutions with a high DDA (>85%) and a molecular weight of 28 kDa to 1671 kDa showed the strongest antibacterial effects.

Wang (1992) reported that increasing the concentration of chitosan from 0.5% to 2.5% with 2% acetic acid increased the effectiveness against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella* typhimurium, *Staphylococcus aureus*, and *Yersinia enterocolitica*, and chitosan showed stronger inhibition ability at pH 5.5 than at pH 6.5. However, the relationship between chitosan concentration and inhibition efficiency still can not be determined. For example, Papineau et al. (1991) showed that 0.02% and 0.1% chitosan lactate was more effective against *E. coli* O517 than 0.5%. Within 1 hour, chitosan lactate at concentrations of 0.02% and 0.1% reduced the population approximately 4 log cycles, whereas a population drop of only 2 log cycles was observed for 0.5% chitosan lactate. Sudarshan et al. (1992) reported that the antibacterial actions of chitosan glutamate and chitosan lactate were more effective against *Staphylococcus aureus* at 0.01% than 0.2% or 0.4%. Sudarshan et al. (1992) considered that this phenomenon was because the polycationic chitosan at low concentration could probably bind to the negative charged bacterial surface to cause agglutination, while at higher concentration, the large number of positive charges may have imparted a net positive charge to the bacterial surface and kept the bacteria in suspension. The minimum inhibitory concentration (MIC) of chitosan towards different microorganism varies widely (Sudarshan et al., 1992; Chen et al., 1998; Roller and Covill, 1999; Tsai and Su, 1999; Rhoades and Roller, 2000). Furthermore, higher concentrations of chitosan is needed in real food systems than in nutrient broths or buffers because the proteins, lipids, salts, and other constituents of foods could interfere with chitosan activity (Tsai et al., 2000). For the influence of different organic acids used as solvents, acetic acid, formic acid and lactic acid were reported to be more effective in inhibiting bacteria than propionic and ascorbic acids (No et al., 2002b).

2.1.3.2 Metal Binding Property of Chitosan

Heavy metal contamination of various water resources is of great concern to the public because of their toxic effect and accumulation in our environment (Wang and Kuo, 2007). It is

well known that chitosan binds with certain metal ions (Muzzarelli, 1977). The potential applications of chitosan are waste water treatment for heavy metal and radio isotope removal, and potable water purification for reduction of unwanted metals (Onsoyen and Skaugrud, 1990). As metal absorbent, chitosan has been reported for the removal of Hg(II) (Kurita et al., 1979; Ohga et al., 1987), Cu(II) (Wu et al., 1999), Cr(III and VI) (Maruca et al., 1982; Modrzejewska and Kaminski, 1999; Vincent and Guibal, 2001), Ag(I) (Asakawa et al., 2000), Fe(III) (Bhatia and Ravi, 2000), Mo(Vi) (Guibal et al., 1998) and Cd(II) (Jha et al., 1988) from waste water. An adsorption capacity of 5.93 mg Cd(II)/g chitosan was reported at a pH range of 4.0-8.3 (Jha et al., 1988). McKay et al. (1989) found the maximum adsorption capacities of chitosan for Hg(II), Cu(II), Ni(II), and Zn(II) were 815, 222, 164, 75 mg/g chitosan, respectively. The interaction between chitosan and hexavalent chromium was also intensively investigated (Modrzejewska and Kaminski, 1999; Ismael Acosta et al., 2004) and an adsorption capacity of 273 mg Cr(VI)/g chitosan was achieved at pH of 4.0 (Udaybhaskar et al., 1990). It was reported that chitosan performed higher metal binding capacity compared to natural substances such as activated sludge, bark and synthetic polymer poly (4-aminostyrene) (Masri and Friedman, 1974). The use of chitosan for potable water purification has been approved by the United States Environmental Protection Agency (USEPA) up to a maximum chitosan concentration of 10 mg/l (Knorr, 1984).

The amount of metal bound to chitosan can be determined by equilibrating chitosan in a metal ion solution of known concentration, followed by determination of the amount of metal ion left in the solution by atomic absorption spectroscopy (Baba et al., 1996; Kawamura et al., 1997), titration with EDTA (Inoue et al., 1993), UV spectra (Muzzarelli and Tanfani, 1982). X-ray photoelectron spectroscopy (XPS) is an effective method to provide direct information regarding the metal complex absorbed on the chitosan (Dambies et al., 2001).

Most literature has focused on the sorption performance of chitosan while little research has dealth on sorption mechanisms. In general, the amine groups at the C-2 position in chitosan molecules are the main reactive groups. The metal chelating mechanism involves the free

electron doublet on nitrogen binding metal cations with ligands in the solution at pH close to neutrality (Randall et al., 1979; McKay et al., 1989; Peniche-Covas et al., 1992; Vasconcelos et al., 1996). Studies on the chelation mechanisms of chitosan have mainly focused on copper sorption (Schlick, 1986; Chiessi et al., 1992; Rhazi et al., 2002). Domard (1987) considered that chitosan forms a unique complex with copper below pH 6.1. Monterio and Airoldi (1999) confirmed this hypothesis by calorimetric measurements. However, few studies have been focused on binding of chitosan with other metals by chelation mechanisms (Guibal, 2004). Another mechanism is based on ion exchange or electrostatic attraction. The protonated amine groups in acidic solutions enable chitosan to attract metal anions (Qian et al., 2000; Ly Arrascue et al., 2001; Guibal et al., 2001). For example, chromium (VI) forms dichromate anion at pH~4. Dobson and McQuillan (1999) found that sorption of Cr (VI) occurred on amine functional groups of chitosan as shown in Figure 2.2. Other mechanisms proposed for metal ion adsorption of chitosan include hydrogen bonding of the metal sorbate or hydroxylated sorbate to hydroxyl groups of chitosan, and weak van der Waals forces (Boddu et al., 2003).

Sorption of a metal may involve different mechanisms depending on the metal ions, the pH, and the matrix of the solution (Guibal, 2004). The chelation of metal cations by ligands may lead to formation of metal anions, causing the chelation mechanism on chitosan turns to electrostatic attraction mechanism on its protonated amine groups (Guibal, 2004).

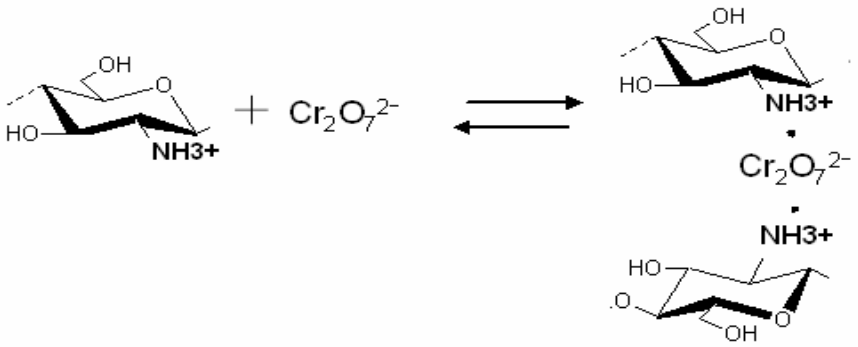


Figure 2.2 Electrostatic attraction between NH_3^+ groups in chitosan and $Cr_2O_7^{2-}$ anions.

Although a number of studies have been conducted on the metal binding capacity of chitosan, it is quite difficult to find similar results even when the experiments were done under similar conditions (metal ions, pH, and sorbent concentration) (Jaworska et al., 2003). This variation is mainly due to the differences in the origin, crystallinity and the DDA of chitosan, which could affect the number of available and accessible sorption sites (Kurita et al., 1979; Miyoshi et al., 1992; Milot et al., 1998; Jaworska et al., 2001). For example, the DDA of chtiosan directly controls the extent of protonation of amine groups in acidic solutions. Yang and Zall (1984) reported that chitosan can chelate five to six times higher amount of metals than chitin. In addition, available free amine groups are more important to be taken into account than the total number of free amine groups. The crystalline regions of chitosan are not accessible to metal ions (Guibal, 2004). Molecular weight of chitosan polymer may be involved in the binding mechanism but with insignificant effect (Milot et al., 1998).

2.1.4 Application of Chitosan in Foods

Many attempts have been conducted to develop functional materials from chitosan. Since chitosan is nontoxic and biofunctional polymer, its effectiveness as a potential food preservative or coating material to enhance quality and shelf life of foods has been studied by numerous researchers. The potential uses of chitosan in food application are various. It has been suggested as an antimicrobial agent against a wide range of foodborne bacteria, fungi and yeast in order to control food-borne pathogens and food spoilage (Sagoo et al., 2002). Chitosan has been proposed as texturing (Manabe et al., 1989), emulsifying (Endo et al., 1990; Hanawa et al., 1990), foaming (Poole, 1989; Noda et al., 1991), and gelling agent (Yamane and Takada, 1991; Hanawa et al., 1991; Fujita and Nishiyama, 1989). Unlike other polysaccharides, chitosan possesses positively ionic charged amino groups in acid systems, which could result in binding with negatively charged proteins and thus serve as a thickener and stabilizer (Filar and Wirick, 1978; Del Blanco et al., 1999). Chitosan coating may offer a protective barrier for moisture

transfer through the food surface, thus reducing the weight loss and keeping texture (Butler et al., 1996; Nadarajah et al., 2006). Chitosan could retard lipid oxidation possibly by chelating the metal ions or by combining with lipids (Xue et al., 1998). Chitosan has also been applied to aid the separation of the colloidal and dispersed particles from food processing wastes (Knorr 1985; No and Meyers, 2000). For example, chitosan was utilized in the clarification of waste water in Japan (Hirano, 1989).

Although some reports indicated that consumers could not differentiate the chitosan-coated foods from the control (Bhale et al., 2003), to some extent, however, the typical bitterness/astringent taste of chitosan limits its use in some foods (Lee and Lee, 2000; No et al., 2007). Lee and Lee (2000) have reported that addition of 0.5% water-soluble chitosan with molecular weight of lower than 10 kDa to milk negatively affected its sensory quality of color, flavor and taste whereas 10-30 kDa chitosan produced the astringent taste.

Chitosan has been approved as a food additive in Japan and Korea since 1983 and 1995, respectively (Weiner, 1992; KFDA, 1995). In Japan, chitosan is used as a preservative in solid food such as noodles, soy sauce, sardines, kamaboko (Li et al., 1997b). In 2005, shrimp-derived chitosan was submitted to the US FDA to be considered as GRAS for use in foods including meat and poultry. However, the US FDA ceased to evaluate the notice, effective October 31, 2005, because the notifier withdrew the application (US FDA/CFSAN 2005). Although the use of chitosan in processed foods is currently limited, many scientific publications have reported the potential applications of chitosan in foods (Table 2.1).

Table 2.1 Scientific publications reporting the food applications of chitosan

Food	Chitosan Application	Effects (compared to control)	References
Bread	Bread coated with 1-2% chitosan (30-493 kDa, DDA>85%)	Less retrogradation; lower total bacterial counts; retarded antioxidation	Park et al., 2002a; Ahn et al., 2003 ; Lee et al., 2002
Egg	Eggs coated with 1-2% chitosan	More desirable albumen and yolk quality; 3-wk longer storage time; consumer acceptance	Lee et al., 1996; Bhale et al., 2003; Caner, 2005
Fruits and vegetables	Strawberry coated with 1, 1.5% chitosan	Reduced the decay of fruit; inhibition of growth of fungi (*Botrytis cinerea*, *Rhizopus stolonifer*); firmer texture, lower respiration rate	El Ghaouth et al., 1991; El Ghaouth et al., 1992a
	Incorporation of calcium or Vitamin E into chitosan coating	Significantly increased the content of these nutrients in strawberries and red raspberries, but reduced the glossiness of strawberries	Han et al., 2004; Han et al., 2005
	Litchi coated with 1,2% chitosan	Delayed the increase in polyphenol oxidase activity	Zhang and Quantick, 1997; Jiang et al., 2005
	Tomato coated with 1,2% chitosan	Reduced the respiration rate and ethylene production; less decay; higher firmness	El Ghaouth et al., 1992b; Kim et al., 1999
Juice	Addition of chitosan solution to fruit juice	Clarifying agent; controlled acidity in fruit juice; prevented enzymatic browning; inhibited growth of spoilage yeasts in apple juice	Chatterjee et al., 2004; Imeri and Knorr, 1988; Sapers, 1992; Roller and Covill, 1999
Meat	Beef, pork dipped into chitosan solution (1%, 30kDa, 120 kDa)	Retarded lipid oxidation; decreased the TBA value of beef by 70%; reduced total viable counts, yeasts, molds and lactic acid bacteria; desirable red color; enhanced storage stability	Darmadji and Izumimoto, 1994a, 1994b; Sagoo et al., 2002; Lee et al., 2003; Juneja et al., 2006
Milk	Addition water-soluble chitosan into milk	Minimized the microbial spoilage of processed milk; but negatively affected its sensory quality	Lee and Lee, 2000; Ha and Lee, 2001
Seafoods & seafood products	Seafood product coated with 1% chitosan	Reduced 50% moisture loss of fillets; delayed lipid oxidation; inhibited the growth of microorganisms	Tsai et al., 2002; Sathivel, 2005
Soybean curd (tofu)	Water-soluble chitosan as a coagulant or as an immersion solution	Increased gel strength; lower turbidity in the immersion solution; strong antibacterial activity against *Bacillus sp.*, *B.cereus*, and *Enterobacter sakazakii*.	Lee et al., 2001; No et al., 2002a ; Chang et al., 2003
Mayonnaise	Addition of chitosan in mayonnaise preparation	Increased emulsifying capacity of egg yolk; emulsion stabilizer; inhibited against both bacteria and yeast in mayonnaise	Lee, 1996; Kim and Hur, 2002; Roller and Covill, 2000

2.2 Chitosan Films

2.2.1 General Biopolymer-based Films

Biopolymer films or edible films are generally designed using biological materials such as polysaccharides (cellulose and derivatives, starch and derivatives, chitosan, carrageenan, alginate, pullulan, pectin, etc.), proteins (gelatin, zein, gluten, whey protein isolates, etc.), and lipids (waxes) (Guilbert et al., 1996). The use of biopolymer films to extend shelf life and improve the quality of fresh, frozen and processed foods has been reviewed (Kester and Fennema, 1986; Labuza and Breene, 1989). Compared to plastic films like those of high density polyethylene films the advantage of biopolymer films is their biodegradable, renewable and nontoxic properties. Many functions of biopolymer films have been widely studied in food applications including controlling moisture transfer between food and surrounding environment, controlling release of chemical agents like antimicrobials and antioxidants, reducing of oxygen permeability, and controlling rate of respiration (Kester and Fennema, 1986; Labuza and Breene, 1989). These properties are dependent on the type of material used. In general, polysaccharide-based and protein-based films have suitable mechanical and optical properties and serve as a good barrier to CO_2 and O_2, but high permeability to water vapor (Park and Chinnan, 1995). In contrast, films composed of lipids have good water vapor barrier properties, but are usually unstable in air, are quite fragile and are subject to rancidity (Guilbert et al., 1996).

Several techniques, including solution casting, thermal gelation, and solidification of melt, have been developed for forming biopolymer films (Cagri et al., 2004). Solution casting method is typically used to produce films. In the process, the biopolymers are dissolved or dispersed in a solvent, such as water, ethanol, or acid solution, which may also contain additives (plasticizers, cross-linking agents, solute), and the film-forming solution is then cast in a thin layer, dried, and peeled from the surface (Cagri et al., 2004). The major mechanism for film formation is that dissolving polymers into the solution breaks apart of polymer segments, and cohesive attractions

between the molecules promote polymer chains to align themselves into a film matrix during water evaporation (Butler et al., 1996; Harris and Ghebre-Sellassie, 1997).

2.2.2 Physical and Mechanical Properties of Chitosan Films

Chitosan films are typically produced by casting of films from a solution composed of 0.5-2% chitosan dissolved in 1-4% organic acid (acetic, lactic, formic, malic, and propionic acids) (Muzzarelli et al., 1974; Butler et al., 1996; Caner et al., 1998; Rhim et al., 1998; Park et al., 1999; Wiles et al., 2000; Park et al., 2002b). Physical and mechanical properties of chitosan films reported in the literature vary due to the differences of chitosan sources, molecular weight, degree of deacetylation, solvent type, methods of film preparation, and use of additives (Kim et al., 2006; Nadarajah et al., 2006).

Chitosan films prepared by solution casting method are normally transparent with slight yellowish color. Nunthanid et al. (2001) reported that chitosan films with lower-molecular weight (50-60 kDa) became dark yellow after storage for a few weeks, which might be due to the accumulation of hydroxymethylfurfural (HMF) in the films (Rajpa et al., 2006). Although chitosan is likely to be involved in Maillard reactions due to its amino groups, color modifications of chitosan films with storage are not related to those reported in protein films due to Maillard reactions or in lipid-based films due to rancidity (Trezza and Krochta, 2000). Chitosan films made from acetic acid solution have slight acidic odor (Nadarajah et al., 2006).

Chitosan exhibits two diffraction peaks at around 11 and 20° with low intensity as examined by X-ray diffraction, which are characteristics of the hydrated crystalline structure of chitosan (Kewon et al., 2001). However, in the chitosan films, the intramolecular interactions between the amino groups and hydroxyl groups in chitosan limit the molecular movement of the chitosan chains and show rather amorphous states with less intensity of the two crystalline peaks (Fuentes et al., 2000; Nunthanid et al., 2001; Xu et al., 2005; Mathew et al., 2006). Wang et al. (2005) considered that the presence of acid solvent residue may hinder the formation of inter-

and intra-molecular hydrogen bonds in the chitosan films and result in less crystallization. In addition, Wang et al. (2005) also regarded that the residual of acid in chitosan films induced degradation of chitosan and that chitosan kept as powder was more stable at elevated temperatures than when kept in the form of films.

Desirable mechanical properties are essential for biopolymer films to be used as food packaging material. Generally, films must be resistant to breakage to protect the food and flexible to adapt to possible deformation, desiring filling, transportation and storage (Guilbert et al., 1996). The value of tensile strength (TS) and percent of elongation at break (%E) for chitosan films relate essentially to the internal material cohesion forces, chitosan molecular weight and concentration, type of solvent, and presence of plasticizer (Silva and Santos, 2007). The tensile strength of chitosan films increased with the increasing molecular weight of chitosan (Chen and Hwa, 1996; Nunthanid et al., 2001; Park et al., 2002b). Kienzle-Sterzer et al. (1982) reported that the mechanical properties of chitosan films change with the type of acid solvent. This may be due to the different interaction between chitosan and acid solution, which might affect both junction density and topological limitations in the films (Kienzle-Sterzer et al., 1982). Caner et al. (1998) showed the TS values of chitosan films formed using lactic or propionic acids were significantly lower than films formed with acetic or formic acid. Acetic acid resulted in the toughest films followed by malic, lactic, and citric acid, respectively (Rhim et al., 1998; Park et al., 2002b). Park et al. (1998) explained the relationship between chitosan film properties and the acid solutions by light scattering method. They showed that molecular weight of chitosan dissolved in acetic acid was larger than that dissolved in the other acids. Chitosan dissolving in acetic acid formed dimers, indicating their intermolecular interaction was stronger and corresponding chitosan films had a tighter structure than those prepared with other acid solutions (Park et al., 1998). The TS of chitosan films are reported to be comparable to many medium-strength commercial polymer films (Butler et al., 1996).

Table 2.2 Scientific publications reporting mechanical properties of chitosan, edible and synthetic films.

Films	Tensile Strength (MPa)	Elongation at break (%)	References
Chitosan (88 kDa, 2%, in 1% acetic acid)	66.70	3.49	Blair et al., 1987
Chitosan (229 kDa, 2%, in 1% acetic acid)	70.30	6.20	Kittur et al., 1998
Chitosan (50-60 kDa, 1%, in 1% acetic acid)	45.07	9.34	Nunthanid et al., 2001
Chitosan (37 kDa, 2%, in 2% acetic acid)	68.80	4.10	Park et al., 2002b
Chitosan (37 kDa, 2%, in 2% malic acid)	27.40	18.2	Park et al., 2002b
Chitosan (49 kDa, 1%, in 1% acetic acid)	82.40	5.20	Suyatma et al., 2004
Chitosan (450 kDa, 1%, in 1% acetic acid)	105.7	5.00	Zivanovic et al., 2005
Chitosan (1%, in 1% acetic acid)	60.70	3.30	Garcia et al., 2006
Edible film			
Starch	47.40	3.60	Garcia et al., 2006
Konjac glucomannan	21	9	Xiao et al., 2000
Whey Protein Isolate (5%)	3.1	7.00	Perez-Gago et al., 1999
Wheat Gluten	2.6	276.2	Gennadios et al., 1993
Pea Protein	7.3	46.8	Choi and Han, 2001
Zein	6.81	3.18	Lai and Padua,1997
Soy Protein Isolate	5.23	90.27	Brandenburg et al., 1993
Synthetic film			
Cellophane	114.0	20	Aydt et al., 1991
LDPE	8.6 - 17.3	500	Brinston, 1988
HDPE	17.3 - 34.6	300	Brinston, 1988

Table 2.2 shows a comparison of data obtained from literature on the mechanical properties of chitosan films, other edible films and synthetic films. Kittur et al. (1998) reported 35 μm-thick films produced with chitosan (229 kDa, 67% DDA) at 2% w/w in 1% acetic acid had TS value of 70.3 MPa. Garcia et al. (2006) reported a TS value of 60.7 Mpa for 15.2 μm-thick films made of 1% chitosan (85% DDA) in 1% acetic acid solution. Compared to the TS values of other biopolymer films and widely used plastic films, such as low density polyethylene (LDPE) and high density polyethylene (HDPE) films, chitosan films possess high tensile strength. However, the molecular rigidity of chitosan makes the forming films to exhibit a brittle behavior with a low percentage (3.3-18.2%) of elongation at break, which is significantly lower than some protein-based films and commercial films (Table 2.2). Addition of plasticizer (polyethylene glycol, glycerol, sorbitol, etc) and blending with flexible materials are usually ways to reduce the brittleness of chitosan films (Aydinli and Tutas, 2000).

Chitosan films exhibit better gas barrier properties than those of LDPE and HDPE (Brody and Marsh, 1997), which could be applied as modified atmosphere packaging of fruits and vegetables (Tharanathan et al., 2002). However, like other polysaccharides, chitosan is hydrophilic by nature and contains large amount of hydroxyl groups which interact with water molecules. This causes plasticization or swelling, therefore chitosan films perform poorly as moisture barriers (Krochta et al., 1994).

Water vapor permeability (WVP) is a measure of the ease with which films can be penetrated by water vapor and indicates film's ability to control water vapor transport between food system and its environment (Gennadios et al., 1994). The reported WVP values of chitosan and other edible and synthetic films are summarized in Table 2.3. The water vapor permeability of chitosan films is comparable with those of hydrophilic polysaccharide-based and protein-based films (Table 2.3). However, it is much higher than the WVP of lipid-based films and synthetic films, such as LDPE and HDPE. The weak moisture barrier property of chitosan films limits their development as food packaging material.

Table 2.3 Scientific publications reporting water vapor permeability (WVP) of chitosan, edible and synthetic films.

Films	WVP ($g \cdot mm/m^2 \cdot h \cdot kPa$ *)	Thickness (µm)	Conditions	References
Chitosan (1%, in 1% formic acid)	0.128	25.4 - 38	-	Wong et al., 1992
Chitosan (3%, in 1% acetic acid)	0.09	51	25°C, 11%RH	Butler et al., 1996
Chitosan (2%, in 2% acetic acid)	1.15	-	25°C, 50/100%RH	Park et al., 2002b
Chitosan (2%, in 2% malic acid)	1.48	-	25°C, 50/100%RH	Park et al., 2002b
Chitosan (2%, in 1% acetic acid)	4.17	28	32°C, 16/95% RH	Miranda et al., 2004
Chitosan (1%, in 1% acetic acid)	0.162	15.2	20°C, 0/75%RH	Garcia et al., 2006
Chitosan (1%, in 2% formic acid)	6.80	2.54	25°C, 50/100%RH	Kim et al., 2006
Chitosan (1%, in 2% acetic acid)	7.2	2.54	25°C, 50/100%RH	Kim et al., 2006
Chitosan (2%)	2.31	100	25°C, 50/100%RH	Kolodziejska and Piotrowska,2007
Edible films				
Corn Starch	0.64	63.10	20°C, 0/75%RH	Garcia et al., 2006
HighlyCarboxymeth-ylated Starch(HCMS)	7.56	94.6	25°C, 50/100%RH	Kim et al.,2002
Methylcellulose	0.27	16	20°C, ΔP=1753Pa	Pinotti et al., 2007
Hydroxypropylmethylc ellulose(HPMC)	0.61	38	27°C, 0/85%RH	Gennadios et al., 1994
Corn Starch (sorbitol)	0.63	63	20°C, ΔP=1753 Pa	Garcia et al., 2006
Whey Protein Isolate (5%)	5.06	139	25°C, 0/71%RH	Perez-Gago et al., 1999
Wheat Gluten (glycerin)	8.31	140	26°C, 50/100%RH	Aydt et al., 1991
Fish Gelatin	2.54	100	25°C, 50/100%RH	Kolodziejska and piotrowska, 2007
Corn Zein	1.90	89	26°C, 50/100%RH	Aydt et al., 1991
Beeswax	0.00209	40 - 50	25°C, 0/100%RH	Greener, 1992
Carnauba wax	0.00187	90 - 110	25°C, 0/100%RH	Greener, 1992
Synthetic films				
Cellophane	0.30	22	25°C, 22/84%RH	Phan et al., 2005
LDPE	0.0033	-	38°C, 0/90% RH	Smith, 1986
HDPE	0.0008	-	38°C, 0/90% RH	Smith, 1986

* All the WVP values reported here were recalculated in the unit of $g \cdot mm/m^2 \cdot h \cdot kPa$.

Wong et al. (1992) reported a chitosan film (1%, in formic acid) with WVP value of 0.128 g·mm/m^2·h·kPa. Butler et al. (1996) prepared chitosan films with 1% acetic acid and 0.25% glycerol and reported WVP values of 0.09 g·mm/m^2·h·kPa at 25°C and 11% RH. Miranda et al. (2004) measured a higher WVP value of 4.17 g·mm/m^2·h·kPa for 28 μm-thick chitosan films cast from 2% chitosan (130 kDa, >70% DDA) solution in 1% acetic acid (28°C, 50/100% RH). Rhim et al. (1998) also reported high WVP values of chitosan films prepared from commercial chitosan ranging from 2.99 - 6.44 g·mm/m^2·h·kPa.

The main factors causing the difference of WVP for the hydrophilic films are not the preparation conditions such as molecular weight of chitosan, types of solvent and concentration, but the addition of plasticizers, the method used, experimental condition such as RH gradient and temperature, correction for the air gap effect, and film thickness (Rhim et al., 1998). Park et al. (2002b) determined that increased molecular weight of chitosan and solvent acids did not significantly affect WVP of chitosan films (Table 2.3).

In the Gravimetric method standardized by ASTM E96 (ASTM, 2005), an air gap exists between a film and the surface of the liquid inside the permeability cup to avoid film contact with the liquid. ASTM E96 assumes that the resistance from this air gap to water vapor transport is negligible, however, for hydrophilic and moisture sensitive films, neglecting the effect of this air resistance can lead to significant underestimation of actual WVP values (Gennadios et al., 1994). Researchers have developed the WVP Correction Method for highly permeable and hydrophilic edible films (McHugh et al., 1993; Gennadios et al., 1994). Miranda et al. (2004) reported that the McHugh correction method could change WVP of 2% chitosan films from 1.28 ± 0.02 to 4.17 ± 0.09 g·mm/m^2·h·kPa (32ºC, 16/95% RH).

Although ideal polymeric films exhibit no thickness effect on WVP, hydrophilic films generally exhibit a positive relationship between thickness and water vapor permeability (Miranda et al., 2004). McHugh et al. (1993) reported that a positive relationship existed between thickness and WVP of hydrophilic films since the increased film thickness could increase the

relative humidity and equilibrium water vapor partial pressure at the inner film surface and provide an increased drive to mass transfer across it.

Since chitosan films swell under acidic conditions due to the ionization of amino groups but remain in a shrunken state under neutral condition, and due to their excellent biodegradable and biocompatible characteristics, chitosan film matrix has been exploited widely for sustained drug delivery in stomach (Graham, 1990; Shu et al., 2001; Dhanikula and Panchagnula, 2004; Jin and Song, 2006; Wang et al., 2007).

2.2.3 Antimicrobial and Metal Binding Properties of Chitosan Films

2.2.3.1 Antimicrobial Effects of Chitosan Films

The antimicrobial and film-forming properties of chitosan have led to extensive number of studies on chitosan films as potential antimicrobial food packaging (Chen et al., 1996; Ouattara et al., 2000a, b; Coma et al., 2002). Chen et al. (1996) have reported that chitosan films made from dilute acetic acid solutions could inhibit the growth of *Rhodotorula rubra* and *Penicillium notatum*. Coma et al. (2002) reported that chitosan films made from 1% chitosan in 1% aqueous acid solution prevented *Listeria monocytogenes* for at least 8 days especially when applied during the first 12 hours of culture. A decrease in antibacterial effect with time was observed probably because the amino groups of chitosan binding to cell components were no longer available to attach to other cell surfaces (Sudarshan et al., 1992). Zivanovic et al. (2005) also showed that chitosan films could reduce the number of *L. monocytogenes* by 1-3 logs, but *Escherichia coli* O157:H7 was less susceptible to the chitosan films. Chitosan films could reduce microorganism counts by 3 or less log units (Joerger, 2007). However, Fernandez-Saiz et al. (2006) reported higher reductions (6.6 log CFU/ml) against *Staphylococcus aureus* by a freshly cast chitosan films. Durango et al. (2006) also showed chitosan films made by 5% film-forming solution caused a reduction of 4-6 log cycles in the population of *Salmonella* enteritidis.

In vitro tests and application tests are common methods used to examine antimicrobial activity of chitosan films (Davidson and Parish, 1989). *In vitro* tests include agar diffusion to measure the diameters of inhibitory zones surrounding film as well as the contact areas of films with agar surface (Li et al., 2006), buffer/broth dilution test to enumerate the number change of target microorganisms in the buffer/broth over time, and optical density method to measure the turbidity of the microorganism medium. Application tests involve either food model system or actual foods inoculated with target microorganisms (Ouattara et al., 2000b; Coma et al., 2002). Generally, chitosan film is placed between two equal food pieces inculated with test microorganism inoculums to make a "sandwich". The assembly is sealed and stored at 10°C for several days for analysis (Zivanovic et al., 2005; Duan et al., 2007). Agar diffusion test has limits of detection of antimicrobial activity of chitosan films because the hydrophilic nature of chitosan could cause film to swell and bend preventing it to stay in full contact with the inoculated agar (Zivanovic et al., 2005; Rhim et al., 2006). In addition, since chitosan macromolecules are incapable of diffusing through the adjacent agar media, chitosan film only inhibits the organisms in direct contact with the active sites (Coma et al., 2002). Therefore, there are studies reporting that chitosan films did not reveal inhibitory zone in any microorganisms tested (Coma et al., 2002; Pranoto et al., 2005).

2.2.3.2 Metal Binding Property of Chitosan Films

Films have been used to remove heavy metal ions from aqueous solutions (Ujang and Anderson, 1998). The current methods to prepare adsorptive films are mainly through surface modification of commercial films made from relatively inert polymers, such as polysulfone, polypropylene, nylon, etc (Liu and Bai, 2006). However, the harsh physical and chemical treatments for surface modification make the films deteriorate easily. Also modified films are too costly to be used for practical applications in heavy metal removal (Liu and Bai, 2006). Recently, there has been considerable interest in preparing chitosan films as an adsorptive separation

material for removal of heavy metals (Kaminski and Modrzejewska, 1997; Steenkamp et al., 2002).

The metal binding capacity of chitosan films varies with metal, film crystallinity, pH value of the solution, available amino group content, DDA and physical adsorption ability (Kurita et al., 1979; Kaminski and Modrzejewska, 1997; Modrzejewska and Kaminski, 1999). Kaminski and Modrzejewska (1997) have reported that chitosan films made from 7% chitosan dissolved in 2% acetic acid solution could separate metal ions Cu(II), Cd(II), Co(II), Zn(II), and Ni(II) almost completely using the ultrafiltration method, and in the case of Cr(VI) and Mn(II) ions, the separation depended on pH and process conditions. At pH=3, chitosan films showed the highest selectivity for Cr (VI) (Modrzejewska and Kaminski, 1999).

One disadvantage of chitosan flakes and powders as metal adsorbents is their low surface area and nonporosity which could cause a great pressure drop in an industrial scale column (Varma et al., 2004). This can be avoided by casting chitosan films with large surface area. High surface area to mass ratio of chitosan films greatly increases the availability of chitosan molecules entrapped within the chitosan matrix and contributes to an increase in number of available $-NH_3^+$ active groups to bind metal ions. However, the scope of applying pure chitosan films as metal binding materials has been largely limited due to their poor resistance to water. To overcome this problem, addition of crosslinkers into chitosan films has been evaluated (Jones and Zivanovic, 2006).

2.2.4 Application of Chitosan Films in the Food Industry

Chitosan films have been extensively studied for their potential use as active packaging materials to extend shelf life of food products due to their unique functionalities (Butler et al., 1996; Jeon et al., 2002; No et al., 2007). The antimicrobial property of chitosan film could inhibit or reduce growth of spoilage and pathogenic microorganisms during food storage (Zivanovic et al., 2005; Duan et al., 2007). For example, Agullo et al. (1998) evaluated the capacities of

chitosan films to extend shelf life of precooked pizza and showed all increased shelf life which was due to the antimicrobial property rather than water vapor barrier. Since chitosan films are more selectively permeable to O_2 than to CO_2 (Bai et al., 1988), using chitosan films to pack fruits and vegetables is likely to modify the internal atmosphere without causing anaerobic respiration (No et al., 2007). Chitosan films used for glazing the skinless pink salmon fillets delayed the lipid oxidation of salmon fillets after eight months frozen storage (Sathivel et al., 2007). Both antioxidant and oxygen barrier properties of chitosan may have contributed to the results. Chitosan films could also be a vehicle for incorporating other functional ingredients such as antioxidants, flavor, colors, antimicrobial agents (acids, lysozyme, nisin, essential oils), and nutraceuticals to prolong the shelf life and enhance the nutritional values of foods (Ouattara et al., 2000a,b; Han et al., 2004; Park et al., 2004; Pranoto et al., 2005; Zivanovic et al., 2005).

2.2.5 Disadvantages of Chitosan Films

Antimicrobial and metal binding properties and film-forming ability of chitosan make chitosan films an ideal biopolymer for use as biodegradable food packaging material. However, one major drawback of chitosan films is their high sensitivity to moisture which negatively affects their application in direct contact with high moisture foods (No et al., 2007). Although the intensity of yellowness of chitosan films is small compared to protein-based films, like whey protein, their yellowish color appearance is still undesirable especially on certain food products (Butler et al., 1996). In addition, the brittleness of chitosan films also limits their wide application (Xiao et al., 2000).

Furthermore, the typical chitosan production process (deproteinization, demineralization, decolorization, and deacetylation) is costly which limits wider food application of chitosan films (No et al., 2007). More research is needed to produce less expensive chitosan or improve the ratio of performance/cost of current chitosan products.

2.3 Chitosan Co-polymer Films

2.3.1 Advantages of Chitosan Co-polymer Films

The blending of natural-natural polymers or natural-synthetic polymers is one of the effective methods for preparation of new desirable materials. Blends are often used to improve mechanical and physical properties of the original polymers, such as hydrophobicity, melt temperature, and glass transition temperature (Rathke and Hudson, 1994). Blends of polymers reported in recent years include silk fibroin/polyacrylamide (Freddi et al., 1999), soy protein isolate/poly (ethylene oxide) (Ghorpade et al., 1995), konjac glucomannan/poly (vinylpyrrolidone) (Xiao et al., 2001a). Poly (vinyl alcohol) blending with starch and LDPE as packaging materials for intermediate moisture foods has also been reported (Holton et al., 1994).

The functional properties of chitosan films could be improved when chitosan is combined with other film-forming materials. There is a large number of research publications on the blending of chitosan with various polymers, including natural polymers (cellulose and its derivatives, starch, konjac glucomannan, guar gum, keratin, etc) (Tanabe et al., 2002; Xiao et al., 2003; Xu et al., 2005; Yin et al., 2006) and synthetic polymers (N-methyl nylon 6, poly (ethylene oxide), poly (lactic acid), poly (ethylene glycol), etc) (Shieh and Huang, 1998; Alexeev et al., 2000; Zhang et al., 2002; Sebastien et al., 2006). The strong hydrogen bonds between components can provide blends with the desired mechanical strength and physical properties (Xiao et al., 2003; Liu and Bai, 2006). For example, one strategy to overcome the drawback of high sensitivity to moisture of chitosan films is to blend with a more moisture resistant polymer (Sebastien et al., 2006). Wong et al. (1992) have reported that the water resistance of chitosan films was improved by the incorporation of hydrophobic materials such as fatty acids to increase hydrophobicity of the films.

On the other hand, functionalities of chitosan can also contribute to improved properties of the blends. Kuo et al. (2006) reported that the blending of chitosan with nylon 11 obstructed the growth of spherical crystals in nylon 11 and changed their physical properties. The blends

also exhibited good biodegradability to soil environments due to the weakening of the hydrogen bonding between the polyamide chains in nylon 11.

The final properties of the blends are significantly related to the miscibility or compatibility of the polymers determined by the extent of the formation of intermolecular hydrogen bonds between polymer components (Coleman and Painter, 1995; Jiang et al., 1999).

The miscibility of two polymers in the blends could be examined by Fourier Transform Infrared Spectroscopy (FTIR). If two polymers are compatible, the IR spectrum of the blends should be considerably different from the spectra of each component due to the chemical interactions, resulting in band shifts, intensity changes, and broadening (Xiao et al., 2003). Other methods for evaluzation include Wide-angle X-ray diffractograms, Raman spectra, and Thermal gravimetric analysis (Yin et al., 2006).

2.3.2 Blend Films from Chitosan and Natural Polymers

The most common method for preparing chitosan/natural polymers blend films is solution casting method. Numerous studies have been carried out to study the properties of chitosan blend films with natural polymers with different blend ratios (Table 2.4).

The physical and mechanical properties of chitosan films have been improved by blending with natural polymers. Garcia et al. (2004) reported that incorporation of methylcellulose (MC) in chitosan films could decrease the yellow coloration, lower WVP, and increase percentage elongation at break from 4.3% for pure chitosan films to 8.3% for 0.25/0.75 chitosan/MC films. An increase in cellulose content (Hosokawa et al., 1990; Suto and Ui, 1996) or whey protein content (Di Pierro et al., 2006) decreased the degree of swelling and water solubility of the chitosan blend films. An increase of starch content in chitosan/starch blend films increased the average percentage of elongation values to 49.12%, and also enhanced the thermal stability of films with decomposition temperature increasing from 294°C to 308°C (Mathew et al., 2006).

Table 2.4 Scientific publications reporting blend films from chitosan and natural polymers.

Blend Films	Film forming conditions	Blend ratios (Chit/others)	Properties tested	References
Chitosan/ Cellulose	SC*, total polymer concentration: 1-7% TFA as co-solvent	10/0, 8/2, 7/3, 5/5	Crystallinity	Isogai and Atalla, 1992
Chitosan/ Homogenized Cellulose	SC, chitosan in 1% acetic acid, 0.8% cellulose	0/100, 10/90, 20/80, 30/70, 40/60	TS*, thermal analysis, water adsorption	Hosokawa et al., 1990
Chitosan/ Methylcellulose (MC)	SC, 1,2% chitosan in 1% acetic acid, 1% MC	0.25/0.75, 0.5/0.5, 0.75/0.25, 1/0	%E*, WVP*, color, water solubility	Garcia et al., 2004
Chitosan/ Hydroxypropyl cellulose(HPC)	SC, total polymer concentration: 1% 5% acetic acid	100/0, 90/10, 70/30, 50/50, 30/70, 90/10	TS, Water solubility, swelling	Suto and Ui, 1996
Chitosan/ Starch	SC, 2% chitosan in 1% lactic acid, 1, 2, 3, 4% starch	1/2, 1/1.5, 1/1, 1/0.5, 1/0	TS, crystallinity, WVTR*, FTIR*	Xu et al., 2005
Chitosan/ Potato Starch	SC, 2% chitosan in 1% acetic acid, 1, 2, 3% starch	2/1, 2/2, 2/3	TS, %E, swelling, thermal analysis, crystallinity	Mathew et al ., 2006
Chitosan/ Corn Starch	SC, 1% chitosan in 1% acetic acid 1%, 3.5, 5, 6% starch	1/1	TS, WVP, optical properties, water solubility	Garcia et al., 2006
Chitosan/ Hydroxypropyl Guar Gum(HGG)	SC, 3% chitosan in 2% acetic acid, 3% HGG	100/0, 80/20, 60/40, 40/60, 20/80, 0/100	TS, %E, crystallinity, thermal analysis	Xiao et al., 2003
Chitosan/ Konjac Gluco-mannan (KG)	SC, 1% chitosan in 0.8% acetic acid, 1% KG	9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9	TS, WVTR, water solubility, antimicrobial	Li et al., 2006
Chitosan/ KonjacGluco-mannan (KG)	SC, 2% chitosan in 2% acetic acid, 7% KG	9/1, 7/3, 5/5, 3/7, 1/9	Mechanical property, crystallinity, thermal analysis, swelling	Xiao et al., 2000
Chitosan/ Pectin	SC, 1-2% chitosan in 1% lactic acid, 1-2% pectin	1/2, 1/1	Mechanical property, WVP	Hoagland and Parris, 1996
Chitosan/ Whey protein	SC, 2.5% chitosan in HCl, 5% whey protein	0.125/9.2, 0.25/9.2, 0.37/9.2, 0.5/9.2, 1.0/9.2	TS, %E, WVP swelling, water solubility	Di Pierro et al., 2006
Chitosan/ Keratin	SC, 2.5% chitosan in 75% acetic acid, 6.5% keratin	1/20, 1/25, 1/5, 1/3, 4/4	Mechanical property, swelling	Tanabe et al., 2002
Chitosan/ Fatty acid	SC, 1% chitosan in 1% formic acid	2/1	WVP, GP*, surface property	Wong et al., 1992

*SC: Solution Casting Method; TS: Tensile Strength; %E: Elongation at Break; WVP: Water Vapor Permeability; WVTR: Water Vapor Transmission Rate; FTIR: Fourier Transform Infrared; GP: Gas Permeability.

Chitosan content in the blends also contributes to the enhanced properties of the blends. The presence of chitosan in the blend films limited the crystallinity of the cellulose (Isogai and Atalla, 1992; Hasegawa et al., 1992), potato starch (Mathew et al., 2006), and increased the tensile strength of cellulose films (Hosokawa et al., 1990; Suto and Ui, 1996), whey protein films (Di Pierro et al., 2006), and keratin films (Tanabe et al., 2002). In chitosan/starch films, chitosan content decreased the opaque appearance from starch content (Garcia et al., 2006).

The miscibility of chitosan and natural polymers in the blends have been widely studied. Xiao et al. (2000) reported good miscibility between chitosan and konjac glucomannan in the blend films by the strong intermolecular hydrogen bonds formed between the amino groups of chitosan and the hydroxyl groups of konjac glucomannan. On the other hand, Hasegawa et al. (1992) and Yin et al. (2006) assessed the miscibility of chitosan and cellulose and cellulose ethers----hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) in the blends and showed that although weak hydrogen bonding existed between the polymer functional groups, these interactions were not predominant and the polymers were not fully miscible.

The altered properties of blend films are related to their blend ratios. Xiao et al. (2003) reported that increasing hydroxypropyl guar gum (HGG) content to 60% could increase the tensile strength and elongation at break to the highest value of 58.94 MPa and 17.25%, respectively. When HGG content further increased, tensile strength was gradually reduced. Similarly, 40/60 chitosan/HGG films also achieved the greatest thermal stability and miscibility compared with films with other blend ratios.

2.3.3 Blend Films from Chitosan and Synthetic Polymers

A combination of chitosan and synthetic polymers has also been extensively studied for their potential beneficial effects on the physical, mechanical and biological characteristics of complex films. Table 2.5 lists the available literature on chitosan/synthetic polymer blend films made by solution casting method.

Table 2.5 Scientific publications reporting blend films from chitosan and synthetic polymers.

Blend Films	Film forming conditions	Blend ratios (Chit/others)	Properties tested	References
Chitosan/ poly (ethylene glycol) (PEG)	SC*, 1% chitosan in 1% acetic acid	2/1, 4/1	Morphology, thermodynamic, wettability, biocompatibility	Zhang et al., 2002
Chitosan/ poly (vinyl alcohol) (PVA)	SC, 2.5% chitosan in 1% acetic acid, 2.5% PVA	10/0, 8/2, 6/4, 5/5, 4/6, 2/8, 0/10	DSC*, TGA*, DMA*, FTIR*, X-ray, contact angle, SEM*	Chen et al., 2007
Chitosan/ poly (vinyl alcohol) (PVA)	SC, 1% chitosan in 1% acetic acid, 10% PVA	100/0, 75/25, 50/50, 25/75, 0/100	TS*, water uptake, Surface tension, contact angle	Bahrami et al., 2003
Chitosan/ poly (lactic acid) (PLA)	SC, 1% chitosan in 1% acetic acid, 1% PLA	100/0, 90/10, 80/20, 70/30	WVTR*, bioactivity, contact angle, water solubility, TS	Sebastien et al., 2006
Chitosan/ poly (lactic acid) (PLA)	SC, 1% chitosan in 1% acetic acid, 2% PLA	75/25, 50/50, 25/75	SEM, FTIR, DSC, TGA, x-ray, swelling, contact angle	Wan et al., 2006
Chitosan/ N-methylol nylon 6	SC, 0.5% chitosan in 88% formic acid, 10% nylon 6	100/0, 75/25, 50/50, 25/75, 0/100	Pervaporation	Shieh and Huang, 1998
Chitosan/ Nylon 11	SC, 5% chitosan and nylon 11 in TFA	0/100, 10/90, 20/80, 30/70, 40/60, 50/50, 100/0	FTIR, SEM, x-ray, biodegradability	Kuo et al., 2006
Chitosan/ poly (acrylamide) (PAAm)	SC, 2% chitosan in 2% acetic acid, 2% PAAm	100/0,90/10, 80/20, 70/30, 60/40, 50/50, 0/100	FTIR, x-ray, SEM, mechanical, thermal, water adsorption	Xiao et al., 2001b
Chitosan/ poly (vinyl acetate) (PVAc)	Graft copolymerization	1/100, 1/20, 1/10, 3/20	Thermal analysis, mechanical property, swelling	Don et al., 2002
Chitosan/ poly (caprolactone) (PCL)	SC, 2% chitosan in 77% acetic acid, 0.2, 0.6, 1.8% PCL	100/0, 75/25, 50/50, 25/75	Mechanical property, FTIR, AFM*, DMTA*, antibacterial property	Sarasam et al., 2006
Chitosan/ silk fibroin (SF)	SC, 0.15-0.75% chitosan in 2% acetic acid, SF 0.12-0.6%	10/0, 8/2, 6/4, 4/6, 2/8, 0/10	FTIR, x-ray, TGA, DSC, SEM	Kweon et al., 2001

*SC: Solution Casting Method; DSC: Differential Scanning Calorimetry; TGA: Thermogravimetric Analysis; DMA: Dynamic Mechanical Analysis; FTIR: Fourier Transform Infrared; SEM: Scanning Electron Microscopy; TS: Tensile Strength; WVTR: Water Vapor Transmission Rate; AFM: Atomic Force Microscopy; DMTA: Dynamic Mechanical Thermal Analysis.

The success of synthetic polymers as biomaterials relies mainly on their wide range of mechanical properties, a variety of shapes to be easily obtained from transformation process and low production costs compared to natural polymers (Cascone, 1997). A number of synthetic polymers, especially for those owning biodegradable and biocompatible properties and offering good tensile strength, flexibility and/or barrier properties, have been selected as blend materials.

Chitosan may be potentially miscible with some synthetic polymers because of the formation of intramolecular and intermolecular hydrogen bonds between hydroxyl groups of synthetic polymer and hydroxyl and amine groups of chitosan (Xiao et al., 2001b; Wan et al., 2006). However, the miscibility of the two polymers depends on the polymers and their blend ratios. Khoo et al. (2003) indicated that chitosan/poly (ethylene oxide) (PEO) and chitosan/ poly (vinylpyrrolidone) (PVP) blends showed evidence of miscibility in all blend ratios, while the chitosan/poly (vinyl alcohol) (PVA) blend only showed evidence of interaction for 80/20 and 50/50 blends, but not for 20/80 blends. Zhang et al. (2002) also reported that interaction between chitosan and poly (ethylene glycol) (PEG) molecules was very small and did not significantly affect their properties.

Like chitosan/natural polymer blend films, blending chitosan with synthetic polymers also produce films with altered properties. The flexibility of chitosan films could be improved by incorporating PVA (Miya et al., 1983; Bahrami et al., 2003; Srinivasa et al., 2003) and poly (acrylamide) (PAAm) (Xiao et al., 2001b). Blending with *Antheraea pernyl* silk fibroin can enhance the thermal stability of chitosan (Kweon et al., 2001). Although hydrophilic synthetic polymers, such as PEO, PVP, PVA, could not significantly reduce the water moisture barrier of blend films (Bahrami et al., 2003; Srinivasa et al., 2003), polymers with hydrophobic nature, such as poly (lactic acid) (PLA) (Sebastien et al., 2006), N-methylol nylon 6 (Shieh and Huang, 1998), poly (vinyl acetate) (PVAc) (Don et al., 2002), and poly (caprolactone) (PCL) (Olabarrieta et al., 2001) reduced the hydrophilic nature of chitosan-based films, improved their moisture barrier properties, and decreased overall the water/matrix interaction. On the other hand, the presence of chitosan

in the blend films can limit the crystallinity of PVA (Chen et al., 2007), poly (3-hydroxybutyric acid) (Ikejima and Inoue, 2000), nylon 11 (Kuo et al., 2006), and polycaprolactone (PCL) (Sarasam et al., 2006) in the blends. This could be explained by the fact that the stiff chitosan molecular chains affected the overall mobility in the blend and impeded the rate of crystal growth (Zhao et al., 1995).

2.3.3.1 Poly (Ethylene Oxide) (PEO)

Poly (Ethylene Oxide) (PEO) is a low-toxic, biodegradable, hydrophilic synthetic polymer with molecular weight range from 20 kDa to 8000 kDa (Herold et al., 1989). With a flexible molecular chains of $(-CH_2CH_2O-)_n$ (Figure 2.3), PEO can easily form hydrogen bonds with other bioactive substances and possess good biocompatibility. Due to their bioadhesive and mucoadhesive properties, high viscosity, thermo-plasticity, and flocculation ability, PEO has been widely used in detergents, cosmetic formulations, contact lens solutions, and drug delivery systems (Clinton and Matlock, 1990). The high molecular weight PEO has good film forming property (Manolova et al., 1997). Casting blend films from natural polymers, such as soy protein isolate, and PEO have shown their potential for production of compostible plastible materials (Ghorpade et al., 1995).

Blending chitosan and PEO is a possible way to produce new materials through the interaction between free hydroxyl groups of PEO and amine groups of chitosan (Yilmaz et al., 2002).

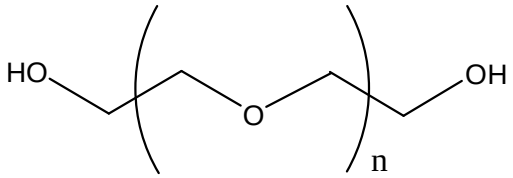


Figure 2.3 Chemical structure of poly (ethylene oxide) (PEO).

Since chitosan is a biocompatible and bioactive polymer whereas PEO is a biologically inert material, their blends have been prepared and studied in the biomedical areas as hydrogels for site-specific antibiotic delivery in the stomach (Patel and Amiji, 1996), and as films for drug release (Manolova et al., 1997; Khoo et al., 2003; Jin and Song, 2006) and for haemodialysis (Amiji, 1995). The swelling and permeability of chitosan/PEO blends have been also evaluated (Neto et al., 2005).

Due to flexible chains of PEO, it has potential to improve the mechanical properties of blends containing rigid chains, such as of chitosan. Alexeev et al. (2000) have examined the mechanical properties of chitosan/PEO blend films and reported that a chitosan/PEO films with weight ratio of 10/2 improved the mechanical properties compared to pure chitosan films. On the other hand, chitosan could potentially impede the PEO crystallization in the films (Angelova et al., 1995; Mucha et al., 1999).

Alexeev et al. (2000) considered that uncharged molecules of PEO ensured the homogenization of their structure, because incorporation of charged polymers could cause too strong interaction with chitosan resulting in either segregation or complicated complexes. However, Budtova et al. (2002) proved that chitosan and PEO were slightly incompatible and the best mechanical properties of films were achieved at chitosan/PEO blend ratio of 83/17 when two polymer components started to be incompatible. Based on the results of thermal behavior and morphology of the blends, Zhao et al. (1995) reported that the compatibility and morphology of chitosan/PEO blends were closely related to the composition. When chitosan content was below 50%, the blends were miscible; otherwise the blends were phase separated.

2.3.3.2 Poly (N-Vinyl-2-Pyrrolidone) (PVP)

PVP is a non-toxic water-soluble synthetic polymer with excellent transparency, biocompatibility and film-forming properties (Yeh et al., 2006). Figure 2.4 shows the chemical structure of PVP.

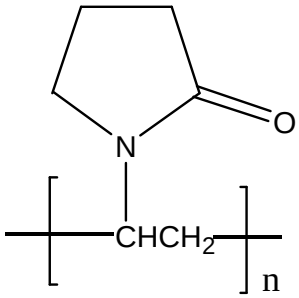


Figure 2.4 Chemical structure of poly (N-vinyl-2-pyrrolidone) (PVP).

PVP has been utilized in a broad range of areas including biomedical, biochemical, food, textile, and other fields (Xiao et al., 2001a). For example, because of its outstanding absorption and complex-forming abilities, PVP has been used as a main component of temporary skin covers or wound dressing (Nho and Park, 2002). As a food processing aid, PVP is a stabilizer and has E number E1201.

The carbonyl groups of PVP favor miscibility with other functional polymers. Researches on the characteristics of blend films of PVP with cellulose derivatives (Kozakiewicz and Maginess, 1987), konjac glucomannan (Xiao et al., 2001a), and collagen (Sionkowska, 2003) have been reported. The blending of PVP with chitosan creates a possibility to produce a new material. Chitosan may form different types of interactions with PVP, including hydrogen bonds between hydroxyl groups of chitosan and carbonyl groups of PVP, and between hydrogen from the amino groups of chitosan and carbonyl groups of PVP (Cao et al., 1998; Marsano et al., 2004; Caykara et al., 2006). Researches have proved that the blend system of chitosan and PVP was miscible (Fang and Goh, 2000; Sakurai et al., 2000). Sakurai et al. (2000) also suggested that since the chitosan and PVP were well miscible, the molecules can not crystallize. Although PVP chains are as rigid as chitosan, their fragile nature enables the forming films perform undesirable mechanical properties (Yeh et al., 2006). Addition of chitosan could possibly reinforce the tensile strength of blending films.

Most of the researches on chitosan/PVP blends focused on their compatibility and miscibility properties. Only a few reported chitosan/PVP films applied to separation of methanol from organic mixtures (Cao et al., 1999) and nylon dyeing process (Yeh et al., 2006). Ignatova et al. (2007) reported that quaternized chitosan/PVP electrospun fibers showed high antibacterial activity against *S. aureus* and *E.coli*, however, few studies discussed about these functionalities of chitosan/PVP films as affected by blend ratios.

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CHAPTER 3. EFFECT OF MOLECULAR WEIGHT AND BLEND RATIOS ON PHYSICAL AND MECHANICAL PROPERTIES OF THICK CHITOSAN/PEO FILMS

Abstract

Chitosan has been widely investigated as a bioactive polycationic material that easily forms films and fibers. A combination of chitosan and synthetic polymers has been extensively studied for their potential beneficial effects on the physical and mechanical characteristics of complex films. Poly (ethylene oxide) (PEO) is a biodegradable and biocompatible polymer that easily forms flexible films. The objective of this study was to investigate the effect of molecular weight and blend ratios on physical and mechanical properties of thick chitosan/PEO films. The blend films were made by solution casting method with chitosan/PEO ratios of 100/0, 75/25, 50/50, 25/75, 0/100. The films were analyzed for thickness, water vapor permeability (WVP), puncture strength (PS), tensile strength (TS) and % elongation at break (%E). Potential development of PEO crystallization was monitored by polarized microscope, and film uniformity was assessed by FTIR spectroscopy. FTIR analysis indicated that hydrogen bonding interaction existed between chitosan and PEO polymers. Incorporation of PEO into chitosan films resulted in decreased yellowish color of the films, decreased thickness (from 91.56 ± 2.23 μm to 57.26 ± 6.71 μm), decreased puncture and tensile strength, but had no significant effect on flexibility and water vapor permeability of the films. Chitosan content apparently decreased tendency to spherulitic crystallization of PEO. Regardless of the molecular weight of the polymers, films with 50% or more chitosan content showed better physical and mechanical properties.

3.1 Introduction

Naturally renewable biopolymers have attracted much research interest in recent years (Lai and Padua, 1997; Perez-Gago et al., 1999; Choi and Han, 2001; Garcia et al., 2006; Lawrie et al., 2007). Chitosan, (1→4)-2-amino-2-deoxy-β-D-glucan, is a natural polycationic polymer obtained by a full or partial N-deacetylation of chitin, which is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans (Roberts, 1992). Due to its reactive amino groups, chitosan exhibits extraordinary functionality such as antimicrobial and metal binding properties (Roller and Covill, 1999; Dutta et al., 2004; Gamage and Shahidi, 2007). Chitosan has been evaluated for various uses in the food, medical, pharmaceutical, agricultural, and chemical industries because of its functionality as well as its nontoxic, biocompatible, mucoadhesive, and biodegradable properties (Angelova et al., 1995; Agnihotri et al., 2004; Zivanovic et al., 2005). Dissolved chitosan in acidic aqueous solvents can form gels, films, sutures, beads, and fibers and provide more application opportunities (Hirano et al., 1999; Ghanem and Skonberg, 2002).

Chitosan films exhibits selective permeability to gases and relatively high tensile strength (Butler et al., 1996; Brody and Marsh, 1997). However, the molecular rigidity of chitosan makes the films brittle (Blair et al., 1987; Garcia et al., 2006). Moreover, the hydrophilic nature of chitosan and high water vapor permeability of its films limite their development as packaging material (Krochta et al., 1994).

Blends of two or more polymers are often used to improve mechanical and physical properties of the original polymer (Rathke and Hudson, 1994). It has been reported that starch/chitosan blend films exhibited a higher flexibility and improved the percentage elongation compared to films produced from single polymers (Mathew et al., 2006). Recently chitosan and poly (ethylene oxide) (PEO) blends have been used for preparation of films for hemodialysis and pH-sensitive drug delivery (Amiji, 1995; Patel and Amiji, 1996). PEO is a flexible synthetic polymer with low-toxic, hydrophilic, biodegradable and biocompatible properties (Herold et al.,

1989). PEO has been widely used for medical purpose (Jin et al., 2004). The high molecular weight PEO has good film forming property (Manolova et al., 1997). However, pure PEO films have relatively poor mechanical characteristics and high crystallinity which limit their application.

Chitosan/PEO blend films may provide additional functionality compared to the pure polymer films. Alexeev et al. (2000) have examined the mechanical properties of chitosan (MW~400 kDa) blend films with PEO (MW~600 kDa, 200 kDa) with PEO weight percent from 4.8% to 66.7%. They reported that addition of 16.7% w/w PEO improved the mechanical properties of chitosan films. However, very limited literature is available on physical and mechanical properties of chitosan/PEO blend films affected by molecular weight and blend ratios.

The objective of this study was to investigate the effect of molecular weight (MW of chitosan: 150 kDa, 450 kDa; MW of PEO: 300 kDa, 900 kDa) and blend ratios (100/0, 75/25, 50/50, 25/75, 0/100) on physical and mechanical properties of thick chitosan/PEO films.

3.2 Materials and Methods

3.2.1 Materials

Chitosan of low molecular weight (LMW ~150 kDa) with a degree of deacetylation 85.7%, medium molecular weight (MMW ~ 450 kDa) with a degree of deacetylation 90.4%, and PEO of medium molecular weight (MMW ~300 kDa) and high molecular weight (HMW ~900 kDa) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

3.2.2 Rheological Characterization of LMW Chitosan/HMW PEO Blend Solutions

1% (w/w) of the mixture of LMW chitosan and HMW PEO with different ratio (100/0, 75/25, 50/50, 25/75, 0/100) were dissolved in 1% (w/w) acetic acid and stirred over night. Rheological characterization of chitosan/PEO solutions was performed in an AR 2000 rheometer (TA Instrument Inc, New Castle, DE) with concentric cylinder, at controlled constant temperature 25°C. Viscosity was determined as a function of shear rate between 0.1 and 100 s^{-1}.

3.2.3 Preparation of Thick Chitosan/PEO Films

Two types of blend solutions were made: MMW chitosan with MMW PEO, and LMW chitosan with HMW PEO. 1% (w/w) of the mixture of chitosan and PEO with different ratio (100/0, 75/25, 50/50, 25/75, 0/100) were dissolved in 1% (w/w) acetic acid and stirred over night. After filtration through Miracloth® (Calbiochem-Novabiochem Corp., San Diego, CA), 20 g of film forming solutions were poured into 50mm-diameter Petri-dishes and dried at 38°C under 17 kPa pressures for 48 hours. The films were kept in a desiccator at 25ºC and 20% RH and tested within one month of preparation.

3.2.4 Thickness

Film thickness (μm) was determined on 6 films per ratio treatment averaging measurements at 5 points for each film using a hand-held microcaliper (Mitutoyo Corp, Kawasaki, Kanagawa, Japan). Similarly, thickness measurements were taken for films used for determination of water vapor permeability, puncture strength and tensile strength, and the values were accordingly calculated.

3.2.5 Film Crystallization

Crystallization of polymers in the films was monitored under a polarized microscope (Olympus-BX51, Melville, NY) with 100x magnification within 24 hours after casting.

3.2.6 Mechanical Properties

The mechanical properties of films were determined using a TA.XT*plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). Puncture strength (PS) was measured using the TA-108S fixture and 2 mm-diameter needle probe (TA-52) moving with a test speed of 1 mm/s. The PS was calculated by dividing the maximum force at break (N) by the thickness (mm) at the broken areas. Tensile strength (TS) and percent elongation at break (*%E*) were determined by following the standard procedures of ASTM D882-02 (ASTM, 2002). Film specimens per ratio treatment were cut by specimen cutting die (Qualitest USA LC, Plantation, FL) to the strips with uniform width of 6 mm and were tested

by using a double clamp (TA-96) at a test speed of 1 mm/s. Initial grip separation was set as 20 mm. The TS was expressed in MPa and was calculated by dividing the maximum load (N) by the cross-sectional area (m^2). Percent elongation at break was determined by dividing the extension at the moment of rupture by the initial gage length of the samples and multiplying by 100. PS, TS and *%E* measurements were replicated 3 times per ratio treatment.

3.2.7 Water Vapor Permeability

Gravimetric techniques are commonly used to determine water vapor permeability (WVP) of edible films. This test has been standardized by ASTM E96-05 (ASTM, 2005). Fisher permeability cups (Fisher Scientific, Pittsburgh, PA) filled with 5 ml distilled water were sealed by the tested films. Silicon sealant (High Vacuum Grease, Dow Corning, Midland, MI) and the ring covers with three screws around the cup circumference were used for attaching the films to the cups tightly. After measuring the initial weight, the cups were placed in an environment chamber (Yamato Scientific America, Inc., Orangeburg, NY) equipped with a fan for air circulation and set at 25°C and 50% RH. Steady state was obtained after 2 hours and cups were weighed 5 times at 1 hour intervals. Linear regression analysis of the weight loss versus time curves was performed to obtain the accurate steady state slopes. WVP ($g \cdot mm/m^2 \cdot h \cdot kPa$) was calculated with the following equations.

$$WVP = [G/t/A] \times [L/ (Pw_0 - Pw_2)] \quad (1)$$

Where G/t = the slope of the water weight loss versus time curves (g/h); A = cup mouth area (m^2); L = film thickness (mm); P_{w0} = water vapor partial pressure at water surface (Pa); Pw_2 = water vapor partial pressure at film surface outside the cup (Pa).

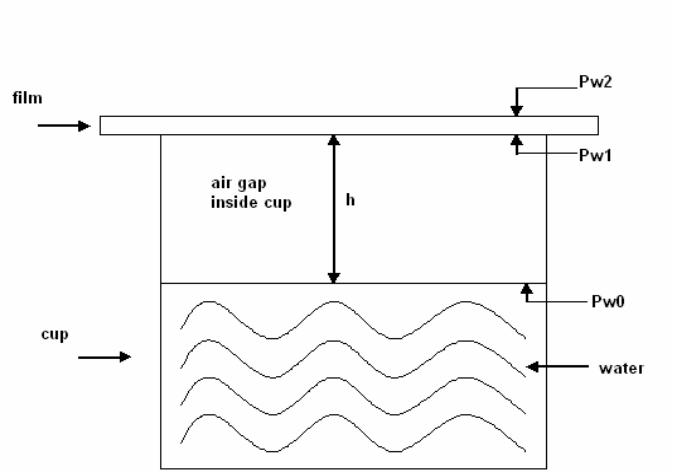


Figure 3.1 Schematic diagram of water vapor permeability measurement cup indicating locations of water vapor pressure values and air gap.

As shown in Figure 3.1, theoretically, partial water vapor pressure difference Pw_1-Pw_2 between the two sides of films provides the driving force for water vapor flux through the film. ASTM E96 method assumes that the air gap offers negligible resistance to water vapor transport and use Pw_0 instead of Pw_1 for the calculation. This method works well for testing films of low WVP, such as LDPE and HDPE plastic films. However, for polysaccharide- and protein-based films, which are hydrophilic and moisture sensitive, resistance provided by this air gap is significant (Gennadios et al., 1994). Neglecting the effect of air stagnant inside the cup between the film and water surface can lead to underestimation of actual WVP values (Gennadios et al., 1994; Miranda et al., 2004). Therefore, it is important to use WVP correction method which has addressed this problem and provided an equation to calculated Pw_1.

WVP correction method was used to calculate WVP ($g \cdot mm/m^2 \cdot h \cdot kPa$) with the following equations (Gennadios et al., 1994).

$$WVP = [G/t/A] \times [L/ (Pw_1 - Pw_2)] \quad (2)$$

Where G/t = the slope of the water weight loss versus time curves (g/h); A = cup mouth area (m^2); L = film thickness (mm); Pw_1 = water vapor partial pressure at film inner surface in

cup (Pa); Pw_2 = water vapor partial pressure at film surface outside the cup (Pa); Pw_1 is calculated as

$$P_{w1}=P_T-(P_T-P_{w0}) \exp [N_w \times h_i \times R \times T/ (P_T \times D)] \quad (3)$$

Where P_{w0} = water vapor partial pressure at water surface (Pa); P_T= total atmosphere pressure (Pa); N_w = measured value of water vapor transmission rate = G/t/A (g/h m^2); h_i = air gap height inside cup (mm); R= gas law constant (8.31 Pa m^3/mol K); T = absolute temperature (K); D = diffusivity of water vapor in air at 25ºC (0.26 cm^2/s).

3.2.8 Fourier Transform Infrared (FTIR) Spectra

Fourier transform infrared (FTIR) spectra were performed to evaluate the uniformity of the film surfaces. The spectra of the films were recorded using FTIR-ATR (Nexus 680, Thermo Nicolet Corp, Madison, WI) with the wavenumber range 500-4000 cm^{-1}.

3.2.9 Statistical Analysis

All measurements were done in triplicate with individually prepared films as the replicated experimental units. Significant differences between two groups were determined using least significant difference (LSD) test in the SAS program (SAS Institute, Cary, NC). On figures with bars, means within the same group with a different letter are significantly different ($p < 0.05$).

3.3 Results and Discussions

3.3.1 Rheological Property of LMW Chitosan/ HMW PEO Blend Solutions

Figure 3.2 presents the shear viscosity of LMW chitosan/ HMW PEO blend solutions at 25°C as a function of shear rate between 0.1 and 100 s^{-1}. Apparently, the flow curve of 1% LMW chitosan solution showed non-Newtonian shear thinning behavior, whereas 1% HMW PEO solution behaved as Newtonian fluid and showed extremely low viscosity due to their low concentration. Our results of viscosity of chitosan/PEO films agreed with those in the literature (Mucha, 1998; Duan et al., 2004).

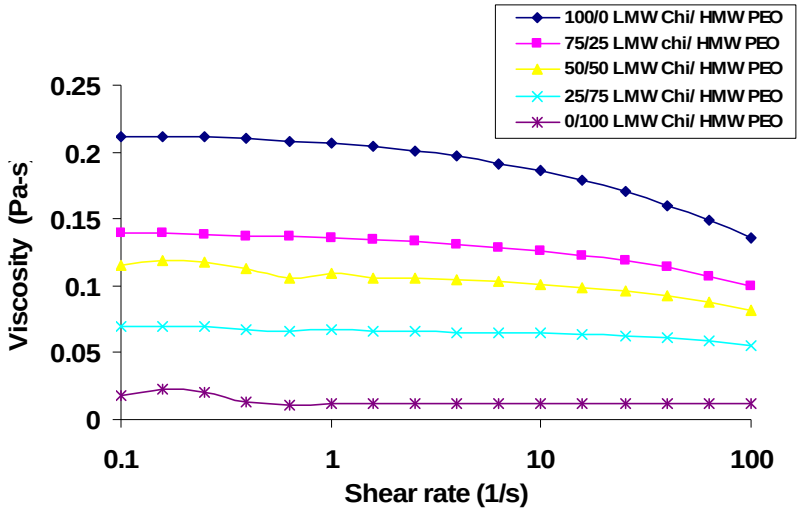


Figure 3.2 Shear viscosity as a function of shear rate at 25°C of LMW chitosan/HMW PEO solutions with different weight ratio: 100/0, 75/25, 50/50, 25/75, 0/100.

With the increase of PEO content, the viscosity of the blend solutions greatly decreased from 0.21 Pa·s for 100/0 chitosan/PEO solution to 0.018 Pa·s for 0/100 chitosan/PEO solution. Viscosity of polymer solution is the characteristics of intermolecular interactions between polymer chains (Bhattarai et al., 2005). The high viscosity of chitosan solution is due to the strong hydrogen bonding between NH_2 and OH groups of chitosan chains. The decrease of viscosity with addition of PEO could be attributed by the change of these interactions of chitosan chains (Bhattarai et al., 2005). It has been reported that chitosan can form complexes with PEO by forming strong hydrogen bonds between ether groups in PEO and hydroxyl groups in chitosan (Mucha, 1998). PEO molecules, which have already bound on chitosan backbones, could disrupt the self-association of chitosan chains by forming new hydrogen bonding between its hydroxyl groups and water molecules and decrease the solution viscosity (Bhattarai et al., 2005).

3.3.2 Film Appearance and Thickness

Films with higher chitosan content were easier to peel off of the Petri dishes. Under the same blend ratios, there were no apparent differences on the appearance between MMW/MMW and LMW/HMW chitosan/PEO films. Figure 3.3 shows the appearance of LMW chitosan/ HMW PEO films with different blend ratios. 100/0 and 75/25 chitosan/PEO films showed satisfactory transparency and PEO content reduced the film yellowish color. However, further increase of PEO content above 50% greatly increased the opacity of films. Mucha et al. (1999) reported that chitosan films prepared with 0-80% weight fraction of PEO had good transparency what was possibly due to the lower molecular weight of PEO (10 kDa) they used. In our films with PEO content of 50% or higher, PEO crystals were visible and were actually the reason for opacity (Figure 3.3). Since the film transparency is important for the food application, films with high PEO content may not be suitable.

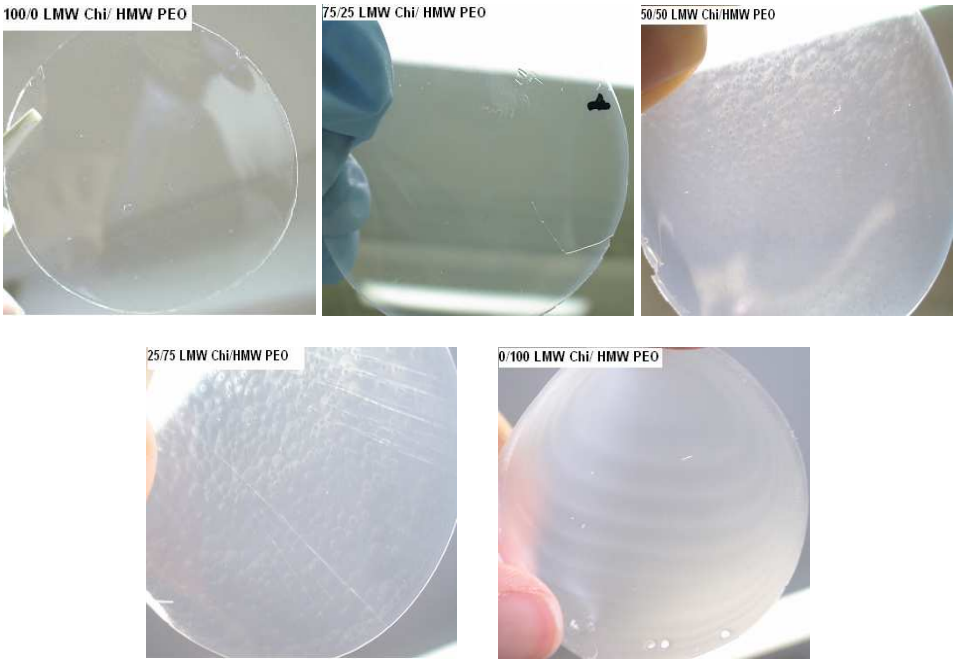


Figure 3.3 Photographs of LMW chitosan/HMW PEO blend films of various ratios (100/0, 75/25, 50/50, 25/75, 0/100).

Table 3.1 Thickness of chitosan/PEO blend films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100[a].

Film (w/w chitosan/PEO)	Thickness (μm) of MMW/MMW films	Thickness (μm) of LMW/HMW films
100/0	90.65 ± 6.32[a]	91.56 ± 2.23[a]
75/25	82.93 ± 1.93[ab]	89.09 ± 4.18[a]
50/50	84.51 ± 2.58[a]	80.73 ± 4.26[b]
25/75	75.44 ± 4.18[bc]	73.69 ± 4.62[c]
0/100	72.35 ± 4.63[c]	57.26 ± 6.71[d]

[a] Values reported are means and standard deviation. Superscript letters within one column indicate significant difference at $p<0.05$ by LSD test (SAS, 2000).

Thickness of the two types of chitosan/PEO films is shown in Table 3.1. The thickness of MMW/MMW films ranged from 72.35 ± 4.63 μm for 0/100 chitosan/PEO films to 90.65 ± 6.32 μm for 100/0 films, whereas for LMW/HMW chitosan/PEO films, thickness ranged from 57.26 ± 6.71 μm to 91.56 ± 2.23 μm. Interestingly, HMW PEO films were thinner than MMW PEO films, possibly because MMW PEO formed more and larger crystals than HMW PEO during film formation. With increase of PEO content in the blend, thickness of both types of the films significantly decreased ($p<0.05$). The reason for this tendency could be explained by the contraction of the three dimensional film matrixes after the strong intermolecular interactions between chitosan and PEO molecules polymer interactions (Pierro et al., 2007). In addition, decreasing tendency in the viscosity of film-forming solution with addition of PEO may also attribute to thinner film (Affrossman et al. 1999).

3.3.3 Film Crystallization

Figure 3.4 illustrates the shape and distribution of the crystals within the LMW chitosan/ HMW PEO films as observed under polarized microscope with 100X magnification. PEO is a semi-crystalline polymer. It crystallizes from solutions in spherulitic structures which appear as monoclinic unit cells of crystals with four radially oriented PEO chains (Mucha et al., 1999). On the other hand, when chitosan films are formed from the solution, the intramolecular interactions between the NH_3^+ and hydroxyl groups limit the molecular movement of the chitosan chains and

result in rather amorphous states of the films (Fuentes et al., 2000; Nunthanid et al., 2001; Xu et al., 2005; Mathew et al., 2006).

According to Figure 3.4, it was noticed that the increase of chitosan content in the blend decreased the extent of PEO crystallization, which was in agreement with those reported in the literature (Angelova et al., 1995; Mucha et al., 1999). In general, copolymer blends could reduce the crystallinity from the initial polymer. Chitosan has been reported to retard the crystallinity of films blending with cellulose (Isogai and Atalla, 1992), poly (3-hydroxybutyric acid) (Ikejima and Inoue, 2000), nylon 11 (Kuo et al., 2006), polycaprolactone (Sarasam et al., 2006), and poly (vinyl alcohol) (Chen et al., 2007). It could be explained by that the stiff chitosan molecular chains affected the overall mobility in the blend and impeded the rate of crystal growth (Zhao et al., 1995).

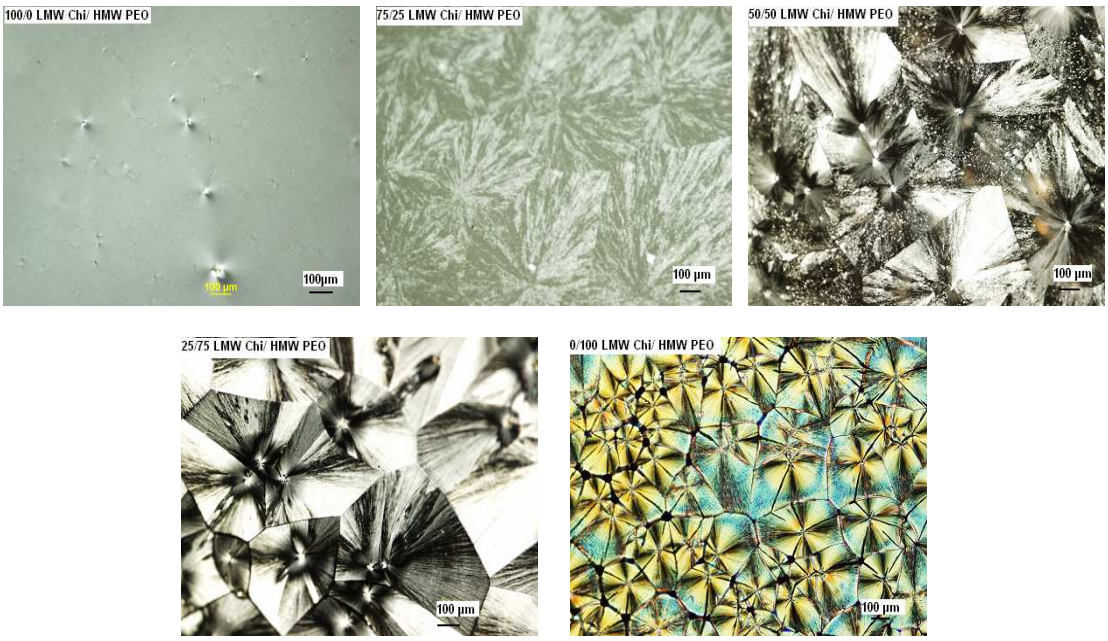


Figure 3.4 Polarized micrographs of thick films with LMW chitosan/HMW PEO blend ratios of 100/0, 75/25, 50/50, 25/75, 0/100 with 100X magnification within 24 hours after casting.

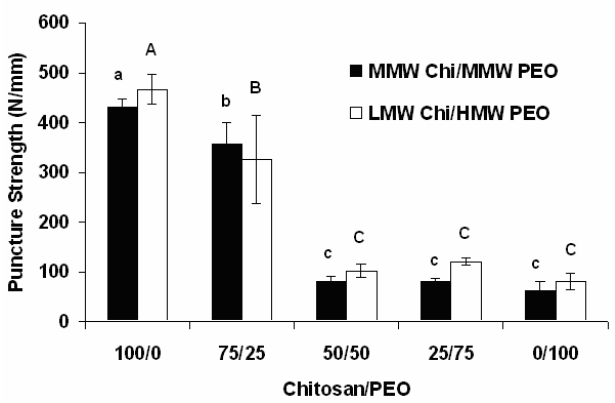
3.3.4 Film Mechanical Properties

The results for puncture strength (PS), tensile strength (TS) and elongation at break (%E) for two types of chitosan/PEO films with different blend ratios are shown in Figure 3.5.

Pure chitosan films exhibited puncture strength (PS) of 431.28-466.89 N/mm, what was higher than the values from the literature. Nadarajah (2005) reported that the puncture strength of their chitosan films was 342.75 N/mm. This difference was possibly because the molecular weight of chitosan they used (3584 Da) was far lower than that used in the prevent study (150 kDa, 450 kDa). Nunthanid et al. (2001) and Park et al. (2002) reported that mechanical properties of polymeric films are significantly affected by molecular weight of the polymers. Regardless on molecular weight of two polymers, the PS of two types of chitosan/PEO films decreased significantly as the chitosan fraction decreased, from 431.28 ± 16.55 N/mm to 63.92 ± 16.39 N/mm for MMW/MMW chitosan/PEO films, and from 466.89 ± 29.72 N/mm to 81.82 ± 15.11 N/mm for LMW/HMW chitosan/PEO films (Figure 3.5 A). Interestingly, when PEO content increased from 25% to 50%, PS greatly decreased from 325.79-358.33 to 80.39-102.33 N/mm, but further increase in PEO content did not make difference in PS. Obviously the PS of chitosan/PEO films was closely related to film composition. The addition of PEO made the blend films easier to puncture.

In Figure 3.5 B, pure chitosan films showed high value of tensile strength (TS) of 78.06 MPa and 83.01 MPa for MMW and LMW chitosan films, respectively. The TS of our films are comparable with the reported values (60.70-82.4 MPa) of chitosan films in the literature (Blair et al., 1987; Kittur et al., 1998; Suyatma et al., 2004; Garcia et al., 2006).

A



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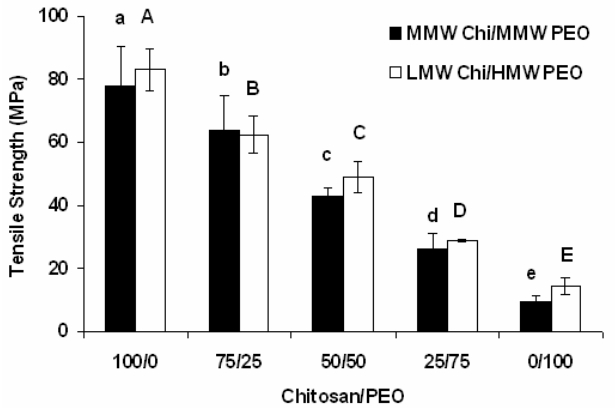

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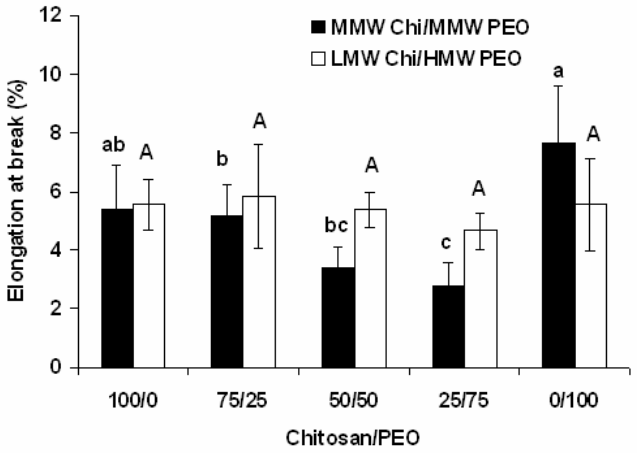


Figure 3.5 Comparison of (A) puncture strength, (B) tensile strength and (C) elongation at break of MMW chitosan/MMW PEO films and LMW chitosan/ HMW PEO films with blend ratio from 100/0 to 0/100. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$.

Alexeev et al. (2000) observed that chitosan/PEO blend films containing 16.7 wt% PEO had TS doubled compared to pure chitosan films. However, our results did not show this phenomenon at the related ratio. On the contrary, the increasing PEO content decreased the TS of blend films significantly (Figure 3.5 B). The both types of blend films with different chitosan and PEO molecular weight combination exhibited the similar decreasing tendency. Pure PEO films showed extremely low values of TS at 14.45 MPa for HMW PEO and 9.74 MPa for MMW PEO.

Similarly to TS, PEO content did not attribute to the flexibility of the films as expected (Figure 3.5 C). The percentage of elongation at break of chitosan/PEO blend films showed low values at all ratios. Even for pure PEO films, 7.6% of elongation at break was still much lower than for other synthetic films, such as LDPE (500%) and HDPE (300%) (Brinston, 1988), and edible films, such as wheat gluten films (276.2%) (Gennadios et al., 1993), soy protein isolate (90.27%) (Brandenburg et al., 1993).

Since PEO has flexible chains, it has potential to improve the mechanical properties of blends containing rigid chains of chitosan. However, PEO did not improve the mechanical properties of chitosan films as expected. This is possibly due to the high crystallization of PEO during film formation. The numerous large PEO crystals broke the integrated structure of the films and impaired their mechanical strength.

3.3.5 Water Vapor Permeability

Table 3.2 shows the effect of molecular weight and blend ratios of chitosan and PEO on the water vapor permeability (WVP) of the films. WVP values were calculated based on both ASTM E96 method and WVP correction method reported by Gennadios et al. (1994). Apparently, double higher WVP values of same film were obtained using the equation of WVP correction method than using the equation of ASTM E96 method.

Table 3.2 Comparison of water vapor permeability of MMW/MMW and LMW/HMW chitosan/PEO films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100 in the environmental chamber at 25°C and 50% RH, calculated by ASTM E96 method and correction method*.

Film (w/w chitosan /PEO)	WVP (g·mm/m^2·h·kPa) of MMW/MMW Chiotsan/PEO Films		WVP (g·mm/m^2·h·kPa) of LMW/HMW Chitosan/PEO films	
	ASTM E96	Correction Method	ASTM E96	Correction Method
100/0	3.14 ± 0.35	6.36 ± 1.05^a	3.60 ± 0.70	8.19 ± 2.99^A
75/25	3.40 ± 0.09	7.03 ± 0.34^a	2.88 ± 0.42	5.76 ± 1.29^A
50/50	3.07 ± 0.27	6.12 ± 0.57^a	2.95 ± 0.10	6.32 ± 0.80^A
25/75	2.54 ± 0.12	5.47 ± 0.25^a	2.53 ± 0.25	5.38 ± 0.57^A
0/100	3.12 ± 0.28	6.83 ± 0.55^a	2.12 ± 0.55	5.09 ± 2.22^A

*Superscript letters within one column indicate significant difference at $p<0.05$ by LSD test (SAS, 2000).

As shown in Table 3.2, WVP values based on WVP correction method ranged between 5.47 and 6.83 g·mm/m^2·h·kPa for MMW/MMW chitosan/PEO films and between 5.09 and 8.19 g·mm/m^2·h·kPa for LMW/HMW chitosan/PEO films. In spite of hydrophilic nature of PEO, the blending of high or medium molecular weight PEO into chitosan films did not increase the WVP of films significantly. On the contrary, the WVP of LMW/HMW chitosan/PEO films showed a slightly but not-significant decreasing tendency with increasing PEO content. The impeded water diffusivity might be due to the intermolecular interactions between chitosan and PEO molecules resulting in more compact films.

Compared to the WVP values of other polysaccharide-based or protein-based films and synthetic films reported in the literature (Table 2.3), our chitosan/PEO films based on WVP correction method performed a high level of WVP value. These differences may come from the method used, measuring conditions such as RH gradient and temperature, and consideration of air gap effect or not. For example, Wong et al. (1992) reported a relatively low WVP value of 0.128 g·mm/m^2·h·kPa for 38 μm-thick chitosan films without considering the air gap effect. In addition, different RH gradient would also affect the water vapor permeability of edible films, especially of hydrophilic ones. Water vapor permeability has been reported to vary with the applied water pressure gradient for cellulose films (Woodruff et al., 1972), and amylose films

(Rankin et al., 1958), because water molecules would interact with polar groups in the film structure causing polasticization or swelling (Gennadios et al., 1994). In addition, since McHugh et al. (1993) had pointed out the positive relationships between thickness and WVP of hydrophilic films, our high WVP value may also be due to their high thickness (57.26-91.56 μm).

3.3.6 FTIR Analysis

FTIR spectra of two types of the films at different blend ratio were taken (Figure 3.6). In the FTIR spectrum of chitosan films, the relative broad band at 3346.09 cm^{-1} was due to the stretching vibration of N-H and O-H (Miya and Iwamoto, 1984). The weak peak at 2882.56 cm^{-1} was typical C-H stretching vibration (Wang et al., 2004). The C=O stretching (amide I) peak and the N-H bending (amide II) peak were observed near 1635 cm^{-1} and 1550 cm^{-1} regions, respectively (Nunthanid et al., 2001). A peak of NH_2 stretching near 1600 cm^{-1} was obviously overlapped with amide I and amide II bands. As to PEO films, the peak at 2884.06 cm^{-1} was also attributed to C-H stretching vibration (Mishra and Rao, 1999).

When two or more polymers were mixed, the characteristic spectra peaks of IR spectrum of the blends should be considerably different from the spectra of each component due to the chemical interactions, resulting in band shifts, intensity changes, and broadening (Yin et al., 1999; Xiao et al., 2003). For our two types of films, with the increase PEO in the blends, the absorption band at 3346.09 cm^{-1} shifted to lower frequencies at 3214 cm^{-1} for 50/50 MMW chitosan/MMW PEO films and at 3291 cm^{-1} for 50/50 LMW chitosan/HMW PEO films, indicating the formation of more hydrogen bonds between the chitosan hydroxyl and PEO ether groups (Wrzyszczynski et al., 1995; Yilmaz et al., 2003). Additionally, their intensity decreased with the increase of PEO ratio, suggesting that parts of the hydrogen bonds in chitosan were broken by the addition of PEO. At lower wavenumbers, both the amide I and amide II bands shifted increasingly to higher wavenumbers from 1635.02 cm^{-1} to 1652.84 cm^{-1} (MMW/MMW films) and 1640.57 cm^{-1} (LMW/HMW films) for amide I band, and from 1550 cm^{-1} to 1558 cm^{-1} (MMW/MMW films) and

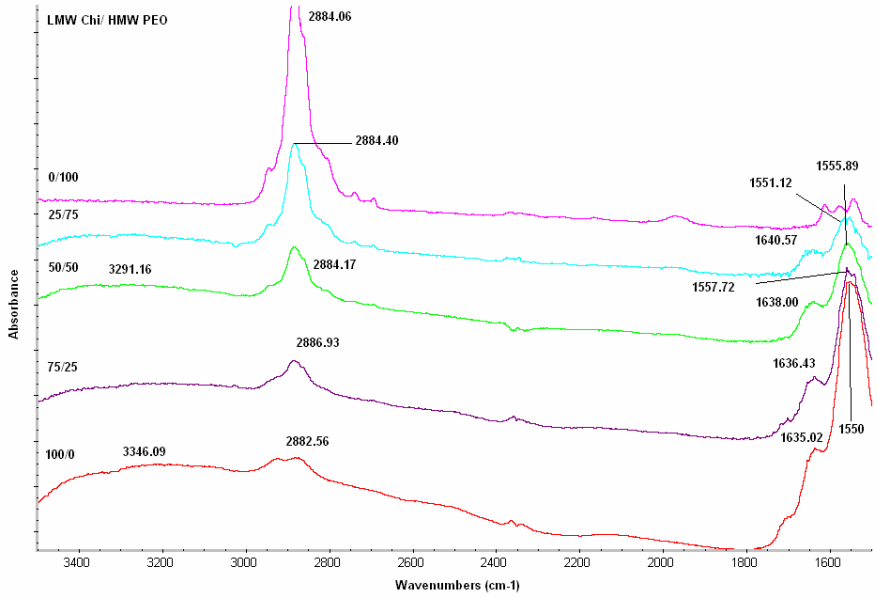
1557.72 cm^{-1} (LMW/HMW films) for amide II band. Since amide I and II bands represented the structure of N-acetyglucosamine (-NH-CO-CH_3), the results indicated that the undeacetylated groups in chitosan were forming less hydrogen bond with PEO than the hydroxyl groups (Khoo et al., 2003). The interaction between –NH_2 groups of chitosan and PEO could not be recognized by the present results.

FTIR analysis indicated that hydrogen bonding interaction existed between chitosan and PEO polymers. The two types of chitosan/PEO films showed similar behavior on the physical blend and chemical interactions.

3.4 Conclusions

Chitosan and PEO with different molecular weights and different ratios were used to prepare films by casting method. FTIR analysis indicated that hydrogen bonding interaction existed between chitosan and PEO polymers. Regardless on molecular weight of the polymers, incorporation of PEO into chitosan films resulted in less coloration of the films, reduced thickness, puncture and tensile strength, but had no effect on flexibility and water vapor permeability of films. Chitosan content apparently decreased tendency to spherulitic crystallization of PEO. Regardless of molecular weight of the polymers, films with 50% or more chitosan content showed better physical and mechanical properties.

A



B

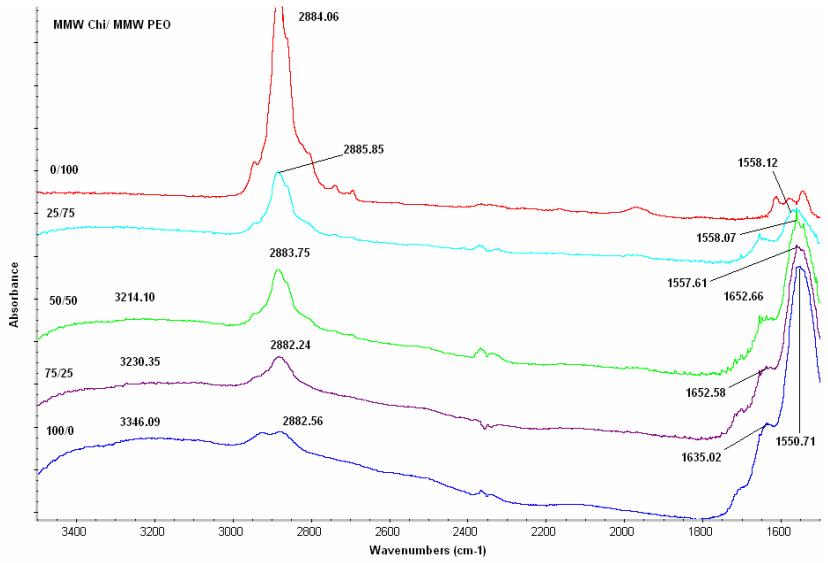


Figure 3.6 A portion (3500-1500 cm-1) of FTIR spectra of LMW chitosan/ HMW PEO films (A), MMW chitosan/ MMW PEO films (B) at blend ratios of 100/0, 75/25, 50/50, 25/75, 0/100. All spectra are plotted in absorbance mode.

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CHAPTER 4. EFFECT OF BLEND RATIOS ON PHYSICAL, MECHANICAL AND ANTIBACTERIAL PROPERTIES OF THIN CHITOSAN/PEO FILMS

This chapter is a lightly revised version of the following paper: Zivanovic S, Li J, Davidson PM, Kit K. 2007. Physical, mechanical and antibacterial properties of chitosan/PEO blend films. Biomacromolecules. 8 (5): 1505-1510.

Abstract

Films formed by blending of two polymers usually have modified physical and mechanical properties compared to films made of the individual components. Our preliminary studies indicated that regardless on molecular weight of the polymers, chitosan/poly (ethylene oxide) (PEO) blend films with the thickness of 57-91 μm showed better physical and mechanical properties when they had 50% or more chitosan content. The objective of this study was to determine the correlation between chitosan/PEO weight ratio and the physical, mechanical, and antibacterial properties of corresponding films with the thickness of 25-44 μm. Films with chitosan/PEO weight ratios from 100/0 to 50/50 in 10% increments were characterized by measuring thickness, puncture strength (PS), tensile strength (TS), elongation at break (%E), water vapor permeability (WVP), and water solubility (WS). Additionally, the films were examined by polarized microscopy, wide-angle X-ray diffraction (WAXD), and Fourier transform infrared (FTIR) spectroscopy, and their antibacterial properties were tested against *Escherichia coli* K12. The chitosan fraction contributes to antimicrobial effect of the films, decreases tendency to spherulitic crystallization of PEO, and enhances puncture and tensile strength of the films, while addition of the PEO results in thinner films with lower water vapor permeability. Films with 90/10 blend ratio of chitosan/PEO showed the most satisfactory PS, TS, %E, and antibacterial properties of all tested ratios.

4.1 Introduction

There has been a growing interest over the past few years in the development of biopolymers partly because of their renewable, sustainable, and biodegradable properties. As one of the candidates for such biopolymers, chitosan has attracted much attention mainly because of its antimicrobial and metal-binding properties. Chitosan is a cationic biopolymer obtained by a full or partial N-deacetylation of chitin, which is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans (Roberts, 1992). Chitosan may be regarded as a binary heteropolysaccharide containing β (1-4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose residues (Angelova et al., 1995). Chitosan has been evaluated for various uses in the food, medical, pharmaceutical, agricultural, and chemical industries because of its nontoxic, biocompatible, mucoadhesive, and biodegradable properties (Angelova et al., 1995; Agnihotri et al., 2004; Zivanovic et al., 2005). Dissolved chitosan has antimicrobial and metal-binding properties and has been used as an antimicrobial additive to bind metals from food-processing wastewaters (Sudarshan et al., 1992; Selmer-Olsen et al., 1996; Zivanovic et al., 2004). In addition, because of its free amino groups, chitosan can be dissolved in acidic aqueous solutions and form gels, films, sutures, beads, and fibers (Angelova et al., 1995).

Poly (ethylene oxide) (PEO) is a synthetic uncharged polymer with a molecular formula $(-CH_2CH_2O-)_n$. It is low-toxic, semicrystalline, bioadhesive, and mucoadhesive because of its water solubility, hydrophilicity, high viscosity, ability to form hydrogen bonds, and biocompatibility with other bioactive substance. PEO has been widely used in a variety of dosage forms in the pharmaceutical industry such as for the production of hot-melt extruded capsules. However, since PEO is a flexible-chain polymer, pure PEO films have relatively poor mechanical and physical characteristics and high water solubility which limit their application. A convenient and effective method to improve PEO film properties is blending PEO with other polymers. The films formed by blending two or more polymers usually result in modified physical and mechanical

properties compared to films made of the individual components. It has been reported that starch-chitosan blend films exhibited a higher flexibility and improved the percentage elongation than films produced from single polymers (Mathew et al., 2006). The blend of chitosan and quaternized poly (4-vinyl-*N*-butyl) pyridine showed stronger tensile strength and breaking elongation than films of pure chitosan (Liu and Xiao, 2004).

Chitosan/PEO blend films may provide additional functionality compared to the pure polymer films. Chitosan may improve mechanical properties and decreased water solubility of the PEO films, while PEO may contribute to the formation of colorless films that are more flexible. Our preliminary data showed that thick films with chitosan content of more than 50% decreased the PEO tendency to spherulitic crystallization. Therefore, the objective of this research was to evaluate the physical, mechanical, and antibacterial properties of thin films composed of blends of low molecular weight chitosan and high molecular weight PEO.

4.2 Materials and Methods

4.2.1 Materials

Chitosan of low molecular weight (LMW ~150 kDa) with a degree of deacetylation above 85% and PEO of high molecular weight (HMW ~900 kDa) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

4.2.2 Film Preparation

Mixtures of chitosan and PEO with different weight ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50) were dissolved in 1% w/w acetic acid and stirred over night at room temperature. The film forming solutions, regardless on the chitosan/PEO ratio, contained 1% w/w polymer solids in 1% w/w acetic acid. After filtration through Miracloth® (Calbiochem-Novabiochem Corp., San Diego, CA), 10 g of film-forming solutions were poured into 50mm-diameter polystyrene Petri dishes and the solvent was evaporated in a vacuum oven at 38°C under 17 kPa pressure for

24 hours. The dried films were peeled from the Petri dishes and kept in desiccators at 20% relative humidity at room temperature.

4.2.3 Thickness

Film thickness (μm) was determined on 6 films per ratio treatment averaging measurements at 5 points for each film using a hand-held microcaliper (Mitutoyo Corp, Kawasaki, Kanagawa, Japan). Similarly, thickness measurements were taken for films used for determination of water vapor permeability, puncture strength and tensile strength, and the values were accordingly calculated.

4.2.4 Film Crystallization

Crystallization of polymers in the films was monitored by a polarized microscope (Olympus-BX51, Melville, NY). Films were observed under polarized microscope with 100x magnification within 24 hours after casting. X-ray diffractometer was used to inspect the diffractograms of the films at ambient temperature. It was conducted using a Molecular Metrology SAXS/WAXD system equipped with a monochromic CuKα (1.5418 Å) X-ray source, a three-pin-hole alignment, and two-dimensional detector operating at 45 kV and 0.66 mA with the beam size of 30 μm. The WAXD patterns were recorded on reusable Fuji image plates, with the sample-to-film distance for 36.52 mm. The image plate was scanned in a Fuji X BAS-1800II image analyzer and the resultant image was converted to intensity versus 2θ; or q plot using Polar X-ray analysis software. The data were collected over an angular range from 5° to 40° 2θ in a continuous mode. Relative crystallinity percentage (Xc) of the films were calculated by the following equation (Helander et al., 2001):

$$Xc = [Ac/(Ac + Aa)] \times 100\%$$

Where Ac and Aa are the areas of the crystalline and amorphous regions, respectively.

4.2.5 Film Mechanical Properties

The mechanical properties of films were determined using a TA.XT*plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK).

Puncture strength (PS) was measured using the TA-108S fixture and 2 mm-diameter needle probe (TA-52) moving with a test speed of 1 mm/s. The PS was calculated by dividing the maximum force at break (N) by the thickness (mm) at the broken areas. Tensile strength (TS) and percent elongation at break (*%E*) were determined by following the standard procedures of ASTM D882-02 (ASTM, 2002). Film specimens per ratio treatment were cut by specimen cutting die (Qualitest USA LC, Plantation, FL) to the strips with uniform width of 6 mm and were tested by using a double clamp (TA-96) at a test speed of 1 mm/s. Initial grip separation was set as 20 mm. The TS was expressed in MPa and was calculated by dividing the maximum load (N) by the cross-sectional area (m^2). Percent elongation at break was determined by dividing the extension at the moment of rupture by the initial gage length of the samples and multiplying by 100. PS, TS and *%E* measurements were replicated 3 times per ratio treatment.

4.2.6 Water Solubility

The dried films were immersed in 40 ml methanol for 24 hours to remove acetic acid existed in the films and then taken out and dried in the vacuum oven for 24 hours at room temperature. The dried films were weighed (W_1), immersed in distilled water for 24 hours, dried in vacuum oven for 24 hours at room temperature, and then stored in a dessicator for 5 hours for weight balance. The equilibrium weights of the films were weighed (W_2). Water solubility was calculated as the following equation:

$$Ws = [(W_1 - W_2)/W_1] \times 100\%.$$

4.2.7 Water Vapor Permeability

Water vapor permeability (WVP) was measured using gravimetric techniques. Fisher permeability cups (Fisher Scientific, Pittsburgh, PA) filled with 5 ml distilled water were sealed by the tested films. Silicon sealant (High Vacuum Grease, Dow Corning, Midland, MI) and the ring covers with three screws around the cup circumference were used for attaching the films to the cups tightly. After measuring the initial weight, the cups were placed in an environment chamber (Yamato Scientific America, INC., Orangeburg, NY) equipped with a fan for air circulation and set

at 25°C and 50% R.H. Steady state was obtained after 2 hours and cups were weighed after 3 to 7 hours at 1-hour intervals as well as after 17 hours. Two types of steady state slopes were calculated by linear regression analysis of the curves of weight loss versus time (after 3-7 hours) and time (after 3-17 hours), respectively. WVP was calculated using both ASTM E-96 standard method (ASTM, 2005) and the WVP correction method described by Gennadios et al. (1994).

4.2.8 Fourier Transform Infrared (FTIR) Spectra

Fourier transform infrared (FTIR) measurements were performed to evaluate the uniformity of the film surfaces. The spectra of the films were recorded using FTIR-ATR (Nexus 680, Thermo Nicolet Corp, Madison, WI) with the wavenumber range 500-4000 cm^{-1}. Film uniformity was assessed by observing both top and bottom sides of the same films.

4.2.9 Antibacterial property in the liquid system

Escherichia coli K-12, the test microorganism, was grown in Brain Heart Infusion (BHI; Difco) broth for 48 hours at 35°C. Test films of 50 mm diameter were submerged into culture tubes containing 9 ml sterile phosphate buffer (0.05 M, pH=7.08) inoculated with ca. 10^6 CFU/ml bacteria, mixed by vortexing and incubated for 6 hours at 25°C. Phosphate buffer with the same *E. coli* K-12 inoculum but with no film was used as positive control and phosphate buffer with film but no inoculum was the negative control. The survival of *E. coli* K-12 was determined using the pour-plate method on Trypticase Soy Agar (TSA) medium (Swanson et al., 2001). All measurements were performed with 3 replications.

4.2.10 Statistical Analysis

All measurements were done in triplicate with individually prepared films as the replicated experimental units. Significant differences between two groups were determined using least significant difference (LSD) test in the SAS program (SAS, 2000). On figures with bars, means within the same group with a different letter are significantly different ($p < 0.05$).

4.3 Results and Discussions

4.3.1 Appearance and thickness

Figure 4.1 shows the appearance of chitosan/PEO thin films with the ratio from 100/0 to 50/50. All tested films were transparent. The films with chitosan/PEO ratios of 100/0 and 90/10 had slightly yellowish color, but as the fraction of PEO increased, the 80/20 to 50/50 films became colorless. Our previous research indicated that more than 50% PEO resulted in opacity of the films due to the spherulitic crystallization characteristics for PEO.

Thickness of the films is shown in Table 4.1. Thickness ranged from 25.4 ± 1.2 μm for 50/50 blend ratio to 44.6 ± 1.7 μm for 90/10 blend ratio, indicating that thickness of the films significantly increased ($p<0.05$) with increase of chitosan content in the blend.

4.3.2 Film Crystallization

Since PEO is a semi-crystalline polymer, it is necessary to study its crystallization within the films as it may affect structure and physical properties of the films. Figure 4.2 illustrates the shape and distribution of the crystals within the films as observed under polarized microscope with 100X magnification. Similar to the results of thick chitosan/PEO films, the increase of chitosan fraction in the thin chitosan/PEO films reduced spherulitic crystallization of PEO and no crystallization was observed in the films with 90/10 blend ratio (Figure 4.2).

Table 4.1 Thickness of chitosan/PEO blend films with weight ratio from 100/0 to 50/50[a].

Film (w/w chitosan/PEO)	Thickness (μm)
100/0	38.1 ± 4.1[b]
90/10	44.6 ± 1.7[a]
80/20	34.5 ± 0.6[c]
70/30	30.2 ± 0.6[d]
60/40	29.4 ± 1.2[d]
50/50	25.4 ± 1.2[e]

[a] Values reported are means and standard deviation. Superscript letters indicate significant difference at $p<0.05$ by LSD test (SAS, 2000).

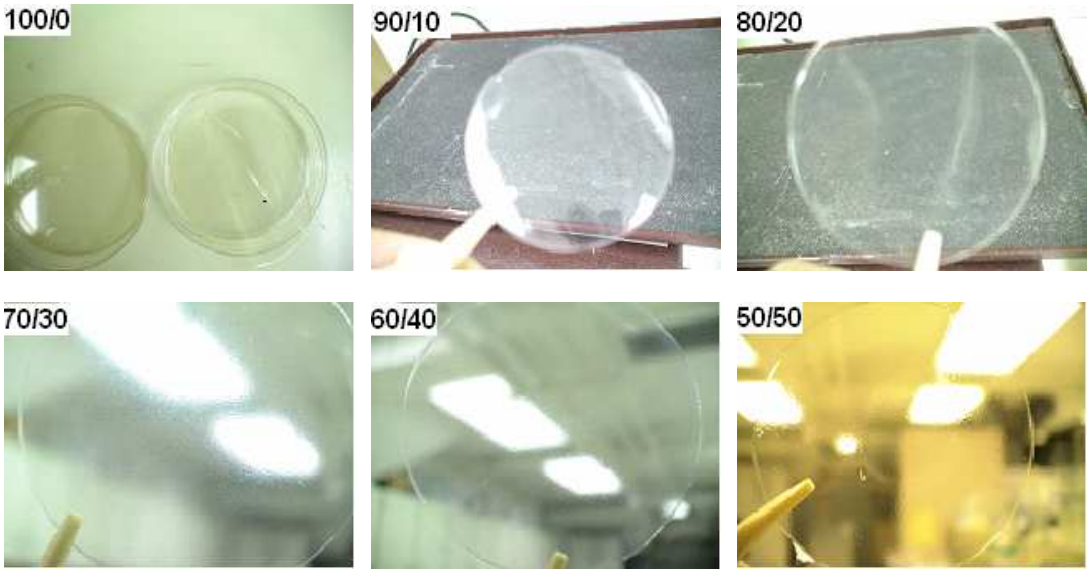

Figure 4.1 Photographs of LMW chitosan/HMW PEO blend films of various ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50).

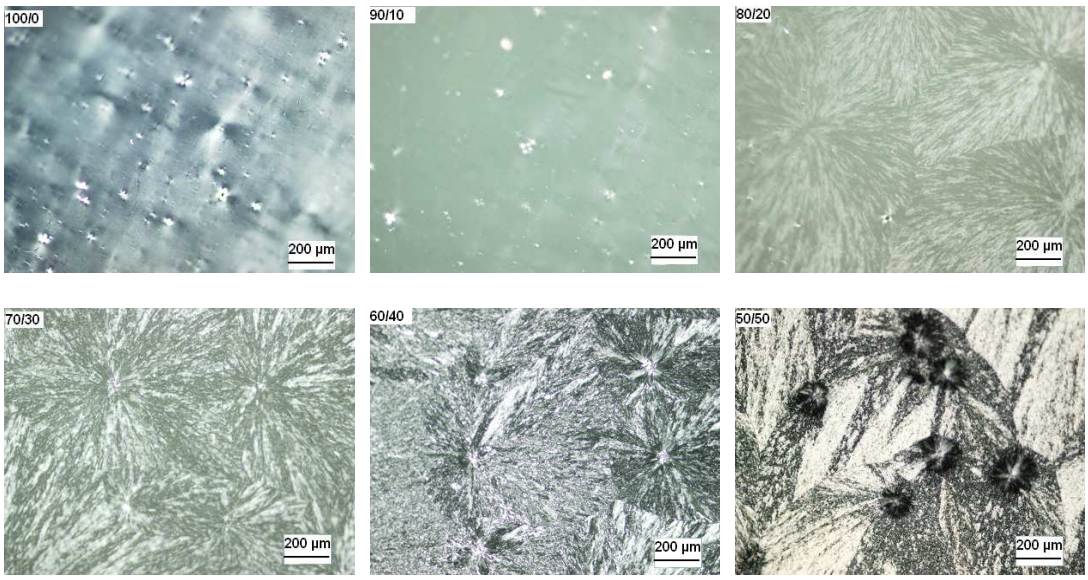

Figure 4.2 Polarized micrographs of films with chitosan/PEO blend ratios of 100/0, 90/10, 80/20, 70/30, 60/40, 50/50 with 100X magnification within 24 hours after casting. Scale bar: 200 µm.

Compared the polarized micrographs of thick chitosan/PEO films (Figure 3.4), the number of PEO crystals formed in the thin films was less than those in thick films.

Wide-angle X-ray diffraction (WAXD) patterns of 100/0 to 50/50 chitosan /PEO blend films are presented in Figure 4.3. The diffractogram of both 100/0 and 90/10 films did not show characteristic peaks but a broad hump in the range of 10° to 40° 2θ, which indicated a predominantly amorphous form of the chitosan in the films (Figure 4.3 A, B). In contrast, all of the diffraction patterns of 80/20 to 50/50 chitosan/PEO blend films showed two sharp peaks with the highest intensity at 19.0° and 23.3° 2θ, resulting from PEO crystals (Figure 4.3 C to F).

The corresponding diffraction angle (2θ) and relative crystallinity percentage (X_c) are listed in Table 4.2. As expected, the crystallinity of the films from 80/20 to 50/50 blend ratio increased significantly having the value of X_c increasing from 14.9% to 34.3%, respectively. The reduction in PEO crystallization by chitosan is probably a result of interruption of PEO-PEO interactions due to formation of hydrogen bonds between ether and amino groups from PEO and chitosan, respectively. Similar to findings of Angelova et al. (1995), our results of X-ray diffraction analysis confirmed that 90% or more chitosan inhibits the crystallization of PEO in the cast films, while the clear phase separation happens when chitosan content is 80% or less.

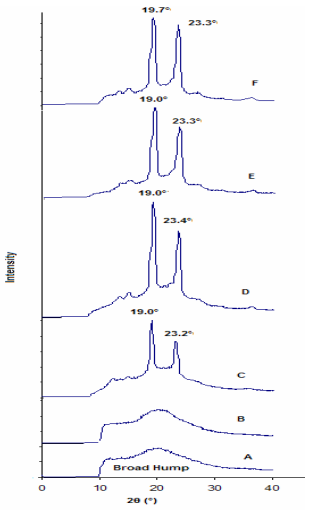

Figure 4.3 2θ-scan Wide-angle X-ray diffraction(WAXD) patterns of films with chitosan/PEO blend ratio of (A) 100/0, (B) 90/10, (C) 80/20, (D) 70/30, (E) 60/40, (F) 50/50.

Table 4.2 Diffraction angle (2θ) of sharp peaks and relative crystallinity percentage (Xc) of the chitosan/PEO blend films with weight ratio from 100/0 to 50/50.

Film (w/w chitosan/PEO)	Peak I (°)	Peak II (°)	*X*c(%)
100/0	a*	a	a
90/10	a	a	a
80/20	19.0	23.2	14.9
70/30	19.0	23.4	28.7
60/40	19.0	23.3	36.7
50/50	19.7	23.3	34.3

*a indicates there is no readable crystalline peak.

4.3.3 Film Mechanical Properties

The results for puncture strength (PS), tensile strength (TS) and elongation at break (*%E*) for films with different chitosan/PEO blend ratios are shown in Table 4.3.

The PS of the film increased from 158.9 ± 17.0 N/mm for 50/50 blend ratio to 416.0 ± 24.7 N/mm for 100/0 blend ratio. Interestingly, the PS increased significantly as the chitosan fraction increased from 50 to 80%, but there was no significant difference in the puncture strength among the films with 80% and more chitosan. Therefore, it appears that the addition of minimum 80% chitosan would be needed to increase the puncture strength of the blend films. Compared the PS of thick films we measured before, we found that decreasing the thickness of films did not significantly reduce the PS of films. Pure thick chitosan films (91 μm) exhibited the PS of 466.89 N/mm, while the PS of pure thin chitosan films (38 μm) was 416 N/mm. In addition, 50/50 thick chitosan/PEO films (80 μm) had the PS of 102.33 N/mm, while 50/50 thin chitosan/PEO films (25.4 μm) showed even higher value of the PS at 158.9 N/mm.

Similarly to PS, TS values increased from 47.0 ± 8.8 MPa for 50/50 films to 73.5 ± 7.1 MPa for 100/0 films. The TS of 90/10 chitosan/PEO films was similar to TS of pure chitosan films, but with 20% or more PEO in the blend, the tensile strength of the films significantly decreased relatively to the PEO fraction.

Table 4.3 Puncture strength (PS), tensile strength (TS) and elongation at break (%E) of the chitosan/PEO blend films with weight ratio from 100/0 to 50/50*.

Film (w/w chitosan/PEO)	PS (N/mm)	TS (MPa)	Elongation at break (%)
100/0	416.0 ± 24.7[a]	73.5 ± 7.1[a]	17.7 ± 13.3[a]
90/10	416.2 ± 54.9[a]	67.0 ± 2.3[ab]	11.1 ± 4.2[ab]
80/20	441.0 ± 21.6[a]	55.4 ± 14.6[bc]	10.8 ± 5.0[ab]
70/30	324.7 ± 31.8[b]	52.7 ± 9.8[bc]	8.0 ± 4.5[ab]
60/40	269.8 ± 6.3[c]	54.3 ± 3.8[bc]	8.1 ± 2.6[ab]
50/50	158.9 ± 17.0[d]	47.0 ± 8.8[c]	4.2 ± 1.4[b]

*Means of three replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly different ($p > 0.05$) by LSD test.

Compared to the TS values of the widely used plastic films, such as LDPE and HDPE with values of 8.6 - 17.3 MPa and 17.3 - 34.6 MPa, respectively (Brinston, 1998), our thin chitosan/PEO films still retained good tensile strength. The TS of thin chitosan/PEO films were comparable to thick films as illustrated by 83.01 MPa and 73.5 MPa for pure thick and thin chitosan films respectively, and 48.98 MPa and 47.0 MPa for 50/50 thick and thin chitosan/PEO films respectively.

Mean values for the elongation at break (%E) are also presented in Table 4.3. The results indicated that addition of PEO to the blend reduced extensibility of the films, creating more brittle products. Compared to the elongation values of LDPE and HDPE of 500% and 300%, respectively (Brinston, 1998), all the thin chitosan/PEO films exhibited poor elongation of 4.2 ± 1.4% for 50/50 films to 17.7 ± 13.3% for 100/0 films. Since crystallinity development of PEO in the films may be the main reason for impaired elongation, further studies will examine effects of film forming conditions on extensibility of the chitosan/PEO blend films.

4.3.4 Water Solubility

The results for water solubility of thin chitosan/PEO blend films are shown in Figure 4.4.

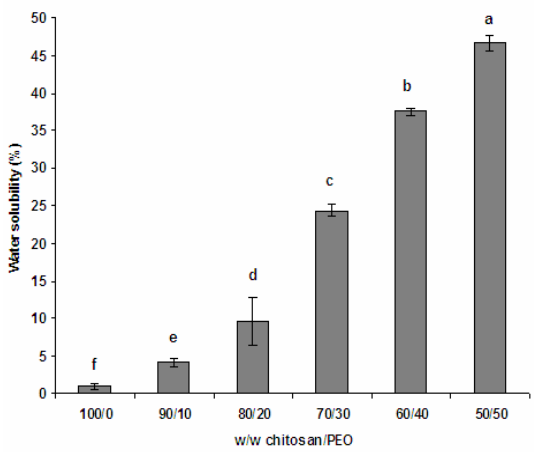

Figure 4.4 Effect of chitosan/PEO blend ratio from 100/0 to 50/50 on water solubility of the corresponding films. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$.

Chitosan is soluble in dilute aqueous acidic solution (pH<6.5) but insoluble in pure water, while PEO is water soluble polymer. Although all the films maintained the original shape after being immersed in methanol for 24 hours at room temperature, the 50/50 films dissolved the most, 46.6 ± 0.5% in water. However, with the increase of chitosan content, the water solubility significantly decreased, with only a 0.9 ± 1.0% loss for 100/0 films ($p<0.05$).

Although high water resistance of plastic films may be desired for many industrial applications, controlled solubility of biodegradable films offers advantages for use in the food pharmaceutical and agricultural industries. For example, it has been reported that microporous chitosan/PEO membranes can control rate of drug release by step-wise solubilization of PEO in water and altering the mesh size of the membranes (Jin and Song, 2006).

4.3.5 Water Vapor Permeability

Table 4.4 shows the water vapor permeability (WVP) of the chitosan/PEO films calculated based on both ASTM E96 method and WVP correction method reported by Gennadios et al. (1994).

Table 4.4 Comparison of water vapor permeability of thin chitosan/PEO films with weight ratio of 100/0, 90/10, 80/20, 70/30, 60/40, 50/50 in the environmental chamber at 25°C and 50% RH, calculated by ASTM E96 method and WVP correction method.

Film (w/w chitosan/PEO)	WVP* ($g \cdot mm/m^2 \cdot h \cdot kPa$)	
	ASTM E96	WVP Correction Method
100/0	1.85 ± 0.31	4.37 ± 0.70[a]
90/10	1.84 ± 0.09	4.44 ± 0.29[a]
80/20	1.64 ± 0.07	3.83 ± 0.15[a]
70/30	1.40 ± 0.10	2.69 ± 0.21[b]
60/40	1.40 ± 0.11	2.67 ± 0.82[b]
50/50	1.32 ± 0.05	2.43 ± 0.09[b]

*Steady state slopes were obtained by linear regression analysis of the curves of weight loss versus time (after 3-7 hours).

Similar to the comparison results in Chapter 3, the WVP values based on correction method were higher than those based on ASTM E96 method due to considering the effect from the air gap between the film and liquid surface. As shown in the Table 4.4, WVP correction values of chitosan/PEO films were significantly affected by the ratio of the polymers from 4.37 ± 0.70 $g \cdot mm/m^2 \cdot h \cdot kPa$ for 100/0 chitosan/PEO films to 2.43 ± 0.09 $g \cdot mm/m^2 \cdot h \cdot kPa$ for 50/50 films. Interestingly, films with 30%, 40%, and 50% PEO had similar and unexpectedly low WVP values compared to films with high chitosan content.

As suggested by Gontard and Guilbert (1994), water vapor transmission through a hydrophilic film depends on solubility and diffusivity of water molecules in the film matrix. However, although the increase of the PEO fraction enhanced the solubility of the films, it did not cause higher permeability, which indicates that the main factor in water vapor permeability is diffusivity of water molecules through the film matrix. The impeded diffusivity could be due to promotion of strong intermolecular interactions between chitosan and PEO molecules which decreased intermolecular distance resulting in more compact films and/or crystallinity of PEO what caused the percolation of water molecules around the insoluble crystals resulting in longer diffusion paths. On the other hand, the water vapor permeability changed with film thickness. The films with 90/10 chitosan/PEO ratio had the highest WVP value partly because these were

the thickest films. McHugh et al. (1993) has reported that the positive relationships exist between thickness and WVP since the increased film thickness could increase the relative humidity in vicinity of the films and alter the water sorption kinetics. These results indicate that the diffusion of our polymer blending films shows non-Fickian behavior (Crank, 1975).

Figure 4.5 compares the difference of WVP correction values of chitosan/PEO films based on different measurement periods. Apparently, a longer measurement period when the water weight loss were measured after 3-17 hours resulted in much higher WVP values than those measured in a shorter period (3-7 hours). Higher water weight loss especially after 17 hours was probably because the long time interaction between water molecules and hydrophilic chitosan/PEO films during diffusivity might have broken the films' structure and enabled more water to pass through the films. Therefore, we considered that WVP values measured under shorter period (3-7 hours) can better reflect the ability of chitosan/PEO films to control water vapor transport. In the following WVP studies, all the water weight loss was measured after 3-7 hours at 1-hour intervals. Comparing the WVP values of thick and thin chitosan/PEO films under the same blend ratios (Table 3.2, Table 4.4), we found that thin chitosan/PEO films had lower WVP values of 4.37 g·mm/m^2·h·kPa and 2.43 g·mm/m^2·h·kPa for 100/0 and 50/50 films than thick films with WVP values of 8.19 g·mm/m^2·h·kPa and 6.32 g·mm/m^2·h·kPa for 100/0 and 50/50 films, respectively. Thickness was still the main factor causing the difference.

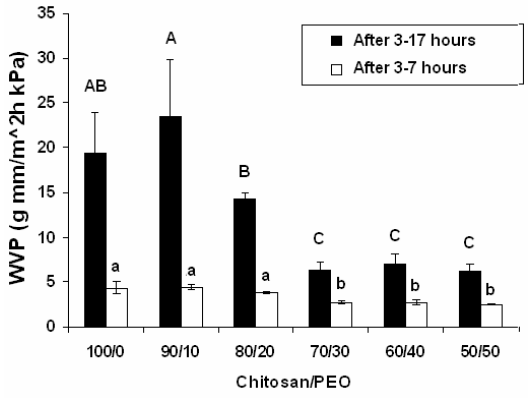

Figure 4.5 Effect of measurement period on water vapor permeability of chitosan / PEO blend ratio from 100/0 to 50/50 in the environmental chamber at 25°C and 50% R.H. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$.

4.3.6 FTIR Analysis

FTIR spectra of chitosan/PEO films are shown in Figure 4.6. Pure PEO (0/100) films were used for comparison. Four characteristic absorption bands of pure chitosan films are as follows (Figure 4.6): (1) a broad band at around 3500-3100 cm^{-1} attributed to N-H and OH···O stretching vibration, as well as intermolecular hydrogen bonding of chitosan molecules (Jiang et al., 1997; De Vasconcelos et al., 2006), (2) a very weak band at 2884 cm^{-1} from CH stretch, (3) the one at 1655 cm^{-1} was due to the amide I (Wan et al., 2006), and (4) the amine $–NH_2$ absorption band (amide II) at around 1550 cm^{-1}, respectively (Duan et al., 2004). As to the pure PEO film (Figure 4.6), the typical absorption band is at 2885 cm^{-1} attributed to the CH_2 stretching vibration (Duan et al., 2004), which can overlap with that of chitosan. Since a high molecular weight (900 kDa) PEO was used, absorption bands of hydroxyl groups from PEO could be considered negligible in the spectra (Sawatari and Kondo, 1999).

With the increase of PEO content, the absorbance intensity of CH_2 stretching vibration at 2885 cm^{-1} increased and absorbance intensity of $–NH_2$ stretching at around 1558 cm^{-1} decreased. Similar to the FTIR spectra of thick chitosan/PEO films, with the increase PEO in the blends, the broad absorption band shifted obviously to lower frequencies from 3277.02 cm^{-1} for 100/0 chitosan/PEO films to 3259.53 cm^{-1} for 50/50 films, indicating the formation of more hydrogen bonds between the chitosan hydroxyl and PEO ether groups (Wrzyszczynski et al., 1995; Yilmaz et al., 2003). Also the decreasing intensity of the broad band near 3259-3277 cm^{-1} showed us that part of the hydrogen bonds in chitosan was broken by the addition of PEO. The peak change of amide I and II groups towards higher frequency confirmed that the undeacetylated groups in chitosan was less favorite as hydrogen bonding sites than hydroxyl groups (Khoo et al., 2003).

To detect the surface composition and evaluate stability of the cast films, the films were observed from top and bottom side with FTIR spectroscopy (Figure 4.7). In films with less than 50% PEO, chitosan was apparently oriented towards the top side of the films and PEO towards the bottom side, while in 50/50 films the FTIR scans were almost the same.

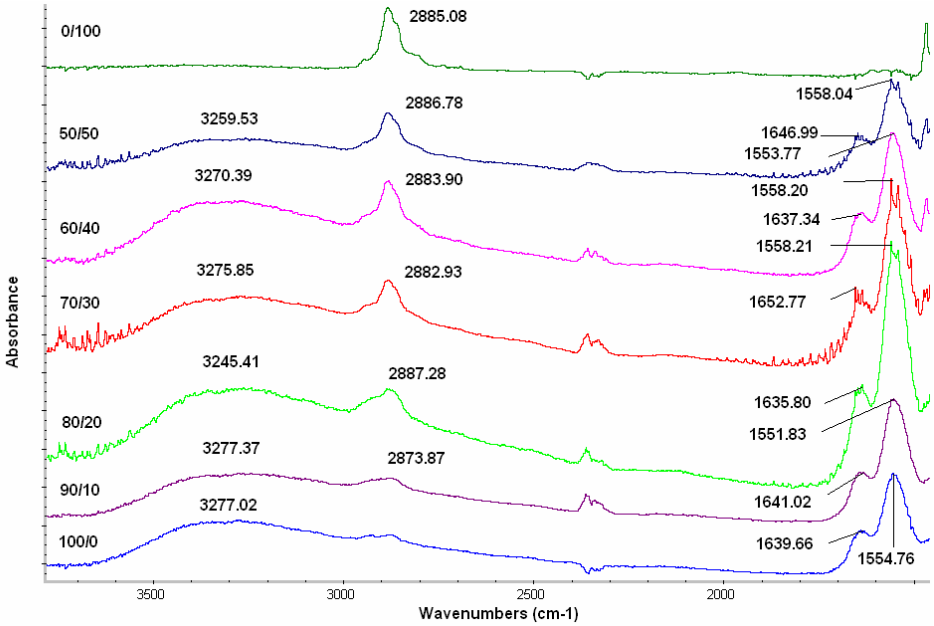

Figure 4.6 A portion (3600-1500 cm-1) of FTIR spectra of chitosan/PEO blend films with ratio of 0/100, 50/50, 60/40, 70/30, 80/20, 90/10, and 100/0. All spectra are plotted in absorbance mode.

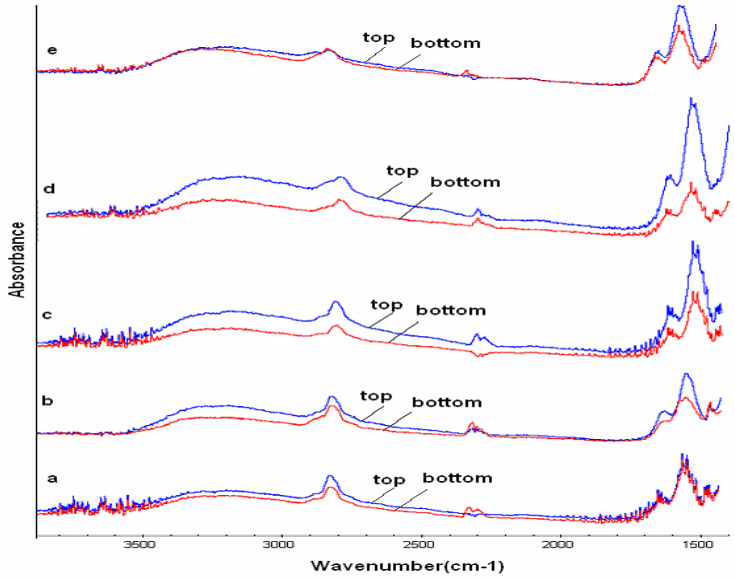

Figure 4.7 A portion (4000-1500 cm-1) of FTIR spectra of chitosan /PEO blend films with ratio: (a) 50/50, (b) 60/40, (c) 70/30, (d) 80/20, (e) 90/10, observing on both top and bottom sides on an identical point.

4.3.7 Antibacterial property in the liquid system

As shown in Table 4.5, all mixtures of chitosan/PEO films reduced the number of *E. coli* K-12 by approximately 3 log CFU/ml from the positive control of 9.63 log CFU/ml in 6 hours. This indicated that the chitosan/PEO films had satisfactory antibacterial properties. As expected, the antibacterial activity of the films was approximately proportional to chitosan fraction. Thus a 3.10 log CFU/ml reduction was observed for 90/10 and 2.54 log CFU/ml for 60/40 chitosan/PEO blend ratio. However, 50/50 chitosan/PEO films showed a strong antibacterial activity with reduction in viable cells of 3.27 log CFU/ml which was similar to that of 100/0 chitosan/PEO films ($p<0.05$). This may be explained by the compatibility of chitosan/PEO blends. As we discussed earlier, the phase separation may start when concentration of PEO is 20% or more.

With the increased content of PEO (80/20 to 60/40 films), the distribution of PEO crystals on the surface of the films may decrease the possibility of interaction of chitosan with *E. coli* K-12 and reduce lethality of the films. However, as suggested by Zhao et al. (1995), the compatibility of chitosan /PEO blends is closely related to the composition and chitosan and PEO may become miscible again at 50% or less chitosan. As for the 50/50 blend ratio, chitosan and PEO are miscible and chitosan molecule chains would have more chance to combine with and kill the bacteria than from immiscible films.

Table 4.5 Inhibitory effects of chitosan /PEO blend films toward Escherichia.coli K-12 inoculated in sterile phosphate buffer (0.05 M, pH=7.08) and stored for 6h at 25°C.

Film (w/w chitosan/PEO)	Surviving *E.coli* K12 (Log_{10} CFU */ml)	*E.coli* K12 reduction (Log_{10} CFU/ml)	pH in negative control tubes after 6 h
Positive Control (no film)	9.63 ± 0.09	0	7.08
100/0	6.20 ± 0.07[e]	3.43	6.57
90/10	6.53 ± 0.26[cd]	3.10	6.84
80/20	6.77 ± 0.15[bc]	2.86	6.85
70/30	6.86 ± 0.27[ab]	2.77	6.87
60/40	7.09 ± 0.04[a]	2.54	7.00
50/50	6.36 ± 0.10[de]	3.27	7.01

*CFU=colony-forming unit. Means of three replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly different ($p>0.05$) by LSD test.

4.4 Conclusions

Films formed by blending of chitosan and PEO have altered properties than films produced from either polymer alone. The chitosan fraction contributes to antimicrobial effect of the films, decreases tendency to spherulitic crystallization of PEO, and enhances puncture and tensile strength of the films, while addition of the PEO results in thinner films with lower water vapor permeability.

Films with 90/10 blend ratio of chitosan/PEO exhibited the best mechanical properties (puncture and tensile strength), no crystallization, high water vapor permeability, and significant antibacterial effect.

Thin chitosan/PEO films performed non-significant difference of puncture strength and tensile strength compared with thick films, but better percentage of elongation at break. Although thin chitosan/PEO films had high water solubility, generally they showed a lower WVP than thick films. These results indicated that it was possible to use less chitosan and PEO materials to make thinner films with same or even better performance compared to the thick films.

Based on our results, chitosan/PEO blend films have the potential to be used in the food industry as active packaging materials to inhibit food borne pathogens and in the pharmaceutical industry for controlled release of active components. The future study will be focused on evaluation of film forming techniques on mechanical, physical and antimicrobial properties of spin coated chitosan/PEO films.

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CHAPTER 5. PRODUCTION AND CHARACTERIZATION OF ULTRA-THIN CHITOSAN/PEO FILMS

Abstract

Films made of two or more polymers have been widely studied. In our previous research, we characterized chitosan and poly (ethylene oxide) (PEO) films produced with different blend ratios. The results showed that thin (25-44 μm) chitosan/PEO films had comparable physical and mechanical properties to thick (57-91 μm) films, indicating that it was possible to use less chitosan and PEO materials to produce thinner films with same or even better performance than the thick films. The chitosan fraction decreased tendency to spherulitic crystallization of PEO. In this study, we focused on producing and testing chitosan/PEO films with the thickness less than 100 nm. Ultra-thin chitosan/PEO films with blend ratio from 100/0 to 50/50 were made by spin-coating method. The thickness was measured using an ellipsometer and the surface morphology of the films was monitored by a multimode Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM). The ultra-thin chitosan/PEO films were 60-70 nm thick and blend ratio apparently did not have effect on film thickness. The surface of pure ultra-thin chitosan films was uniform and smooth, but the incorporation of PEO content increased the surface roughness of the blend films.

5.1 Introduction

There are numerous reports on the preparation of ultra-thin films of synthetic (Affrossman et al., 1999; Dalnoki-Veress et al., 2001) and natural polymers (Baumgart and Offenhausser, 2003). Ultra-thin chitosan films have also been studied because of their potential to be used as sensors, coatings, and layers incorporated into multilayer packaging (Jiang et al., 1996; Schauer et al., 2003; Murray and Dutcher, 2006; Shih et al., 2006). The physical and chemical properties of ultra-thin chitosan or chemically modified chitosan films have been published (Ligler et al., 2001; Nosal et al., 2005a-b; Murray and Dutcher, 2006).

The physical and mechanical properties of blend films made by natural polymer chitosan and synthetic polymer poly (ethylene oxide) (PEO) have been studied as affected by the molecular weight, thickness and blend ratios in the previous chapters of this dissertation (Chapter 3, 4). Our results showed that chitosan/PEO films with the thickness of 25-44 μm (thin films) had comparable physical and mechanical properties to those of thick films, with the thickness of 57-91 μm. 50/50 thick chitosan/PEO films (80 μm) had the PS of 102.33 N/mm, while 50/50 thin chitosan/PEO films (25.4 μm) showed even higher value of the PS at 158.9 N/mm. The TS of thin chitosan/PEO films were comparable to thick films as illustrated by 83.01 MPa and 73.5 MPa for 100/0 thick and thin films, and 48.98 MPa and 47.0 MPa for 50/50 thick and thin chitosan/PEO films, respectively. Thin chitosan/PEO films had lower WVP values of 4.37 $g \cdot mm/m^2 \cdot h \cdot kPa$ and 2.44 $g \cdot mm/m^2 \cdot h \cdot kPa$ for 100/0 and 50/50 films than thick films with WVP values of 8.19 $g \cdot mm/m^2 \cdot h \cdot kPa$ and 6.32 $g \cdot mm/m^2 \cdot h \cdot kPa$ for 100/0 and 50/50 films, respectively. The chitosan fraction decreased tendency to spherulitic crystallization of PEO and the number of PEO crystals formed in the thin chitosan/PEO films was less than those in thick films. These data indicate possibility to produce even thinner chitosan/PEO films with similar or even better performances than the regular films. Due to the functionality of chitosan, thinner chitosan/PEO films have potential to be used as coatings, in sensors, or as layers incorporated into packaging to inhibit food borne pathogens and to bind heavy metal in the environment.

Presently, the most common method to make ultra-thin polymer films is by spin-coating. This is a simple and time-saving procedure that allows preparation of films and coatings of an extremely wide range of thicknesses (Baumgart and Offenhausser, 2003). The films are produced from polymer-containing aqueous solution and coatinged on silicon wafers. Uniformity and thickness of ultra-thin films vary depending on polymer composition, molecular weight, solution concentration, angular velocity and acceleration of the spinner (Thompson et al., 1983), but is not significantly affected by amount of dispensed solution. Excess amount of the dispensed solution will fly out during the spinning. Typically, less than 1% of the dispensed amount remains on the silicon wafer after spinning (Mishra, 2002). High viscosities and lower spin speeds will produce thicker films (Mishra, 2002). For a given polymer-based system with constant molecular weight and solution concentration, the film thickness depends on polymer composition and spinning parameters.

The morphology of polymer blends with at least one crystallizable component has attracted a lot of attention over the years (Kalfoglou et al., 1988; Chen et al., 1998; Lim et al., 1999; Huang and Chen, 2001). Especially, polymer crystallization in confined nano-environments, such as in ultra-thin films (thickness < 100 nm), on solid substrate can lead to monolayer lamellae and has received considerable attention recently (Frank et al., 1996; Reiter et al., 1999; Hong et al., 2001; Schonherr and Frank, 2003; Zhai et al., 2005). Crystallization in ultrathin films of semicrystalline PEO (Ok and Demirel, 2003; Schonherr and Frank, 2003; Okerberg et al., 2007; Zhu et al., 2007) as well as PEO blends with other amorphous polymers, such as poly (methyl methacrylate) (Wang et al., 2004; Okerberg and Marand, 2007), poly (vinyl acetate) (Huang and Chen, 2001), poly {2,5-bis[(4-methoxyphenyl) oxycarbonyl]-styrene} (Huang et al., 2006), were widely studied. The crystallization rate and crytallinity decrease with the decreasing of the film thickness (Sawamura et al., 1998; Dalnoki-Veress et al., 2001a; Massa et al., 2003). With the rapid solvent evaporation during formation of an ultra-thin film, intermediate phase structures may become "frozen in" due to the sudden onset of restricted mobility of the polymer chains

(Affrossman et al., 1999), which allows detecting the crystalline structure of the materials. The surface morphology of chitosan/PEO ultrathin films has not been reported in the literature.

The objective of this study was (1) to prepare ultra-thin chitosan/PEO films with controlled thickness and good uniformity, and (2) to evaluate the surface morphology of films with different blend ratios.

5.2 Materials and Methods

5.2.1 Materials

Chitosan of low molecular weight (LMW ~150 kDa) with a degree of deacetylation 85.7%, and PEO of high molecular weight (HMW ~900 kDa) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

5.2.2 Preparation of Ultra-thin Chitosan/PEO Films

Chitosan/PEO blend film-forming solutions were prepared as 1% (w/w) of the mixture of chitosan and PEO with different ratio (100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 0/100) dissolving in 1% (w/w) acetic acid after stirring over night at room temperature. The film forming solutions were filtrated through Miracloth® (Calbiochem-Novabiochem Corp., San Diego, CA) and centrifuged for 1 hour at relative centrifugal force of 28,714 g to remove any possible impurities and/or undissolved particles.

The 4 cm^2 silicon chips (n-type doped with arsenic, 100 orientation) (Crysteco Inc., Wilmington, OH) were pre-cleaned by immersing in a solution of concentrated H_2SO_4 / 30% H_2O_2 (3:1) for 1 hour, followed by rinsing with d.i. water, and drying under a nitrogen stream. Ultra-thin chitosan/PEO films were prepared by placing 0.5 ml chitosan/PEO blend solutions on the silicon chips and spun using a spin coater (P-6708 D, Special Coating Systems Inc., Indianapolis, IN) at spin speed of 2500 rpm for 60 seconds.

5.2.3 Thickness

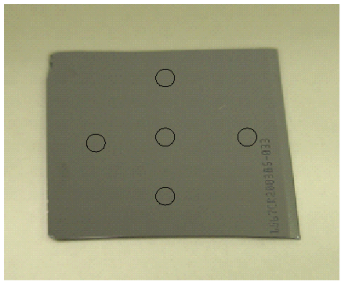

Figure 5.1 A photograph of ultra-thin film on one silicon chip used to measure the thickness. O stands for the places where thickness was measured.

Thickness of ultra-thin films was assessed on 4 films per ratio treatment averaging measurements at 5 points for each film using an ellipsometer (Gaertner Scientific Corp., Skokie, IL) (Figure 5.1). The manually entered indexes of refraction for the chitosan and PEO were 1.50 and 1.45, respectively (Jiang et al., 1996).

5.2.4 Atomic Force Microscope (AFM)

Atomic force microscope (AFM) was used to study the surface roughness and morphology of ultra-thin chitosan/PEO films. AFM topographic images were obtained in air at room temperature using a multimode AFM with a Nanoscope III controller (Digital Instruments Veeco Metrology Group, Santa Barbra, CA) in tapping mode. The scan size was 1 μm square and the scan rate was 1.001 Hz with 512 pixels collected per line. The roughness of the surface was determined by measuring the root-mean-square (RMS) roughness parameter.

5.2.5 Scanning Electron Microscope (SEM)

The morphology of the ultra-thin chitosan/PEO films was also characterized using a field emission scanning electron microscope (FESEM, LEO 1525). The SEM samples were sputter-coated with gold to prevent charging during SEM imaging.

5.2.6 Statistical Analysis

All measurements were done in triplicate with individually prepared films as the replicated experimental units. Significant differences between groups were determined using

Tukey-Kramer HSD test in the JMP program (JMP 2007). In tables means within the same group with a different letter are significantly different ($p < 0.05$).

5.3 Results and Discussions

5.3.1 Appearance and Thickness

Ultra-thin films of all blend raios were successfully produced without defects. All the films appeared purple. All ratios of ultra-thin films on the clean silicon chips showed good adhesion. As suggested by Jiang et al. (1996), good adhesion was obtained when the substrates were processed under clean conditions. However, the spin-coating processing made part of the film-forming solution to aggregate on the edge of the films and resulted in thicker films with blue color. Figure 5.2 demonstrates the appearance of 100/0 ultra-thin chitosan/PEO films as an example. The chitosan/PEO films were hard to peel off from the substrate due to their extremely low mass. Thus, all the measurements were done on films still attached to the wafers.

The ellipsometry data for the ultra-thin chitosan/PEO films showed that thickness varied from 32.12 to 73.85 nm (Table 5.1). The relationship between film color and thickness was consistent with the results from Schauer et al. (2003), who reported that 110 nm thick chitosan-Resimene/TEG films reflected blue color.

Figure 5.2 Photographs of ultra-thin chitosan films prepared by placing 0.5 ml chitosan solutions on the silicon chips and spun using a spin coater at spin speed of 2500 rpm for 60 seconds. The size of film with silicon chip was compared to a quarter.

Table 5.1 Thickness of ultra-thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50 and pure PEO films*.

Chitosan/PEO	Thicknesss (nm)
100/0	72.33 ± 1.98[a]
90/10	69.40 ± 0.70[b]
80/20	73.85 ± 0.37[a]
70/30	72.95 ± 0.39[a]
60/40	66.04 ± 1.31[c]
50/50	68.23 ± 1.33[b]
0/100	32.12 ± 0.13[e]

* Values reported are means and standard deviation. Superscript letters indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP, 2007).

The thickness of each ratio of ultra-thin films had low standard deviations, indicating that spin-coating produced uniform films. As expected, the increase of PEO content in the blend generally resulted in thinner films. As described before, this lower thickness of the films with increased PEO fraction was probably the result of lower viscosity of film-forming solution with higher PEO content. The viscosity of the blend solutions greatly decreased from 0.21 Pa·s for 100/0 chitosan/PEO solution to 0.018 Pa·s for 0/100 chitosan/PEO solution (Chapter 3). Similarly, Affrossman et al. (1999) found the PEO nano film made by 1% solution had a 3-fold greater overall thickness (621 nm) compared to that of PMMA films (173 nm) made by the solution with even lower viscosity than PEO.

5.3.2 Surface Morphology

Tapping mode atomic force microscope (AFM) was used and the images of 100/0, 90/10, 80/20 and 50/50 ultra-thin chitosan/PEO films are given in Figure 5.3. Pure PEO ultra-thin films were investigated as the comparison. The surface morphology of pure ultra-thin chitosan films was more uniform and smooth (Figure 5.3 A). This is in agreement with the conclusions from Murray and Dutcher (2006) who found that ultra-thin chitosan films were very smooth when produced with spin speeds > 1000 RPM.

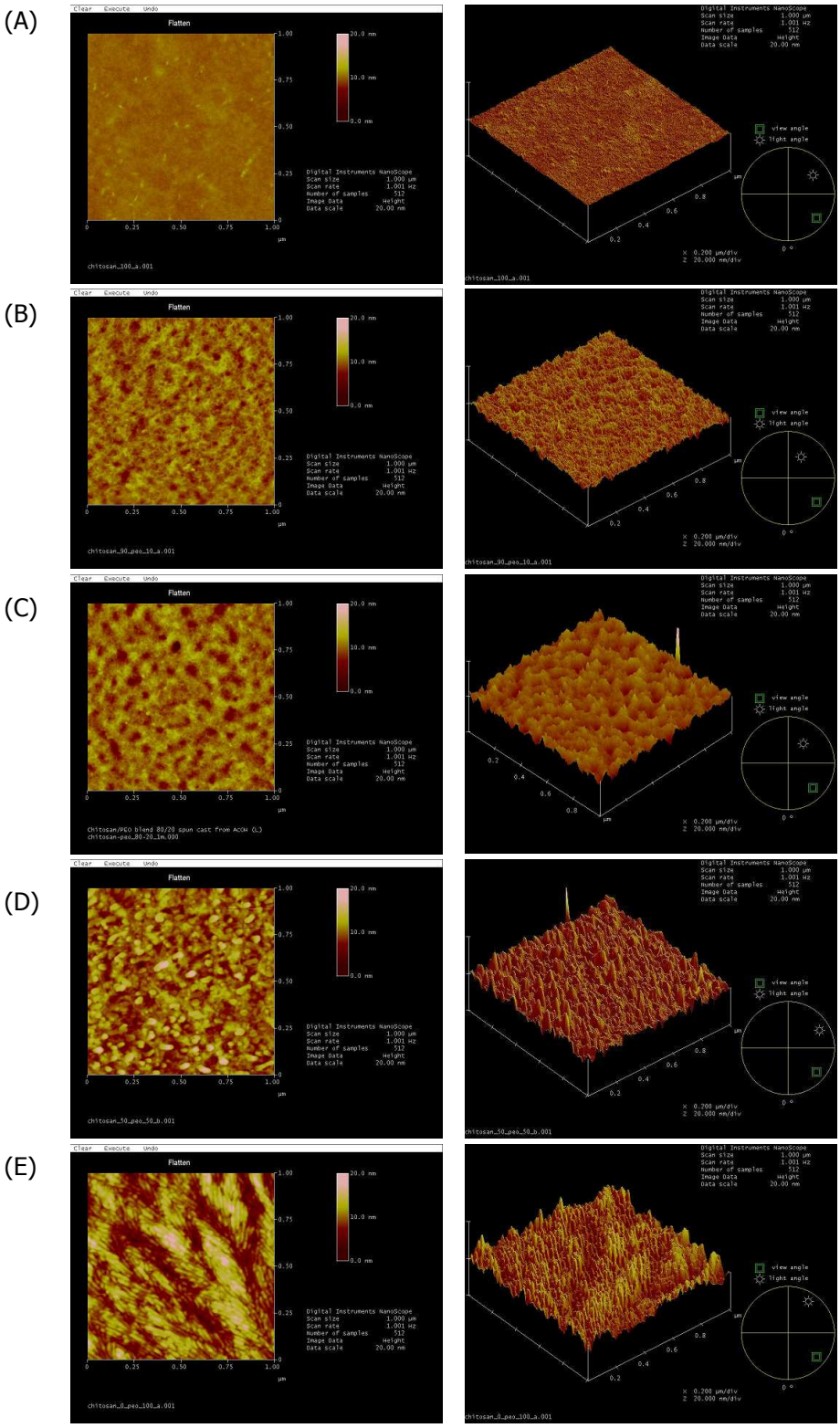

Figure 5.3 Tapping mode AFM two-dimensional images (left) and three-dimensional images (right) of 1 μm scans for ultra-thin chitosan/PEO blend films with ratios of (A) 100/0, (B) 90/10, (C) 80/20, (D) 50/50, (E) 0/100.

Table 5.2 The RMS roughness parameters for surfaces of ultra-thin chitosan/PEO films with blend ratios of 100/0, 90/10, 80/20, 50/50 and 0/100.

Chitosan/PEO	RMS roughness (nm)
100/0	0.377
90/10	1.110
80/20	1.564
50/50	2.047
0/100	2.660

The root mean square (RMS) values, which present the roughness of ultra-thin chitosan/PEO films surface, were shown in Table 5.2. Pure chitosan films showed the RMS roughness of only 0.377 nm (Table 5.2), which was consistent with the results from Nosal et al. (2005a) who reported that spin-coating chitosan films had RMS roughness of 0.33 nm. Comparable RMS values from other polysaccharide-based ultra-thin films were reported in the literature. Maciel et al. (2007) produced cashew gum ultra-thin films on silicon wafer with the RMS values of 0.18 nm. Kosaka et al. (2007) reported cellulose acetate films with the thickness of only 5.7 nm led to RMS of 0.5 nm. This smooth topographic property was probably because their rigid molecular size and high intrinsic stiffness due to abundant intra-molecular hydrogen bonds would not change greatly during the instant drying process (Brugnerotto et al., 2001; Cai et al., 2005).

On the other hand, the AFM images of PEO ultrathin films with thickness of 32.12 nm showed that the needle-like crystals packed parallel to each other (Figure 5.3 E), which were similar to those described in the literature (Ok and Demirel, 2003; Huang et al., 2006). In sufficiently thick PEO films (>1 μm), both perpendicular and parallel lamellae oritentation were favored for nucleation and spherulitic crystal growth (Schonherr et al., 2003; Zheng et al., 2007). However, for most ultrathin films with thickness in the range of 15-300 nm, since film-formation during spin-coating is almost instantaneous (60 seconds compared to 24 to 48 hours in casting), PEO crystalline lamellae preferentially oriented parallel to the substrate to reduce the surface energy and resulted in needle-like crystals (Schonherr and Frank, 2003; Huang et al., 2006;

Zheng et al., 2007). The RMS roughness of PEO film was much higher (2.660 nm) than the pure chitosan film (0.377 nm) due to its crystallization (Table 5.2).

The crystallization in polymer blend films would be more complex than homopolymer films due to film composition, film structure, and interaction between components (Wang et al., 2004). As seen in Figure 5.3 B-D, the topography of chitosan/PEO blend films changed with the increase of PEO content, from the initially smooth to rougher. The white spots on the two-dimensional images as well as the three-dimensional images clearly illustrated increasing peaks on the surface. The RMS values of the blend ultra-thin chitosan/PEO films increased from 1.110 nm for 90/10 films to 2.047 nm for 50/50 films (Table 5.2). The increase of PEO content to 50% made the surface of corresponding films become rougher due to PEO crystallites.

The images of ultra-thin chitosan/PEO films obtained from scanning electron microscope (SEM) under the same condition (Magnification=25 KX) are illustrated in Figure 5.4. Although a nanometer scale resolution as obtained with AFM was not achievable with the SEM instrument (Malwitz et al., 2004), PEO crystals with the needle-like structures were present near the surface. SEM observations were consistent with the results from AFM and also indicated that the increased content of PEO in ultra-thin chitosan films enhanced formation of microfractures and resulted in increased surface roughness (Figure 5.4).

5.4 Conclusions

Ultra-thin chitosan/PEO films with thickness between 65 and 75 nm could be produced by spin-coating on silicon wafers. Higher chitosan fraction in the blend resulted in thicker and smoother films. As the PEO content in the ultra-thin films increased, the surface roughness increased. High crystallization tendency of PEO was probably the reason for rough topography of the ultra-thin chitosan/PEO blend films.

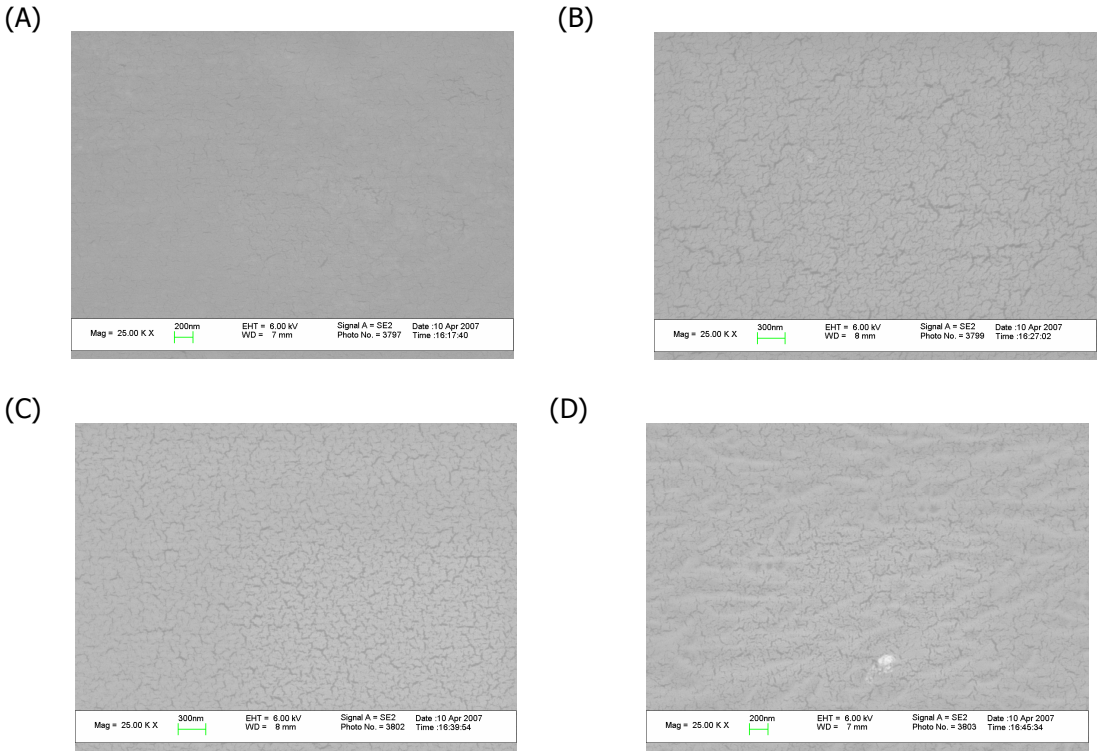

Figure 5.4 SEM images of 1 μm scans for ultra-thin chitosan/PEO blend films with ratios of (A) 100/0, (B) 90/10, (C) 50/50, (D) 0/100.

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CHAPTER 6. COMPARISON OF FUNCTIONAL PROPERTIES OF THICK, THIN AND ULTRA-THIN CHITOSAN/PEO FILMS

Abstract

Chitosan, as a natural polycationic polysaccharide, has been gaining considerable attention for its functional properties including antibacterial and metal binding properties. Our previous research has shown that chitosan/poly (ethylene oxide) (PEO) films had altered physical and mechanical properties at different thickness and blend ratios. Our current study focused on the functional properties of chitosan/PEO films as affected by films thickness, composition and preparation methods. Mixtures of low-molecular-weight chitosan and high-molecular-weight water-soluble PEO with different blend ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50) were dissolved in 1% acetic acid to form 1% blend solutions. Films prepared by casting of 20g or 10g film forming solution per 50-mm diameter polystyrene Petri dish and by using spin-coating method had average thickness of 80.8-94.1 μm (thick), 34.7-41.5 μm (thin) and 66.0-73.9 nm (ultra-thin). Potential development of polymer crystallization was monitored by X-ray diffractometer at ambient temperature. The metal-binding capacity of the films was assessed based on removal of chromium ions from K_2CrO_4 solution, and the antibacterial properties of the films were tested against *Escherichia coli* K-12. The crystallization extent of thick, thin and ultra-thin chitosan/PEO films showed a decreasing tendency because of the decreased film-forming time. Decreasing the thickness of films was a possible way to increase the surface area to mass ratio, further increase the number of available active ($–NH_3^+$) sites on the film surface per unit weight, and promote the metal binding and antibacterial efficiency of chitosan. Ultra-thin chitosan/PEO films showed a significantly higher chromium binding capacity [26.47-70.86 mg Cr (VI)/g film] compared to the regular cast films; however, they did not show significant antibacterial properties. The lack of antibacterial efficiency was due to their extremely low concentration of chitosan (0.00025%) in the inoculated test tubes insufficient to kill significant number of bacteria.

6.1 Introduction

As a nontoxic, renewable and biodegradable polymer, chitosan, a cationic polysaccharide obtained by alkaline deacetylation of chitin, has been widely investigated over the last two decades (Kumar, 2000; Marsano et al., 2004). Due to the presence of reactive amino groups as well as hydroxyl groups, chitosan exhibits its tremendous functionalities including antimicrobial activity (Kendra and Hadwiger, 1984; Sudarshan et al., 1992; Fang et al., 1994; Sekiguchi et al., 1994; Chen et al., 1998; Roller and Covill, 1999; Tsai and Su, 1999; No et al., 2002; Tsai et al., 2004) and metal-binding property (Babel et al., 2003; Dutta et al., 2004; Nomanbhay and Palanisamy, 2005; Gamage and Shahidi, 2007). Many attempts have been conducted to develop functional materials from chitosan, such as films and fibers, and to apply them in the metal removal from waste water (Deans and Dixon, 1992; Selmer-Olsen et al., 1996; Kaminski and Modrzejewska, 1997), and as antimicrobial food packaging (Zivanovic et al., 2005; Duan et al., 2007; Ye et al., 2008).

Although chitosan films have significant metal binding and antibacterial effects, these effects are less intensive compared to chitosan solutions because chitosan molecules entrapped within the films and possible crystallization of the polymer molecules could reduce the availability of active amino groups. Studies have reported that increasing the surface area to mass ratios of chitosan by making chitosan nanofibers could improve their functionality (Sams et al., 2004; Desai et al., 2008). Besides nanofibers, decreasing the thickness of the films to nano-scale is also a good way to increase the surface area to mass ratios. Schauer et al. (2003) have studied the color change of cross-linked chitosan and poly (allyl amine) hydrochloride ultra-thin films upon metal binding. However, there is no data on comparing properties of ultra-thin films with regular cast films from chitosan.

In addition, blend of natural and synthetic polymers is a possibility for development of optimally functional materials. Our previous study proved that chitosan/poly (ethylene oxide) (PEO) films performed an altered physical and mechanical properties compared to pure chitosan

and PEO films. Although PEO has not been reported to have antimicrobial and metal-binding properties, chitosan/PEO blend films may exhibit combined effects of both polymers and result in good appearance, desirable mechanical properties and antimicrobial and metal binding efficiency. Since PEO is less expensive than biopolymer chitosan, blending of these polymers films may potentially reduce the cost.

The objective of this study was to investigate the crystallinity, antimicrobial properties and metal binding capacities of chitosan/PEO films as affected by the thickness, compositions and preparation methods.

6.2 Materials and Methods

6.2.1 Materials

Since chitosan material used in previous studies obtained from Aldrich Chemical Co. (Milwaukee, WI) was not available any more, another chitosan material from Sigma-Aldrich, Inc. (St. Louis, MO) with silmilar low molecular weight (~150 kDa) was used in this study. High molecular weight PEO (900 kDa) was the same as before and obtained from Aldrich Chemical Co. (Milwaukee, WI).

6.2.2 Preparation of Chitosan/PEO Films

1% of chitosan/PEO blend film-forming solutions with different ratio (100/0, 90/10, 80/20, 70/30, 60/40, 50/50) were prepared as described in Chapter 4. After the filtration, the solutions were centrifuged for 1 hour at relative centrifugal force of 28,714 g to remove any possible impurities and/or undissolved particles. Thick and thin chitosan/PEO films were prepared by pouring 20 g and 10 g film forming solutions into 50mm-diameter Petri dishes and the solvent was evaporated in a vacuum oven at 38°C under 17 kPa pressure for 48 and 24 hours, respectively. The dried films were peeled from the Petri dishes and conditioned in desiccators at 25ºC and 20% RH prior to testing.

For preparation of ultra-thin chitosan/PEO films, the 4 cm^2 silicon chips were pre-cleaned by immersing in a solution of concentrated H_2SO_4 / 30% H_2O_2 (3:1) for 1 hour, followed by rinsing with d.i. water, and drying under a nitrogen stream. Ultra-thin chitosan/PEO films were prepared by placing 0.5 ml chitosan/PEO blend solutions on the silicon chips and spun using a spin coater (P-6708 D, Special Coating Systems Inc., Indianapolis, IN) at spin speed of 2500 rpm for 60 seconds.

6.2.3 Thickness

Thickness of the cast films was determined on 6 films per ratio treatment averaging measurements at 5 points for each film using a hand-held microcaliper (Mitutoyo Corp, Kawasaki, Kanagawa, Japan). Thickness of ultra-thin films was assessed on 4 films per ratio treatment averaging measurements at 5 points for each film using an ellipsometer (Gaertner Scientific Corp., Skokie, IL). The manually entered indexes of refraction for the chitosan and PEO were 1.50 and 1.45, respectively (Jiang et al., 1996).

6.2.4 X-ray Diffractometer

Film crystallization of thick and thin films was monitored by X-ray diffractometer at ambient temperature using a Molecular Metrology SAXS/WAXD system equipped with a monochromic CuKα (1.5418 Å) X-ray source, a three-pin-hole alignment, and two-dimensional detector operating at 45 kV and 0.66 mA with the beam size of 30 μm. The WAXD patterns were recorded on reusable Fuji image plates, with the sample-to-film distance for 36.52 mm. The image plate was scanned in a Fuji X BAS-1800II image analyzer and the resultant image was converted to intensity versus 2θ; or q plot using Polar X-ray analysis software. The data were collected over an angular range from 5° to 40° 2θ in a continuous mode. Relative crystallinity percentage (Xc) of the films were calculated by the following equation (Helander et al., 2001):

$$Xc = [Ac/(Ac + Aa)] \times 100\%$$

Ultra-thin films were analyzed using grazing angle pattern of X-ray diffractometer.

6.2.5 Metal-binding Capacity

Film metal-binding properties of chitosan/PEO films were determined as change in concentration of Cr (VI) before and after adding films to the solution. Concentration of Cr (VI) in solutions was monitored following NIOSH method (NIOSH Manual of Analytical Methods (NMAM), 1994). Thick and thin films (4.9 cm^2) were soaked into 25 ml of 5 mg/l K_2CrO_4 solution purchased from Sigma and ultra-thin films (4 cm^2, still attached to silicon wafer) were soaked into 25 ml of 1 mg/l K_2CrO_4 solutions. All the samples were continuously shaken on a shaker for 3 hours. Solutions of K_2CrO_4 with no films were equally treated and used as control. For ultra-thin films, K_2CrO_4 solutions with blank silicon chips were used as control. After 3 hours of contact, 1 ml of sample solution was taken and mixed with 7 ml 0.5 N H_2SO_4 in 25 ml volumetric flask, 0.5 ml sym-diphenylcarbazide solution in 50 % acetone was added as an indicator, and the volume was adjusted with 0.5 N H_2SO_4 to 25 ml. The absorbance of these solutions was measured at 540 nm in 10-cm cuvette (UV-2102PC, Shimadzu, Kyoto, Japan). The chromium binding capacities of chitosan/PEO films was calculated on films weight basis [mg chromium/ g film], and on area basis [mg chromium/ cm^2 film]. The areas of thick and thin films were calculated from double sides.

6.2.6 Antibacterial Property in the Liquid System

Antibacterial test was carried out by submerging thick and thin chitosan /PEO films (4.9 cm^2) and ultra-thin films (4 cm^2 on silicon wafers) into culture tubes containing 10 ml sterile phosphate buffer (0.05 M, pH=7.08) inoculated with ca. 10^6 CFU/ml *Escherichia coli* K-12. The tubes were vortexed and incubated for 6 hours at 25°C. The survival of *E. coli* K-12 was determined using the pour-plate method on Trypticase Soy Agar (TSA) medium (Swanson et al., 2001).

6.2.7 Statistical Analysis

All measurements were done in triplicate with individually prepared films as the replicated experimental units. Significant differences between groups were determined using

Tukey-Kramer HSD test in the JMP program (JMP 2007). In tables means within the same group with a different letter are significantly different ($p < 0.05$).

6.3 Results and Discussions

6.3.1 Appearance and Thickness

All cast chitosan/PEO films were transparent and the yellowish coloration from chitosan decreased with increased PEO content. Incorporation of other polymers into chitosan films is a good way to reduce the yellowish color. Garcia et al. (2006) has reported that addition of corn starch into chitosan films significantly decreased the yellowness. Srinivasa et al. (2003) measured a decreasing tendency of hunter *b* (yellowness) values of chitosan/ poly (vinyl alcohol) (PVA) films with the increase of PVA content. The mass of 50-mm diameter films produced from 20 g and 10 g film-forming solution was 0.19-0.20 g and 0.09-0.10 g, respectively. The corresponding thicknesses were 80.80-94.07 μm for thick and 34.67-41.47 μm for thin films (Table 6.1). The thickness of thin films was in a good agreement with our previous results in Chapter 4.

The ultra-thin chitosan/PEO films had thickness of 66.04-73.85 nm (Table 6.1). The mass of ultra-thin films was estimated at 0.025 mg, based on weight changes of silicon chips before and after formation of the films. The mass of chitosan used per 1 cm^2 thick, thin and ultra-thin films was calculated and showed in Table 6.1.

Table 6.1 Thickness and mass per area of thick, thin and ultra-thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50*.

Chitosan/PEO	mg chitosan/ 1 cm^2 film (Thick/Thin/Ultra-thin)	Thick films (μm)	Thin films (μm)	Ultra-thin films (nm)
100/0	10.81 / 5.31 / 0.0062	94.07 ± 10.70^{a}	35.80 ± 0.60^{ab}	72.33 ± 1.98^{a}
90/10	9.23 / 4.58 / 0.0056	92.93 ± 7.60^{a}	41.47 ± 4.64^{a}	69.40 ± 0.70^{b}
80/20	8.00 / 4.04 / 0.0050	89.07 ± 6.31^{a}	38.20 ± 0.87^{ab}	73.85 ± 0.37^{a}
70/30	7.09 / 3.48 / 0.0044	84.47 ± 1.42^{a}	38.07 ± 2.12^{ab}	72.95 ± 0.39^{a}
60/40	5.91 / 2.80 / 0.0038	83.13 ± 2.04^{a}	39.07 ± 1.55^{ab}	66.04 ± 1.31^{c}
50/50	5.05 / 2.41 / 0.0031	80.80 ± 4.66^{a}	34.67 ± 0.76^{b}	68.23 ± 1.33^{b}

* Values reported are means and standard deviation. Superscript letters within a column indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP, 2007).

Ultra-thin films with area of 1 cm^2 used extremely low mass of chitosan material (0.0031-0.0062 mg) compared to thick (5.05-10.81 mg) and thin films (2.41-5.31 mg).

6.3.2 Film Crystallization

Wide-angle X-ray diffraction (WAXD) patterns and the corresponding diffraction angle (2θ) of 100/0 to 50/50 thin chitosan/PEO blend films and their relative crystallinity percentage (χc) values have been studied in Chapter 4 of this dissertation. Figure 6.1 and Table 6.2 present them again in order to compare to the crystallinity of thick chitosan/PEO films. Similar to the diffractograms of thin films (Figure 6.1b), 100/0 and 90/10 thick films did not show characteristic peaks but a broad hump in the range of 10° to 40° 2θ, indicating a predominantly amorphous form of the chitosan in the films (Figure 6.1a: A,B). All of the diffraction patterns of 80/20 to 50/50 chitosan/PEO thick films illustrated two sharp peaks from PEO crystals at 19.0° and 23.3°2θ, which was consistent with the results from our thin films (Figure 6.1a: C to F) and PEO blend films in the literature (Huang and Chen, 2001).

PEO is a semi-crystalline polymer and easily forms crystals with spherulitic structure from the solution (Mucha et al., 1999). It was clear that the peak positions on the diffractograms of both thick and thin blend samples all appear at 19.0° and 23.3°2θ, indicating that the PEO crystals formed in the films possessed similar unit-cell structure.

Chitosan content in the blends limited the crystallinity extent from the PEO in both thick and thin films. The stiff chitosan molecular chains affected the overall mobility in the blend and impeded the rate of crystal growth (Zhao et al., 1995). However, no significant shift in the diffraction peaks was observed, suggesting that the presence of chitosan did not affect the ordered structure of PEO but limited the nucleation process of PEO (Mucha et al., 1999; Peesan et al., 2005).

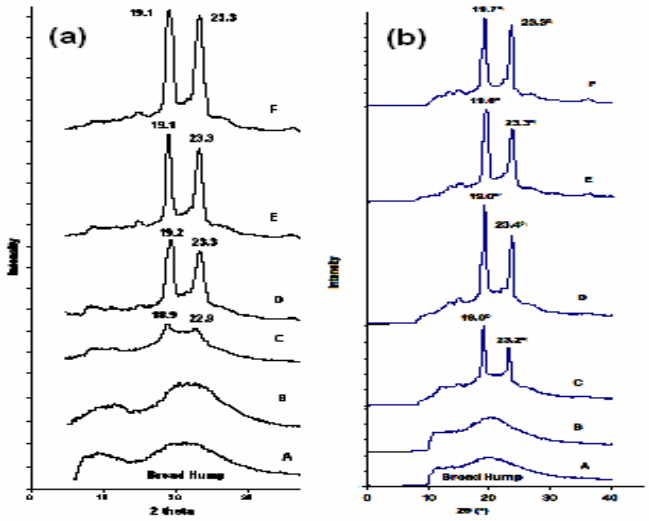

Figure 6.1 2θ-scan Wide-angle X-ray diffraction (WAXD) patterns of films with thick (a) and thin (b) chitosan/PEO blend ratio of (A) 100/0, (B) 90/10, (C) 80/20, (D) 70/30, (E) 60/40, (F) 50/50.

Table 6.2 Relative crystallinity percentage (Xc) of the thick and thin chitosan/PEO blend films with weight ratio from 100/0 to 50/50.

Chitosan/PEO	Thick films	Thin films
100/0	a*	a
90/10	a	a
80/20	32.2%	14.9%
70/30	40.6%	28.7%
60/40	42.1%	36.7%
50/50	45.9%	34.3%

* a indicates there is no readable crystalline peak.

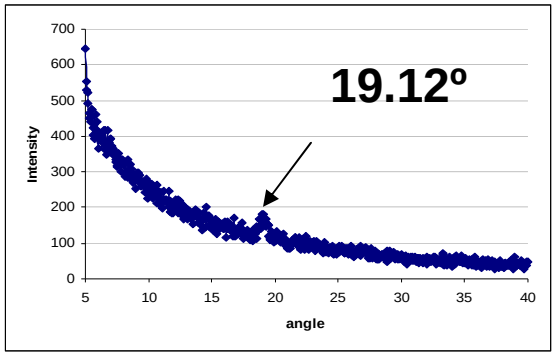

Figure 6.2 X-ray Diffractogram of grazing angle pattern on 50/50 ultra-thin chitosan /PEO films.

Polymer morphology, degree of crystallinity, preferred polymer chain orientation, and crystal growth rates, may vary with film thickness (Despotopoulou et al., 1996; Sakai et al., 1996; Dalnoki-Veress et al., 2001; Schonherr and Frank, 2003; Hu et al., 2004). According to Table 6.2, the relative crystallinity percentage of the films from both chitosan and PEO was 32.2-45.9% for 80/20 to 50/50 thick films and 14.9-34.3% for 80/20 to 50/50 thin films. The overall higher crytallinity of thick films was probably due to the longer drying period of thick films what allowed prolonged mobility of polymer chains and promoted formation of PEO crystals. On the other hand, no apparent crystal peaks were detected from ultra-thin chitosan/PEO films under grazing angle pattern. Only a very small diffraction peak at 19.12° was found on 50/50 films (Figure 6.2). Although our previous AFM images (Chapter 5) illustrated that roughness of chitosan/PEO ultrathin films increased with increased PEO content indicating possible PEO crystallization, the present results from X-ray diffraction have proved that spin-coating method significantly reduced the crystallization extent of chitosan and PEO in films compared to solution casting method.

As suggested by Affrossman et al. (1999), the rapid solvent evaporation during spin-coating process caused the sudden onset of restricted mobility of the polymer chains and froze in the phase structures, resulting in a minimum extent of crystallization in films. Similar results were reported by Jiang et al. (1996) and confirmed that chitosan nano-thin films with the thickness of 300 nm had an amorphous structure.

6.3.3 Metal-binding Capacity of the Films

Figure 6.3 shows the percentage of chromium bound by thick, thin and ultra-thin chitosan/PEO films under different blend ratios. For thick and thin films, 100/0 and 90/10 chitosan/PEO films swelled in chromium solutions due to the hydrophilic nature of chitosan and PEO and their weak intermolecular interactions between polymer chains, resulting in the failure to determine their metal binding property.

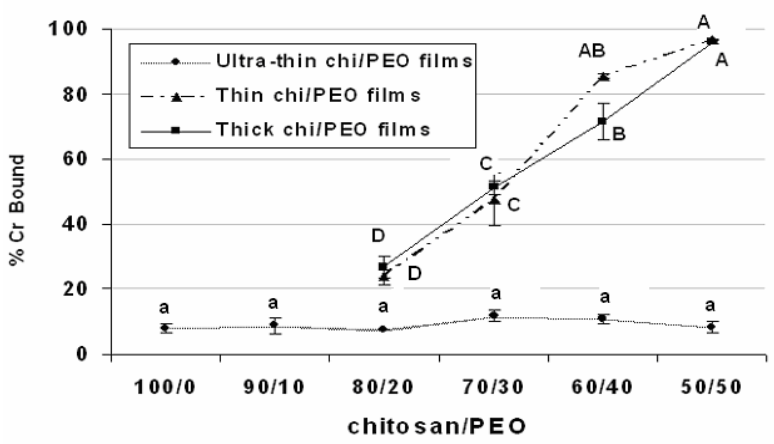

Figure 6.3 Percentage of Cr (VI) bound by thick, thin and ultra-thin chitosan/PEO films with blend ratio from 100/0 to 50/50 in 25 ml 5 mg/l chromium solution for thick and thin films and in 25 ml 1 mg/l chromium solution for ultra-thin films. Error bars represent standard deviation (n=3). Letters indicate significant difference at p< 0.05.

Table 6.3 Thickness of ultra-thin chitosan/PEO films before and after metal-binding tests*

Chitosan/PEO	Thickness before binding (nm)	Thickness after binding (nm)	% decrease
100/0	72.33 ± 1.98^{a}	62.72 ± 0.58^{a}	13.28
90/10	69.40 ± 0.70^{b}	55.41 ± 0.93^{b}	20.16
80/20	73.85 ± 0.37^{a}	52.08 ± 0.65^{c}	29.48
70/30	72.95 ± 0.39^{a}	42.98 ± 0.57^{d}	41.09
60/40	66.04 ± 1.31^{c}	29.18 ± 0.83^{e}	55.82
50/50	68.23 ± 1.33^{b}	27.22 ± 0.41^{f}	60.10

*Values reported are means and standard deviation. Superscript letters indicate significant difference at p<0.05 by Tukey-Kramer HSD test (JMP, 2007).

Table 6.4 Comparison of Cr (VI) binding capacities of thick, thin and ultra-thin chitosan/PEO films*.

Chit/ PEO	mg Cr (VI) bound by 1g film			10^{-3} mg Cr (VI) bound by 1 cm^2 film		
	Thick films	Thin films	Ultra-thin films	Thick films	Thin films	Ultra-thin films
100/0	-	-	32.82 ± 14.19^{a}	-	-	0.21 ± 0.09^{a}
90/10	-	-	40.24 ± 24.00^{a}	-	-	0.25 ± 0.15^{a}
80/20	0.73 ± 0.19^{b}	1.04 ± 0.08^{c}	26.47 ± 0.53^{a}	3.42 ± 0.43^{d}	3.04 ± 0.30^{c}	0.17 ± 0.01^{a}
70/30	1.67 ± 0.10^{ab}	2.51 ± 0.91^{bc}	70.86 ± 20.39^{a}	6.54 ± 0.28^{c}	7.07 ± 1.05^{b}	0.44 ± 0.13^{a}
60/40	2.25 ± 0.90^{ab}	7.59 ± 1.51^{ab}	59.10 ± 12.47^{a}	9.12 ± 0.71^{b}	10.89 ± 0.07^{a}	0.37 ± 0.08^{a}
50/50	2.86 ± 0.17^{a}	6.21 ± 0.98^{a}	36.65 ± 17.32^{a}	12.21 ± 0.04^{a}	12.32 ± 0.01^{a}	0.32 ± 0.11^{a}

*Values reported are means and standard deviation. Superscript letters within a column indicate significant difference at p<0.05 by Tukey-Kramer HSD test (JMP, 2007).

Thick films of 80/20 to 50/50 ratio bound 25.8-95.9% of chromium from the solution while thin films bound 23.8-96.7%. Under the same ratio level, the binding percentage values between thick and thin films had no significant difference. With estimated film weight of only 0.025 mg per film, ultra-thin chitosan/PEO films still bound 8.1-11.9% of chromium ions in the solution.

The thickness of ultra-thin films before and after metal-binding tests is shown in Table 6.3, suggesting that although the thickness of the films significantly decreased 13.28 to 60.1% due to the increasing content of water-soluble PEO, all the ultra-thin films had good adhesion to the silicon chips even after soaking in chromium solutions for 3 hours.

The chromium binding capacity of three types of films calculated on weight and area basis are presented in Table 6.4. When calculated on a basis of amount of chromium bound by 1 cm^2 film, the thick and thin films had similar performances under the same blend ratio. Both of them performed significantly higher values of 3.42-12.21 $\times$ 10^{-3} mg chromium/cm^2 film for thick films and 3.04-12.32 $\times$ 10^{-3} mg chromium/cm^2 film for thin films than ultra-thin films of 0.17-0.44 $\times$ 10^{-3} mg chromium/cm^2 film (Table 6.4). However, when calculated on a basis of amount of chromium bound by per gram film, the ultra-thin chitosan/PEO films showed extremely higher chromium binding capacity with values of 26.47-70.86 mg chromium/g film compared to the regular thick films of 0.73-2.86 mg chromium/g film and thin films of 1.04-6.21 mg chromium/g film (Table 6.4). Although hexavalent chromium binding capacity of ultra-thin chitosan/PEO blend films was lower than those of composite chitosan biosorbent with adsorption capacities of 153.8 mg/g chitosan (Boddu et al., 2003), and chemically modified crosslinked xanthated chitosan flakes and beads with adsorption capacities of 625 mg/g and 256 mg/g (Sankararamakrishnan et al., 2006), respectively, they were higher than the reported chromium binding capacities of 27.3 mg/g for plain chitosan powder (Udaybhaskar et al., 1990), and 3-16 mg/g chitosan for high molecular weight chitosan/PEO nanofibers (Desai et al., 2008).

Though hydroxyl groups on chitosan may be involved in bonds with chromium ions, the main active groups remain the amine functions (Guibal, 2004). The dominant mechanism of metal binding is due to the electrostatic attraction between the dissociated CrO_4^{2-} ions in solution and NH_3^+ ions on the chitosan film surface (Qian et al., 2000). The metal binding capacity of chitosan films varies with metal, film crystallinity, pH value of the solution, available amino group content and physical adsorptive ability (Kurita et al., 1979; Kaminski and Modrzejewska., 1997; Modrzejewska and Kaminski., 1999). We found that there was not a large change in pH of 5 mg/l and 1 mg/l K_2CrO_4 solution before (pH=7.3) and after (pH=7.0) immersing blend films for 3 hours. Considering chitosan's pKa of 6.3 and DDA of 85%, we could calculate that a maximum of only 14% of total NH_3^+ groups on chitosan chains were available to bind with chromium ions at pH=7.0. However, unlike chitosan solutions, chitosan films can pack chitosan molecules within the films and/or make possible crystallization of the polymer molecules, both of which could reduce the availability of active amino groups. Theoretically, films with larger surface area could have more amino groups on chitosan chains available to interact with chromium ions and perform better metal binding. This means that the metal binding effect of chitosan films was not only related to the mass of active materials in the films but also their surface area. In our study, the calculated surface area to mass ratio of ultra-thin chitosan/PEO films was 16 m^2/g, which was much higher than the thin films (0.036-0.058 m^2/g) and thick films (0.017-0.026 m^2/g). Also according to the results from WAXD, the crystallinity of ultra-thin chitosan/PEO films greatly decreased compared to the regular cast films due to the rapid solvent removal and increased more available amine groups. Thus, the extremely high surface area to mass ratio and low extent of crystallinity of ultra-thin chitosan/PEO films greatly increased the availability of chitosan molecules and contributed to an increase in number of available $-NH_3^+$ active groups to bind chromium ions compared to regular cast films.

Interestingly, presence of PEO in the films up to 50% content did not significantly reduce the chromium binding performance of films. This might be explained by the effect of physical

adsorption properties of films. 100/0 and 90/10 chitosan/PEO cast films dissolved in chromium solutions due to their weak inter and intramolecular interactions and due to residual acetic acid that acidified solution promoted dissolving chitosan. However, with the increase of PEO content in the films from 20% to 50%, the increasing intermolecular interactions between chitosan and PEO molecules as well as decreasing acidic residuals provided the films with desired stability in the solution, which was important for adsorbing chromium ions onto film surface. Additionally, films with porous surfaces provided the advantages of high metal ions binding and adsorption capacity (Denizli et al., 2001; Beppu et al., 2004; Liu and Bai, 2006; Xi et al., 2006). Water-soluble PEO molecules may prevent high packing density of the polymer within the films and contributed to their porosity and adsorptive ability for the removal of heavy metal ions. There was no significant difference in performance of chromium bound by ultra-thin chitosan/PEO films with different blend ratio due to their extremely low thickness and weight.

6.3.4 Antibacterial Property of the Films

The inhibitory effects of regular thick and thin chitosan/PEO films toward *E. coli* K-12 are shown in Table 6.5. All the films had the same total surface area of 9.8 cm^2 having both sides available for contact. As shown in Table 6.5, the thick chitosan/PEO films with the weight of 0.038-0.058 g reduced the number of *E. coli* K-12 from 2.51 to 3.11 log CFU/ml whereas the thin chitosan/PEO films with the weight of 0.017-0.027 g reduced 1.80 to 2.62 log CFU/ml of bacteria. The antibacterial property of our chitosan/PEO cast films was comparative with those in the literature. Park et al. (2004) have reported that 0.03 g chitosan films (average thickness 70 μm, surface area of 6.28 cm^2) reduced 1.8 log CFU/g of *E.coli*. A point needed to be metioned was that the log reduction values of the current thin chitosan/PEO films were lower than the results of films presented in Chapter 4. This was because the weight of current films (0.017-0.027 g) used was far lower than those films (0.086-0.095 g).

Table 6.5 Inhibitory effects of thick and thin chitosan/PEO films toward *Escherichia.coli* K12 inoculated in sterile phosphate buffer (0.05 M, pH=7.08) and stored for 6h at 25°C*.

Chit/ PEO	Thick Films			Thin Films		
	Weight (g)	Chitosan in inoculated test tubes (%)	*E.coli* K12 Reduction (Log_{10} CFU/ml)	Weight (g)	Chitosan in inoculated test tubes (%)	*E.coli* K12 Reduction (Log_{10} CFU/ml)
100/0	0.047	0.470	2.67 ± 0.05^{ab}	0.023	0.230	2.37 ± 0.08^{abc}
90/10	0.058	0.522	3.11 ± 0.21^{a}	0.026	0.235	2.20 ± 0.15^{bc}
80/20	0.055	0.440	2.90 ± 0.10^{ab}	0.025	0.200	2.40 ± 0.45^{abc}
70/30	0.044	0.308	2.75 ± 0.07^{ab}	0.021	0.147	2.56 ± 0.01^{abc}
60/40	0.038	0.228	2.68 ± 0.29^{ab}	0.027	0.162	2.62 ± 0.12^{abc}
50/50	0.047	0.235	2.51 ± 0.16^{abc}	0.017	0.085	1.80 ± 0.10^{c}

*Means of three replicates ± standard deviation. Superscript letters in both columns of thick and thin films indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP, 2007).

Many studies have reported the inhibitory effect of chitosan against *E.coli* (Tsai and Su, 1999; Tsai et al., 2004; Duan et al., 2007). One general consideration of the mechanism was the cationic amino groups ($-NH_3^+$) on chitosan chains could interact with negative charged cell surface and change membrane permeability, causing the leakage of intracellular components and final death of cells (Tsai and Su, 1999). Like metal binding property, the antibacterial property of chitosan/PEO films was also significantly related to their surface area to mass ratio. As seen in Table 6.5, under the same blend ratio, the inhibition property of all thick films, except for 90/10 chitosan/PEO films, was slightly but non-significantly better than thin films. The results suggested that with the same surface area, the thick chitosan/PEO films with higher amount of chitosan did not show a significantly stronger antibacterial ability compared with the thin chitosan/PEO films with lower amount of chitosan. In other words, it could be estimated that thinner chitosan/PEO films with larger surface area to mass ratio would potentially perform better than thick films with the same blend ratio.

Increasing the surface area to mass ratio of chitosan made it possible to use minimum amount of chitosan material to inhibit microorganisms. Sams et al. (2004) have produced chitosan/PEO composite fibers with the diameters on nanometer range with high surface area to mass ratio and showed that these fibers reduced *Listeria monocytogenes* count by 1.85 log

CFU/ml with estimated chitosan concentration in the inoculated samples of only 0.008%. In our experiment, reduction of *E.coli* at the level of 1.8 log CFU/ml was achieved with the regular cast films with the lowest chitosan concentration of 0.085% (Table 6.5). Therefore the antibacterial ability of ultra-thin chitosan/PEO films was tested as well. Unfortunately, these films did not show significant antibacterial properties because of extremely low concentration of chitosan (c. 0.00025%) in the inoculated test tubes that was insufficient to kill significant number of bacteria.

In addition, the increasing of PEO content up to 50% in regular cast chitosan/PEO films did not significantly reduce their antibacterial property (Table 6.5). This indicates that addition of PEO to chitosan films can reduce the chitosan usage with no effect on the antibacterial property of the films.

6.4 Conclusions

Thick, thin, and ultra-thin chitosan/PEO films were successfully produced and their performances were compared. The results showed that chitosan/PEO films thickness, composition and preparation methods were significantly related to the surface morphology, antimicrobial activity and metal binding capacity of the forming films.

The crystallization extent of thick, thin and ultra-thin chitosan/PEO films decreased as the thickness of the films decreased because of reduced film-forming time, especially in the spin-coating method. Rapid evaporation of a solvent greatly restricts the mobility of the polymer chains and, thus, prevents crystallization within the films. Decreasing the thickness of films was a possible way to increase the surface area to mass ratio, further increase the number of available active $-NH_3^+$ sites on the film surface, and promote the metal binding and antibacterial efficiency of chitosan. Ultra-thin chitosan/PEO films showed a significantly higher chromium binding capacity compared to the regular cast films; however, these films did not show significant antibacterial properties due to extremely low concentration of chitosan (c. 0.00025%) in the inoculated test tubes that was insufficient to kill significant number of bacteria.

The incorporation of PEO content in the chitosan films slightly decreased the film thickness but increased the film crystallinity and the surface roughness of the ultra-thin films. Presence of PEO in the films up to 50% did not significantly reduce the metal-binding capacity and antibacterial property of chitosan. On the contrary, the intermolecular interactions between chitosan and PEO molecules provided the films with desired stability in the solution. In addition, water-soluble PEO molecules may prevent high packing density of the polymer within the films and contributed to their porosity and adsorptive ability for the removal of heavy metal ions. These results indicated that partial replacement of chitosan with PEO in the films could reduce the chitosan usage with no effect on the functional properties of the films.

Chitosan/PEO films have potential to be used as functional packaging material in the food and pharmaceutical industries. In particularly, due to their excellent metal-binding capacity, ultra-thin chitosan/PEO films have potential to be used as coatings, in sensors, or as layers incorporated into packaging.

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CHAPTER 7. CHARACTERIZATION AND COMPARISON OF CHITOSAN/PEO AND CHITOSAN/PVP FILMS

Abstract

Blending of synthetic and natural polymers may produce films with improved properties compared to films prepared with the counterpart single polymer only. In our previous studies, we have determined the physical, mechanical and functional properties of thick, thin and ultra-thin chitosan films prepared with blended poly (ethylene oxide) (PEO). The objective of this study was to investigate the functional performance of chitosan films prepared with another synthetic polymer, poly (N-vinyl-2-pyrrolidone) (PVP), and compare their characteristics with those of chitosan/PEO films. Physical and mechanical properties of chitosan/PVP and chitosan/PEO films with various blend ratios (100/0, 75/25, 50/50, 25/75, 0/100) were evaluated by measuring color, water vapor permeability (WVP), puncture strength, tensile strength and elasticity. Potential development of polymer crystallization in films was monitored by polarized microscope. The metal-binding capacity of the films was assessed as percentages of chromium ions removed from a 10 mg/l Cr (VI) solution by one film, and the antibacterial properties of the films were tested against *Escherichia coli* K-12. Similar to chitosan/PEO films, increasing the PVP content in chitosan/PVP films reduced yellowish coloration and resulted in films susceptible to be punctured and fractured. However, PVP contributed less significantly to elasticity of the blending films than PEO. Contrary to chitosan/PEO films, WVP of the chitosan/PVP films significantly increased when PVP content reached 75%. Both chitosan/PVP and chitosan/PEO films with 25% chitosan content still maintained satisfactory chromium binding capacity and antibacterial activity. Incorporation of either PVP or PEO in chitosan films could significantly reduce the price with no adverse effects on functionality of the films. Both chitosan/PVP and chitosan/PEO films have potential to be used as functional packaging material in the food industry.

7.1 Introduction

Hydrophilic films such as those of chitosan have been widely studied in membrane based application (Kittur et al., 1998; Nadarajah, 2005; Zivanovic et al., 2005). Because of their high impermeability to oxygen and antimicrobial property, chitosan films have been studied in the food industry for their potential as active packaging materials to extend shelf life of food products (Butler et al., 1996; Jeon et al., 2002; No et al., 2007). However, their wide application is limited by the yellowish color, low flexibility in regulating the mechanical profile and relatively poor water vapor barrier characteristics (Butler et al., 1996).

Use of blend polymers is the most effective way to produce new multipurpose materials (Yeh et al., 2006). In addition, since synthetic polymers are easily obtained and have low production cost, the blending of natural and synthetic polymers may improve the cost to performance ratio of the film (Fried, 1995). Studies on blend films of chitosan with synthetic polymers, such as poly (vinyl alcohol) (PVA) (Bahrami et al., 2003), N-methylol nylon 6 (Shieh and Huang, 1998), polycaprolactone (PCL) (Sarasam et al., 2006), have shown improved properties compared to pure chitosan films. Generally chitosan is blended with other hydrophilic polymers to overcome the disadvantage of the low mechanical strength in the wet state (Smitha et al., 2006).

In our previous study (Chapter 3, 4, 5), we investigated the physical, mechanical and functional properties of thick, thin, and ultra-thin films produced by blending chitosan and poly (ethylene oxide) (PEO), a low-toxic, hydrophilic, biocompatible synthetic polymer. The results showed that incorporation of PEO into regular cast chitosan films could decrease the yellowish color from chitosan, lower water vapor permeability, and decrease mechanical properties. In the present study, we studied the characterization of films formed by chitosan with another synthetic polymer --- poly (N-vinyl-2-pyrrolidone) (PVP). Like PEO, PVP is a non-toxic polymer with excellent transparency, biocompatibility and film-forming ability and has been utilized in a broad range of areas, such as medical, cosmetics, food, and health-related products (Yeh et al., 2006).

It was reported that annual world wide consumption of PVP reached beyond ten thousand tons in 2001 (Xiao et al., 2001). As a food processing aid, PVP is a stabilizer and has food additives number code in European Union of E1201. However, PVP containing the pyrrolidone rings have more rigid nature than PEO.

Most of the published articles related to chitosan and PVP blends focuse on their compatibility and miscibility. The blend system of chitosan and PVP was miscible because the pyrrolidone rings in PVP chains contain a proton accepting carbonyl groups, which can possibly interact with amino groups and hydroxyl groups in chitosan chains by hydrogen bondings (Cao et al., 1998; Fang and Goh, 2000; Sakurai et al., 2000; Marsano et al., 2004). Due to the rigid nature of PVP molecules, chitosan/PVP films exhibited low crystallinity (Sakurai et al., 2000). Other studies on chitosan/PVP blends included the surface energy (Caykara et al., 2006), thermoresistance property (Yeh et al., 2006), and methanol permeability for fuel cell applications (Smitha et al., 2006). Very limited literature could be found regarding the physical and functional properties of chitosan/PVP films.

The objectives of this research were (1) to evaluate physical and mechanical properties and functional performance of thin chitosan/PVP films prepared with various polymer ratios with the aim to produce new materials possessing the benefits of both; and (2) to compare the properties of chitosan/PVP films with chitosan/PEO films.

7.2 Materials and Methods

7.2.1 Materials

Low molecular weight chitosan (~150 kDa) and high molecular weight PVP (1300 kDa) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). High molecular weight PEO (900 kDa) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

7.2.2 Preparation of Chitosan/PVP and Chitosan/PEO Films

The chitosan solution was prepared by dissolving 1% w/w chitosan in 1% v/v acetic acid, stirred overnight at room temperature, and filtrated through Miracloth® (Calbiochem-Novabiochem Corp., San Diego, CA) to remove impurities. PVP and PEO were dissolved separately in d.i. water to form 1% w/w solutions separately. Aqueous solutions of the individual polymer were mixed to prepare a series of chitosan/PVP and chitosan/PEO blend solutions with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100. 10 g of film-forming solutions were poured into 50mm-diameter polystyrene Petri dishes and the solvent was evaporated in a vacuum oven at 38°C under 17 kPa pressures for 24 hours. The dried films were peeled from the Petri dishes and conditioned in desiccators at 25ºC and 20% RH prior to testing.

7.2.3 Thickness

Film thickness (μm) was determined from 6 films for each treatment; each film was measured at 5 locations using a hand-held microcaliper (Mitutoyo Corp., Kawasaki, Kanagawa, Japan).

7.2.4 Film Color

Hunter color scale of lightness (*L*), chromatic parameters *a* (red-green), and *b* (yellow-blue) of chitosan/PVP and chitosan/PEO films were measured using a Hunter colorimeter (Hunterlab, Reston, VA) at room temperature. Color measurements of films were replicated five times.

7.2.5 Film Crystallization

Crystallization of polymers in the films was observed under a polarized microscope (Olympus-BX51, Melville, NY) with 400x magnification within 24 hours after casting. Wide-angle X-ray diffraction (WAXD) photographs of samples were obtained using a Molecular Metrology SAXD/WAXD system (Northampton, MA) equipped with a monochromic CuKα (1.5418 Å) X-ray source, a three-pin-hole alignment, and two-dimensional detector operating at 45 kV and 0.66 mA with the beam size of 30 μm. The WAXD patterns were recorded on reusable Fuji image

plates, with the sample-to-film distance for 36.52 mm. The image plate was scanned in a Fuji X BAS-1800II image analyzer.

7.2.6 Mechanical Properties

Puncture strength (PS) and Tensile strength (TS) were determined using a TA.XT*plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) and calculated as described in Chapter 3 (ASTM, 2002). Instead of elongation at break, elasticity, which was valued by the distance of the penetration before breaking the films, was examined. PS, TS and elasticity measurements were replicated 3 times per ratio treatment.

7.2.7 Water Vapor Permeability

Water vapor permeability (WVP) was measured gravimetrically using the ASTM E-96 standard method (ASTM, 2005) and calculated using the WVP correction method (Gennadios et al., 1994) as described in Chapter 3.

7.2.8 Metal-binding Capacity

The metal-binding capacity of films was determined by the concentration change of Cr (VI) following NIOSH method (NIOSH Manual of Analytical Methods (NMAM), 1994) before and after adding films. Based on the experience with metal-binding test presented in Chapter 6, we improved the testing method. Previously we found that 100/0 and 90/10 chitosan/PEO films dissolved in the aqueous chromium solution due their weak intermolecular interaction in the film matrix and due to residual acetic acid that acidified solution and promoted dissolving chiotsan. Thus, for the current study, all the films were treated with the methanol to remove acetic acid from the films. Chitosan/PVP and chitosan/PEO films with different ratios were pretreated in 20 ml methanol for 24 hours, dried in vacuum oven for 24 hours. After the methanol treatment, both chitosan/PVP and chitosan/PEO films with a blend ratio from 100/0 to 25/75 maintained a good shape after being soaked in the aqueous chromium solution for 3 hours. However, pure PVP or PEO films still dissolved in the solutions because of their hydrophilic nature. In addition, due to the good binding performance of previously tested films, we increased the chromium

concentration from 5 mg/l to 10 mg/l. Films were separately placed into 25 ml K_2CrO_4 solution for 3 hours. 1 ml sample solution was taken and mixed with 6-7 ml 0.5 N H_2SO_4 in 25ml volumetric flask, 0.5 ml sym-diphenylcarbazide in 50 % acetone was added, and the volume was adjusted with 0.5 N H_2SO_4 to 25 ml. Absorbance was read at 540 nm using spectrophotometric method (UV- 2102PC, Shimadzu, Kyoto, Japan).

7.2.9 Antibacterial Property in the Liquid System

Antibacterial test was carried out by submerging films (19.6 cm^2) into culture tubes containing 9 ml sterile phosphate buffer (0.05 M, pH=7.08) inoculated with ca. 10^6 CFU/ml *Escherichia coli* K-12, mixed by vortexing and incubated for 6 hours at 25°C. The survival of *E. coli* K-12 was determined using the pour plate method on Trypticase Soy Agar (TSA) medium (Swanson et al., 2001).

7.2.10 Statistical Analysis

All measurements were done at least in triplicate with individually prepared films as the replicated experimental units. Significant differences between groups were determined using Tukey-Kramer HSD test in the JMP program (JMP 2007). On figures with bars, means within the same group with a different letter are significantly different ($p < 0.05$).

7.3 Results and Discussions

7.3.1 Film Color and Appearance

Homogeneous, thin films with thickness from 34.05 ± 2.89 μm to 44.52 ± 3.71 μm were formed from 10 g chitosan and PVP blend solutions (Table 7.1). The thickness of chitosan/PEO films were from 33.60 to 38.26 μm, which were consistent with the previous results (Chapter 4). All of chitosan/PVP films were more easily removed from the Petri dishes than chitosan/PEO films. The chitosan/PVP films were transparent (Figure 7.1).

Table 7.1 Thickness of and mass per area of chiosan/PVP and chitosan/PEO blend films with weight ratio of 100/0, 75/25, 50/50, 25/75, 0/100*.

Film (w/w chi/PEO or PVP)	Chitosan/PVP films		Chitosan/PEO films	
	mg chi/ 1 cm^2 film	Thickness (μm)	mg chi/ 1 cm^2 film	Thickness (μm)
100/0	4.85	44.52 ± 3.71[a]	4.85	44.52 ± 3.71[a]
75/25	3.49	42.24 ± 4.03[a]	3.49	38.26 ± 2.06[b]
50/50	2.27	43.27 ± 3.84[a]	2.27	33.86 ± 2.81[b]
25/75	1.05	35.84 ± 3.49[b]	1.05	33.60 ± 1.82[b]
0/100	0	34.05 ± 2.89[b]	0	36.31 ± 2.22[b]

* Values reported are means and standard deviation. Superscript letters within one column indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP 2007).

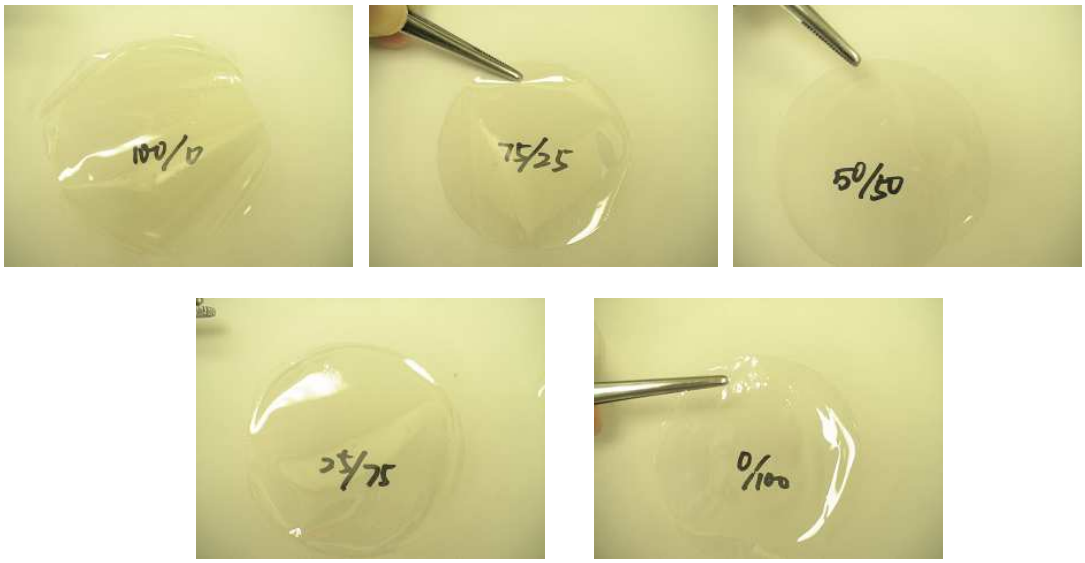


Figure 7.1 Photographs of LMW chitosan/HMW PVP blend films of various ratios (100/0, 75/25, 50/50, 25/75, 0/100).

Table 7.2 Optical properties of chitosan/ PVP and chitosan/PEO films*

Film composition	Film color		
	L	***a***	***b***
chitosan	88.36 ± 0.25[df]	-2.50 ± 0.10[e]	5.54 ± 0.67[b]
chitosan/PVP (75/25)	87.87 ± 1.17[f]	-2.29 ± 0.04[d]	4.01 ± 0.58[c]
chitosan/PVP (50/50)	89.05 ± 0.17[bcde]	-2.08 ± 0.04[c]	2.33 ± 0.26[d]
chitosan/PVP (25/75)	89.11 ± 0.06[bcde]	-1.95 ± 0.02[bc]	1.53 ± 0.03[de]
PVP	89.71 ± 0.55[bc]	-1.75 ± 0.02[a]	0.56 ± 0.03[e]
chitosan/PEO (75/25)	88.35 ± 0.23[def]	-2.77 ± 0.06[f]	9.42 ± 0.55[a]
chitosan/PEO (50/50)	89.10 ± 0.20[ce]	-2.37 ± 0.06[d]	5.27 ± 0.64[b]
chitosan/PEO (25/75)	89.93 ± 0.35[b]	-2.02 ± 0.03[c]	2.15 ± 0.19[d]
PEO	91.78 ± 0.25[a]	-1.84 ± 0.04[ab]	0.53 ± 0.08[e]

* Values reported are means and standard deviation. Superscript letters within one column indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP 2007).

The Hunter color scale of *L, a, b* values of chitosan/PVP and chitosan/PEO films are listed in Table 7.2. According to the change of *b* values, increasing PVP or PEO content significantly reduced film yellowish coloration. An exception was the unexpected higher *b* value of 75/25 chitosan/PEO films which should be colorless according to the results in Chapter 4. This abnormal yellow color of 75/25 films may come from possible impurities either from raw chitosan flakes or from the environment during the film formation process. Lightness (*L*) values showed that there was no siginifcant difference between chitosan/PEO and chitosan/PVP films of the ratio from 75/25 to 50/50. Similar to results described in Chapter 3, films with a PEO content of 50% and more, the *L* value greatly increased and films started to show opaque appearance due to PEO crystallization (Figure 3.3).

7.3.2 Film Crystallization

Figure 7.2 shows the shape and distribution of the crystals within chitosan/PVP films and compares with the crystallinity of chitosan/PEO films as examined under polarized microscope. Similar to chitosan, PVP is a partially crystalline polymer (Sakurai et al., 2000). As illustrated in Figure 7.2, the extent of crystallization in chitosan, PVP and their blend films was much lower compared to PEO and chitosan/PEO films.

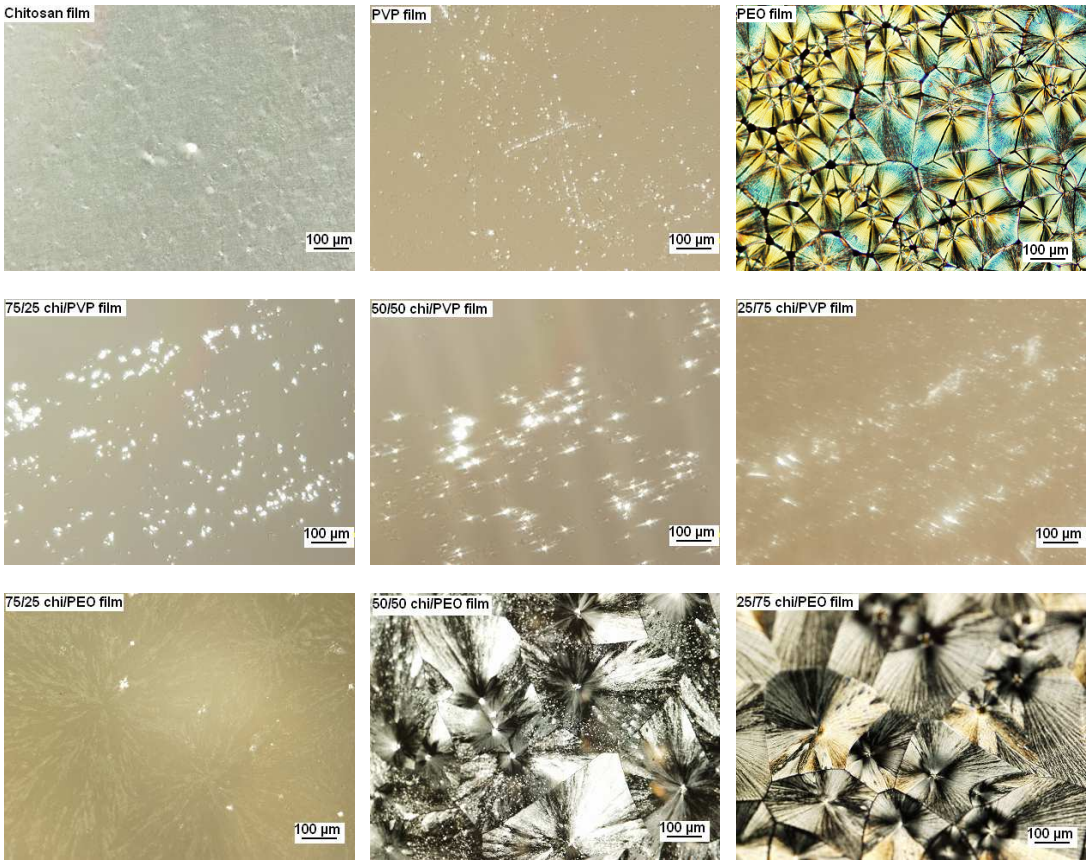
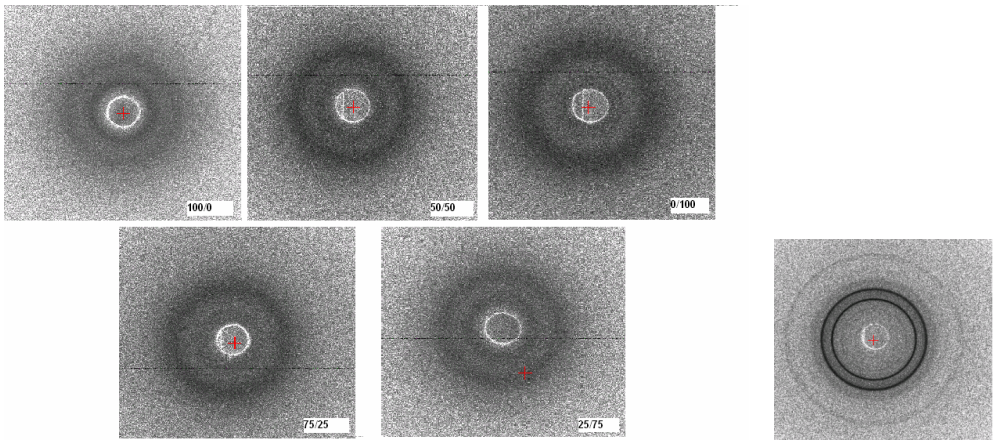


Figure 7.2 Polarized micrographs of chitosan, PVP and PEO films (top), chitosan/PVP films (middle), and chitosan/PEO films (bottom) with ratios of 75/25, 50/50, 25/75 with 400X magnification within 24 hours after casting.



50/50 Chitosan/PEO films

Figure 7.3 WAXD photographs of chitosan/PVP blend films with ratios of 100/0, 75/25, 50/50, 25/75, 0/100 as well as WAXD photograph of 50/50 chitosan/PEO films as comparison.

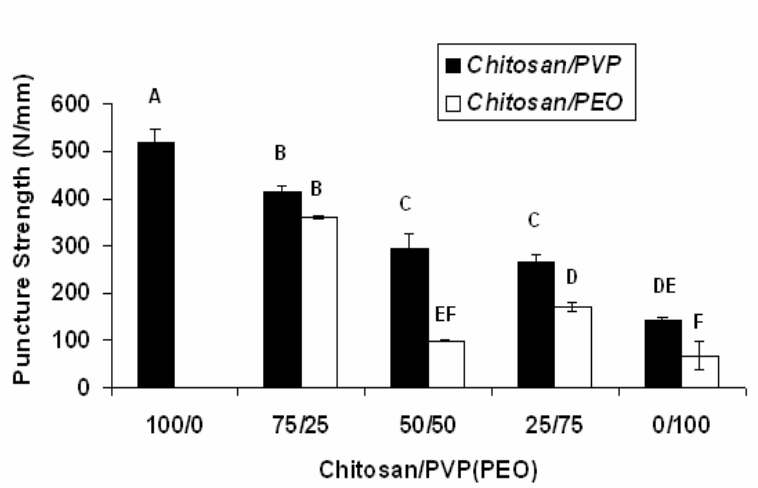
Being different from semi-crystalline polymer PEO (Mucha et al., 1999), PVP does not have spherulitic crystallization property. The crystallinity of chitosan/PVP films was further analyzed in WAXD photographs shown in Figure 7.3. Both chitosan and PVP gave some crystalline diffraction rings on WAXD photographs. However, they were neither strong nor sharp, compared to WAXD photographs of 50/50 chitosan/PEO films that were highly crystalline. These results indicate that although the linear molecules of the polymers aligned to some extent, chitosan and PVP predominantly existed in amouphous form in the films (Sakurai et al., 2000).

7.3.3 Film Mechanical Properties

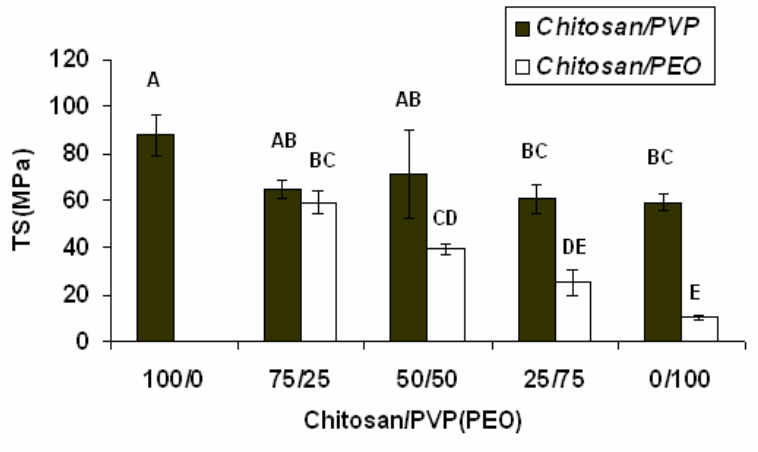
The puncture strengths (PS) of chitosan/PVP and chitosan/PEO films with different ratios are compared in Figure 7.4 A. The PS of the films decreased significantly as the chitosan fraction decreased, from 520.69 ± 25.13 N/mm for pure chitosan films to 144.58 ± 5.90 N/mm for 0/100 chitosan/PVP films and 66.78 ± 28.69 N/mm for 0/100 chitosan/PEO films. Addition of either PVP or PEO made the blend films easier to puncture. However, when the fraction of synthetic polymer in the films was 50% or higher, effect of PEO was more pronounced. This is probably due to the extensive crystallization of PEO in the films (Fugure 7.2) what disturbed the whole structure of film matrix and decreased its ability to undergo the stresses.

Similarly to the effect on PS, PEO content significantly contributed to the decreasing TS values of films from 87.68 ± 8.86 MPa to 9.89 ± 1.05 MPa for 100/0 and 0/100 chitosan/PEO films (Figure 7.4 B). With increasing PVP fraction, the TS of films showed decreasing tendency, however, the decreasing change was not significant and pure PVP films still maintained the high value of 59.13 ± 3.31 MPa. Chitosan/PVP films generally showed higher TS than chitosan/PEO films under the same ratio. In addition to the absence of crystallinity in chitosan/PVP films, the rigid chains of chitosan and PVP were another factor to attribute to the high TS.

A



B



C

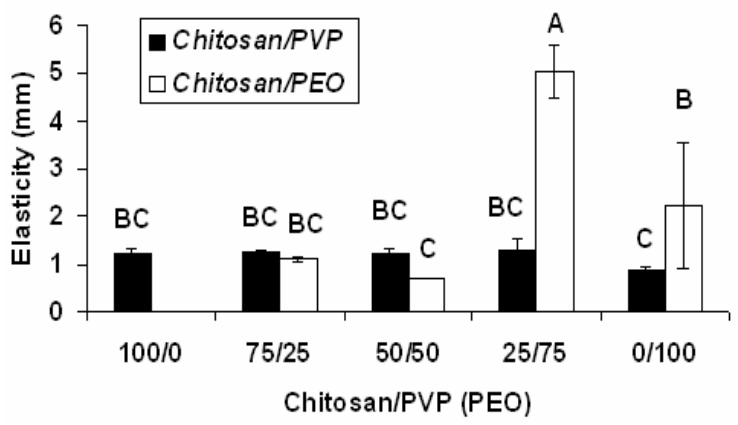


Figure 7.4 Comparison of puncture strength (A), tensile strength (B) and elasticity (C) of chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 0/100. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$.

Similarly, PVP has been used to increase the tensile strength of glucomannan films (Xiao et al., 2001). Compared to the TS values of other biopolymer films, such as whey protein isolate films with the value of 13.9 MPa (McHugh and Krochta, 1994), zein films with the value of 6.81 MPa (Lai and Padua, 1997), and widely used plastic films, such as LDPE and HDPE with the values of 8.6-17.3 MPa and 17.3-34.6 MPa (Brinston, 1988), respectively, our chitosan/PVP films possessed higher tensile strength and thus have advantage for applications where resistance to tear is required for packaging material.

The elasticity of films is presented in Figure 7.4 C. The elasticity was assessed as the distance of the penetration before breaking the films, which reflected the flexibility but was different from elongation at break.

One disadvantage of pure chitosan films is their lack of flexibility. In spite of the flexible chains of PEO, our previous study has shown that incorporation of PEO in chitosan films could not attribute to the value of elongation at break due to their low tensile strength. The present study proved that the change of film flexibility could be better illustrated by measuring the elasticity value during puncture test. Incorporation of 75% of PEO in chitosan films could increase the elasticity of chitosan films significantly from 1.22 ± 0.11 mm to 5.04 ± 0.56 mm. However, the addition of PVP in the blends could not increase the elasticity of the films due to their brittle nature. Our results indicate that the films formed by blending chitosan with PVP could not improve the flexibility of films but addition of 75% PEO can significantly increase it.

7.3.4 Water Vapor Permeability

Table 7.3 shows the WVP values of chitosan, PVP, and their composite films as well as PEO and chitosan/PEO films. Similar to the results presented in Chapter 4, addition of hydrophilic polymer PEO caused decrease in WVP values. Interestingly, another hydrophilic polymer PVP showed an opposite performance in the blends and attributed to significantly higher WVP values.

Table 7.3 Comparison of water vapor permeability of chitosan/PVP films, chitosan/PEO films and other edible, synthetic films*

Film composition	Water vapor permeability (g mm/m^2 h kPa)	Thickness (µm)	References
chitosan	3.53 ± 0.45^{bcd}	39.91 ± 1.78	Present study
chitosan/PVP (75/25)	4.43 ± 0.41^{b}	42.22 ± 2.27	
chitosan/PVP (50/50)	4.30 ± 0.68^{bc}	37.02 ± 2.56	
chitosan/PVP (25/75)	6.36 ± 0.49^{a}	38.89 ± 2.26	
PVP	8.06 ± 1.69^{a}	32.78 ± 1.84	
chitosan/PEO (75/25)	3.82 ± 0.79^{bcd}	37.81 ± 3.63	
chitosan/PEO (50/50)	2.42 ± 0.14^{cd}	35.74 ± 0.81	
chitosan/PEO (25/75)	2.68 ± 0.62^{bcd}	34.93 ± 2.08	
PEO	1.81 ± 0.31^{d}	35.66 ± 0.56	
Edible films			
Highly Carboxymethyl- ated Starch (HCMS)	7.56	94.6 ± 12.2	Kim et al., 2002
Whey Protein Isolate	5.06	139	Perez-Gago et al., 1999
Wheat gluten	4.52	140	Aydt et al., 1991
Zein	1.93	200	Ghanbarzadeh et al., 2007
Beeswax	0.00209	40 - 50	Greener, 1992
Carnauba wax	0.00187	90 - 110	Greener, 1992
Synthetic films			
Cellophane	0.30	22 ± 0.41	Phan et al., 2005
LDPE	0.0033	-	Smith, 1986
HDPE	0.0008	-	Smith, 1986

* Values reported are means and standard deviation. Superscript letters indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP, 2007).

Although the strong interaction between amino groups and hydroxyl groups of chitosan and carbonyl groups of PVP has been recognized (Cao et al., 1998; Fang and Goh, 2000; Sakurai et al., 2000; Marsano et al., 2004), this apparently did not efficiently impede the diffusivity of water molecules.

Hydrocolloid films are generally characterized by their poor water vapor permeability (Krochta and De Mulder-Johnson, 1997; Mali et al., 2002). Comparing to the WVP values of other edible films and synthetic films reported in the literatures (Table 7.3), both chitosan/PVP and chitosan/PEO films in our present study had similar WVP values to those polysaccharides-based or protein-based films but showed significantly higher values than lipid-based and synthetic films. Addition of PEO in our experiments did reduce water vapor permeability of chitosan films.

7.3.5 Metal-binding Capacity of the Films

The chromium-binding capacity of chitosan/PVP films was measured and compared to those of chitosan/PEO films as shown in Figure 7.5 and Figure 7.6. All the films showed excellent ability to remove chromium ions from aqueous solutions. Figure 7.5 showed the percentage of chromium ions bound by one piece of film in the 25 ml of 10 mg/l solutions. Both chitosan/PVP and chitosan/PEO films with only 50% content of chitosan absorbed almost 100% of chromium ions. Although further decrease of the chitosan content to 25% started to decrease the efficiency to remove chromium from the solution, the bound amount was still as high as 80% for chitosan/PVP films and 60% for chitosan/PEO films.

Chromium binding capacity was calculated based on one gram of the films and shown in Figure 7.6. As it can be seen, 1 g chitosan/PVP films bound 3.106 - 3.496 mg chromium ions whereas 1 g chitosan/PEO films bound 1.888 - 3.272 mg chromium ions.

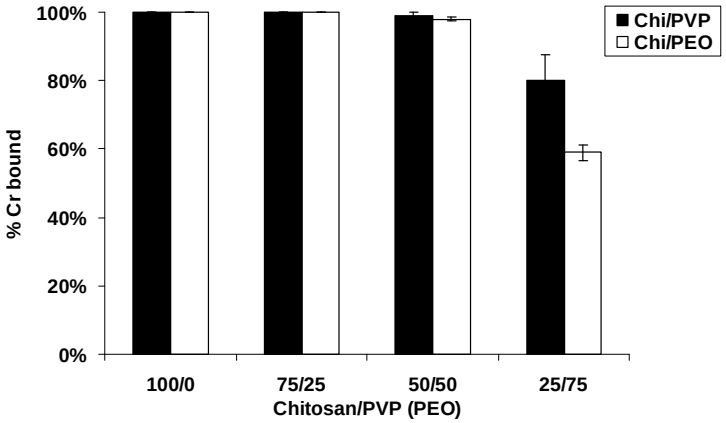


Figure 7.5 Percentage of Cr (VI) bound by chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 25/75 in 25 ml 10 mg/l chromium solution. Error bars represent standard deviation (n=3).

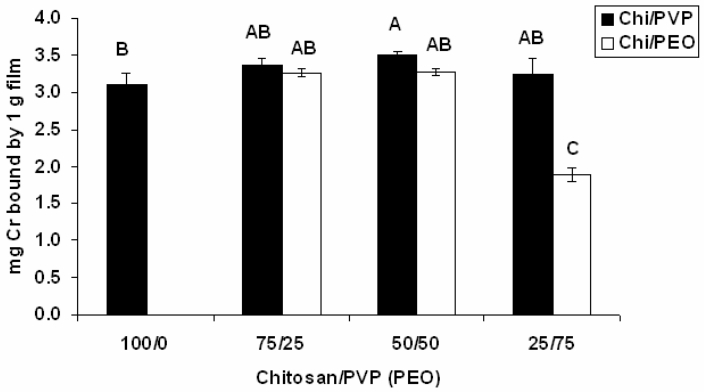


Figure 7.6 Weight of Cr (VI) (mg) bounded by 1 g chitosan/PVP and chitosan/PEO films with blend ratio from 100/0 to 25/75 in 25 ml 10 mg/l chromium solution. Error bars represent standard deviation (n=3). Letters indicate significant difference at $p<0.05$.

The chromium binding capacity of our chitosan/PVP and chitosan/PEO films were at still lower level compared to reported 27.3 mg/g for plain chitosan powder (Udaybhaskar et al., 1990), 3-16 mg/g for high molecular weight chitosan/PEO nano-fibers (Desai et al., 2008), and 26.47-70.86 mg/g for our ultra-thin chitosan/PEO films (Chapter 6).

However, similar to the results of cast chitosan/PEO films in 5 mg/l chromium solution described in Chapter 6, 50/50 chitosan/PVP and chitosan/PEO films showed comparable chromium binding capacity (3.496 mg/g film, 3.106 mg/g film) with pure chitosan films (3.106 mg/g film) in 10 mg/l chromium solutions. Thus, our results showed that replacing even 50% of chitosan with PVP or PEO in chitosan films did not significantly decrease the chromium binding properties of the films. This indicates that although the total number of functional amino groups decreased with decreasing chitosan content in the blends, incorporation of hydrophilic PVP or PEO into chitosan films made the films more porous in the aqueous chromium solution, and allowed chromium ions to penetrate into the volume of the films and interact with NH_3^+ ions of chitosan within the films, resulting in blend films maintaining comparable or even higher metal binding efficiency than pure chitosan films.

Since the price of synthetic polymers were lower than chitosan, for example, the price of chitosan ($15-16/kg) was twice higher than that of PVP ($6-8/kg) (Babel and Kurniawan, 2003), the current results may suggest that blending chitosan with synthetic hydrophilic polymers could produce new materials with metal-binding properties but low cost.

7.3.6 Antibacterial Property of the Films

The inhibitory effects against *E. coli* K-12 of chitosan/PVP films under different blend ratios were shown and compared with chitosan/PEO films in the Table 7.4. Chitosan/PVP films showed satisfactory antibacterial efficiency. The films reduced the number of *E. coli* K-12 by 2.82 log CFU/ml for 75/25 films and 2.15 log CFU/ml for 25/75 films compared to 3.32 log CFU/ml for pure chitosan films.

Table 7.4 Inhibitory effects of chitosan/PVP and chitosan/PEO blend films toward *Escherichia.coli* K-12 inoculated in sterile phosphate buffer (0.05 M, pH=7.08) and stored for 6h at 25°C.

Film composition	Weight (g)	*E.coli* K-12 reduction (Log_{10} CFU[a]/ml)
chitosan	0.0951	3.32 ± 0.26^{ab}
chitosan/PVP (75/25)	0.0914	2.82 ± 0.38^{bc}
chitosan/PVP (50/50)	0.0891	2.48 ± 0.08^{cd}
chitosan/PVP (25/75)	0.0826	2.15 ± 0.15^{d}
chitosan/PEO (75/25)	0.0922	3.42 ± 0.10^{ab}
chitosan/PEO (50/50)	0.0865	3.75 ± 0.20^{a}
chitosan/PEO (25/75)	0.0752	2.93 ± 0.48^{bc}

[a] CFU=colony-forming unit.
Means of three replicates ± standard deviation. Superscript letters indicate significant difference at $p<0.05$ by Tukey-Kramer HSD test (JMP 007).

Chitosan/PVP films only showed significant decreasing tendency when PVP content was 50% and more. Our previous study (Chapter 4, 6) has shown that the increasing PEO content till 50% in regular cast chitosan/PEO films did not significantly reduce their antibacterial property. 50/50 chitosan/PEO films even showed unexpected high inhibition against *E. coli* K-12 compared to pure chitosan films (Chapter 4). The current study further prove these conclusions and further shows that chitosan/PEO films with blend ratio of even 25/75 still possess antibacterial ability (Table 7.4). Chitosan/PEO films had better inhibition ability against *E. coli* K-12 than chitosan/PVP films with the same blend ratio.

As described before (Chapter 4,6), the main accepted mechanism of chitosan's antimicrobial activity is in interaction of the cationic amino groups NH_3^+ on chitosan with negative charged cell surface what interrupts metabolism and kills the microorganism (Fang et al., 1994; Chen et al., 1998). PVP and PEO do not have antimicrobial ability. And also they could not attribute to enhance the opportunity to the interactions of NH_3^+ and bacteria cells as they did in the metal binding process because the larger size of cells (0.5-5 μm in diameter) can not penetrate into the films like metal ions. However, the current results showed that incorporation of PVP or PEO polymers less than 50% in chitosan films did not significantly reduce antibacterial property of chitosan (Table 7.4). Chitosan blend films with only 25% chitosan content still

showed over 2 log CFU/ml reduction, indicating that there still existed a number of active positive NH_3^+ groups on the film surface to interact with cells.

7.4 Conclusions

Films formed by blending chitosan with PVP had altered physical properties compared to films formed by single polymer. All chitosan/PVP blending films with ratio from 100/0 to 0/100 appeared transparent, which was much better than the opaque appearance of chitosan/PEO films with PEO content 50% and more. Similar to PEO, incorporation of PVP into chitosan films reduced the yellowish color and made films easier to puncture and tear by decreasing the puncture and tensile strength of the films. PVP affected PS and TS of blend films to less extent than PEO. PEO, when added in concentration of 75%, significantly improved elasticity of the films. Although chitosan/PEO blend films showed lower WVP values than chitosan/PVP films, blending chitosan with hydrophilic polymers was not an effective way to significantly improve the water vapor permeability.

Our study showed that replacing even 50% of chitosan with PVP or PEO in chitosan films did not significantly decrease the chromium binding and antibacterial properties from chitosan. Especially, hydrophilic PVP or PEO polymer made the blend films more porous in the aqueous chromium solution, and allowed metal ions to penetrate into the volume of the films and interact with NH_3^+ ions of chitosan within the films, resulting in blend films maintaining comparable or even higher metal binding efficiency than pure chitosan films.

Since synthetic polymers are less expensive than biopolymer chitosan, production of films by blending chitosan and synthetic polymer could reduce the price and have no effect on functionality of the films. Based on our results, chitosan/PVP and chitosan/PEO blend films have the potential to be used in the food industry as active packaging materials to inhibit food borne pathogens and as absorbent to bind heavy metal from contaminated water.

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CHAPTER 8. CONCLUSIONS

The physical, mechanical and functional properties of chitosan films produced by blending with poly (ethylene) (PEO) or poly (N-vinyl-2-pyrrolidone) (PVP) were examined as affected by molecular weight, thickness, and blend ratios.

The results demonstrated that chitosan/PEO films formed by the solution casting method have altered properties compared to films produced from either polymer alone. Regardless of molecular weight, chitosan decreased the spherulitic crystallization tendency of PEO, and enhanced puncture and tensile strengths of the films, while the PEO addition resulted in reduction of yellow coloration and film thickness and did not significantly alter water vapor permeability. However, films with PEO content of more than 50% showed opaque appearance due to the high crystallinity of PEO. At a 90/10 ratio of low molecular weight chitosan to high molecular weight PEO, the formed films had the best physical and mechanical properties. These films exhibited the best mechanical properties (puncture and tensile strengths), no crystallization, and significant antibacterial efficacy. Thin chitosan/PEO films with thickness of 25-44 μm had similar puncture and tensile strength but better percentage of elongation at break compared to thick films of 57-91 μm. Based on these results, it may be possible to replace a fraction of chitosan by PEO to produce thinner films with similar or better performance than the thick films. Production of ultra-thin chitosan and chitosan/PEO films with thickness below 80 nm was possible by spin-coating on silicon wafers. The increase of PEO content to 50% did not affect thickness of the films but the surface of corresponding films became rougher probably due to the formation of PEO crystallites.

Characterization of thick, thin, and ultra-thin chitosan/PEO showed that a decreased film-forming time, especially in the spin-coating method, greatly restricted the mobility of the polymer chains and decreased the crystallization extent. Decreasing the thickness of the films was a possible way to increase the surface area to mass ratio, further increase the number of available active $-NH_3^+$ sites on the film surface, and enhance the metal binding capacity and antibacterial efficacy of chitosan. Ultra-thin chitosan/PEO films showed a significantly higher chromium binding

capacity than regular cast films; however, they did not show significant antibacterial properties because their extremely low weight and consequently low concentration of chitosan (c. 0.00025%) in the inoculated test tubes was insufficient to kill significant number of bacteria.

All chitosan/PVP blend films with a ratio from 100/0 to 0/100 appeared transparent. Similar to PEO, incorporation of PVP into chitosan films reduced the yellowish color and resistance against puncturing and tearing. Addition of PVP lowered puncture and tensile strengths and improved films elasticity to a lower degree than addition of PEO. Although chitosan/PEO blend films showed lower WVP values than chitosan/PVP films, blending chitosan with hydrophilic polymers was not an effective way to significantly improve the water vapor permeability.

Our study showed that replacing up to 50% of chitosan by PVP or PEO in chitosan films did not significantly decrease the metal-binding and antibacterial properties of the films. Since synthetic polymers are less expensive than biopolymer chitosan, blend films of chitosan and synthetic polymers could reduce the use of chitosan and lower the production cost without compromising film functionalities.

Chitosan/PEO and chitosan/PVP films have potential to be used as functional packaging materials in the food and pharmaceutical industries. Based on our results, chitosan/PVP and chitosan/PEO blend films have the potential to be used in the food industry as active packaging materials to inhibit food borne pathogens and as absorbent to bind heavy metals from the environment. Ultra-thin chitosan/PEO films showed high metal binding capacity and have potential to be used as coatings, sensors, or layers incorporated into packaging.

VITA

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