

## CHAPTER I

### INTRODUCTION

Increasingly, natural polymers are becoming more important because they are renewable resources and have low costs. Due to their interesting properties, including nontoxicity, biocompatibility, and biodegradability, natural polymers have been investigated with a view to expanding their utilization. Chitin is the most abundant naturally occurring biopolymer and is found in crustacean shells and lower plants such as mushroom and cell wall of bacteria. Chitin is mainly poly ( $\beta$ -(1-4)-2-acetamido-D-glucose), which is structurally similar to cellulose except that a secondary hydroxyl group on the second carbon atom of the hexose repeat unit is replaced by an acetamide group (Andrady and Xu, 1997; Brack *et al.*, 1997). Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of poly ( $\beta$ -(1-4)-2-acetamido-D-glucose) unit and ( $\beta$ -(1-4)-2-amino-D-glucose) unit with the latter usually greater than 75% (Li *et al.*, 1997). Chitosan is a white, odorless, and biodegradable substance. Because of highly crystallinity structure, chitosan is insoluble in common organic solvents, acids, and alkalis. However, at ambient temperature it dissolves readily in weak inorganic acids over a wide range of concentrations because of salt formation (Li *et al.*, 1992). Viscous solutions of chitosan in acetic and formic acids can be used to cast films. Its film forming properties have been investigated. Chitosan can be blended with synthetic polymers, such as poly(vinyl alcohol) (Chandy and Sharma, 1992; Nakatsuka and Andrady, 1992), poly(acrylic acid) (Wang *et al.*, 1997), and poly(vinyl pyrrolidone) (Quarashi *et al.*, 1992) to yield products whose physical and chemical properties have some potential applications. In addition, chitosan can be blended with biomaterials. like cellulose (Hasegawa *et al.*, 1992, 1994), silk

fibroin (Chen *et al.*, 1997), pectin (Yao *et al.*, 1996), collagen (Fang *et al.*, 1991), alginate (Kim *et al.*, 1992) etc. A blend of chitosan with poly(vinyl alcohol) was studied as a carrier for riboflavin and insulin for medical application (Shen *et al.*, 1998). In addition, a blend of chitosan/poly(vinyl alcohol) was investigated to improve a blood compatibility (Fang *et al.*, 1991). Chen *et al.* (1997) investigated a conformational transition of silk fibroin induced by blending with chitosan. The rigid chain of chitosan can induce transformation of the random coil conformation of silk fibroin to the  $\beta$ -sheet conformation because of the occurrence of hydrogen bonding between chitosan and silk fibroin. In addition, chitosan can be prepared as a hydrogel that is very in the medical area. Genpeng *et al.* (1991) developed a collagen-chitosan composite hydrogel for contact lens application.

*Bombyx mori* silk fibroin, a naturally occurring high-molecular weight fibrous protein, has recently been received a considerable interest. The majority of silk fibroin has highly periodic regions containing various types of amino acids. The basic, highly repetitive sections consist mainly of glycine, alanine, and serine (Shen *et al.*, 1998). The sum of these amino acids is greater than 80 mol% of the total amino acid composition. Silk fibroin has been considered for application as a biomedical material because of its microbial resistance, biocompatibility, and nontoxicity (Mathur and Narang, 1990). It can be prepared in the forms of powder, gel and film. Silk fibroin films often lose their flexibility and elasticity, so in the dry state, they are very brittle and unsuitable for practical uses (Freddi *et al.*, 1995). To solve this problem, silk fibroin has to be blended with other synthetic or natural polymers to improve the mechanical properties. Among the great number of polymeric materials potentially suitable for blending with silk fibroin, natural polymers are preferred due to the favorable impact of naturally based polymer blends in various applications, especially in the medical field. Blends of silk fibroin with chitosan, an aminopolysaccharide, are of great interest. It was reported that

chitosan can induce a conformational transition of silk fibroin from random coil to  $\beta$ -sheet structure (Chen *et al.*, 1997) and these two biopolymers can also form a hydrogel having a semi-interpenetrating polymer network morphology that is sensitive to pH and ion concentration changes. Chen, *et al.* (1997) studied the swelling behavior of chitosan/silk fibroin blend film in pH buffer solutions, showing that the blend films were remarkably swollen in acidic solutions. In addition, the degree of swelling of the blend films in various concentrations of  $\text{AlCl}_3$  solution was also reported. However, the effect of concentrations of other salt types on the swelling behavior of chitosan/silk fibroin blend film has not been widely investigated.

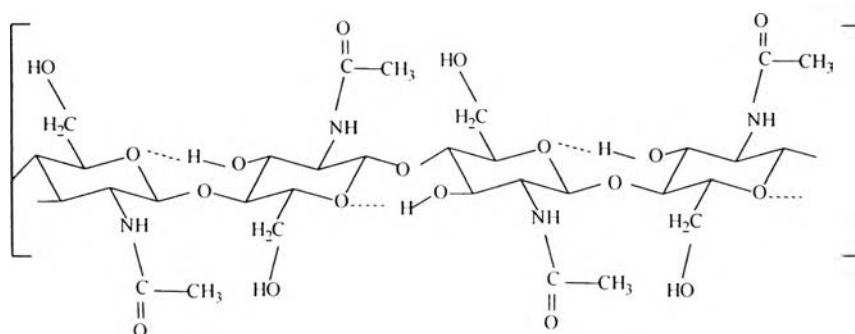
In this study, chitosan/silk fibroin blend films were prepared with varying chitosan content. The effect of blend composition on the physical properties, mechanical properties, and swelling behavior of films were studied. The swelling behavior of the blend films was determined in pH buffer solutions and various types of salt solutions. The effect of varying concentration of various salt types on the swelling property of the blend films was determined. Furthermore, the effect of crosslinking agent on the mechanical properties, physical property, and swelling property was also investigated.

## **1.1 Theoretical background**

### **1.1.1 Chitin**

Chitin, poly- $\beta$ -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine, a cellulose-like biopolymer, is the second most abundant renewable organic resource on earth. Chitin is often considered as a cellulose derivative although it does not occur in organisms producing cellulose. It is the most abundant organic skeletal component of invertebrates. In nature, chitin serves as a “glue” for chemical components making up the delicate wings of insects and the crunchy

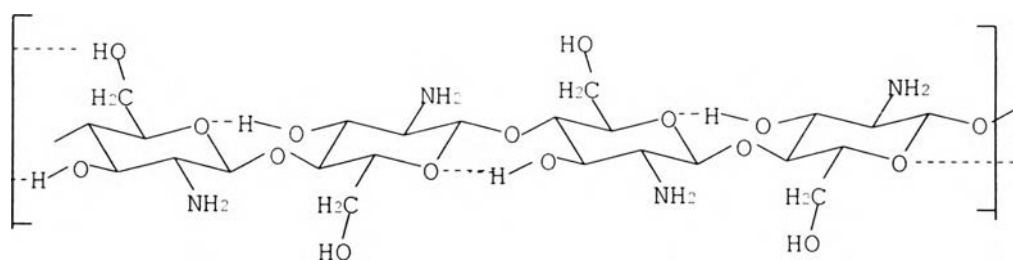
integuments of crustaceans such as crabs and shrimps. It is the characteristic polysaccharide of several important phyla, e.g., *Arthropoda*, *Annelida*, *Mollusca*, and *Coelenterata*, and also occurs in many fungi, e.g., *Eusomycetes*, *Zygomycetes*, *Basidiomycetes*, and *Deuteromycetes*. Chitin is commercially manufactured from crustacean shell waste that provided  $1.2 \times 10^5$  metric tons annually accessible on a worldwide basis (Knorr, 1991). Shell of crabs, lobster, shrimp, prawn, and krill are the best available sources of chitin. It represents 20 % to 50 % of the dry weight of shrimp and crab processing waste. Moreover, chitin also represents 12 % of fresh water crayfish meal, 13 % of crab meal, and 8 % of shrimp meal. Since crab and shrimp are harvested in different seasons, raw materials for chitin production are always readily available. To obtain chitin from crustacean shell waste, it is usually ground and mixed with a dilute aqueous sodium hydroxide solution to dissolve the protein. The residual material is then treated with a dilute aqueous hydrochloric acid solution to dissolve the calcium carbonate as calcium chloride, leaving behind chitin as a white powder. The chitin powder is soaked in an aqueous 40-50 % sodium hydroxide solution at 110-120 °C for several hours to hydrolyze N-acetyl linkages, then rinsed, pH-adjusted, and the product is dewatered. This treatment converts chitin into chitosan, preferably with a nitrogen content higher than 7 % by weight. Enzymatic protein removal and deacetylation yield a less degraded chitosan product ( Li *et al.*, 1992).



**Figure 1.1** Chemical structure of chitin.

### 1.1.2 Chitosan

Chitosan, a deacetylated chitin, is a cation-active poly-primary amine with diverse applications in fields such as dispersing agents, adhesives, pesticides, wastewater treatment, food processing and wound healing. Chemically, chitosan is  $\beta$ -(1  $\rightarrow$  4)-2-amino-2-deoxy-D-glucan. The molecular weight of chitosan for commercial products depends on the processing conditions. Chitosan with molecular weight in the range of 10,000 -1,000,000 daltons are available. The mole fraction of deacetylated unit (glucosamine), defined as the degree of deacetylation, is usually 70-90 %. Normally chitosan is prepared from chitin by alkaline hydrolysis whereby the chitin is sufficiently deacetylated to become soluble in 1-4 % acetic or formic acid. The parent chitin isolate is derived, for example, from crustacean shells by decalcification and deproteinization with acid and alkali solutions, respectively.



**Figure 1.2** Chemical structure of chitosan.

Chitosan is biocompatible with its degradation products being known natural metabolites and can be produced in powder, film, bead, fiber, and fabric formats. It was evaluated in a number of medical applications including as a potential wound dressing where it was shown that it could enhance wound healing and /or blood clot formation. Many of chitosan properties depend upon its cationic nature. At acidic pH, it is a linear polyelectrolyte with a high

charge density, one positive charge per glucosamine residue and so will interact with negatively charge molecules including proteins, anionic polysaccharides and nucleic acids, many of which are located in skin. It was shown that in the area of wound healing chitosan can reduce scar tissue (fibroplasia) by inhibiting the formation of fibrin in wounds, it is hemostatic and can form a protective film/coating. One reason postulated for the ability of chitosan to enhance wound healing is its biodegradability. It is a substrate for lysozyme with the degradation products being absorbed and possibly even having some nutrient value. Also chitin, chitosan, and chitosan derivatives affect macrophage activity, which will influence the wound healing process (Lloyd *et al.*, 1998).

Another useful property of chitosan is for chelation. Chitosan can selectively bind desired materials such as cholesterol, fats, metal ions, proteins and tumor cells. Chelation has been applied to areas of food preparation, health care, water improvement, and pharmaceuticals. It is also a good cationic polymer for membrane formation. In early research, it was shown that membranes formed from the polymer could be exploited for water clarification, filtration, fruit coating, surgical dressing, and controlled release. The applications of chitosan are summarized in table 1.

**Table 1.1** Applications of chitosan (Li *et al.* , 1992).

Applications	Examples
Water treatment	Removal of metal ions Flocculant/Coagulant: -Proteins -Dyes

Applications	Examples
Pulp and Paper	Amino acid Filtration Surface treatment Photographic Paper Carbonless Copy Paper
Medical	Bandages, Sponges Artificial blood vessel Blood Cholesterol Control Tumor Inhibition Membranes Dental/Plaque Inhibition Skin Burns/Artificial Skin Eye Humour Fluid Contact Lens ControlledRelease of Drugs
Cosmetics	Bone Disease Treatment Make-up Powder Nail Polish Moisturizers Fixtures Bath Lotion Face, Hand and Body Creams Toothpaste
Biotechnology	Foam Enhancing Enzyme Immobilization Protein Separation

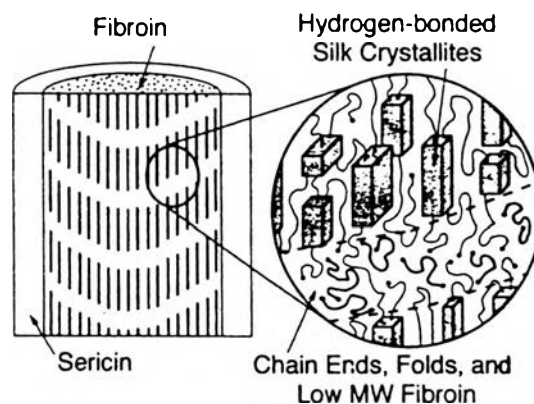
Applications	Examples
Agricultural	Chromatography
	Cell Recovery
	Cell Immobilization
	Glucose Electrode
	Seed Coating
	Leaf Coating
	Hydroponic/Fertilizer
Food	Controlled Agrochemical Release
	Removal of Dyes, Solids, Acids
	Preservatives
	Color Stabilization
	Animal Feed Additive
Membranes	Reverse Osmosis
	Permeability Control
	Solvent Separation

Chitosan is a high molecular weight polysaccharide, which can be chemically modified to alter its physical and biological properties. Chemical modifications of the amino group and both the primary and secondary hydroxy groups are possible. Possible derivatizations include etherification, graft copolymerization, and cross-linking. The crosslinked chitosan can be prepared by the addition of bifunctional reagents such as aldehydes, carboxylic anhydrides, and glutaraldehyde to chitosan solution. Glutaraldehyde is the most commonly used as a crosslinking agent that can form a schiff's base for chitosan and crosslinking is occurred through the imine bond formation.



### 1.1.3 Silk fibroin

Silk fibroin (*Bombyx Mori*) is a fibrous protein obtained from the cocoon of the silkworms. Its chemical composition is characterized by the presence of few types of amino acid residues with small side chains, the sum of the three simplest amino acids (glycine, alanine, and serine) accounting for more than 80 mol %. The primary structure arising from this characteristic amino acid composition contains many  $-(\text{gly-ala})_n-$  repeating units, which form the highly specific secondary structure, known as antiparallel  $\beta$ -sheet structure.



**Figure 1.3** Model of microstructure of silk fibroin.

Besides its textile application, silk has recently been investigated as a starting material for the preparation of bio-based polymeric materials potentially interesting for applications in the biotechnological and biomedical fields. Silk fibroin can be prepared in the form of powder, gel, and film from either fibers, after dissolution with concentrated salt solution (regenerated fibroin), or liquid silk taken directly from the mature silk gland (native fibroin). Silk membranes have proved to be an excellent substrate for enzyme immobilization, because of their good mechanical and physical properties, thermal stability, microbial resistance, and absence of interactions with the enzyme immobilized. Chen *et al.* (1994) reported the transport of

pharmaceutical through silk fibroin membrane. The permeability of the pharmaceutical could be controlled by the external pH value. The silk fibroin membrane was an amphoteric ion exchange membrane and it was expected to be used as a pH-sensitive drug delivery system. Asakura *et al.* (1988) prepared a glucose biosensor by immobilizing glucose oxidase (GOD) within silk fibroin membranes. A noticeable increase in biosensor sensitivity has been reported concerning GOD immobilized on the surface of nonwoven fabrics by means of silk fibroin gel. Silk fibroin membranes can be used to separate water from water-methanol solutions by pervaporation. The high oxygen permeability in the wet state, similar to that of other synthetic hydrogel membranes currently used to produce contact lenses, make silk fibroin attractive as a biomaterial. Moreover, the good *in vivo* blood compatibility of silk fibroin has currently been reported (Freddi *et al.*, 1995).

The good thermal stability is one of attractive characteristics that makes silk fibers suitable for several applications. In fact, it has been investigated that the thermal decomposition of silk occurred at around 310 - 320°C, about 100°C higher than wool (230-240°C), another protein fiber. Therefore, the purpose of improving the thermal resistance of silk by suitable treatments should be considered as a new approach for the research in the field of structured proteins in order to make new materials and expand their utilization (Tsukada *et al.*, 1992).

Crystallization of silk fibroin films cast from aqueous solution can be promoted by suitable thermal, mechanical, and chemical treatments, which induce the conformational transition from random coil to  $\beta$  structure. Fibroin films in the dry state are very brittle and almost unsuitable for practical use, while in the wet state the elongation is considerably higher, in such a way that they can be applicable as biomaterial in the biomedical fields. The inferior tensile properties of silk fibroin films can be improved by blending with natural or synthetic polymer. Blends of silk fibroin with chitosan, sodium

poly-glutamate, sodium alginate and polyvinyl alcohol have been reported. Water absorption, mechanical properties, and thermal stability were improved by blending silk fibroin with sodium alginate (Freddi *et al.*, 1995). Silk fibroin/syndiotactic-rich poly(vinyl alcohol) blend films showed an increased permeability to neutral salt, the permeability being influenced by hydration radius of the ions (Yamaura *et al.*, 1990). Chen *et al.* (1997) studied the swelling behavior of chitosan/silk fibroin blend film in pH buffer solutions, showing that the blend films were remarkably swollen in acidic solutions. In addition, the degree of swelling of the blend films in various concentrations of  $\text{AlCl}_3$  solution was also reported. However, the effect of concentrations of other salt types on the swelling behavior of chitosan/silk fibroin blend film has not been widely investigated.