CHAPTER I

INTRODUCTION

Silk fibroin is a fibrous protein that is composed of 17 amino acids. The highly repetitive sections consist mainly of glycine, alanine and serine (Minoura *et al.*, 1995). The sum of these repetitive amino acids is greater than 80 mol% of the total amino acid composition. The primary structure arising from this characteristic amino acid composition contains many $-(\text{-gly-ala-})_n$- repeating unit. Silk fibroin can be prepared in the form of powder, gel and film from either silk fiber, after dissolution with concentrated salt solution, or liquid silk taken directly from the nature silk gland. Silk fibroin has become more important because of it properties, such as non-toxicity, biodegradability and good biological compatibility. It has been investigated for biomaterials in biotechnological and biomedical fields. For example, the glucose sensor prepared by using silk fibroin as enzyme substrate to immobilize glucose oxydase showed high stability against pH and temperature changes (Asakura *et al.*, 1989). Silk fibroin has been studied for biomedical applications such as surgical sutures (Asakura *et al.*, 1990), wound covering materials (Wu and Tian, 1996), wound-repairing, and bone binding function (Tamada, 1997). However, silk fibroin is very brittle and almost unsuitable for practical use in the dry state (Minoura *et al.*, 1990). Some inferior physical and mechanical properties of silk fibroin membranes can be improved by blending with other natural or synthetic polymers. Water absorption, mechanical properties, and thermal stability of silk fibroin films were improved by blending with sodium alginate (Liang and Hirabayashi, 1992a). The addition of cellulose to silk fibroin permitted preparation of films with excellent elastic behavior (Freddi *et al.*, 1995). Silk fibroin/PVA blend films showed increased permeability to neutral salts (Tsukada *et al.*, 1994).

Chitin is a natural abundant polysaccharide that is widely distributed in crustaceans, insects, mushrooms and in the cell walls of bacteria. Chitin consists of 2-acetamido-2-deoxy-D-glucose through a β (1→ 4) linkage. Chitin has become attractive because of its rich resources and some interesting properties such as biocompatibility, biodegradability and non-toxicity. Chitin has been found to be useful as a biodegradable pharmaceutical carrier (Copazza, 1975), a blood anticoagulant (Whistler and Kosik, 1971), and a wound-healing accelerator (Balassa, 1975). Chitin has also proved to be a highly effective antigen (Porter, 1971). However, chitin has a limitation. It is known that chitin is insoluble in most common solvents except for strong acids such as methanesulfonic, sulfuric and formic acids. The insolubility of chitin has been suggested to be due to its rigid crystalline structure through intra-and inter-molecular hydrogen bonds (Pearson *et al.,* 1960). This property can be improved by chemical modification of chitin. Chitin could be modified by introducing carboxymethyl groups to enhance its solubility in water. Carboxymethyl-chitin (CM-chitin) is a water soluble chitin derivative. CM-chitin is soluble not only in acidic media but at any pHs. CM-chitin has been investigated as polymeric drug (Nishimaru *et al.,* 1984), wound healing (Muzzarelli, 1988), cosmetic ingredients for hair and skin cares (Imamura, 1991), and chelating agent (Tokura *et al.,* 1991).

However, silk fibroin and CM-chitin blend films still have not been investigated. In this study, CM-chitin/silk fibroin blend films were prepared by varying blend composition of CM-chitin and silk fibroin. The effects of blend compositions on physical properties, mechanical properties, as well as swelling behavior, of the blend films were investigated.

Theoretical Background

1.1 Chitin

Chitin is a polysaccharide consisting predominantly of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) residues. It can be regarded as a derivative of cellulose, in which the C-2 hydroxyl groups have been replaced by acetamido residues. Chitin is not found alone, and usually forms a part of very complex systems; thus, insect exoskeletons are largely composed of chitin-protein complexes, whereas crustacean shells usually contain large proportions of calcium carbonate in addition to protein. As the major, organic, skeletal substance of invertebrates, chitin is found principally in the phyla *Annelida* (segmented worms), *Arthropoda* (crustaceans, insect, etc.) and *Mollusca* (snails, squid, etc.)

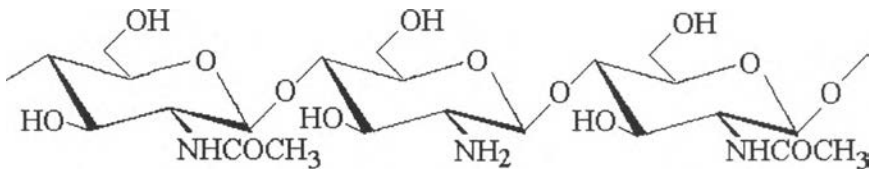

Figure 1.1 Chemical structure of chitin.

Like cellulose, chitin functions naturally as a structural polysaccharide, but differs from cellulose in its properties. Chitin is highly hydrophobic and is insoluble in water and most organic solvents. It is soluble in hexafluoroisopropanol, hexafluoroacetone, chloroalcohols in conjugation with aqueous solutions of mineral acids and dimethylacetamide containing 5% lithium chloride.

Chitin is known as a potential useful biomedical material for would healing, artificial skin, suture, and drug carrier (Lee *et al.*, 1996). The unique properties of chitin such as biocompatible, non-toxic, non-allergic and antifungal properties make it be a promising polymer not only in the biomedical field but also in the other industrial area as summarized in Table 1.1.

Table 1.1 Some applications of chitin-based materials

Area	Application
Biomedical	Biodegradable pharmaceutical (Copazza, 1975) Blood coagulant (Whistler and Kosik, 1971) Would-healing accelerator (Balassa, 1975)
Cosmetics	Skin-care product (Elizabeth, 1993) Hair stiffener (Elizabeth, 1993)
Environmental	Absorbent for waste-water treatment (Yang and Zall, 1984)

1.2 Carboxymethyl-Chitin (CM-chitin)

CM-chitin, a water soluble chitin derivative, is a polyelectrolyte with properties resembling those of carboxymethyl-cellulose (CMC).

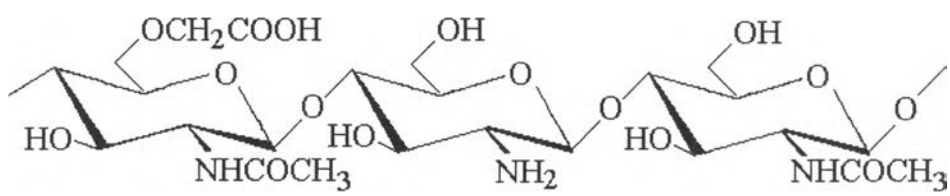

Figure 1.2 Chemical structure of CM-chitin.

CM-chitin is soluble not only in acidic media but at any pHs, making it an attractive option in connection with its use in food products and cosmetics. Some applications of CM-chitin are summarized in Table 1.2.

Table 1.2 Current practical uses of CM-chitin

Applications	References
Drug delivery system	Nakano *et al.*, 1980
Cosmetic ingredients for hair and skin cares	Imamura *et al.*, 1991
Would healing	Muzzarelli, 1988
Chelating agent	Uraki and Tokura, 1988

CM-chitin has been considered to be one of advanced carriers for the polymeric drug, since CM-chitin was reported as highly biodegradable and non-toxic mucopolysaccharide in animal body (Nishimura *et al.*, 1984). However, intraperitoneal injection of CM-chitin was reported to active mouse peritoneal macrophages for short periods and to induce the mitogenic activity very faintly

(Nishimura *et al.*, 1984). Because of these properties, CM-chitin has been applied to the carriers both for the induction of hepten-specific antibody and for the controlled release of the drug. CM-chitin has been investigated to prepare adsorption or entrapping type of polymeric drug, because CM-chitin trends to adsorb phenyl group specifically in the presence of calcium ion and to precipitate gradually in the presence of trivalent iron ion following to gel formation. Two-step hydrolysis of drug has been investigated which the amino or hydroxyl groups of drug was bound to the carboxylic terminal of peptide spacer through amide (amide drug) or ester (ester drug) linkage. The amide drug was protected by the CM-chitin molecule from enzymatic attack until CM-chitin became oligomeric with a molecular weight of less than 4000. As these phenomena have been observed for the adsorption or wrapping type polymeric drugs as well as the pendent type, the stabilization of the drug would be achieved by concealing the drug part in the CM-chitin molecule (Tokura *et al.*, 1994).

1.2 Silk Fibroin

Silk fibroin (*Bombyx mori*) is a fibrous protein obtained from the cocoon of the silk worms. Silk fibers consist primarily of two components, fibroin and sericin. Fibroin is the structural protein of the silk fiber and sericin is the water-glue soluble that serves to bond fiber together. The majority of the fibroin is highly periodic with simple repeating sections broken by more complex regions containing amino acids with bulkier side chains. The compositions of amino acid are glycine 45%, alanine 30%, and serine 12%. The primary structure arising from this characteristic amino acid composition contains many –(gly-ala)$_n$- repeating units, which form the highly specific secondary structure, known as antiparallel β-sheet structure.

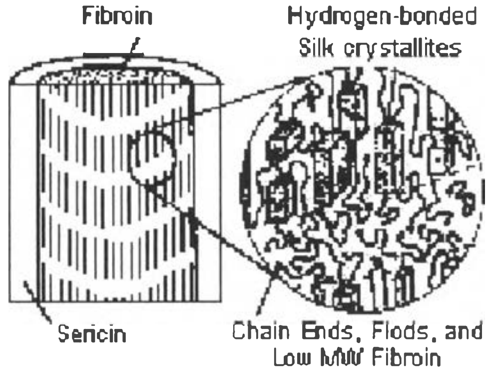

Figure 1.3 Model of microstructure of silk fibroin.

Besides its textile application, silk fibroin is considered an interesting starting material for biotechnological and biomedical utilization. Silk fibroin can be prepared in the form of powder, gel, compact and porous membrane after dissolution with suitable solvents. Silk membranes have proved to be an excellent substrate for enzyme immobilization, because of their good physical and mechanical properties, thermal stability, microbial resistance, and absence of interactions with the enzyme immobillized. Asakura *et al.,* 1990 prepared a glucose biosensor by immobilizing glucose oxidase (GOD) within silk fibroin membranes. A noticeable increase in biosensor sensitivity has recently 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, makes silk fibroin attractive as a biomaterial. Moreover, the good *in vivo* blood compatibility of silk fibroin has recently been reported.

Crystallization of silk fibroin films cast from aqueous solution is promoted by suitable thermal, mechanical, and chemical treatments, which induce the conformational transition from random coil to β-sheet 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 biomaterials in the medical fields. The inferior tensile properties of silk fibroin films can be improved by blending with other natural or synthetic polymers.