CHAPTER I
INTRODUCTION

Nowadays, natural polymers such as protein and polysaccharide have become increasingly important as a rich resource for low cost raw materials. Especially, they provide useful materials for biomedical applications, due to their nontoxicity, biodegradability, and biocompatibilty.

Silk fibroin is a fibrous protein that is composed of 17 amino acids, of which the main components are nonpolar species such as glycine, alanine, and serine. Silk fibroin can exist in 2 molecular conformations, random coil and β-sheet form. The conformational transition of silk fibroin from random coil to β- sheet structure can be induced by treatments such as heating, stretching or immersion in polar solvents. This transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β-sheet structure is resistant to water and has good mechanical properties (Park *et al.*, 1999). Silk fibroin is considered a potential precursor to new materials and devices for biotechnological and biomedical utilizations. It has been reported that silk fibroin film has good oxygen permeability in the wet state, which suggests promising applications as a wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures, in biocompatible devices with controlled drug release (Tsukada *et al.*, 1994) and for bone binding functions. However, silk fibroin in the dry state is very brittle and unsuitable for practical use (Freddi *et al.*, 1995). To overcome this limitation, silk fibroin has been blended with other synthetic polymers, such as polyacrylamide (Freddi *et al.*, 1999) and poly(vinyl alcohol) (Yamaura *et al.*, 1990), or natural polymers, such as cellulose (Freddi *et al.*, 1995) and sodium alginate (Liang *et al.*, 1992), to improve mechanical and physical properties.

Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of β(1→4)-linked 2-acetamido-D-glucose unit and β(1→4)-linked 2-amino-D-glucose unit with the latter usually greater than 75% (Li *et al.*, 1997). Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-

dimensional crosslinked network with the ability to absorb significant amount of water. Crosslinked chitosan hydrogels can swell extensively due to the positive charges on the network and respond to changes in the pH of the medium. Due to the benefits of being nontoxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for sustained drug delivery. For example, it was used for the delivery of drugs such as, prednisolone (Kofuji *et al.*, 2001) and diclofenac sodium (Gupta *et al.*, 2000). Furthermore, it has been reported that chitosan can induce a conformational transition of silk fibroin from random coil to β-sheet structure and that, using glutaraldehyde as a crosslinking agent, a polymer blend of these biopolymers can also form a hydrogel, having a semi-interpenetrating network structure (Chen *et al.*, 1997).

Until now, there has been no report on using chitosan/silk fibroin blend film as a drug delivery vehicle. This research is a preliminary study on using a glutaraldehyde cross-linked chitosan/silk fibroin blend film as a matrix for a drug delivery system. The model drugs used were theophylline, diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effect of blend composition, degree of crosslinking, and pHs of the external swelling media on drug release from the blend films was investigated.

1.1 Theoretical Background

1.1.1 Controlled Drug Delivery

Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which the chemists and chemical engineers are contribute to human health care. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from material in a predesigned manner. The release of the active agent may be constant over a long period, or it may be triggered by the environment or other external events.

The benefit behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. Other advantages of using controlled delivery systems can include the maintenance of drug level within a desired range, the need for fewer administrations, optimal use of the drug in question, and increase the patient compliance.

The goal of many of the original controlled-release release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional tablets or injections, the drug level in the blood follow the profiles shown in Figure, in which the level rises after each administration and then decreases until the next administration. The key point with traditional drug administration is that the blood level of the agent should remain between a maximum value, which may represent a toxic level, and a minimum level, below which the drug is no longer effective. In controlled release system designed for long term administration, the drug level in the blood follows the profile shown in Figure1.1, remaining constant, between the desired maximum and minimum, for an extended period of time.

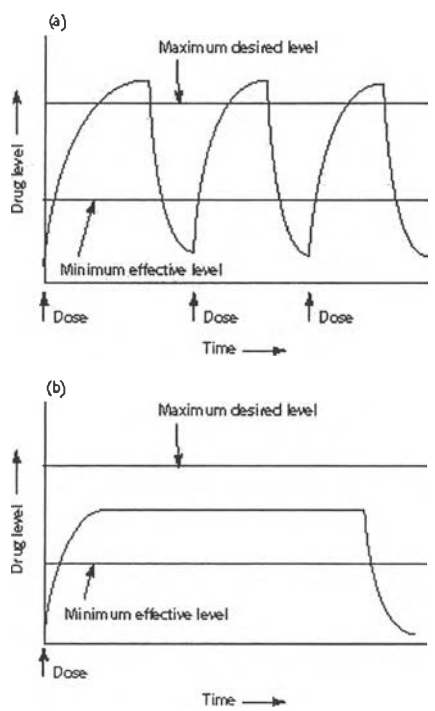

Figure 1.1 Drug level in the blood from (a) traditional drug administration and (b) controlled delivery dosing.

There are three primary mechanisms by which active agents can be released from a delivery system described as follows.

1.1.1.1 Diffusion controlled release

Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled-release device. The diffusion can occur on a macroscopic scale—as through pores in the polymer matrix—or on a molecular level, by passing between polymer chains. Examples of diffusion-release systems are shown in Figures 1.2. In this Figure, a polymer and active agent have been mixed to form a homogeneous system, also referred to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

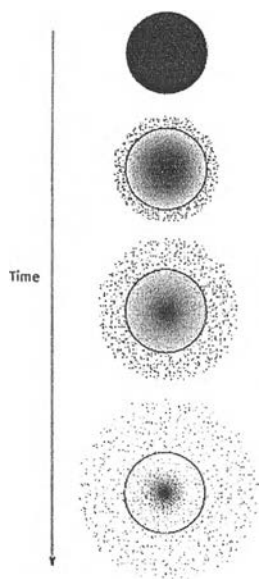

Figure 1.2 Drug delivery from a typical matrix drug delivery system.

For the diffusion-controlled systems described thus far, the drug delivery device is fundamentally stable in the biological environment and does not change its size either through swelling or degradation. In these systems, the

combinations of polymer matrices and bioactive agents chosen must allow for the drug to diffuse through the pores or macromolecular structure of the polymer upon introduction of the delivery system into the biological environment without inducing any change in the polymer itself.

1.1.1.2 Swelling controlled release

It is also possible for a drug delivery system to be designed so that it is incapable of releasing its agent or agents until it is placed in an appropriate biological environment. Swelling-controlled release systems are initially dry and, when placed in the body, will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment. Most of the materials used in swelling-controlled release systems are based on hydrogels, which are polymers that will swell without dissolving when placed in water or other biological fluids. These hydrogels can absorb a great deal of fluid and, at equilibrium, typically comprise 60–90% fluid and only 10–30% polymer. One of the most remarkable, and useful, features of a polymer's swelling ability manifests itself when that swelling can be triggered by a change in the environment surrounding the delivery system. Depending upon the polymer, the environmental change can involve pH, temperature, or ionic strength, and the system can either shrink or swell upon a change in any of these environmental factors. A number of these environmentally sensitive or "intelligent" hydrogel materials are listed in Table 1. For most of these polymers, the structural changes are reversible and repeatable upon additional changes in the external environment. The diagrams in Figure 1.3 illustrate the basic changes in structure of these sensitive systems. Once again, for this type of system, the drug release is accomplished only when the polymer swells. Because many of the potentially most useful pH-sensitive polymers swell at high pH values and collapse at low pH values, the triggered drug delivery occurs upon an increase in the pH of the environment. Such materials are ideal for systems such as oral delivery, in which the drug is not released at low pH values in the stomach but rather at high pH values in the upper small intestine.

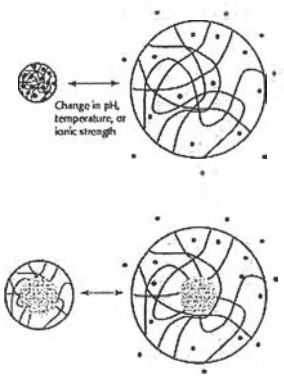

Figure1.3 Drug delivery from environmentally sensitive release systems.

Table 1.1 Environmentally sensitive polymers for drug delivery

Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH ------swelling-------- Release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength, change in concentration of ions inside gel ----change in swelling--- release of drug
Chemical species	Hydrogel containing electron accepting groups	Electron donating compounds, formation of change/ transfer complex ----change in swelling--- release of drug
Enzyme-substrate	Hydrogel containing immobilized enzymes	Substrate present, enzymatic conversion product changes ----change in swelling--- release of drug

1.1.1.3 Erosion controlled release

All of the previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort are being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller compounds. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 1.4a. For some degradable polymers, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 1.4b).

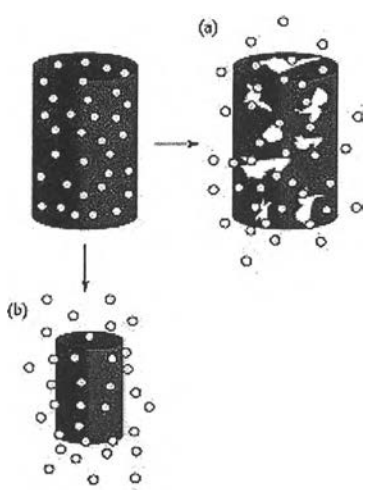

Figure 1.4 Drug delivery from (a) bulk erosion and (b) surface erosion systems.

1.1.2 Chitin and Chitosan

. Chitin, a naturally abundant mucopolysaccharide, and the supporting materials of crustaceans, insects, etc, is well known to consist of 2-acetamido-β-D-

glucose through a β(1→4)linkage Kumar, 2000). Chtin is often considered as a cellulose derivative although it does not occur in organisms producing cellulose. It is the second 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. To obtain chitin from crustacean shell waste, it is usually ground and mixed with a dilute aqueous sodium hydroxide solution to dissolve protein. The residual material is then treated with a dilute aqueous hydrochloric acid solution calcium carbonate as calcium chloride, leaving behind chitin as a white powder.

Chitosan is a polyaminosaccharide, [β(1 4)-2-amino-2-deoxy,β-D-glucan] normally obtained by alkaline deacetylation of chitin, a naturally occurring polymeric material (Gupta and Kumar, 2000). For preparation of chitosan, chitin powder is soaked in an aqueous 40-50% sodium hydroxide solution at 110°C-120°C

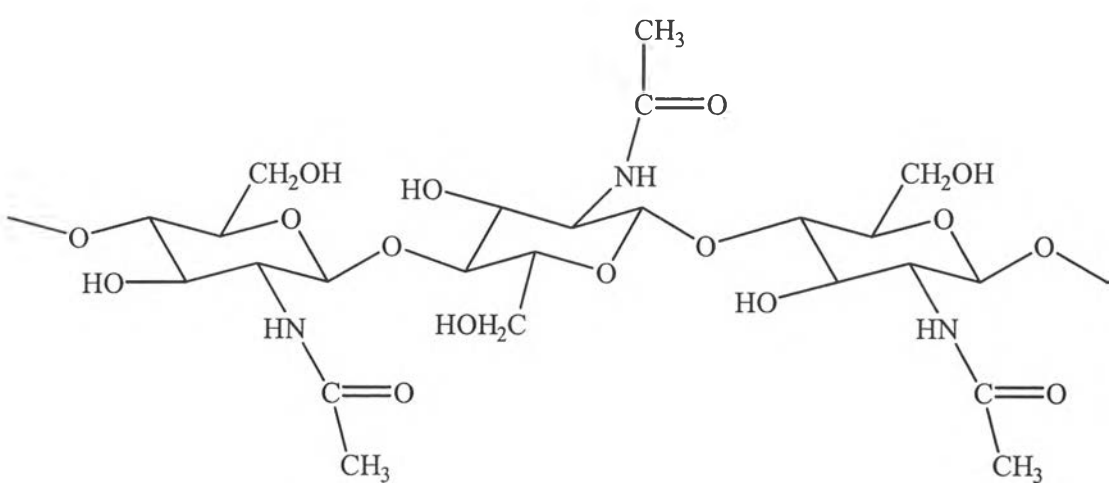

Figure 1.5 Chemical structure of chitin.

for several hours to hydrolyze N-acetyl linkages, then rinsed, pH adjusted, and the product dewatered. This treatment converts chitin into chitosan, preferably with a nitrogen content higher than 7% by weight.

Chtiosan is biocompatible with its degradation products being know natural metabolites and can 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 if it could enhance wound healing and/or blood clot formation. Many of chitosan properties depend upon its cationic nature.At acidic pH

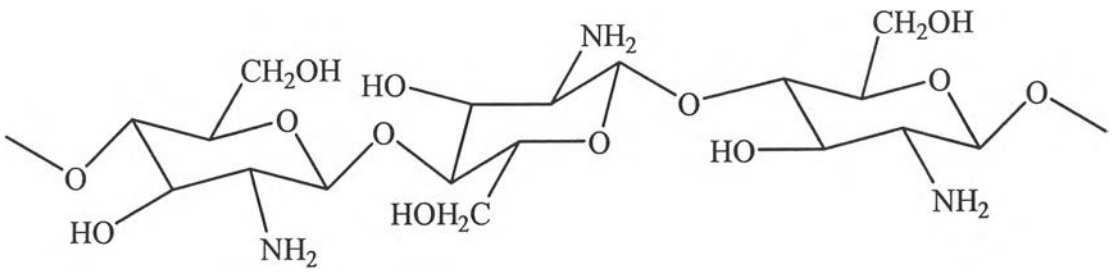

Figure 1.6 Chemical structure of chitosan.

it is a linear polyelectrolyte 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 the scar tissue (fibroplasia) by inhibiting the formation of fibrin in the wounds, it is hemostatic and can form a protective film/coating. One reason postulated for the ability of chitosan to enhance is its biodegradability. It is a substrate for lysozyme with the degradation products being adsorbed 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)

Chitosan is non-toxic and easily bioabsorbable with gel-forming ability at low pH. Moreover, chitosan has antacid and antiucler activities, which prevent or weaken drug irritation in the stomach. Also, chitosan matrix formulations appear to float and gradually swell in an acid medium. All these interesting properties of chitosan make this natural polymer for controlled drug release formulations(Kumar, 2000).

Chitosan is a natural polycationic polymer which posses valuable properties as a metal recovering and water purifying agent (Onsoyen and Skaugrud, 1990). The other applications are wastewater treatment for heavy metal and radioisotope removal and valuable metal recovery, potable water purification for

reduction of unwanted metals, complex binding of iron in precooked food to reduce 'warmed –over flavour'.

1.1.3 Silk Fibroin

Silk Fibroin is the main part of natural silk, obtained from the cocoon of silkworms, and is a fibrous protein whose major amino acid composition consists of glycine, alanine and serine residues over 80 mol%. The primary structure arising from this characteristic amino acid composition contains many $-(gly\text{-}ala)_n-$ repeating units, which form the highly specific secondary structure, known as antipararelle β-sheet structure.

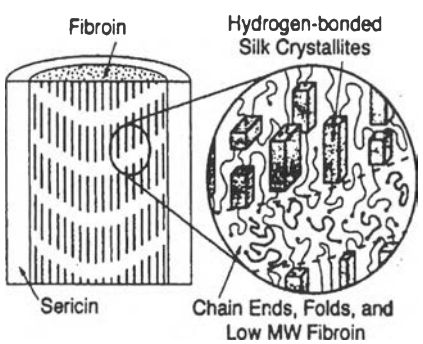

Figure 1.7 Model of microstructure of silk fibroin.

Besides silk use as a textile fiber, silk is considered as an interesting starting material for nontextile application. It is used for developing new materials and devices for biotechnological and biomedical utilizations. As it is well known, silk fibroin is available not only in form of fiber, but can also prepared in the form of gel, powder, and porous membrane after dissolution with suitable solvents. Silk fibroin films in the dry state are very brittle and unsuitable for practical uses, while in the wet state the elongation is considerable higher in such a way that they can be applicable as biomaterials in the medical fields. Silk membranes have proved to be an excellent substrate thanks to their good mechanical and physical properties, thermal stability, microbial resistance and absence of interactions with enzyme immobilized. Chen *et al.* (1994) reported the transport pharmaceutical through silk fibroin membrane. The permeability of the pharmaceutical could be controlled by

the external pH value. The silk fibroin was an amphoteric ion exchange membrane and it was expected to be use as a pH-sensitive drug delivery system. 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 synthetic hydrogel membranes currently used to produced contact lens. In addition he good in vivo blood compatibility of silk fibroin has recently been reported (Freddi et al., 1995).