CHAPTER I
INTRODUCTION

Hydrogels are three-dimensional hydrophilic polymeric networks that can absorb large amounts of water without changing their shapes. Early investigations on synthetic hydrogels had been concentrated in the field of contact lenses application (Corkhill and Tighe, 1990). Currently, studies on hydrogels have been diversified into several other areas, such as medical and agricultural applications. One of the main interests concerns synthesis and characterization of hydrogels as matrices for controlled release of drugs due to the ability of these hydrogels to release an entrapped drug in aqueous medium and to regulate the release of such drug by control of swelling. Releasing of drugs from hydrogels depends on several factors, such as the hydrophilic/hydrophobic balance of hydrogels, degree of crosslingking and, especially, the degree of swelling which is an important parameter to consider in the design of hydrogels for drug delivery system (Lloyd *et al.*, 1998). Using hydrogels as potential candidates for drug delivery has many advantages, such as their biocompatibility, ability to respond to external stimuli under various physiological conditions, importing patient compliance and decreasing incidence of adverse drug reactions. In addition, water retention in hydrogels provides a suitable drug diffusion partway by a pore mechanism (Yin *et al.*, 2002). Moreover, pH-sensitive hydrogels have a potential use in the site-specific delivery of drugs to specific regions of the gastrointestinal tract (GIT) attributed to pH changes throughout the gastrointestinal tract. Either synthetic or natural hydrophilic polymers such as polyvinyl alcohol, polyethylene oxide, polyvinyl pyrrolidone, cellulose, chitin and its derivatives, etc. have been used to prepare hydrogels (Yoshii *et al.*, 2002).

Carboxymethyl-chitin (CM-chitin), a water-soluble chitin derivative and one of hydrogel-forming polymers, can be prepared by carboxymehylation of chitin under alkaline conditions. CM-chitin is a polyelectrolyte with properties resembling to those of carboxymethyl-cellulose (CMC). CM-chitin is soluble not only in water but also in media at any pHs that makes it an attractive option for its use in drug delivery system (Nakano *et al.*, 1980), cosmetic ingredients for hair and skin cares

(Imamura *et al.*, 1991), wound healing (Muzzarelli *et al.*, 1988), chelating agent (Tokura *et al.*, 1993). 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). In addition, intraperitoneal injection of CM-chitin was reported to activate mouse peritoneal macrophages for short periods and to induce the mitogenic activity faintly (Nishimura *et al.*, 1983). Because of its interesting properties, CM-chitin has been investigated as a potential carrier for controlled release of the drug (Watanabe *et al.*, 1992).

Silk fibroin is a linear polypeptide which its main components are nonpolar amino acids such as alanine, glycine and serine. Silk fibroin has two conformations: the random coil and the β-sheet structures. Silk fibroin with a β-sheet structure is resistant to water and has better mechanical properties than silk fibroin with the random coil conformation (Demura *et al.*, 1992). Besides its textile use, silk has recently been investigated as a starting material for preparation of naturally based polymeric materials for applications in biotechnological and biomedical fields such as an enzyme-immobilization material that can be used as a good biosensor (Liu *at al.*, 1996), a cell culture substrate (Tsukada *et al.*, 1994), an oral dosage form (Asakara *at al.*, 1992) and so on. Moreover, silk fibroin film has good oxygen permeability in the wet state, similar to that of human skin, which suggests promising applications of silk fibroin as wound dressing and artificial skin (Minoura *et al.*, 1990). However, the silk fibroin film is too brittle to be used by itself. Poor mechanical properties of silk fibroin could be improved by blending it with other synthetic or natural polymers such as poly(ehtylene glycol) (PEG) (Kweon *et al.*, 2001), poly(vinyl alcohol) (PVA) (Liu *et al.*, 1996), cellulose and chitosan (Tsukuda *et al.*, 1994). Kweon *et al.* 2001 reported that mechanical properties of silk fibroin/chitosan blend films containing 10-40% chitosan were found to be improved as compared to those of silk fibroin itself. In addition, Chen *et al.* (1997) reported that the conformational transition of silk fibroin from random coil conformation to β-sheet structure could be induced by blending with chitosan.

Both CM-chitin and silk fibroin are biopolymers that have potential applications in the biomedical field. In this study, the blend films with various blend ratios of CM-chitin and silk fibroin were prepared and drug release characteristics of CM-chitin/silk fibroin blend films were investigated.

1.1 Theoretical Background

1.1.1 Drug Delivery Systems

Of the several possible routes of introducing controlled release medication into the body, the oral administration of single dose medicinals is one of the simplest and safest; since it does not pose the sterility problem and the risk of damage at the site of administration is at minimal. However, an oral controlled release formulation is subjected to frequently changing environments during transit through the gastrointestinal tract as it passes from the strongly acidic to the weakly alkaline medium in the lower part of the small intestine. The variable absorbing surfaces over the length of the gastrointestinal tract adds further constraint to the design of oral dosage forms. Moreover, the stomach emptying period varies from person to person. These factors collectively introduce considerable variability in the performance of oral controlled delivery systems. Several approaches have been taken in the past to prolong the retention of the dosage form in the stomach

1.1.2 Polymeric Drug Delivery

The polymeric controlled delivery systems are being used for a wide range of reagents in various environments (Tokura *et al.*, 1992). The most popular application is the drug delivery, in which the main objective is to achieve an effective therapeutic administration for an extended period of time. The technique is also termed as sustained release.

In recent years, there has been a rapid growth in the area of drug discovery, facilitated by novel technologies such as combinatorial chemistry and high-throughput screening. These novel approaches have led to drugs which are generally more potent poorer solubility than drugs developed from traditional approaches of medicinal chemistry. The development of these complex drugs has

resulted in a more urgent focus on developing novel techniques, to deliver these drugs more effectively and efficiently.

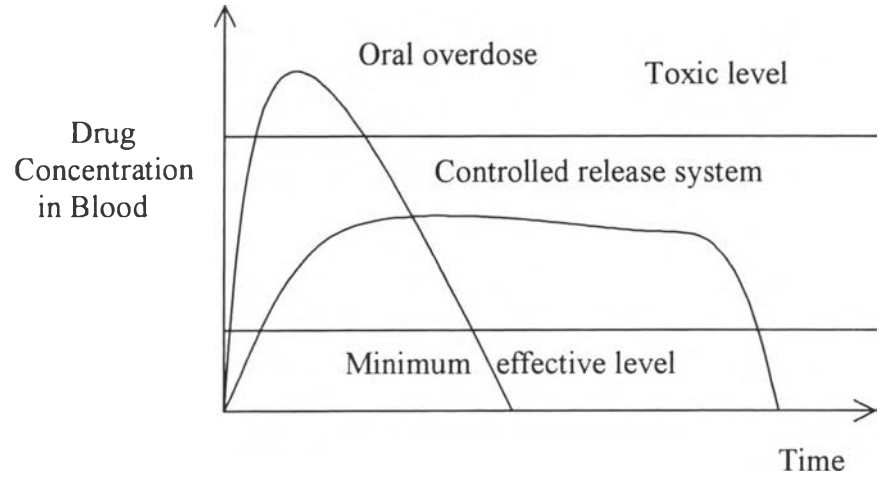

Figure 1.1 Conventional and ideal drug release profiles.

As can be seen in Figure 1.1, the conventional oral and intravenous routes of drug administration do not provide ideal pharmacokinetic profiles especially the drugs which display high toxicity and/or narrow therapeutic windows. For such drugs, the ideal pharmacokinetic profile will be one wherein the drug concentration reaches therapeutic levels without exceeding the maximum tolerable dose and maintains these concentrations for extended periods of time untill the desired therapeutic effect is reached. One of the ways such a profile can be achieved in an ideal case scenario would be by encapsulating the drug in a polymer matrix.

The three key advantages that polymeric drug delivery products can offer are:

1. Localized delivery of drug: The product can be implanted directly

at the site where drug action is needed and hence systemic exposure of the drug can be reduced. This becomes especially important for toxic drugs which are related to various systemic side effects (such as the chemotherapeutic drugs).

2. Sustained delivery of drugs: The drug encapsulated is released over extended periods and hence eliminates the need for multiple injections. This feature can improve patient compliance especially for drugs for chronic indications, requiring frequent injections (such as for deficiency of certain proteins).
3. Stabilization of the drug: The polymer can protect the drug from the physiological environment and hence improve its stability *in vivo*. This particular feature makes this technology attractive for the delivery of labile drugs such as proteins.

1.1.3 Controlled Drug Delivery

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 the material in a predesigned manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose 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 levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. While these advantages can be significant, the potential disadvantages cannot be ignored: the possible toxicity or nonbiocompatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort from the delivery device, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations.

Providing control over the drug delivery can be the most important factor at times when traditional oral or injectable drug formulations cannot be used. These include situations requiring the slow release of water-soluble drugs, the fast release of low-solubility drugs, drug delivery to specific sites, drug delivery using nanoparticulate systems, delivery of two or more agents with the same formulation, and systems based on carriers that can dissolve or degrade and be readily eliminated. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize.

The goal of many of the original controlled-release systems is to achieve a delivery profile that would yield a high level of the drug over a long period of time. With traditional tablets or injections, the drug level in the blood follows the profile shown in Figure 1.1, in which the level rises after each administration of the drug 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 value, below which the drug is no longer effective. In controlled drug delivery systems designed for long-term administration, the drug level in the blood follows the profile shown in Figure 1.1, remaining constant, between the desired maximum and minimum, for an extended period of time. Depending on the formulation and the application, this time may be anywhere from 24 hours.

In recent years, controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated, with the ability to do more than simply extend the effective release period for a particular drug. For example, current controlled-release systems can respond to changes in the biological environment and deliver—or cease to deliver—drugs based on these changes. In addition, materials have been developed that should lead to targeted delivery systems, in which a particular formulation can be directed to the specific cell, tissue, or site where the drug it contains is to be delivered. While much of this work is still in its early stages, emerging technologies offer possibilities that scientists have only begun to explore.

1.1.4 Controlled –Release Mechanism

There are three primary mechanisms by which active agents can be released from a delivery system:

a) 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.

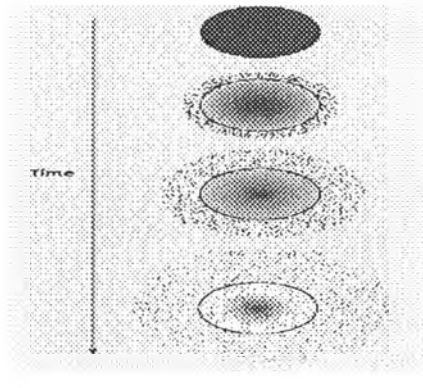

Figure 1.2 Drug delivery from a typical matrix drug delivery system.

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.

b) 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. Features of a polymer's swelling ability, 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.

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

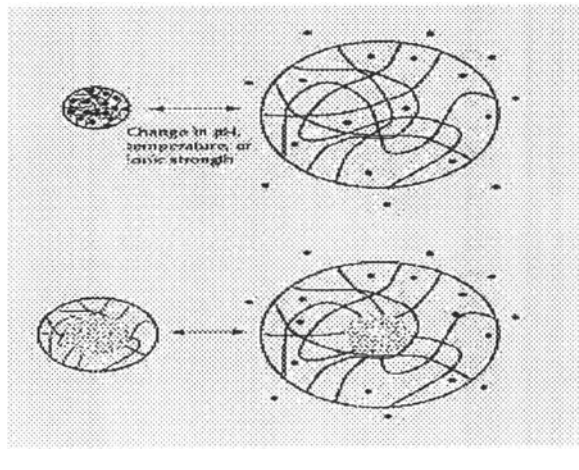

Figure 1.3 Drug delivery from environmentally sensitive release systems.

c) 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 therelease 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).

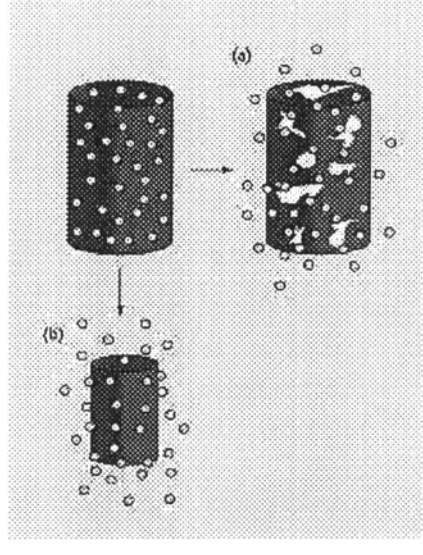

Figure 1.4 Drug delivery from (a) bulk erosion and (b) surface erosion systems.

The release of drug depends on several factors such as the hydrophilicity and the degree of swelling of supporting materials. One of widely used materials is based on hydrogels.

1.1.5 Hydrogel

Hydrogels are highly swollen, hydrophilic polymer networks that can absorb large amounts of water and drastically increase in volume. It is well known that the physicochemical properties of the hydrogel depend not only on the molecular structure, the gel structure, and the degree of crosslinking but also on the content and state of water in the hydrogel. Hydrogels have been widely used in controlled release systems.

Recently, hydrogels, which swell and contract in response to external pH, are being explored. The pH sensitive hydrogels have a potential use in site-specific delivery of drugs to specific regions of gastrointestinal tract and have been prepared for low molecular weight and protein drug delivery. It is known that the release of drugs from the hydrogels depends on their structures or their chemical properties in response to environmental pH. These polymers, in certain cases, are expected to reside in the body for a longer period and respond local environmental stimuli to modulate drug release. On the other hand, it is some times expected that the polymers are biodegradable to obtain a desirable device to control drug release. Thus, to be able to design hydrogels for a particular application, it is important to know the nature of systems in their environmental conditions to design them in proper situation.

Some of the recently developed modes of drug delivery are summarized below:

1. Transdermal Drug Delivery

As the name suggests, transdermal drug delivery involves the delivery of drugs through the skin pores. The commonly used mechanism of drug delivery in this case is diffusion. However the skin pore size limits the use of this technique to small drugs. Hence various techniques such as Iontophoresis (which uses an electrical gradient as a driving force for the delivery of the drug) and Sonication (which uses ultrasound techniques to increase the pore size) are being investigated.

2. Pulmonary Drug Delivery

Pulmonary drug delivery mainly includes aerosol based systems wherein drug delivery is carried out using nasal sprays.

3. Polymeric Implants

Polymeric drug delivery is the most widely studied area of drug delivery in the recent past. A polymeric implant which consists of the drug imbedded in a polymer matrix is surgically planted into the body (in the affected area). The drug is then released directly into the affected site via diffusion or surface erosion.

1.1.6 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 $-(gly\text{-}ala)_n-$ repeating units, which form the highly specific secondary structure, known as antiparallel β-sheet structure.

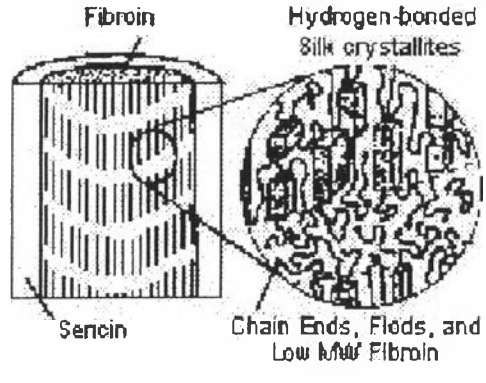

Figure 1.5 Model of microstructure of silk fibroin.

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 (Shen *et al.*, 1998).