CHAPTER I
INTRODUCTION

Among possible routes of introducing controlled release medication into the body, the oral administration of single dose medication is one of the simplest and safest. However, an oral controlled release formulation subjects to frequently changing environments during transit through the gastrointestinal (GI) tract as it passes from the strongly acidic to the weakly alkaline medium in the lower part of the small intestine. One recent effort on eliminating some of the problems of traditional dosage forms is the development of transdermal delivery systems (administration of a drug applied to the skin in ointment or patch form), in which the main objective is to achieve an effective therapeutic administration for an extended period of time. Moreover, the merits of transdermal administration are therapeutic plasma levels (reduced peaks/valleys associated with intermittent drug administrations), avoiding continuous infusion technique difficulties, low side effect incidence (smaller doses) and generally good patient compliance (Ranade and Hollinger, 1995).

Chitosan is a linear polysaccharide formed by β-1,4 linkage of D-glucosamine and N-acetylglucosamine (40% maximum) residues. Some interesting properties of chitosan include biocompatibility (Vandevord *et al.*, 2002), biodegradability (Xu *et al.*, 1996), non-toxicity (Chandy and Sharma, 1991), microbial resistance (Wang and Qian, 1999) and gel-forming ability (Arguelles *et al.*,1998). There are many reports on the applications of chitosan, such as controlled drug-delivery system (Gupta and Kumar, 2000), wound dressings (Mi *et al.*, 2002), sutures (Hirano and Noishiki, 1985), hollow fibers (Vincent and Guibal, 2000), membranes (Matsuyama *et al.*, 1999) and gauze (Tucci *et al.*, 2001). Rocha *et al.* (2002) studied the permeabilities of isoniazid and amitriptyline hydrochloride in chitosan membranes and concluded that chitosan membranes can potentially be used in a controlled-release system.

Silk fibroin is one of the most extensively studied materials among the natural biopolymers. Silk fibroin is a fibrous protein which consists of few types of amino acid residues which are glycine, alanine, and serine. The sum of these amino

acids accounts for more than 80 mol% (Freddi *et al.*, 1995). Silk membrane showed moisture permeability (Li *et al.*, 2000), good mechanical and physical properties (Tsukada *et al.*, 1994a). In addition, silk fibroin membrane is an amphoteric ion exchange membrane composed of both weak acedic and weak basic groups and it is expected to be used as the matrix of the drug delivery system with pH-responsive function (Chen and Minoura, 1994). Nevertheless, silk fibroin films are very brittle in the dry state and almost unsuitable for practical use. It has been reported that both strength and elongation at break of silk fibroin films could be improved by blending with either natural or synthetic polymers. Freddi *et al.* (1995) prepared and characterized silk fibroin/cellulose blend films. It was concluded that the mechanical properties of silk fibroin were improved by blending with cellulose. Wang *et al.* (2003) studied the properties of silk fibroin/poly(ethylene glycol) blend films. The resulting film exhibited much better mechanical properties in dry and wet state than silk fibroin itself, owing to the conformational change of silk fibroin in the blends from random coil to β-sheet structure and intermolecular hydrogen bond formation between silk fibroin and poly(ethylene glycol). It can be seen that the mechanical properties of silk fibroin could be improved by blending. There are two conformations of silk fibroin, random coil and β-sheet structure. Silk fibroin with β-sheet structure has better mechanical properties than that with random coil conformation. Conformation transition from random coil to β-sheet structure can be induced by gamma irradiation (Tsukada *et al.*, 1994b), blending (Chen *et al.*, 1997a), treatment with methanol solution (Kweon and Park, 1999). Chen *et al.* (1997a) prepared the polymer blend of chitosan and silk fibroin using glutaraldehyde as crosslinking agent. It was found that the conformation transition of silk fibroin from random-coil to β-sheet structure occurred by blending with chitosan. In addition, Chen *et al.* (1997b) also reported that crosslinked chitosan/silk fibroin blend film had semi-interpenetrating network and the blend film with 80% chitosan had higher degree of swelling than the pure component.

The aim of this study is to investigate the application of chitosan/silk fibroin blend films as transdermal drug delivery system. The *in vitro* study was carried out using modified Franz diffusion cell and pig skin was used as a material representing

human skin. The effects of blend composition and model drug types on drug release property of chitosan and the blend films were investigated.

1.1 Theoretical Background

1.1.1 Transdermal drug delivery system

Transdermal drug delivery system is administration of a drug applied to the skin in ointment of patch form. Although some drugs have inherent side effects that cannot be eliminated in any dosage form, many drugs exhibit undesirable behavior that is specifically related to a particular route of administration. One recent effort at eliminating some of the problems of traditional dosage forms is the development of transdermal delivery systems. Oral administration of drugs has been practiced for centuries and, most recently, through tablets and capsules, injectables came into being approximately 130 years ago, but have only become acceptable since the development of a better understanding of sterilization. Topical application has also been used for centuries, predominantly on the treatment of localized skin diseases. Local treatment requires only that the drug permeate the outer layers of skin to treat the diseased state with the hope that this occurs with little or no systemic accumulation.

Transdermal delivery systems, on the other hand, are specifically designed to obtain systemic blood levels and have been in the U.S. since the 1950s. Transdermal permeation, or percutaneous absorbtion, can be defined as the passage of a substance, such as a drug, from the outside of the skin through its various layers into the bloodstream. Any time there is systemic access of a drug, unwanted side effects or toxic effects can occur. Certainly, each dosage form has its unique place in medicine, but some attributes of the transdermal delivery system provide distinct advantages over traditional methods. Cleary has listed important advantages and disadvantages of transdermal delivery systems. The advantages are

1. The system avoids the chemically hostile gastrointestinal (GI) environment

2. No GI distress or other physiological contraindications of the oral route

3. Can provide adequate absorption of certain drugs
4. Increased patient compliance
5. Avoids first-pass effect
6. Allows effective use of drugs with short biological half-lives
7. Allows administration of drugs with narrow therapeutic windows
8. Provides controlled plasma levels of very potent drugs
9. Drug input can be promptly interrupted when toxicity occur

Disadvantages of this system include

1. Drug that require high blood levels cannot be administered
2. Adhesive may not adhere well to all types of skin
3. Drug or drug formulation may cause skin irritation or sensitization
4. Uncomfortable to wear
5. May not be economical

In the development of transdermal delivery systems, a series of interrelated elements must be taken into consideration. These factors can be classified into five basic areas: bioactivity of the drug, skin characteristics, formulation, adhesion, and system design. The transport of drugs through the skin is complex, since many factors influence their permeation. To simplify the situation somewhat, one should consider skin structure and its properties, the penetrating molecule and its physical-chemical relationship to skin and the delivery platform, the platform or delivery system carrying the penetrant, and the combination of skin, penetrant, and delivery system as a whole. The major emphasis of this article is to discuss each of these factors, their complexities, and their interdependencies in the development of transdermal delivery system (Ranade and Hollinger, 1995).

1.1.1.1 Design of In vitro Skin Permeation Apparatus

Several designs of *in vitro* membrane permeation apparatus, whose hydrodynamic characteristics have been fully investigated, are discussed in the sections that follow.

(a) Horizontal-Type Skin Permeation System, Small Cell Volume

The skin permeation system (Figure 1.1) has been extensively used for studying the skin permeation kinetics of drugs, using either human cadaver skin or freshly excised animal skin. This cell design has a solution compartment of relative small volume in each half –cell for maximal analytical sensitivity, and a rather small membrane area (0.64 cm^2) to accommodate the skin specimen available. Both the donor and receptor solutions are agitated, under a totally enclosed system, by a matched set of star-head magnets (diameter, 8 mm), which are rotates at a synchronous speed of 600 rpm at a fixed position in the stirring platforms by a specially designed driving unit positioned directly underneath the cells. The temperature of the system can be controlled at isothermal or nonisothermal conditions by circulating thermostated water through the water jacket surrounding the solution compartment.

(b) Horizontal-Type Membrane Permeation System, Large Solution Volume

The second horizontal membrane permeation system is also composed of one pair of donor and receptor compartments in mirror image (Figure 1.2), in which the fluid is agitated by a matched set of bar-shaped magnets (2.54 cm long). The magnets are driven by a pair of synchronous motors located directly underneath the cells to rotate at a synchronous but variable rate in a specially designed stirring platform. Each pair of half-cells has a large effective membrane area for permeation (13.9 cm^2). Each compartment can hold a volume of 140-250 ml of solution. The rotation speed of the magnets con be controlled at a constant level of 60-1000 rpm. Samples may be withdrawn for analysis from a sampling port at various intervals. The donor and receptor compartments are both jacketed and thermostated by an external circulating bath to maintain isothermal conditions, or if designed, the temperature in either donor or receptor compartment can be programmed to simulate any environment variations.

(c) Franz Diffusion Cell

The vertical-type skin permeation system (Figure 1.3) developed by Franz and commercialized by Crown Glass has been frequently used for studying the kinetics of percutaneous absorption. The cell has a receptor compartment with an effective volume of approximately 10-12 ml and an effective surface area for permeation varying from 1.57 to 4.71 cm^2. The solution in the receptor compartment is stirred by a rod-shaped magnet driven by a 3-w synchronous motor. The stirring magnet rotates at a speed of 600 rpm in a low viscosity receptor solution such as saline solution. The temperature in the bulk of the solution can be maintained at a constant level by circulating thermostated water through the water jacket surrounding the receptor compartment. However, the temperature near the upper opening, at which the skin will be positioned, varies as the surrounding temperature varies. The observed variation in receptor solution temperature results because the donor compartment is not thermally controlled. The hydrodynamic characteristics of the Franz diffusion cell recently were established.

(d) Modified Franz Diffusion Cell

Another vertical-type skin permeation cell was recently developed in response to the observation that the Franz cell has rather poor solution hydrodynamics as a result of inefficient mixing. The results are a significant temperature gradient in the diffusion. The Franz diffusion cell (Figure 1.4) improve efficiency of fluid mixing. The modified cell has an effective receptor solution volume of 12 ml and a skin surface area of 3.14 cm^2. The receptor solution is stirred by a star-head magnet rotating at a constant speed of 600 rpm by the same driving unit originally designed for Franz diffusion cell. The hydrodynamic characteristics of the modified Franz cell were recently investigated.

(e) Rotating-Disc-Type Membrane Permeation Cell

One of the advantages of the rotating-disc cell is that the hydrodynamic diffusion boundary layer on the surface of the rotating disc has been well established theoretically (Figure 1.5).

If the rotating disc is assumed to be sufficiently large that the edge effects are negligible, the thickness of the diffusion boundary layer is given by:

$$\delta = 1.61\ (D_f \rho/\mu)^{1/3}\ \{\mu/(\rho\omega)\}^{1/2} \qquad (1.1)$$

where D_f is the drug diffusivity in the fluid,

ρ is the density of the fluid,

μ is the viscosity of the fluid,

ω is the angular velocity ($= \pi Nd$).

In the rotating-disc diffusion cell, if the disc is not large enough the flow pattern in the cell is easily influenced by the wall of the vessel. Then the diffusion layer determined experimentally is usually thicker than that calculated from equation 1.1 due to the dissipation of additional energy on the vessel wall. Therefore, equation 1.1 should not be used a priori for estimating the diffusion boundary layer thickness existing on the surface of the rotating disc.

Recently, a rotating-disc-type membrane permeation cell for studying the release of drug from suppositories was developed. The rotating disc has an effective membrane area of 12.6 cm^2 and can be used together with the one-liter USP dissolution vessel as the receptor compartment. The hydrodynamic characteristics of this diffusion cell were recently investigated (Chien, 1987).

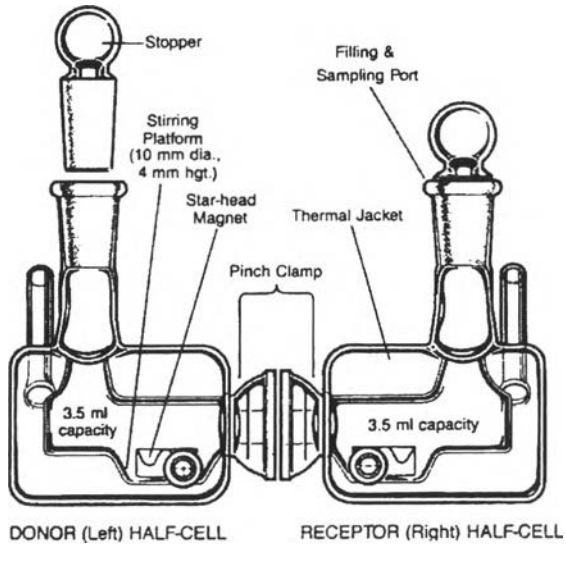


Figure 1.1 Horizontal-type skin permeation system, small cell volume.

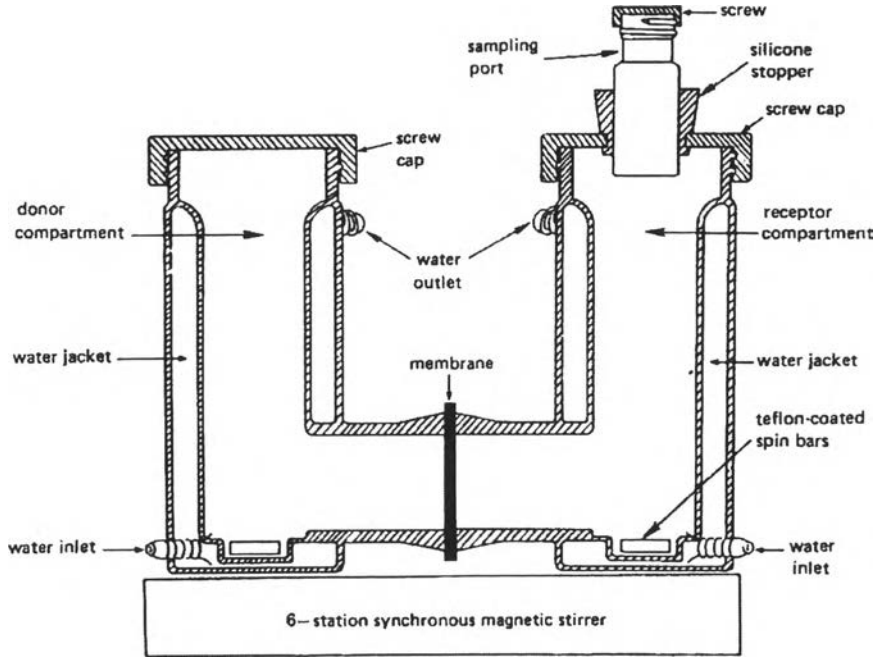


Figure 1.2 Horizontal-type membrane permeation system, large solution volume.

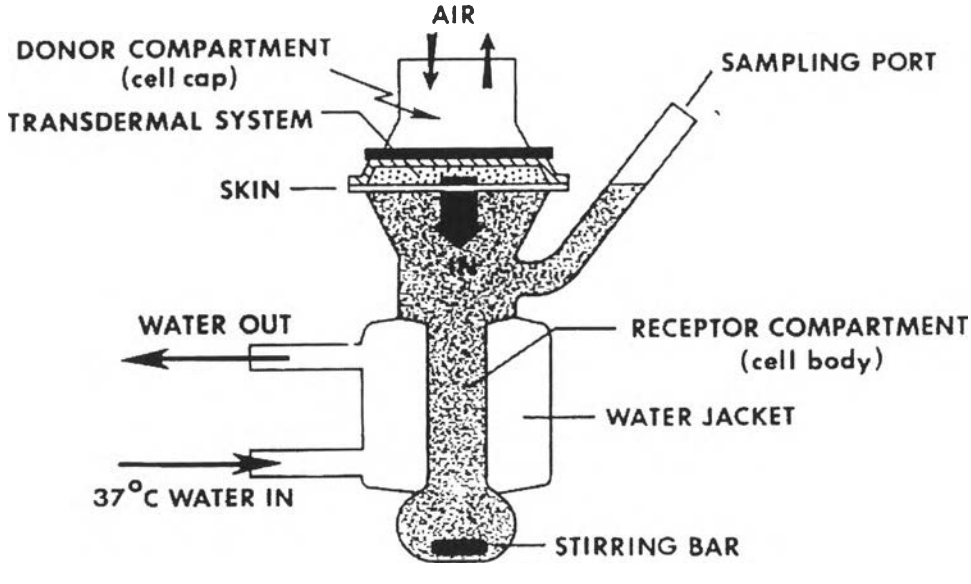


Figure 1.3 Franz diffusion cell.

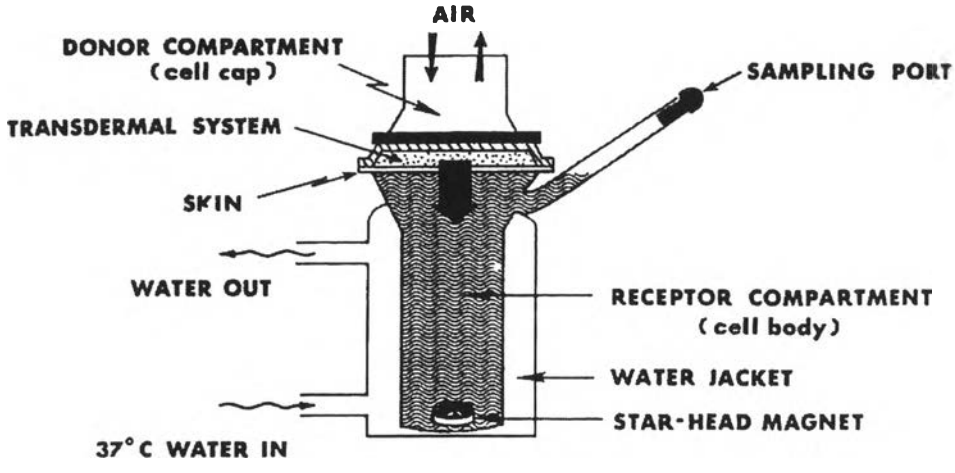


Figure 1.4 Modified Franz diffusion cell.

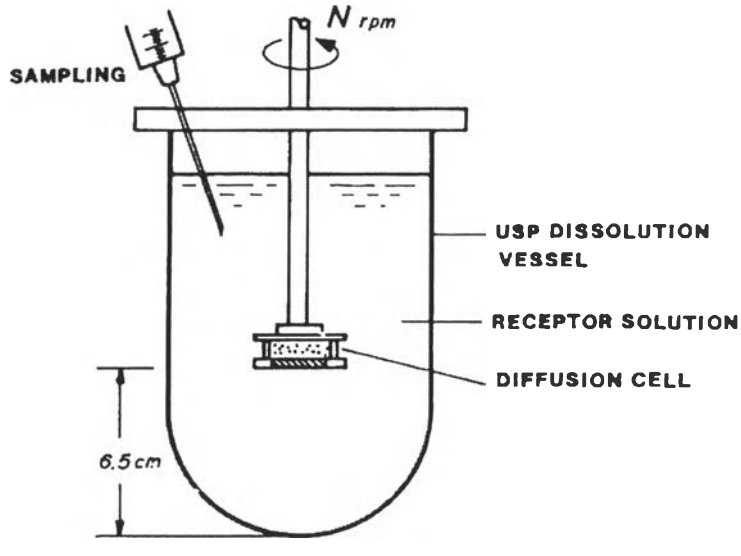


Figure 1.5 Rotating-disc-type membrane permeation cell.

1.1.1 Chitosan

Chitosan is the fully deacetylated chitin, i.e., a pure D-glucosamine polymer, but also polymers with low degree of deacetylation (DD) such as to become soluble in acidic conditions. Chitosan is a polysaccharide obtained by deacetylating chitin, which is the major constituent of the exoskeleton of crustaceous water animals. This biopolymer was traditionally used in the Orient for the treatment of abrasions and in America for the healing of machete gashes.

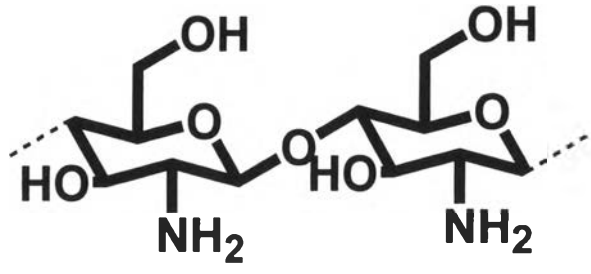


Figure 1.6 Chemical structure of chitosan.

The main driving force in the development of new applications for chitosan lies in the fact that the polysaccharide is not only naturally abundant, but it is also nontoxic and biodegradable. Unlike oil and coal, chitosan is a naturally regenerating resource (e.g., crab and shrimp shells) that can be further enhanced by artificial culturing. It was reported that chitosan and chitin are contained in cell walls of fungi. Chitin, however, is more widely distributed in a nature than chitosan and can be found in mushrooms, yeasts, and the hard outer shells of insects and crustaceans. It was reports, for example, that about 50-80% of the organic compounds in the shells of crustacea and the cuticles of insects consists of chitin. At present, most chitosan in practical and commercial use comes from the production of deacetylated chitin with the shells of crab, shrimp, and krill (the major waste by-product of the shellfish-processing industry) being the most available sources of chitosan.

One of the most useful properties 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 pharmaceutics. Chitosan has also shown affinity for proteins, such as wheat germ agglutinin and trypsin. Other properties that make chitosan very useful include inhibition of tumor cells, antifungal effects, acceleration of wound healing, stimulation of the immune system, acceleration of plant germination.

Chitosan is a good cationic polymer for membrane formation. In early reseach it was shown that membranes formed from the polymer could be exploited for water clarification, filtration, fruit coating, surgical dressing, and

controlled release. In 1978, for example, Hirano showed that N-acetyl chitosan membranes were ideal for controlled agrochemical release. Later, he found that a semi-permeable membrane with a molecular weight cutoff ranging from 2,900 to 13,000 could be formed from chitosan.

This article focuses on various applications of chitosan, as well as current research on its physicochemical properties. Application areas that are covered include water treatment ,pharmaceutics, biotechnology, food processing, and membranes.

1.1.2.1 Application of Chitosan

The industrial production and use of chitosan has been steadily increasing since the 1970s. In Japan, for example, the production of chitosan increased 37% each year from 1978 to 1983, the total amount reaching 311 tons by 1930 and 1,270 tons by 1986. At that time, the major applications of chitosan were centered on sludge dewatering, food processing, and metal ion chelation. The present trend, in industrial applications, however, is toward producing high value products, such as cosmetics, drug carriers, feed additives, semi-permeable membranes, and pharmaceutics. The difference in value between the products and the low- cost polymer is one of the main driving forces pushing studies on new applications of chitosan. Biotechnology is currently attempting large-scale production of high value bioproducts like monoclonal antibodies. Immobilization techniques have been proven to be an effective way to increase cell density, product concentration, and hence, productivity in a culturing system. Chitosan membrane and gels have great potential for use in immobilized cell culture systems. There are

-Water treatment and papermaking

-Pharmaceutics and biotechnology

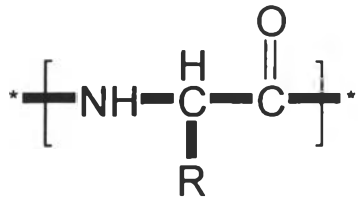
-Agriculture and food processing

-Chitosan membranes and cell encapsulation (Goosen, 1997).

1.1.3 Silk Fibroin

Natural silk is formed by the solidification of liquid silk secreted by the body of the silkworm. Each cocoon filament is made up of two mono filaments arranged parallel to each other. Important ingredients of a mono filament are fibroin and sericin. Fibroin is the staple of natural silk along with the crucial α amino acid containing glycine, alanine, serine and tyrosine. Of these glycine and alanine, which are simple in structure, occupy more than three-fourth of the total. The quality of α RNA having dicarboxyl or diamino (acid), is very poor. The chemical structure of a fibroin molecule is quite simple and it becomes clear through X-ray diffraction. Fibroin contains crystalline natural protein molecule(s).

Silk fibroin which is the main part of natural silk is a fibrous protein obtained from cocoon silkworm. The primary structure arising from this characteristic amino acid composition contains many –(gly-ala)$_n$- repeating units, which from the highly specific secondary structure, known as antiparallel β-sheet structure. Silk fibroin is a fibrous protein whose 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%.



45% Glycine (R = H)

30% Alanine (R = CH_3)

12% Serine (R = CH_2OH)

Figure 1.7 Chemical structure of silk fibroin.

Besides its textile application, silk has 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 valve. The silk fibroin membrane was an amphoteric ion exchange membrane and it was expected to be used as a pH-sensitive drug delivery system.