Application of Silk Fibroin in Controlled-Release of Theophylline

by

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ÖZ

Polimerik malzemeler kullanılarak ilaç salınım proseslerini modellemek amacı ile çalışmalar yapılmaktadır. Organik bazlı polimerik çözeltiler ile ilaçların kaplanması hala çok yaygın olmasına rağmen ,organik bazlı polimerik çözeltilerin toksik ve çevreye zaralı olması nedeni ile ilaç endüstrisi farklı kaplama malzemeleri üzerine çalışmaya başlamıştır. Su bazlı kaplama teknikleri, organik polimerik çözeltilerin neden olduğu toksik özellikleri ortadan kaldırmak amacı ile kullanılmaktadır. Bu çalışmada kontrollü ilaç salınım mekanizmasını elde etmek için kaplama malzemesi olarak su bazlı fibroin çözeltisi ve model ilaç olarak da teophylline kullanılmıştır. İlaç tabletleri ısıl işlem görmüş silk fibroin, polietilenglikol (PEG) ve fibroin çözeltisi karışımı ile kaplanmıştır. Ek olarak EDC ile kroslink edilmiş fibroin çözeltisiden elde edilen kaplamanın ilaç salınımına etkisi araştırılmıştır. Bütün örneklerin ilaç salınım profilleri UV-spectrofotometre kullanılarak dissolusyon testi ile yapılmıştır. Ayrıca PEG konsantrasyonunun ve film kaplama kalınlığının etkileri araştırılmıştır. Taramalı elektron mikroskobu kullanılarak filmlerin morfolojisi ve film kaplama kalınlıkları bulunmuştur. PEG in plastikleştirici etkisinin ve fibroinin EDC ile kroslink edilmesinin, ilacın kontrollü bir şekilde salınmasını sağladığı gözlemlenmiştir. Hedef profile göre %17 PEG içeren fibroin çözeltisi ile kaplanan tabletlerin %70 salınım profilinde çözünme süresi 345 dakika olarak bulunmuştur. İlaç salınım hızı ve tablet kaplama kalınlığı arasında bir bağıntı elde edilmiştir. EDC ile kroslink edilen fibroin çözeltisiyle kaplanan tabletlerde , kaplama kalınlığı arttıkça ilaç salınımı azalmıştır. Film kaplama kalınlığını 7.68 μm'ye getirilerek istenen ilaç salınımı elde edilmiştir.

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CHAPTER 1

INTRODUCTION

Drug delivery systems have previously been developed. In the 1950's, approaches for incorporating drugs into solid polymers began to be developed for agricultural products. In the initial studies, drugs were placed inside silicone rubber tubing, or distributed through a polyethylene matrix. In the 1970's, approaches were developed so that large molecular weight drugs could be continuously released from solid polymers. In the past decade, many applications of polymer drug systems were considered. By using polymers as carriers, drugs could be continuously released for very long periods of time; patient to patient variations in drug administration patterns were decreased because the rate-limiting step for drug release was its removal from the polymer. By using different polymeric systems or altering polymer-drug incorporation procedures, different release rates could be obtained. The performance of these drug delivery devices is evaluated primarily in terms of their release kinetics and overall ease of administration. Devices that release drug with zero order kinetics (a time-independent rate) for an extended time period are usually considered optimal.

A useful classification of controlled-release polymeric systems is based on the mechanism controlling the release of incorporated drug. The rate limiting step of the release process may be pure drug diffusion according to Fick's law (diffusion-controlled systems), chemical reaction at the interface between the polymer and the dissolution medium (chemically-controlled systems), or countercurrent diffusion of the dissolution medium at constant penetration velocity in the polymer (swelling-controlled systems) (Peppas et al., 1981).

Silk fibroin is an attractive natural fibrous polymer produced by different species of silk worms. Among the wide variety of silks, that produced by the species *Bombxy mori* (domestic silk) has been extensively investigated (Tsukada et al., 1999). Silk fibroin is regarded as a block-type copolymer composed of both hydrophobic chains and

hydrophilic chains. Besides the use as a textile fiber, silk fibroin has been recently studied as a starting material for nontextile applications. These include enzyme immobilization for the preparation biosensors and the production of oxygen-permeable membranes. Silk fibroin has some interesting advantages compared to other biopolymers. High-purity silk fibroin can be easily obtained from cocoons in fiber form. Aqueous silk solutions represent a good starting material for the preparation of different kinds of fibroin-based materials, such as gel, powder, porous membranes, and homogeneous membranes. The use of porous substrates is highly attractive for several biotechnological and biomedical applications, because the high surface area available and the presence of a network of interconnected pores can favor processes related to diffusion, permeation, enzyme-substrate reaction. Moreover, porous polymeric materials can be successfully exploited as carriers for the controlled delivery of drugs.

Dissolution testing is used as a quality control procedure in pharmaceutical production, in product development to assist in selection of a candidate formulation, in research to detect the influence of critical manufacturing variables such as binder effect, mixing effect, coating parameters, excipient type.

This study aims to investigate the controlled release of theophylline coated with heat treated silk fibroin solution, polyethylene glycol and fibroin solution, and crosslinked fibroin solution. For this purpose dissolution tests of the coated tablets were performed and the morphologies of the coatings were obtained by scanning electron microscopy.

CHAPTER 2
CONTROLLED-DRUG DELIVERY SYSTEMS

The systems, which are designed to deliver therapeutically active agents at a specified rate, at a desired target organ for a certain period of time, can be defined as controlled drug delivery systems. Figure 2.1 shows the differences in plasma drug levels between conventional and controlled-release systems. In conventional systems, it is very difficult to maintain plasma drug level between minimum effective and maximum desired level. After each dose, fluctuations in plasma drug level cause some harmful side effects and unnecessary drug use. In controlled-release systems, dosing frequency is reduced and plasma drug levels are rarely above the drug's therapeutic range. In order to achieve the drug delivery in a controlled manner, many approaches were developed by making use of polymeric systems.

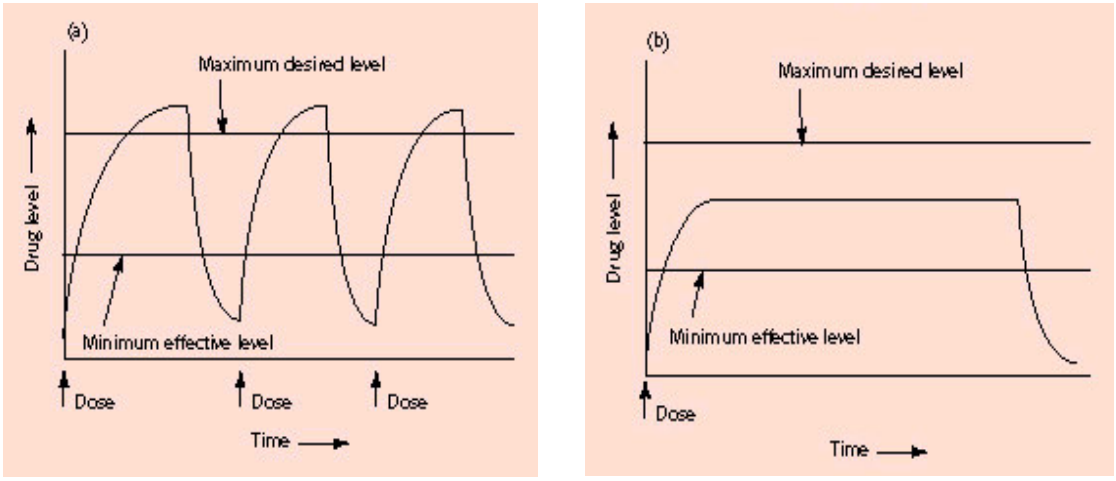


Figure 2.1 Drug concentration levels for conventional system (a); controlled-release system (b)

The techniques used to control drug release include the use of slowly dissolving polymeric coatings such as cellulosic materials, addition of substances which would

form a complex with the drug and decrease its solubility, the use of compressed tablets and employment of emulsion and suspension technologies (Popovich,1990).

Control release delivery system has several advantages compared with single unit dosage forms;

- therapeutically desirable range of plasma drug levels
- reduced harmful side effects by local administration from polymer-drug depot
- protection of drugs that have short in vivo half-lives
- improved patient compliance
- less expensive and less wasteful

Besides the advantages of controlled drug delivery, there are also some disadvantages to be considered. These are:

- toxicity or lack of biocompatibility from the polymer material used
- production of harmful by-products from the polymer if it is biodegradable
- expense of a particular polymer-drug formulation
- pain caused by the presence of the implant (Kojima,2002).

2.1 CLASIFICATION OF CONTROLLED RELEASE SYSTEMS

Controlled release systems can be classified into five main categories. These are diffusion controlled systems, swelling controlled systems, magnetically controlled systems, chemically controlled systems.

2.1.1 Diffusion-Controlled Release Systems

Diffusion controlled systems are most widely used systems, which are mainly divided into two groups: reservoir and matrix systems. Reservoir and matrix tablets are common sustained release dosage forms, in part, due to simplicity of their formulation and production. Reservoir systems consist of a drug/excipient core coated with a controlling membrane, whereas matrix systems comprise drug/excipient embedded

within a matrix. Typically, polymers are used for the coating and matrix materials. They can be either hydrophobic or hydrophilic (Krajacic, 2002).

2.1.1.1. Reservoir Systems

In this system, a core of drug is surrounded by swollen or non-swollen polymer film and diffusion of the drug through the polymer is the rate-limiting step. A critical problem, from a pharmaceutical standpoint, is the ability to achieve zero-order (independent of drug concentration) release rate; the principal advantage of reservoir systems is their simple design to achieve zero-order release kinetics. The most important parameters for zero-order kinetics are membrane thickness, permeability, and release area. The concentration gradient across the membrane and diffusivity must remain constant.

Although reservoir systems have great advantages to obtain zero-order kinetics, they have also several disadvantages. For example, these systems are generally non-biodegradable; therefore, implants must be surgically removed. These systems are generally not useful for long-term delivery of drugs. In addition, if leaks occurred, they could be potentially dangerous because the entire incorporated drug could be rapidly released. Preparation of membrane systems is generally more expensive than that of other types of controlled release systems (Peppas,1981).

Mainly four types of reservoir systems are present. These are microporous membranes, nonporous membranes, microencapsulation and, osmotic pressure types. Drug permeation through polymer membranes is generally described in terms of two mechanisms: the “pore” mechanism and the “partition” mechanism. In the “pore” mechanism, drugs permeate the polymeric membrane by diffusion through pores within the membrane at a rate controlled by mainly by the pore size of the membrane and the molecular volume of the drug. In nonporous membranes, solute transport occurs via a solution diffusion or “partition” mechanism. The “partition” mechanism involves drug dissolution in the polymer structure followed by drug diffusion along and between the polymer segments that make up the membrane structure. In microporous membrane, the solute does not pass through the polymer phase, but diffuses through water-filled pores. In other words, the drug diffuses through pores in the polymer structure, rather than

between macromolecular chains. Active agent is microencapsulated by polymeric material in microencapsulation system. This yields small solid particles or liquid droplets enclosed by intact, thin shell of polymeric material. The release of active agent may be a result of eroded polymeric shell or diffusion through the polymeric shell. Usually release is achieved by erosion and diffusion. Osmotic pumps include a core consists of a solid, water-soluble drug. This is enclosed in water permeable but drug-impermeable polymer membrane, which contains small openings. When the device is bought in contact with aqueous tissue or body fluids, water is transported into the reservoir by permeation through the polymer membrane. Hydrostatic pressure builds up within the reservoir and is periodically removed by discharge of drug solubilized water through openings (Heasen,1982).

Puttipipatkhachorn et al., 2001 used four different grades of chitosan varying in molecular weight and degree of deacetylation to prepare chitosan films. Salicylic acid and theophylline were incorparated into cast chitosan films as model drugs. Crystalline characteristics, thermal behavior, drug – polymer interaction and drug release behaviors of the films were studied. The Fourier transform infared was use to demonstrate the drug polymer interactions between the salicyclic acid and chitosan, resulting in salicylate formation, whereas no drug-polymer interaction was observed between theophylline-loaded chitosan films. They determined that most chitosan films loaded with either salicylic acid or theophylline exhibited a fast release pattern, whereas the high viscosity chitosan films incorporated with salicyclic acid was seen sustained release patterns in distilled water. They evaluated the sustained release action of salicyclic acid from the high viscosity chitosan films was due to the drug-polymer interactions.

Fan et al., 2001 investigated pulsatile release tablets, which can suppress drug release in stomach and release the drug rapidly after a predetermined lag time in intestine. The system consisted of a core containing drug and a swelling agent of cross-linked polyvinylpyrrolidone and a coating film of ehthylcellulose/EudrogitL. Prior to drug release, Eudrogit L was dissolved in an environmental pH above 6 and caused pores in the coating film. They reported that water penetrated through the pores of the film into the core causing a swelling and bursting the film which made the drug rapidly released.

Herbig et al., 1995 studied trimazosin tablets coated with a symmetric membrane cellulose acetate. Release rates were determined by immersing the coated tablets in magnesium sulfate solution and in water. The tablets were analyzed by UV, coating surface and thickness were observed by SEM. Also osmotic pressures were measured using a freezing point depression osmometer. They observed no driving force for trimazosin delivery from the tablets into magnesium sulphate solution whereas, there was a driving force for delivery into water. So the release rate into the magnesium sulphate solution should be negligible compared with that into the water. It was seen that the release rate was very slow into the magnesium sulphate solution, since trimazosin could only be released by diffusion; the release rate was much higher into water to osmotic pumping of the drug from the tablet. They also used the same asymmetric membrane coated tablets to compare with trimazosin tablets with dense cellulose acetate coating where both types coated tablets exhibited constant steady state release kinetics. The steady state release from the asymmetric –membrane coated tablets was about 65 times higher than the release rate from the dense coated tablets. They also observed that the overall thickness of the coating of the tablet did not influence the release rate.

Yenal, 2001 studied the asymmetric type of coating which was applied on the theophylline tablets. Coatings were prepared from cellulose acetate, acetone, water solution by phase inversion technique. In this study, the effects of composition of the polymer/solvent/nonsolvent, coating time, number of coating layers, evaporation conditions and the nonsolvent type on the release rate of the drug and the structure of membrane were investigated.

2.1.1.2 Matrix Systems

The drug is uniformly distributed (dissolved or dispersed), throughout a solid polymer. As in reservoir systems, drug diffusion through the polymer matrix is the rate-limiting step. From the fabrication cost point of view, the ease of accomplishing this distribution pattern allows a significant cost decrease compared to reservoir systems (Peppas,1981). However, because of the different way in which drug is distributed, release characteristics are not generally zero-order. The release depends on active agents' molecular size, solubility, tortuosity, and the fractional volume of the pores

(Heasen,1982). When a matrix tablet is placed in the dissolution medium, initial drug release occurs from the tablets' superficial layers and, consequently, the release is relatively fast. As the time passes, the external layers of the tablet become depleted of the drug and water molecules travel through long, tortuous channels to reach the drug remaining in the deeper layers of the tablet. Similarly, the drug solution that is formed within the tablet diffuses through long capillaries to reach the external dissolution medium. The primary reason for the continuously decreasing rate of drug release is the increasing distance that is traversed by water and drug molecules into, and out of the tablet respectively. Matrix system is shown below in Figure 2.2. (Pather,1997).

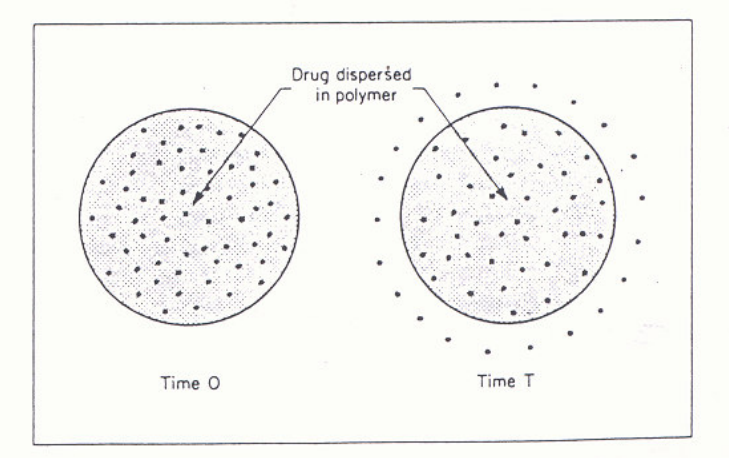


Figure 2.2. Diffusion-controlled matrix systems.

Samani et al., 2003 studied to evaluate the effect of polymer blends on the in vitro release profile of diclofenoc sodium. Several controlled release matrices of diclofenoc sodium with different proportions of hydroxypropylmethylcellulose, carbopol 940 and lactose as water filler were prepared. The studies firstly were done by only HPMC and carbopol 940 individually. At higher HPMC/drug ratio the drug release was observed to be decreased. In spite of prolonged release time, they found that the correlation coefficient did not fit to zero-order kinetic and also at low carbopol 940/drug ratio could sustain the drug release, but the pattern of release was not suitable. At the beginning it was determined that release was very slow but later the release sharply increased. So HPMC and carbopol 940 were used as a matrix form. And it was seen that fluctuations in the formulations were decreased and the kinetic of the drug release approached to zero-order.

Lorenzo et al., 2000 studied the drug release properties of theophylline matrix tablets prepared with different hydroxyproppylcelluloses which have different molecular weight and particle sizes. They observed that rapid drug release with the tablets prepared relatively small amounts of highest molecular weight and highest particle size.
Richard et al., 1981 studied the effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs. Dilute, aqueous polyvinylalcohol solutions containing theophylline were crosslinked with glutaraldehyde. The crosslinking ratio was varied between 0.01 and 0.2 moles glutaraldehyde per mole of PVA repeating unit. Theophylline release from those rubbery matrices was followed as a function of time. It was determined that, within the range of crosslinking ratios studied, the crosslinked macromolecular structure affected the solute diffusion process. Theophylline release from crosslinked PVA slabs, which were originally dehydrated at 30^{o}C, was also measured. They observed that the drug release was significantly impeded in these systems. They explained this behaviour in terms of relaxation of the macromolecular chains and possible existence of ordered chain structure.

Qiu et al.,1997 investigated to overcome the major disadvantage of non-linear release kinetics of matrix systems. Three layered matrix tablets were prepared with the middle layer containing the active compound embedded in water-insoluble materials. The press coated barrier layers contains water soluble and/or water insoluble materials. They concluded that by providing an additional releasing surface with time to compensate for decreasing rate, the linear release profile could be obtained.

Gren et al., 1999 prepared a multiple extended-release matrix by incorporation of hydrophilic drug (paracetanol) and lipophilic release modifiers (cetylalcohol and paraffin) into porous cellulose matrices. The incorporation was performed using a one-step melt method. They controlled the release rate by changing the ratio of cetylalcohol to paraffin. They reported that the release rate was affected by the distribution of drug in the matrices and an increase in porosity during the drug release. The porosity was observed to form cracks and voids as the cellulose swelled.

2.1.2. Swelling Controlled Systems

Drug is originally dissolved or dispersed in a polymer solution; then the solvent is evaporated leaving the drug dispersed in a glassy (solvent-free) polymer matrix. As the dissolution medium penetrates the matrix, the polymer swells and its glass transition temperature is lowered below the temperature of the medium. Therefore, the swollen polymer is in a rubbery state and it allows the drug contained in it to diffuse outward. Figure 2.3 shows the idealized swelling-controlled system.

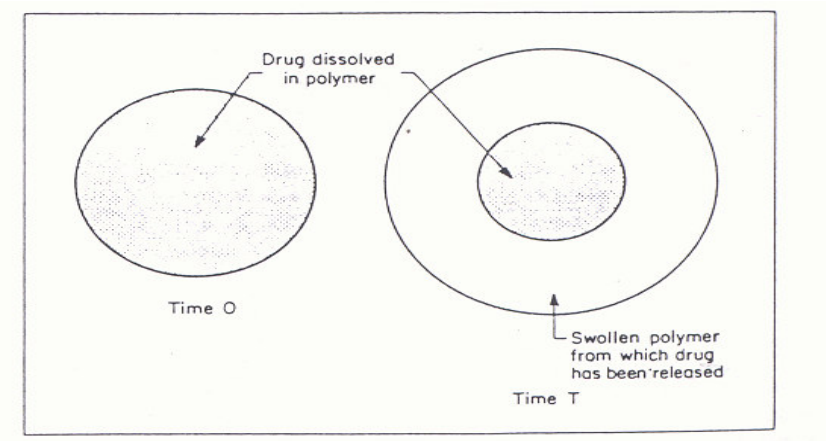


Figure 2.3 Idealized swelling-controlled system

Colombo et al., 1995 investigated the movement of the penetrant and polymer fonts and the drug dissolution in highly loaded swellable matrix tablets to establish the relationships between font position and the drug release kinetics. It was confirmed that in swellable matrix tablets drug release was inversely proportional to the dynamics of gel layer thickness.

Catellani et al.,1998 studied swellable matrix tablet which was partially coated with cellulose acetate, whose permeability to water was altered by mixing increasing amounts of polyethylene glycol. Drug permeability through the polymeric cup and SEM analysis on the films were performed. It was found that the system exhibited drug-release kinetics very close to linearity. The mechanism governing the drug release was explained as drug diffusion through the uncoated gel layer, drug transport through the gel layer due to the osmotic pressure difference, and the drug diffusion through the cup

pores. They reported that the system with a cup of 1%, 13%, and 33% PEG w/w behaved (semipermeable) in part as osmotic systems, whereas the system having a permeable cup behaved as a hybrid reservoir system. They concluded that a large modulation of drug release from a swellable matrix tablet could be obtained by partially coating the tablet with films semipermeable or permeable to drugs and also reported that all the systems coated with cellulose acetate and PEG films present drug release kinetics very close to linear.

2.1.3. Magnetically Controlled Systems

Drug and small magnetic beads are uniformly dispersed within a polymer matrix. The drug is released by leaving the matrix into an aqueous media. Also by oscillating external magnetic field, drug is released at a much higher rate (Herbig,1995).

2.1.4. Chemically Controlled Systems

Chemically controlled release systems can be classified into two main groups: Bioerodible systems and pendant chain systems.

2.1.4.1 Bioerodible Systems

The drug is distributed uniformly, throughout a polymer in the same way as in matrix systems. The differences, however, relates to the fact that while the polymer phase in the matrix systems remains unchanged with time, and drug is released by diffusion, the polymer phase in bioerodible systems decrease with time. Consequently, as the polymer surrounding the drug is eroded, the drug escapes (Figure 2.4). This offers a significant advantage over non-erodible systems in many applications. Since the body eventually absorbs bioerodible polymers, there is no need for surgical removal. But it is important that these products must not be toxic, immunogenic or carcinogenic.

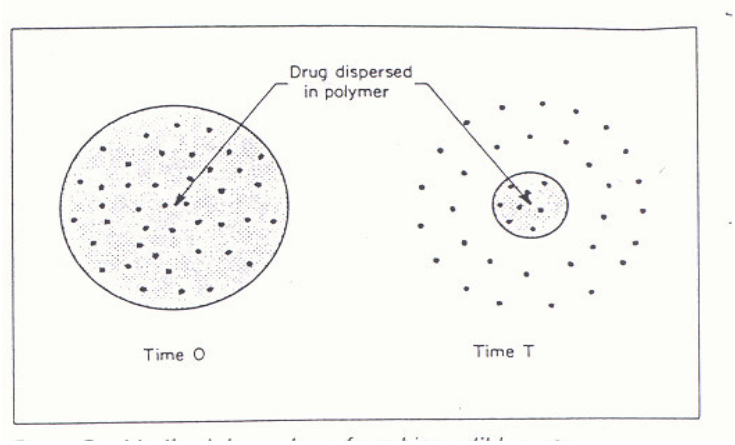


Figure 2.4 Drug release from bioerodible systems

2.1.4.2. Pendant Chain Systems

Drug is chemically bound to a polymer backbone chain, which is released by hydrolytic, or enzymatic cleavage. The polymer system can be either soluble or insoluble. Soluble backbone chains are generally used for transport functions such as cell targeting; insoluble forms are more desirable for long-term controlled released implants. Backbone may also be biodegradable or non-biodegradable. Pendant chain systems offer an important advantage over other controlled release systems in that over 80% by weight of total delivery systems can be drug itself.

2.2 POLYMERIC MATERIALS FOR CONTROLLED-RELEASE FORMULATIONS

In controlled drug delivery systems not only the active agent must be concerned but also the effects of the polymer used must be taken into account. The toxicity, biocompatibility, and immunogenicity of the polymeric device are critical because the device interfaces directly with the biological environment in which it is injected, implanted or inserted. In the case of all systems, it must be demonstrated that the properties of the polymer are not affected by prolonged exposure to the biological environment. Especially in bioerodible systems, all polymeric products must be nontoxic, non-carcinogenic, and excreted without excessive accumulation in tissues (Heasan, 1982).

The chosen biomaterial can also affect diffusion and partition coefficient in diffusion controlled system. This mechanism of release is important because it encompasses nonporous membrane bound reservoir systems as well as nonporous matrix systems in which active agent is dissolved or dispersed. There are a number of properties of the polymer that influence the diffusion of a molecule; which are polymer molecular weight, cross-linking, backbone stiffness, inter-chain interactions, crystallinity, diluents and plasticizers, filler contents (Peppas, 1981).

Consequently, optimum design of biomaterials for drug release systems requires understanding of two types of problems related to the structure and morphology of the polymeric material and problems related to the diffusion process. From material point of view, optimum diffusive conditions can be achieved by controlling the crystalline phase, porous structure, degree of swelling, additive concentration, mesh size of the cross-linked macromolecular chains and thermodynamic transitions related to macromolecular relaxation phenomena. From a diffusion point of view, thermodynamic interactions between the polymer and the diffusion species are important.

Good adhesion between a polymer and the surface of a solid is a major prerequisite for the film coating of pharmaceutical dosage forms. Loss of adhesion may lead to an accumulation of moisture at the film-tablet interface, significantly affecting the stability of drug susceptible to degradation by hydrolytic mechanism. Two major forces that have been found to affect polymer-tablet adhesion include the strength of the interfacial bond and the internal stresses within the film coating. For pharmaceutical products, hydrogen bond formation is the primary type of interfacial bonding mechanism between the tablet surface and polymer. When a polymeric solution or dispersion applied to a substrate, an internal stress develops within the film. The total stress within the film is the sum of all the stresses acting on the polymer, including stresses due to shrinkage of the film on evaporation of the solvent, thermal stresses due to the difference in thermal evaporation of the film and the substrate and volumetric stress due to change in volume when a substrate swells.

Natural or modified polysaccharides such as amylose, dextran, chondroitin sulphate, calcium pectinate, pectin, chitosan and crosslinked guar gum have been used as

potential carriers for the peroral delivery of drugs to the colon as they are safe, biodegradable and widely available. Of these, the use of pectin and chitosan have shown particular promise as they can form polyelectrolyte complexes when exposed to the various media in the gastrointestinal tract (GIT) The formation of a complex would be valuable in minimizing or controlling drug release in the upper GIT. Pectin in the form of matrix tablets, compression coatings and film coatings has been used as a potential carrier for the site specific delivery of drugs to the colon (Macleod, 1999).

The physical and chemical characteristics influence the adhesive properties of polymeric film. The surface roughness of the tablet compact and the force of compression used during the tableting will affect polymer adhesion, by altering the effective area of contact between the film coating and the surface of the solid. Excipients used in tablet formulations can change the chemical properties of the tablet surface as well. In addition to the polymer itself, film-coating formulations generally include a solvent, plasticizing agent, and a pigment, which may influence polymer adhesion (Felten, 1997).

2.3. AQUEOUS-BASED TABLET COATING POLYMER SYSTEMS

The aqueous film coating technology has been widely utilized for the application of the polymer film coating to pharmaceutical dosage forms. Aqueous based polymeric solutions and dispersions are commonly employed for product identification purposes as well as for the preparation of controlled release dosage forms through the application of a rate- controlling polymer membrane to crystalline drug particles, pellets, granules, and pharmaceutical tablet dosage forms. While coating with organic polymeric solutions is still widespread, due to potential environmental safety and toxicity problems associated with some organic solvents, the pharmaceutical industry has been exploring alternatives to organic based tablet coating formulations. Aqueous polymeric dispersions have drawn much attention to overcome the problems in organic polymeric solutions such as; high solvent costs, explosion hazards, potential toxicity, and solvent recovery in production scale.

One of the methods to achieve coating involves spraying the polymer organic solutions onto the dosage forms in coating chamber followed by the solvent evaporation. Several problems such as explosion and health hazards are associated with organic solvents, and therefore, the solvents such as acetone, methylene chloride and methanol must not be expelled into environment (Cole, 1995). The solvent recovery costs escalated steeply, leading to the substantial economic impact on the dosage form manufacturing. Therefore, scientists have shifted the focus from organic based to aqueous-based coating. As a result, several aqueous coating systems such as Aquacoat ® from FMC Corp., and Surelease ® from Colorcon, Inc. are now commercially available. However there is a still need to discover ne w aqueous coating systems. In this research potential use of silk fibroin as an aqueous coating system was investigated.

2.4 PROPERTIES OF THEOPHYLLINE AS A MODEL DRUG

In this study, theophylline anhydrate was used a model drug. Theophylline is one of the most commonly used medications for the treatment of the symptoms of chronic asthma. Theophylline occurs as a white, odorless, crystalline powder with a bitter taste. Molecular formula of anhydrous theophylline is $C_7H_8N_4O_2$ with a molecular weight of 180.17 and molecular structure is shown in Figure 2.5.

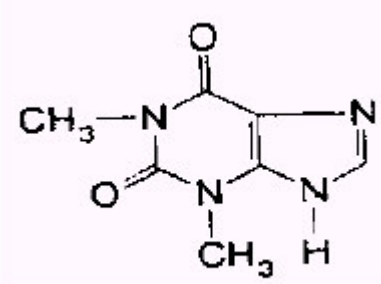


Figure 2.5. Molecular structure of theophylline.

Methyl xanthines have been employed in the treatment of asthma for more than a century, initially in the form of strong coffee. Today the xanthine usually employed in clinical medicine is theophylline. Theophylline promotes diaphragmatic contractility and mucociliary clearance, aids cardiac functions and lowers pulmonary artery pressure.

However, theophylline has the potential for significant adverse effects. Appropriate dosing is essential, as theophylline has a narrow therapeutic index which lies in the range 30–100 μmol l^{-1} , with toxic effects likely at concentrations greater than 110 μmol l^{-1} , Plasma con-centrations in excess of 200 μmol l^{-1} result in serious cardiovascular and central nervous system (CNS) effects, the most serious being arrhythmia, which can be fatal. Theophylline is routinely administered in the form of oral sustained release preparations in order to maintain effective plasma concentrations for periods as long as 8 h. More efficient systems, however, are clinically desirable.

Theophylline was selected as a model drug because it is nearly neutral and has a low aqueous solubility of 8.3 mg/ml in water. Theophylline is an anti-asthmatic with a short half-life; hence there is a therapeutic rationale in formulating it for controlled-release applications (Mickael, 1998).

2.5 RELEASE KINETICS

The release kinetics of the drugs depends on three primary mechanisms by which the release of active agents can be controlled: erosion, diffusion, and swelling followed by diffusion. Erosion may take place due to hydration or hydrolysis of the bulk, the polymer being slowly degraded starting at the periphery of the tablet. Diffusion can occur through the unhydrated polymer matrix but will generally be facilitated as the polymer gradually swells in contact with the body fluids (Peppas , 1995).

The release kinetics from the most systems that depend on membrane diffusion can be grouped in three profiles:

- zero-order kinetics
- $t^{-1/2}$ kinetics
- first order kinetics

In zero-order kinetics, the release rate remains constant until all of the active ingredient has been delivered. The term zero-order means time-independent rate and so as being independent of quantity of drug remaining. In $t^{-1/2}$, the release rate decreases proportionally to the square root of time in controlled release. The release rate is proportional to the quantity of drug remaining in the first order kinetics (Figure 2.6).

For both $t^{-1/2}$ and first-order systems most of the active ingredient is released during the first third of the total duration of release (Peppas, 1997).

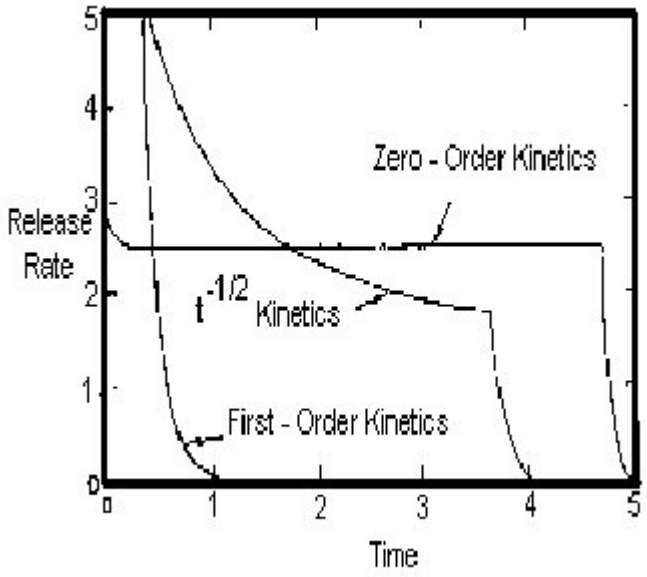


Figure 2.6. Comparison of release kinetics from conventional (first-order) and controlled-release formulations (Herbig, 1992).

Dissolution data were analyzed using Equation 1 proposed by Ritger and Peppas to describe the mechanism of drug release.

$$\frac{M_t}{M_\infty} = Kt^n \qquad (1)$$

Where M_t corresponds to the amount of drug released in time t, M_∞ is the total amount of drug that must be released at infinite time, K is a constant and n is the release exponent indicating the type of drug release mechanism. If n approaches to 0.5 the release mechanism can be Fickean. If n approaches 1, the release mechanism can be zero order and on the other hand if $0.5<n<1$ non-Fickean transport could be obtained (Samani et al., 2003).

Pather et al.,2003 investigated the direct compression sustained release formulations. The theophylline to ethylcellulose ratio and the tablet hardness were found to influence

the rate of drug release. It was found to be possible to sustain the release of a therapeutic dose of the theophylline over 12 hours by changing compression force and theophylline to ethylcellulose ratios. It was observed that zero-order kinetic could not be achieved.

Graham et al., 1998 used electron microscopy and high performance liquid chromatography to quantify the effects of formulation changes on the phase inversion dynamics and in vitro drug release properties of PLGA based drug delivery systems. Gel growth rates and water influx rates were determined from plots of the square of the respective front motion with time. They found that the additives which accelerated the solution gelation rate at constant morphology resulted in high initial release rate. Conversely, additives that slowed the rate of gelation dramatically was observed to reduce the initial drug release rate and leaded to more dense sponge-like morphology.

CHAPTER 3

SILK FIBROIN

Silks are generally defined as protein polymers that are spun into fibers by some *lepidoptera larvae* such as silkworm, spiders, scorpions, mites and flies. Silks differ widely in composition, structure and properties depending on the specific source. The most extensively characterized silks are form of the domesticated silkworm, *Bomyx mori* which is the most abundant and is therefore obtained easily and cheaply (Gregory, 2002).

Silk protein consists of two kinds of proteins, silk fibroin and silk sericin (glue-like protein). The ratio of silk fibroin to silk sericin is about 3:1. Both proteins consist of 18 amino acids, about 85% of them are Glycine, Alaline, Serine and Tyrosine. (Joseph,1998). Many studies on amino acids indicated that Glycine had an effect on reducing the cholesterol level in blood, Alaline could accelarate alcoholic metabolism and Tyrosine served the function of preventing senila dementia. Also it was shown that various degrees of peptide chains in size made of many kinds of amino acids had tangible results of adjusting the physiological action and the immunity system (Luo, 1997).

The primary structure of silk fibroin depicted as (-Gly-Ala-Gly-Ala-X-)n where X=Ala or Ser. Most of the component amino acid residues are hydrophobic, while some sequences including hydrophilic residues were also isolated. Therefore silk fibroin is regarded as a block-type copolymer composed of both hydrohobic chains (crystalline region) and hydrophilic chains(amorphous region). Its average molecular weight is about 300000 (Tsuruta, 1999). Amino acid content for fibroin is shown in Table 3.1.

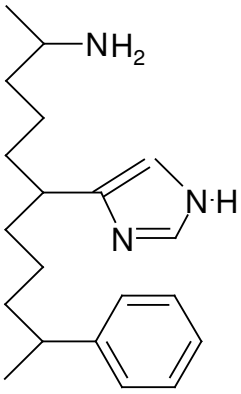


Figure 3.1 Molecular structure of fibrion.

Table 3.1 Amino –acid content, g/100g fibroin

Amino-acid	Li et al.	Mukhamedzhanova et al.
Lys	0.63	0.51
His	-	0.3
Arg	0.95	1
Asp	2.43	2.75
Thr	1.09	1.25
Ser	**9.91**	**13.18**
Glu	1.3	2.09
Pro	-	0.53
Gly	**35.08**	**33.34**
Ala	**30.75**	**26.61**
Val	4.26	2.62
Ile	1.59	0.87
Leu	1.05	0.67
Tyr	8.03	11.42
Phe	1.24	1.2
Met	1.37	-

Macramolecules of fibrillar (fibrous) proteins like fibroin have porous ball conformations in aqueous solutions. These are formed by complicated constructs in that combine in concentrated solutions into complex supermolecular associates. Destruction of the regular structure of the macromolecular fibroin chain during chemical and physical modifications, decrease the molecular weight, changes and decrease the strength of intermolecular interactions, and changes the structure of concentrated solutions.

Silk fibroin is a protein of a natural origin with beta structure. The structural layers in silk thread are bonded to each other only through Van-der-Waals interactions which makes the fibroin flexible. The polypeptide chains in silk fibroin are situated antiparallel. Along the plated beta sheet structure, there are also amorphous regions in the fibroin in which bulky residues of Tyr, Val, Arg. The amorphous region increases the extensibility while diminishing elasticity. The silk film cast from aqueous solution consists of alpha form and beta form (Mukhamedzhanova, 2001).

It is known that besides material for clothing, fibroin has been widely applied to cosmetics, medicine, food and chemical industry, particular in the field of food. Owing to the structure and composition of fibroin, it is entirely possible to develop fibroin into fashionable food of late which possesses specific function (Luo, 1997).

Recently, several researchers have been investigated SF as one of promising resources of biotechnology and biomedical materials due to its unique properties including good biocompatibility, good oxygen and water permeability, biodegradability, and minimal inflammatory reaction. Dissolution applications are demanded in the forms of film, porous membrane, powder, and gel among others (Um, 1997).

Minoura et al., (1994) found that silk fibroin had high dissolved-oxygen permeability and high water-vapor permeability. Therefore they reported that silk fibroin had the potential to find applications in the development of soft contact lenses, artificial skin substitutes, and so on. Because of the permeability characteristic, the silk fibroin might lead to the application of silk fibroin film in the development of controlled drug-delivery devices that respond to the conditions found in the body.

Benefits with the use of silks for biomedical applications:

- novel mechanical properties that are superior to any other natural fiber and rival many high performance polymers,
- natural fiber with a long standing history of use in clinical applications,
- the ability to process silks in aqueous solutions for subsequent formation of films and other material formats, with relatively simple insolubilization via exposure to alcohols and environmental factors,
- easily chemicals modified with surface decorations, such as adhesion sites or cytokines, due to availability of amine and acid side chains on some of amino acids.
- genetically tailorable composition and sequence to moderate specific features, such as molecular weight, crystallinity, solubility,
- no known risk of bioburden.

Silk fibroins can be processed into various forms including gels, powders, fibers, and membranes (Figure 3.2). Although silks have been used for more than 5000 years, interest in membranes has grown only within the last few decades.

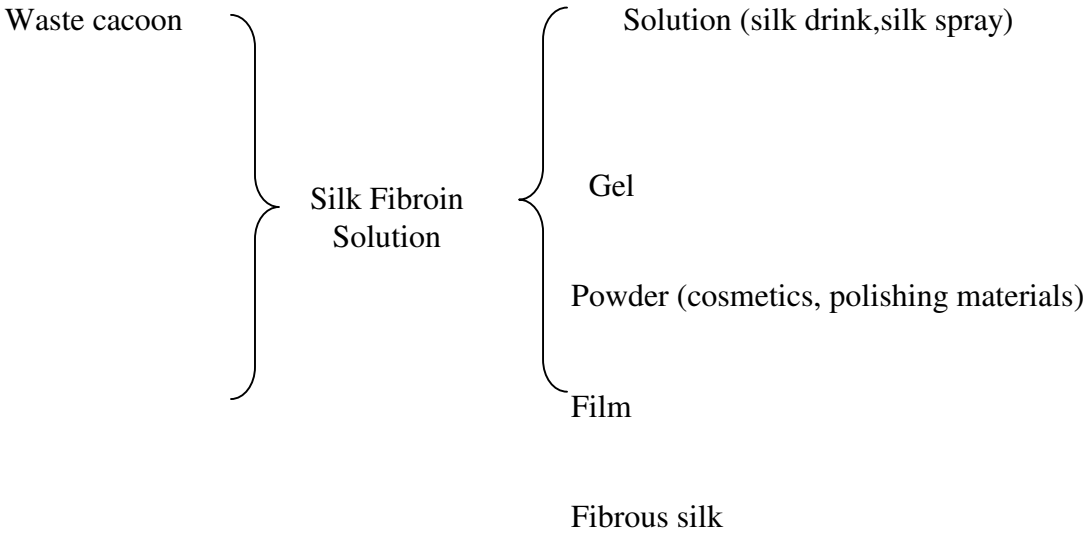


Figure 3.2 Applications of silk fibroin solution

Transparent membranes of silk fibroin have been processed with liquid silk from the gland, as well as solutions made with silk fibers dissolved in alkali salt solutions, other types of solutions, and organic solvents. Blend solutions of fibroin with cellulose, polyvinyl alcohol, polyurethane, cellulose acetate, chitosan, sodium polyglutamate, polyethyleneglycol, and sodium alginate have been used. A variety of processes have been employed. These include casting followed by air-drying or free drying, mechanical shearing, compression, and bubble blowing. Processing conditions such as solution concentration, solution temperature, quenching temperature, drying temperature, drying time, the presence of electric field, Ph, the presence of certain enzymes and the type of solvent can be used to control the molecular conformation in the resulting membrane. Casting onto different surfaces such as polyethylene, glass, poyltetrafluoroethylene, polyproplylene, polycarbonate, polystyrene, acrylics, polyvinylchloride also can affect the conformation.

Post processing treatment parameters have included: dry heat, steam heat, stretching, stretching rate, stretch ratio, and the application ratio. Other parameters included the application of hydrophilic polar organic solvents and their solutions, application of salt solutions and hot water as well as application time.

Membranes have been made for a variety of reasons. One is to hold various molecules such as different enzymes for application in various bio-sensors and other detectors. (Putthanarat, 1995),

Putthanarat et al.,1995 observed the effect of processing temperature on morphological data of the silk membranes. The silk gland of *Bombyx mori* was cast onto glass plates at 20, 50, 60, and 80 ^{o}C. Silk from the anterior and posterior sections was cast at 20^{0}C. Samples cast at 20 ^{0}C exhibited particles, grains, nanofibrils and an irregular morphology. Each exhibited approximately the same dimensions for all the samples. Samples casted above 20 ^{0}C were observed to be not exhibit the irregular morphology. Samples casted above 50 ^{0}C had larger grains and more densely packed nanofibrils. They concluded that all that changes might result from conversion of the amorphous structure to the beta-plated structure. The nanofibrils appeared to be self-assembled bio-nanofibrils. And also the packing densities of the 60^{o}C and 80^{o}C nanofibrils were found to be greater than those for 20 and 50^{o}C.

Tsukada et al., 1999a investigated dissolved oxygen permeability, water content, crystalline structure, crystallinity, and the density of silk fibroin membrane as a function of immersion time of the membrane in 50vol% aqueous methanol solution. Permeability coefficient and water content was observed minimum at the treatment time of 30 min. Also after treatment of methanol, the silk crystalline structure was seen. They indicated that the density of the membrane decreased with increasing treatment time, although the crystallization proceeded in the membrane which defining that an existence of roughly molecular-packed space among the crystals. The appearance of the minimum for water content was attributed to the development of the crystal and to the growth of the roughly molecular-packed space. Tsukada et al., 1999b also reported that the membrane had oxygen permeability, water vapour permeability, transparency and biodegradability.

Um et al., 1997 compared the structural characteristics and solution properties of the regenerated silk fibroin prepared from formic acid (FU) with the SF from water (AU). According to the turbidity and shear viscosity measurement, SF formic acid solution was seen stable and transparent and no molecular aggregation was occurred. The sample FU was observed beta structure while AU random coil conformation by using FTIR, X-ray diffraction, and differential scanning calorimetry. The effects of methanol treatment

on the samples were also examined. According to the measurement of XRD and FTIR, long/short-range ordered structure formation was observed. Long-range ordered crystallites were predominantly formed for methanol treated SF film while SF film cast from formic acid favored the formation of short-range ordered structure.

Magoshi et al., 2000 clarified the crystallization mechanism of silk fibroin from silk fibroin solution by using X-ray diffraction and differential scanning calorimetry. By evaporation of water from liquid silk, they determined that silk fibroin crystallized when weight loss of water reached 40% at the temperature below 120^{o}C and also reported that in the DSC curve
of liquid silk of domestic silk *Bombyx mori*, the alpha to beta transition occurred at 51^{o}C (Figure 3.3).

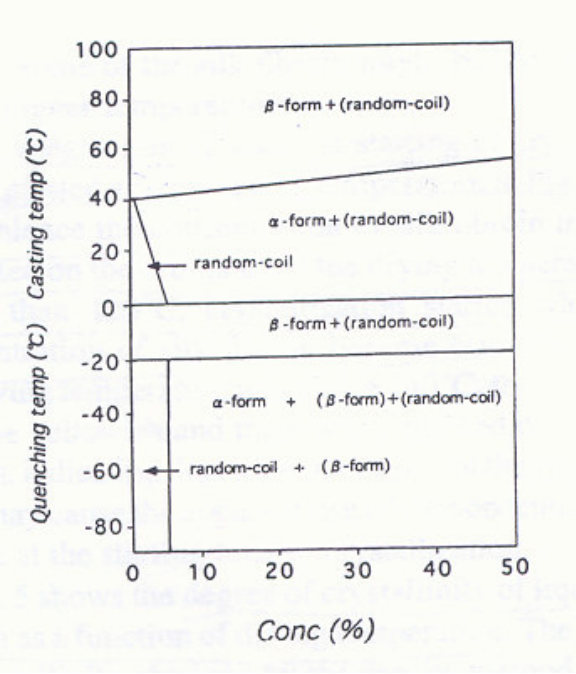


Figure 3.3 The relation between conformation, quenching or casting temperature and concentration of silk fibroin.

Kang et al., 2000 studied the influence of the concentration of poloxamer ($EO_{97}PO_{69}EO_{97}$; EO=Ethylene oxide and PO= propylene oxide), the pH and the temperature on the gelation of silk fibroin. It was found that the gelation of SF occurred in the presence of poloxamer at pH value of 7.0 while gelation did not occur. The

gelation time of SF was shortened with increasing the poloxamer concentration and the temperature. The sol-gel transition of SF became reversible with addition of poloxamer. From IR, it was found that a conformational change of the SF in the SF/poloxamer system from random coil to beta structure was accelerated after forming a polymer complex with the poloxamer. Also it was reported that the crystallinity of the poloxamer was reduced SF from X-ray diffraction measurements.

Minoura et al, 1994 dealt with the preparation and morphological characterization of porous materials silk fibroin solutions. They prepared silk fibroin materials with a variable extent of porosity in different ways, either by changing temperature at which liquid silk is frozen and the pH of the starting fibroin solution by adding some amount fixed amount of organic solvents (methanol) to liquid silk. They confirmed that the average pore size decreased by lowering the freezing temperature at which the silk fibroin aggregates exhibited sheet like structure. By lowering the pH from neutrality to 4.01 and 2.65, the morphology of the solid phase was observed to change from a sheet like to fiber structure. Also they reported that addition of different amounts of methanol to the silk solution hardened the material and decreased the average pore size which exhibited a typical fibrous structure. From the obtained parameters, they incorporated acetylsalicylic acid into a porous silk fibroin carrier. The drug was not covalently bonded to the polymeric substrate, but only physically dispersed into it. The active agent was found to be rapidly diffuse into the internal solution within the first two hours and then during the first stage, attaining a rate of diffusion that remained constant almost constant for several hours. They concluded that their preliminary results demonstrated the potential suitability of porous silk as a carrier for preparation of drug delivery system.

3.1. SF/PEG MEMBRANES

SF was blended with polymers in order to overcome the drawback of SF itself by improving mechanical properties (Um, 1997). So that SF has been blending by agents like sodium alginate, polyvinylalcohol, cellulose, and PEG to improve the properties.

PEG is well known to be non toxic, non antigenic and biocompatible, to be soluble in water and most organic solvents and by itself to have solubilizing properties. Also it is suitable for use as drug carrier in the body and rapidly eliminated form the body. In addition to this, it is reported in the literature that proteins are often modified with PEG to reduce the immunoreactivity and immunogenicity of the proteins (Salamone 1996).

Demura et al., 2000 prepared porous membranes of Bombyx mori silk fibroin by removal polyethyleneglycol from a silk fibroin-PEG membrane in order to obtain high substrate permeability. The structure characterization of the porous membrane was investigated by nuclear magnetic resonance spectra and scanning electron microscopy. According to the NMR spectra obtained, the fraction of the Silk II, which exists as anti-parallel beta sheets in the porous silk fibroin membrane, was observed to be 60-65%. An increase of the permeability of glucose and NaCl by a factor of more than 20 was observed when the weight ratio of PEG to silk fibroin was increased to 3.0. The NMR spin-lattice relaxation times for water in the porous silk fibroin membranes were analyzed as two components (14-26 msec) and slow (274-1385 msec), suggesting constrained motion of the water molecules in the NMR time scale in the silk fibroin membrane. The logarithmic permeability coefficients of glucose and NaCl decrease linearly with increasing relaxation rates of the slow components of the water. Glucose oxidase was immobilized with these porous membranes, and a sensing system for monitoring the glucose concentration was constructed. They reported that glucose concentration data declared that simultaneous reaction and diffusion control occurred in the membranes.

Baba et al., 1997 developed silk fibroin-poly(ethylene glycol) conjugate films. They also examined the mechanical properties of the films. PEG and SF were reacted in the aqueous solution containing 0.1M sodium borate as modifier. After the reaction took place, the membranes were cast on polyethylene films. An SF having a beta sheet

structure was prepared by immersing the film in a 50% (v/v) methanol-water mixture for 4 h at room temperature and drying the SF film at ambient relative humidity at room temperature. They observed a random coil conformation with small amounts of beta sheet and helix structures and also reported that Sf film exhibited only endothermic peak at DSC, while the untreated film had an endothermic peak at 228 °C attributed to the transition from random coil to beta sheet.

Gotoh et al., 1997 investigated the physical properties of the poly(ethylene glycol)-silk fibroin conjugate films. The circular dichroism spectrum exhibited that the conjugate film exhibited both negative and positive extrema while the silk fibroin showed two broad negative bands at about 205 and 220 nm (Figure 3.4). These results confirmed that silk fibroin possessed a random coil confirmation and the conjugate film had a β-sheet structure. Also circular dichroism results indicated that the introduction of the PEG chains into silk fibroin induced the formation of a β-sheet structure. It was estimated that the introduced PEG chains extended perpendicular to the β-sheet plane of silk fibroin without hindering the formation of a β-sheet and form of the PEG-rich phases. The tensile properties of silk fibroin having PEG could be explained by supposing that the sliding between silk fibroin and sheet planes took place due to the ductile PEG-rich phases. Based on these results, the layered structure of the silk fibroin having PEG is shown in Figure 3.5.

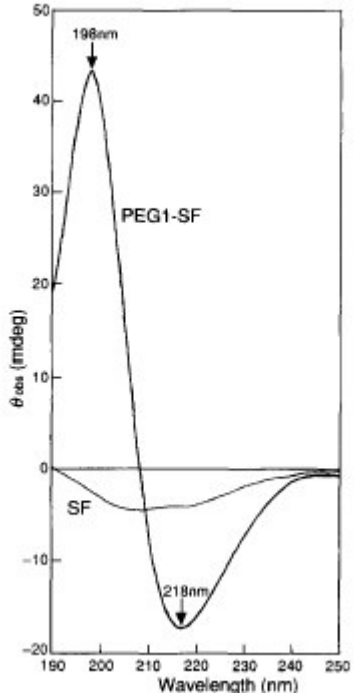


Figure 3.4. Circular dichroism of the silk fibroin and PEG-silk fibroin film.

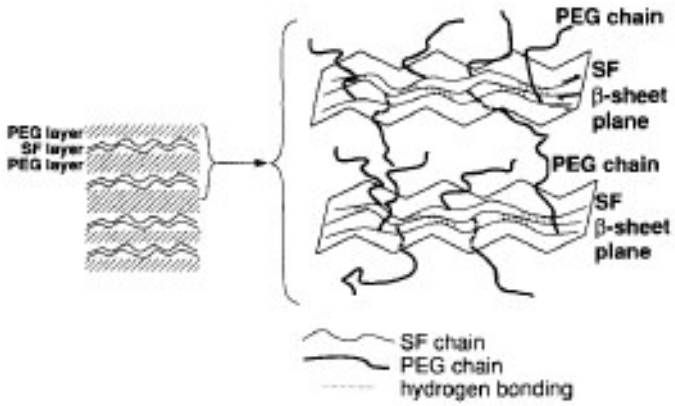


Figure 3.5. Schematic representation of the PEG - silk fibroin conjugate film

In this study PEG was used as plasticizing agent to improve the mechanical and film-formating properties of the silk-fibroin film.

3.2. SF/EDC MEMBRANES

Carbodimides are members of family of zero-length protein cross-linking reagents, which promote the formation of covalent cross-links between side-groups of amino acids, but do not remain as part of the cross-links. Carbodiimides are available in variety structures; however, due to their solubility in water, two particular reagents have been utilized to cross-link collagenous bioprothetic materials; cyanamide and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). These reagents have advantages over industrial standard cross-linking compounds like glutaraldehyde in that:

i-) They do not remain as part of the cross-link, thus precluding depolymerization and possible release of potentially cytotoxic reagent;

ii-) They produce water soluble reaction by-products; which can be easily removed during routine rinsing tissue (Gratzer, 2000).

Park et al., 2002, investigated a scaffolding material for tissue regeneration. For this purpose, they prepared a porous matrix of hyaluronic acid and collagen. Cross-linking was achieved using water-soluble EDC which crosslink collagen and HA by the amide and ester bond formation of side groups on them. It was reported that carboxyl groups of proteins was converted into amides with EDC (Figure 3.6). They immersed the fabricated membrane of collagen and HA into 1-100mM of EDC. FTIR was used to investigate the extent of cross-linking. It was observed that EDC was very efficient at concentrations higher than 10mM.

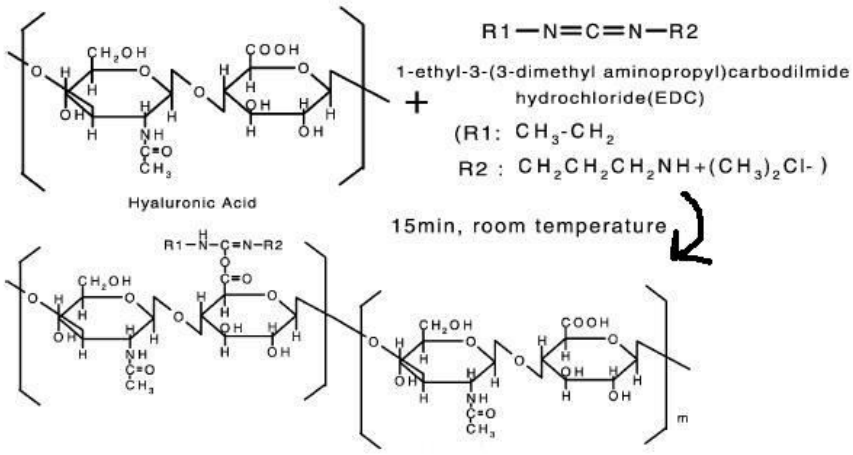


Figure3.6 Schematic Presentation of Crosslinking HA with EDC.

Tomihata et al., 1996 crosslinked hyaluronic acid with EDC to produce low water-content films when brought into contact with water. The crosslinking reaction was performed in two different ways; one was by using HA films and the other by casting HA solution.

Kim et al., 2003 studied the characterization of zein films. They crosslinked the zein molecules with EDC to alter the brittle form of film. The water-soluble carbodiimide EDC was used to form covalent conjugates via amide bonds They reported that the film forming properties, flexibility and tensile strengths of the zein films were improved.

Tropini et al., 2003 also used EDC as crosslinking agent to improve the film forming properties of wheat gluten. They investigated the swelling of films in water and the mechanical properties. They reported that by crosslinking the tensile strength was increased where lowest swelling was achieved.

Ofner et. al.,1996 crosslinked the amino groups of gelatin and used a chemical detection method in order to determine the extent of crosslinking. They observed that EDC was not participate in the bond itself. This is an advantage over glutaraldehyde which places a distance between linked chains.

Recently, preparation and characterization of EDC crosslinked SF films are being studied for their potential use in iontophoric trandermal drug delivery (Malay,2004). In this thesis, EDC crosslinked silk fibroin was used as an aqueous based tablet coating material.

CHAPTER 4

MATERIALS AND METHOD

4.1 MATERIALS

Theophylline anhydrate was used as a model drug which was supplied from Sigma-Aldrich.

The cocoon silk of *Bombyx mori* was obtained in reeled form from Bursa Institute for Silkworm research.

1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDC) was used as a crosslinking agent and supplied from Fluka Chemie. Molecular weight of the material is 191.7 g/mole. The molecular structure of water soluble EDC is shown below.

$$R1\text{-}N{=}C{=}N\text{-}R2$$

R1: $CH_3\text{-}CH_2$ R2: $CH_2CH_2CH_2NH + (CH_3)_2Cl^-$

Polyethyleneglycol (PEG) was soluble in water 100% by weight. It was used as a plasticizing agent to improve the film property. The molecular structure of this material is shown as;$H(OCH_2CH_2)_nOH$. Molecular weight is between 3500-4500 g/mole. It was supplied from Merck.

4.2 METHOD

Experimental methods used in this study include theophylline tablet preparation, silk fibroin solution preparation, tablet dip-coating, dissolution studies and characterization. The experimental procedures followed are shown in Figure 4.1.

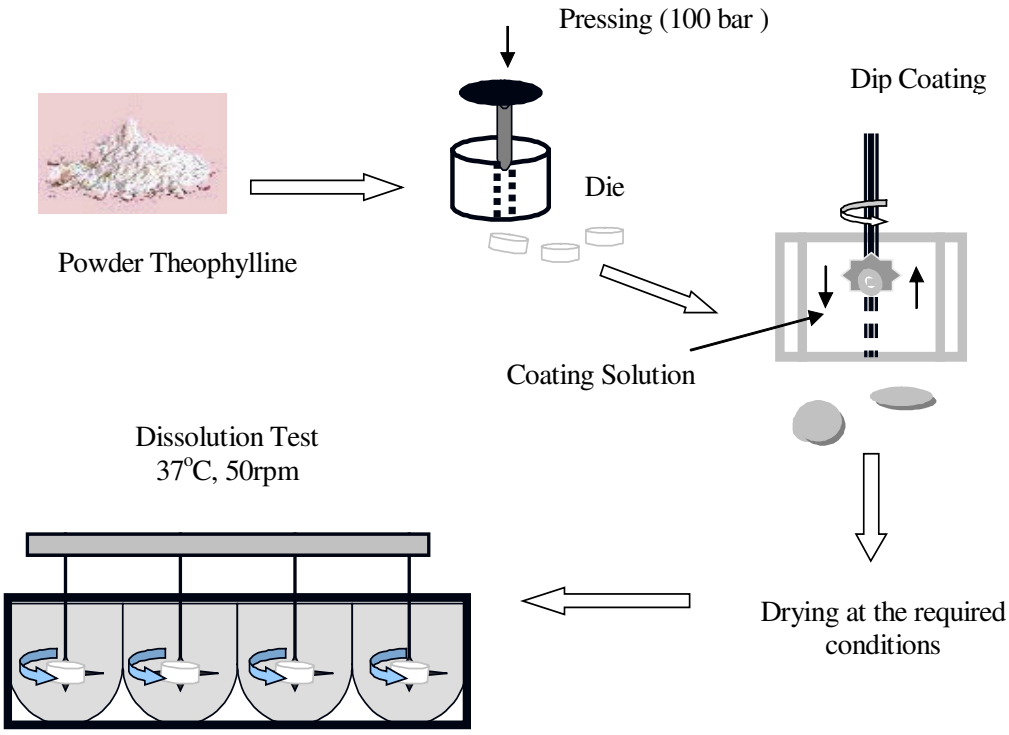


Figure 4.1 Experimental procedure followed in this study.

4.2.1. Tablet Preparation

In this study, powder theophylline was pressed in the die which has diameter of 1.2 cm. To produce 400 mg tablets which having cylindrical shape, were pressed at same pressure of 100 bar.

4.2.2. Preparation of Silk Fibroin Solution

To handle the silk fibroin solution, raw silk was treated three times in boiling 0.5 wt % Na_2CO_3 solution (98 ^oC) for 1 hour to remove the sericin (degumming). At each batch the silk was rinsed with distilled water.

Degummed silk was dissolved in a ternary solvent system of $CaCl_2$ / H_2O / EtOH solution (1:2:8 mole ratio) at 78^0C for 2 hours with shaking in water bath.
The ratio of degummed silk to solvent was 1: 20 (weight/volume respectively). This solution was dialyzed for 3 days at 4 ^oC in cellulose tubing with a molecular weight cut-off value of 12000 against deionized water to remove $CaCl_2$ and ions. The ions check

test was done by silver chloride. Then the solution was concentrated to 10% (w/v) at the rotary evaporator at 45°C, at 60 rpm.

4.2.3. Preparation of Heat Treated Fibroin Solution

The goal of the heat treatment is known to cause β sheet transformation to occur.

After the prepared tablets were coated with the fibroin solution, they were dried at 45°C to make the membrane insoluble. The samples were dried at the ventilated oven for one day without controlling the relative humidity.

4.2.4. Preparation of Fibroin Solution treated with PEG

Polyethyleneglycol was added to the concentrated fibroin solution in the Petri dish at different ratios. The tablets were also coated with these solutions (12.5%,17% 25 % PEG weight/volume) and dried at 45°C for one day without controlling the relative humidity.

4.2.5. Preparation of SF/EDC Solution

5 ml 20% (w/v) fibroin solution was mixed with 5 ml 20 mM EDC in a Petri dish. The tablets coated at the same conditions with dip-coater device. To see the effect of coating thickness on release rate of the model drug, after the tablets were dried at the ambient conditions, some of the tablets were coated successfully to form a thick coating. All coated tablets were left to dry at the ambient conditions.

4.3. DIP-COATING

Coating processes was carried out using by a dip-coater device (Nima Dip-Coater) as shown in Figure 4.2. Coating parameters used in Table 4.1

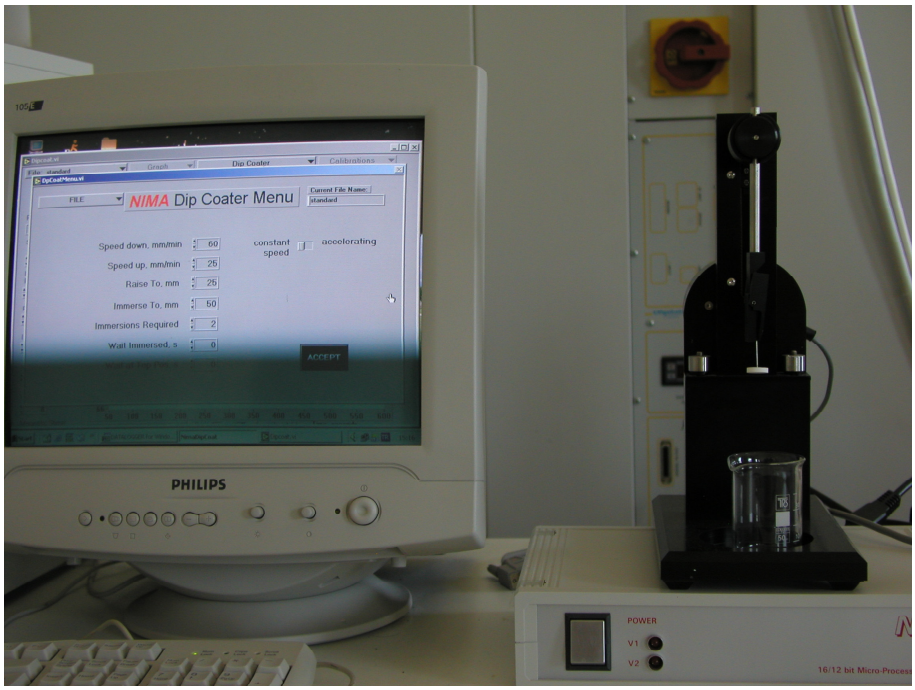


Figure 4.2. Dip coating device.

Table 4.1 Coating parameters

Speed down	150 mm/sec
Speed up	150 mm/sec
Raise to	50 mm/sec
Immerse to	755 mm/sec
Immerse required	10
Waited immerse	4 sec
Waited at top	4 sec

The experimental part of this study includes tablet and silk fibroin preparation, tablet coating, dissolution studies and characterization. The set-up is shown in Figure 4.1.

4.4. DISSOLUTION STUDIES

Dissolution testing is an essential requirement for the development, establishment of release of the model drug. The United State Pharmacopeia XXIII (USP XXIII) standards was used in performing in vitro dissolution studies.

Caleva dissolution device was shown in Figure 4.3 used for dissolution tests. Device has 8 vessels with stirrers. Temperature of the medium, paddle rotation speed and dissolution time was adjusted by a controlling unit of the device. Tablets of each formulation were tested in 900 ml of distilled water maintained at 37 $^{-+}$ 0.5 ^{o}C using a water bath according to standards.

Figure 4.3 Caleva dissolution test apparatus

Drug is expected to remain in stomach 2 to 4 hours and in the small intestines for 4 to 10 hours. The pH of the stomach varies from 1 to 3 and increasing to between pH 7 and pH 8. In this study pH of the dissolution medium was kept at 3 during the first 3.5 hours and then it was increased to 7.4 to simulate the gastrointestinal track (GIT). To reduce

the pH , 8.5 % phosphoric acid was used. 5.3 M NaOH was added to increase the pH to 7.

The samples were taken from the dissolution medium at certain times. The theophylline was determined at 272nm wavelength using a spectrophotometer (Shimadzu, UV-1601).

4.5. CHARACTERIZATION

The characterizations of the samples were performed by scanning electron microscopy (Philips,-30S FG). The morphology and the information about the thickness of the coating was determined by SEM micrographs.

CHAPTER 5

RESULTS AND DISCUSSION

In this section, release data of theophylline tablets which were coated with three different types of coating solutions (heat treated fibroin, fibroin-PEG solution, and fibroin crosslinked with EDC solutions) were evaluated.

Equation 1 is termed the power law model, with where n being diffusional exponent. In this equation M_t and M_∞ are the amounts of drug released at time t and the total amount of drug released, repectively. K is a release constant and is releated to the structural and geometric properties of the dosage form and, n is the release exponent which gives an indication of the mechanism of the drug release (Peppas,1981).This equation has been used frequently in the literature , due to its utility in decscribing the relative importance of Fickian (n=0.5) and zero-order drug mechanism in drug diffusion (see section 2.4).

$$\frac{M_t}{M_\infty} = Kt^n \qquad (1)$$

In this thesis, the mechanism of drug release from the uncoated and film coated tablets was evaluated by means of equation 1.

5.1. COATING WITH HEAT TREATED SF

Processing conditions such as solution concentration, solution temperature, drying temperature, drying time, the presence of electric field, pH, the presence of certain enzymes and type of solvent can be used to control the molecular conformation of the resulting SF membrane.

Insolubilization of membrane around the tablet is required to achieve sustained release of model drug theophylline. After coating the prepared theophylline tablets using SF solution the tablets were dried at 45 °C in a static oven in order to convert the structure from random coil to β sheet form. The conformational change in SF structure was investigated earlier in the literature (Magoshi et al., 2001). By achieving β sheet transformation of the silk fibroin, insoluble membranes could be obtained. To

investigate the drug release kinetics, dissolution tests of the tablets coated with heat treated silk fibroin were performed. The release profiles have been interpreted according to the target profile. The uncoated theophylline tablet, tablet coated with heat treated silk fibroin and the target release profile is shown in Figure 5.1. Dissolution data of uncoated theophylline tablet showed 100% drug release at the end of 210 min. As seen from Figure 5.1, release profile was far from the desired target profile. To sustain the release tablets were coated with heat treated silk fibroin. For this case drug release was sustained to a certain extent up to 100 min, then followed almost similar release pattern with the uncoated theophylline tablet since the SF coating around the tablet partially peeled off at the end of 100min of the dissolution test. As shown in Figure 5.1 the curve which was plotted with fibroin coated theophylline is closer to target linear profile than the uncoated tablet. The K, n, values and regression coefficients of the curves for the tablet coated with heat treated silk fibroin were evaluated for two regions (Table 5.1). In the first region which is up to 34% release, the diffusion occurred through the channels of the membrane. In the second region the coating around the tablet was peeled off, the release occurred without facing any diffusional barrier. Therefore, release rate was enhanced and it was comparable to that of uncoated tablet (Figure 5.2c).

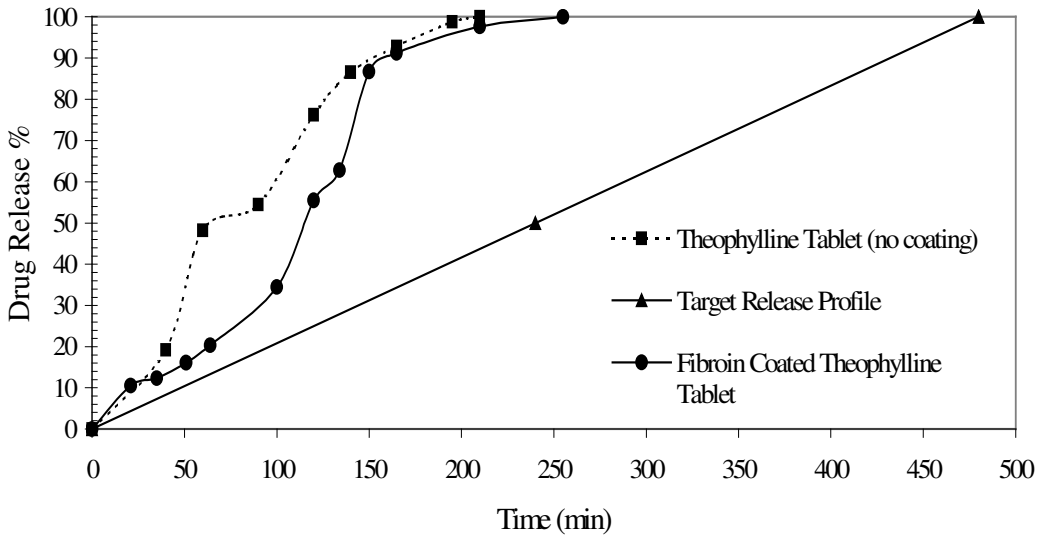
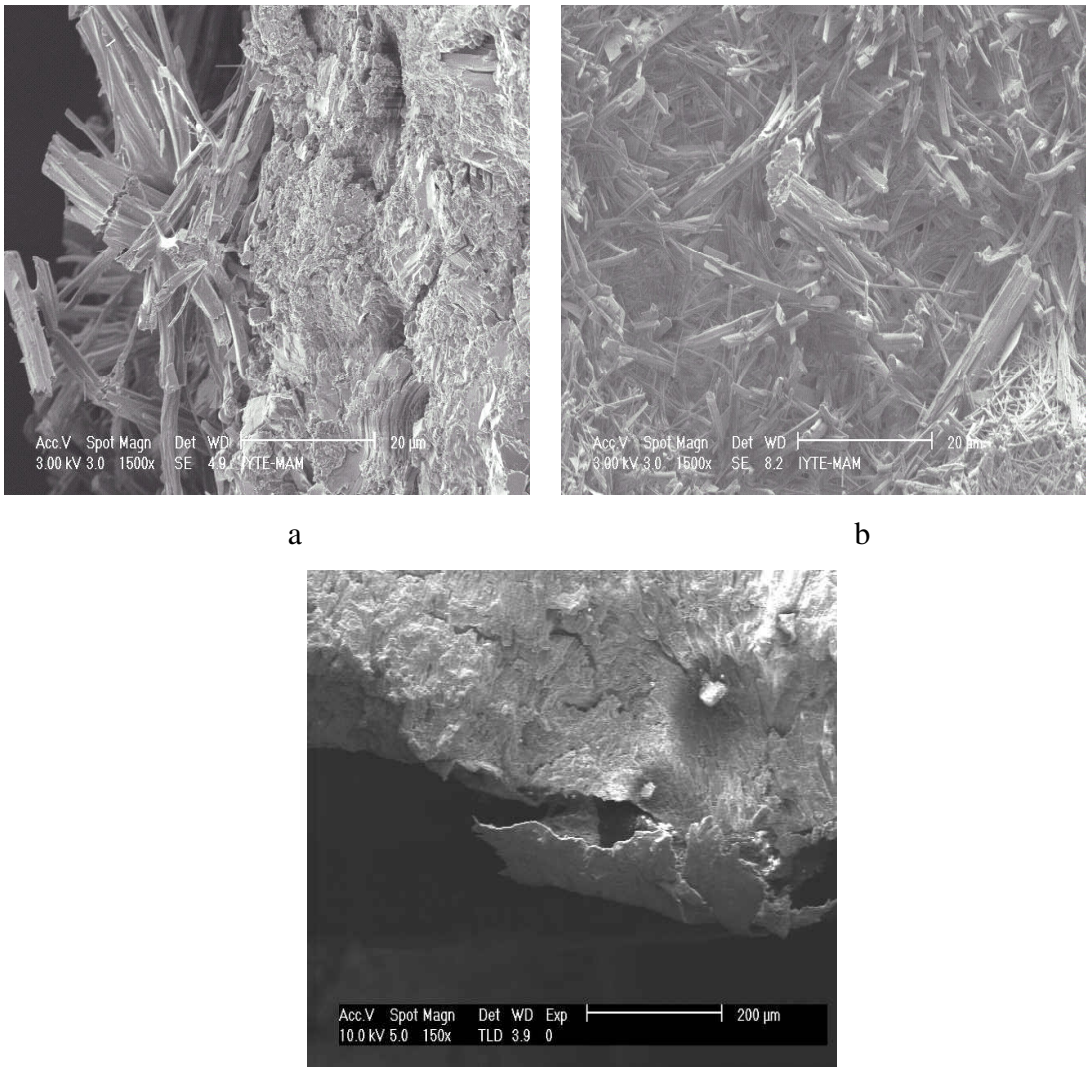


Figure 5.1 Release profile of the theophylline tablet coated with heat treated SF.

Table 5.1. K and n values of the theophylline tablets coated with heat treated SF.

Sample	K	n	R^2	Drug release mechanism
Tablet coated with heat treated SF at the end of 34% release	0.5	0.97	0.98	Zero order
Tablet coated with heat treated SF at the end of 100% release	0.7	0.49	0.91	Fickean

SEM micrographs of heat treated silk fibroin were obtained to characterize the silk fibroin membrane. Heat treated silk fibroin coating possess a crystalline structure. In literature crystalline structure of silk fibroin membranes dried at high temperatures were mentioned (Kuduğ, 2004, Putthanarat, 2002). As shown in Figure 5.2b, the heat treated fibrous coating also have a surface with varying porosity and channels where diffusion mechanism occurred within these channels. In polymer coatings, solute transport might occur via a “pore” mechanism or “partition” mechanism. (Figure 5.2.a and 5.2.b). In practice, drug permeation probably occurred by both mechanism but one was more likely to predominant.



a b

c

Figure 5.2. SEM micrographs of theophylline tablets coated with heat treated silk fibroin a; cross section of tablet, b; surface tablet c; cracked off silk fibroin coating around the tablet

Since the heat treated silk fibroin coating possessed a brittle structure which probably cause poor film forming and poor interaction between SF coating and theophylline particles (Figure 5.2.c), fibroin films in dry state become unsuitable for practical use. The properties can be improved by blending with other natural or synthetic polymers. Silk blends have been extensively studied with respect to film formation. Blends with polyacrylamide, sodium alginate, cellulose, chitosan, poly(vinyl alcohol), poly(ethylene glycol) were investigated for their stability or membrane properties.

In order to improve film making property of SF, PEG was used as a plasticizer in the SF coating solutions.

5.2. COATING WITH SF/PEG SOLUTION

In this part of the study, silk fibroin and polyethylene glycol solution was used as a tablet coating solution. The kinetics of drug release was investigated, after coating theophylline with the SF solution having different amount of PEG. The release profile obtained can be seen in Figure 5.3. While the uncoated sample dissolved almost completely at the end of 210 min, the tablets coated with the SF solution having 12.5% and 25% PEG dissolved completely at the end of 300 min. The tablet coated with 17% PEG-SF solution requires a longer release time (345 min for 70% release) and release rate is slower than the others. In addition to sustained release, the release profile is closer to target release profile than the others. Circular dichroism results in literature (Yohko et al, 1996) indicated that the introduction of PEG chains into silk fibroin induced to formation of a β sheet structure. According to results in this study, it was indicated that the formation β sheet structure was not increasing in direct proportional to almost amount of PEG. The tablet coated with SF having 25% PEG showed similar release profile with the tablet of coated with 12.5% PEG-SF solution. The best release profile was observed with the tablet coated with 17% PEG and fibroin solution. When the concentration of the PEG was high in the solution (25%), the membrane was probably partially dissolved in the dissolution medium due to solubility of the PEG and insufficient β sheet structure formation. After 17% PEG concentration in the coating solution, β sheet formation could be hindered and soluble coating. Therefore the drug might diffuse more rapidly at high PEG ratios. In order to expose the effect of PEG concentration Figure 5.4 was plotted for a constant 60% release profile for each case. It was observed that the best sustained release was achieved when the tablets were coated with SF solution having17% PEG based on the target profile. The cross section and surface SEM micrographs of tablets coated with 17% PEG-SF solution which have channels are shown in Figure 5.5.a and 5.5.b; respectively

Table 5.2 shows the K, n, R^2 values of the samples coated with SF-PEG solution. The tablets coated with 12.5%, 17% and 25% PEG and silk fibroin solution present zero

order release mechanism where the regression coefficients (R^2) of the samples are 0.98, 0.98and 0.99, respectively.

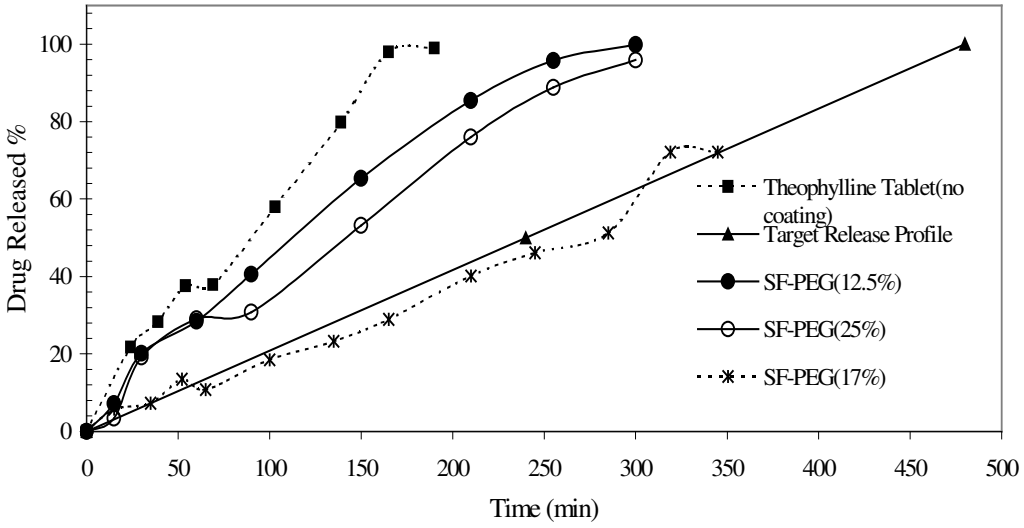


Figure 5.3. Release profile of the theophylline tablets coated with SF solution having polyethylene glycol

Table 5.2. K and n values of the theophylline tablets coated with fibroin and polyethylene glycol solutions.

Sample	K	n	R^2	Drug release mechanism
Tablet coated with 12.5% PEG and fibroin solution	45	0.98	0.98	Zero order
Tablet coated with 17% PEG and fibroin solution	33	1.04	0.98	Zero order
Tablet coated with 25% PEG and fibroin solution	40	1.00	0.99	Zero order

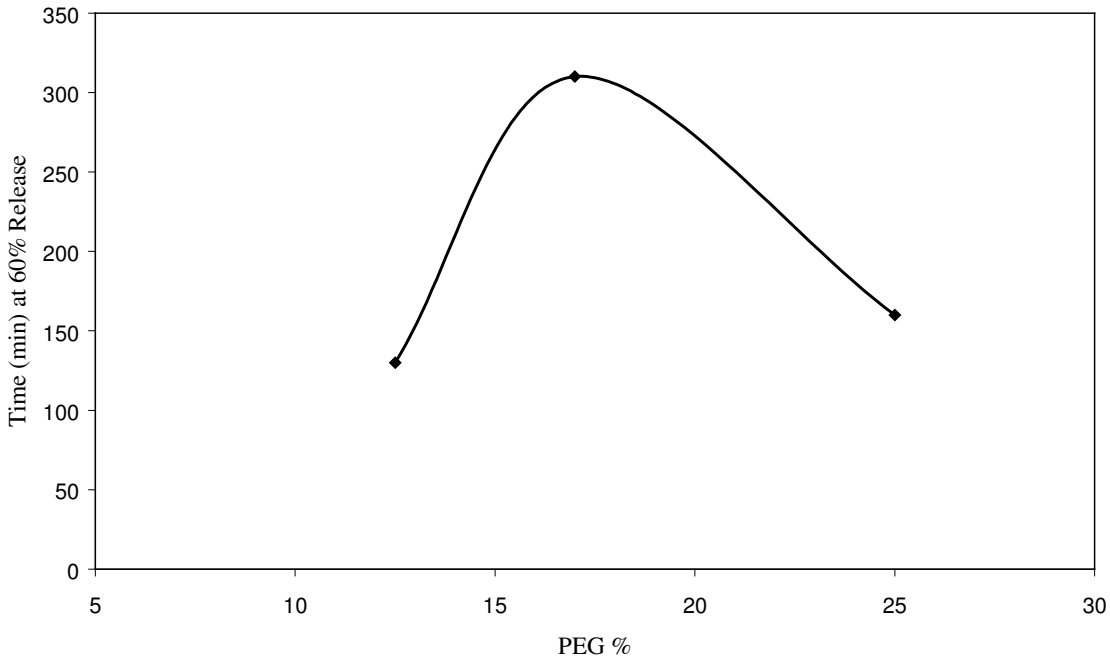


Figure 5.4. 60 % release time for the fibroin solution having different amounts of PEG solutions.

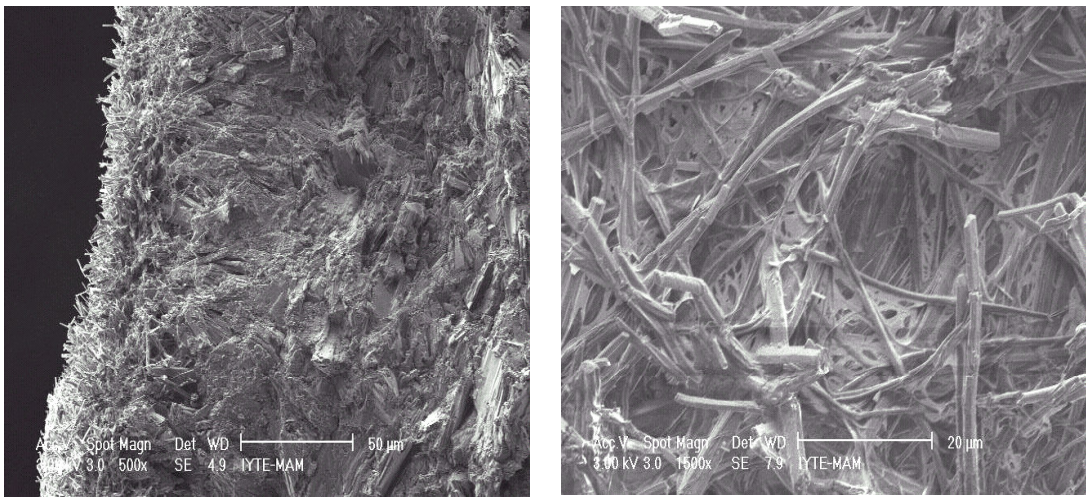


Figure 5.5. SEM micrographs of theophylline tablets coated with silk fibroin solution having 17 % PEG a; cross section of tablet, b; surface of tablet

5.3. COATING WITH SF/EDC SOLUTION

Cross-linking is the process of chemically joining two or more molecules by a covalent bond. Cross-linking reagents contain reactive ends to specific functional groups on proteins or other molecules. Because of the availability of several chemical groups in proteins and peptides that may be targets for reactions, proteins and peptides are readily conjugated and otherwise studied using cross-linking methods. Cross-linkers also are commonly used to modify nucleic acids, drugs and solid surfaces. Carbodiimides are zero-length cross-linkers (e.g., EDC) and effect direct coupling between carboxylates (–COOH) and primary amines ($–NH_2$).

The aim of this part of the study was to improve the film properties of the membrane in order to obtain sustained release of theophylline by crosslinking the silk fibroin by water soluble 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDC). As mentioned in the previous part, the heat treated fibroin membranes had poor film properties and they were in the form of brittle structure, the film properties should be improved. It was observed crosslinking the SF with EDC made the membrane flexible (Malay,2004). In the literature that crosslinking of the proteins with EDC has been used to modify the film properties (Kim,2003, Troponi,2003).

The dissolution tests of the tablets coated with crosslinked silk fibroin were performed by changing coating thickness in order to observe the effect of coating thickness on the release profile of theophylline. Figure 5.6. shows the release profiles of theophylline from prepared by single-coated, double-coated, and triple-coated tablets. The number of immerse time for a single coating was 10, and dipping time for a coating was 4 second. By allowing a drying time between each coating procedure, it was aimed to obtain different coating thicknesses. The release profiles of the tablets coated with EDC crosslinked fibroin solution were compared with the release profile of the theophylline tablet and target release profile in Figure 5.6. As shown in this figure sustained release profiles were achieved for three samples coated with EDC crosslinked fibroin solution since the EDC crosslinked SF membrane prevented rapid diffusion of the drug. It was observed that resistance to transport of drug increased with increasing the length of diffusion path, in other words, increasing the number of coatings. The release rate

constants of coated tablets were much smaller than uncoated tablets. Release rate constants were found 40%, 32%, 21% min^{-1} for single-coated, double coated, triple coated tablets, respectively.

The release rate of theophylline from coated tablets was significantly decreased due to more barrier hinderance by thicker coating materials. The thickness of the film influences the rate of dissolution of the drug. For this reason thickness is an important factor. As shown in Figure 5.6, the double coated tablet is more similar to the target release profile than the others. When the coating thickness was increased, the release profile decreased at the same time. Therefore the decrease in the release % was expected as increasing the coating thickness. Table 5.3 shows the K, n and R^2 values of the samples coated with crosslinked solution. When the tablets coated with crosslinked silk fibroin solution, it is not only reached a sustained release profile but also followed a zero order drug release mechanism. Because n values are close to 1,for all cases it is safe to say, the release profile presents the zero order mechanism.

SEM micrographs of these samples before and after dissolution tests are shown in Figure 5.7. These micrographs were obtained to observe the morphology of the tablet coating and to determine the coating thickness. After dissolution test obtained at 300 min, as shown in Figure 5.7, the EDC crosslinked silk fibroin film on the surface tablet did not dissolve even after 300 of dissolution test. This is a clear indication of the drug release occurring by diffusion mechanism through the interchannels of the coating. Besides this, SEM micrographs of the double coated tablets at different magnifications were obtained in order to observe morphology of the cross section of this sample. Porous structure of the coating can be seen in Figure 5.8a. A dense structure above the porous structure was observed in Figure 5.8c. This dense structure might be the depression of the theophylline powder due to the high pressure applied during the preparation of tablet. The coating thicknesses were measured at various parts of the each tablets' cross section and the average values of them were plotted against % release in Figure 5.9. The release profile is inversely proportional to the thickness of the coatings as shown in Figure 5.9. For the single coated tablet 80% of drug was dissolved through the membrane at the end of 300 min increasing the coating thickness from 4.11μm to 7.68μm by double coating, caused only the %58 percent of the tablet to dissolve at the end of the same minute of 300 min. Again by increasing the thickness of

the coating from 7.68µm to 11.41µm by triple coating, 48 % release profiles was achieved at the end of 300 min. Since resistance to transport of drug increased with increasing the length of diffusion path, the decrease in release rate with increasing number of coating was an expected result. This result is consistent with the literature (Yenal, Herbig et al., 1995). Besides this Figure 5.10 shows the photograph of double coated tablet after dissolution test. This photograph clearly shows the presence of the coating after dissolution test was performed (at the end of 300 min).

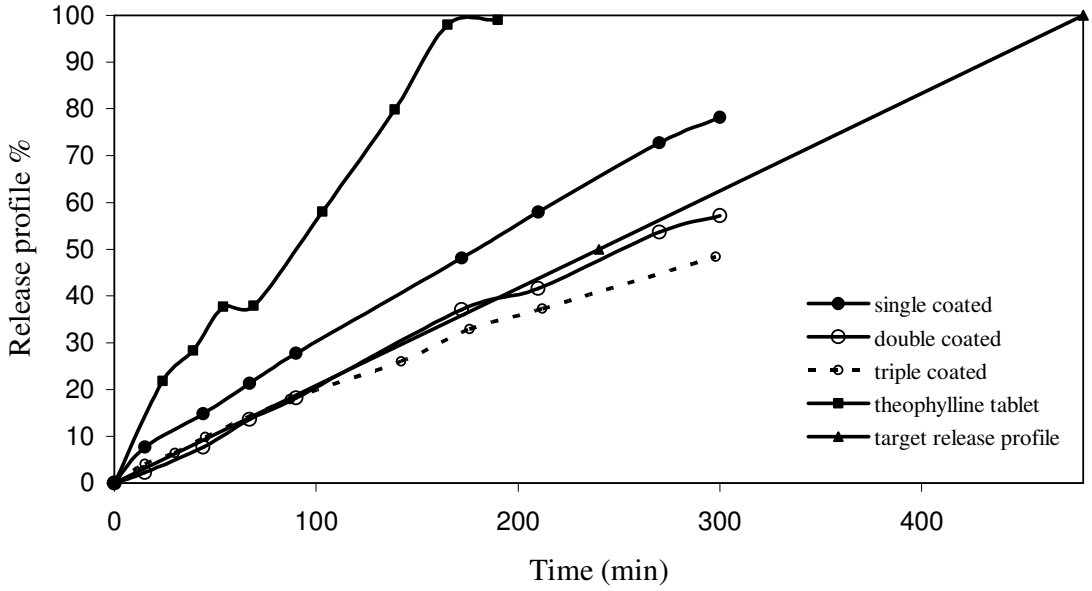
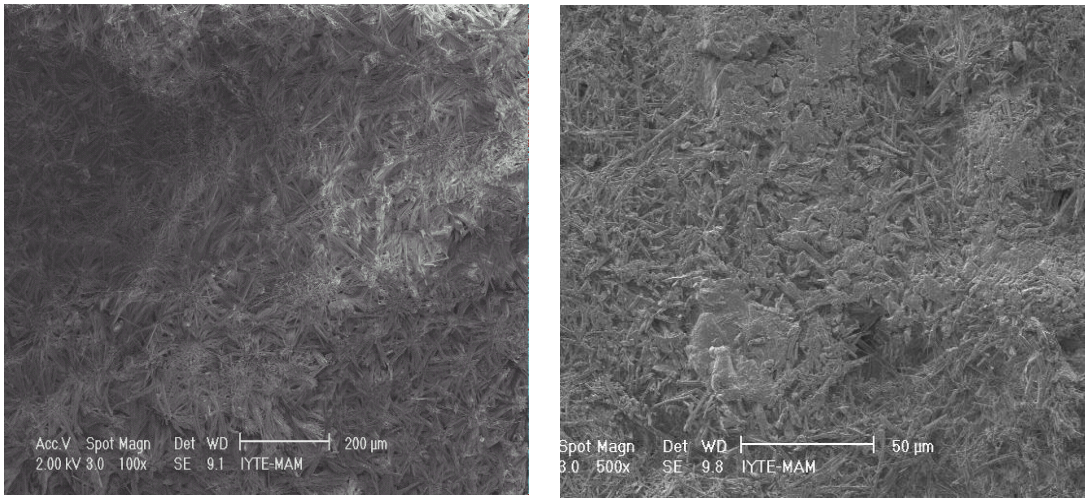


Figure 5.6. Release profile of the theophylline tablets coated with crosslinked fibroin solution.

Table 5.3. K and n values of the theophylline tablets coated with crosslinked fibroin solution.

Sample	K	n	R^2	Drug release mechanism
Tablet single coated with crosslinked fibroin solution	4	0.97	0.99	Zero-order
Tablet double coated with crosslinked fibroin solution	32	1.08	0.99	Zero-order
Tablet triple coated with crosslinked fibroin solution	21	1.04	0.99	Zero-order



a b

Figure 5.7. SEM micrographs of surfaces of double coated theophylline tablets (a; before dissolution, 100x, b; after dissolution, 500x).

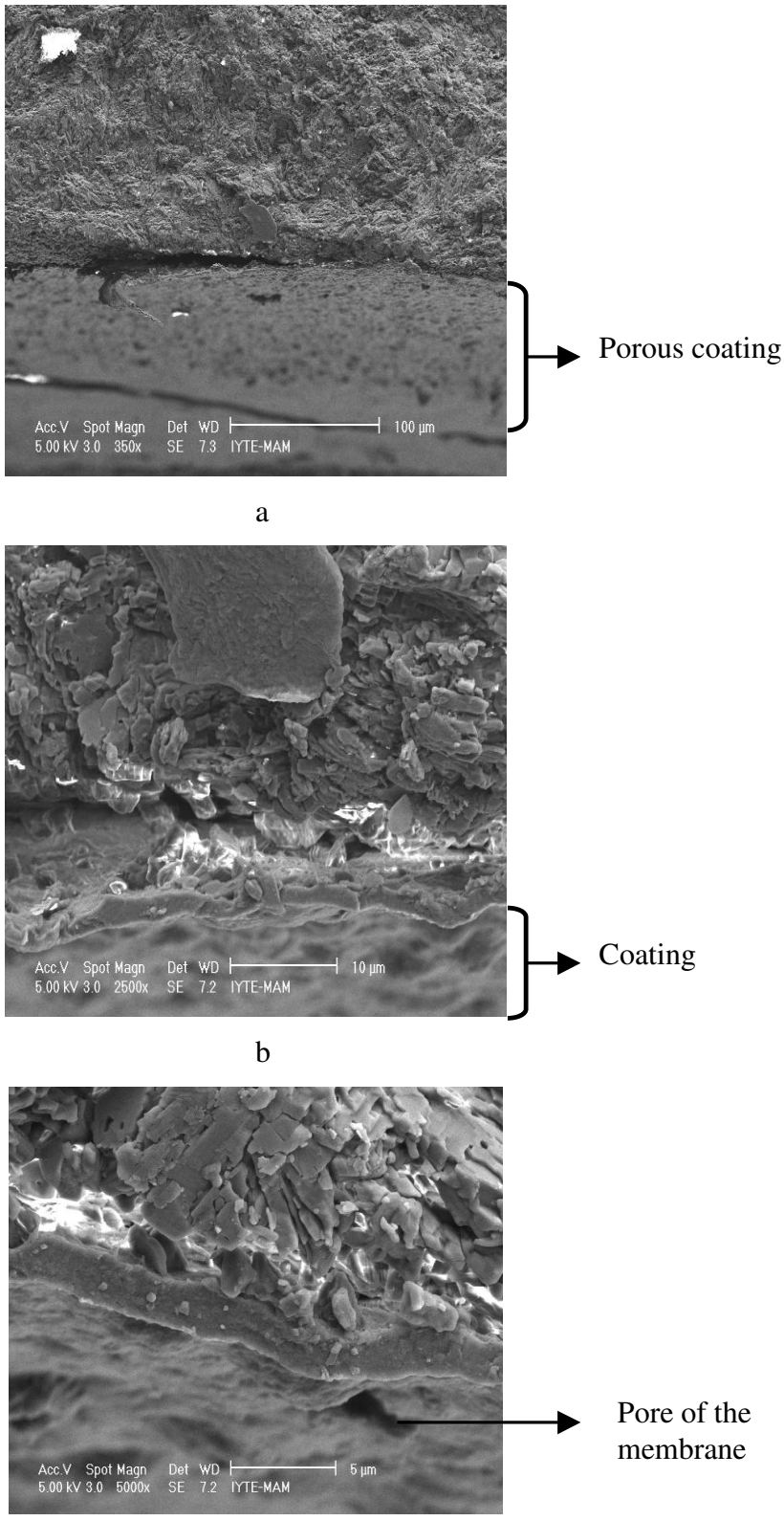


Figure 5.8. SEM micrographs of the cross sections of double coated theophylline tablets (a; 350x, b; 2500x, c; 5000x)

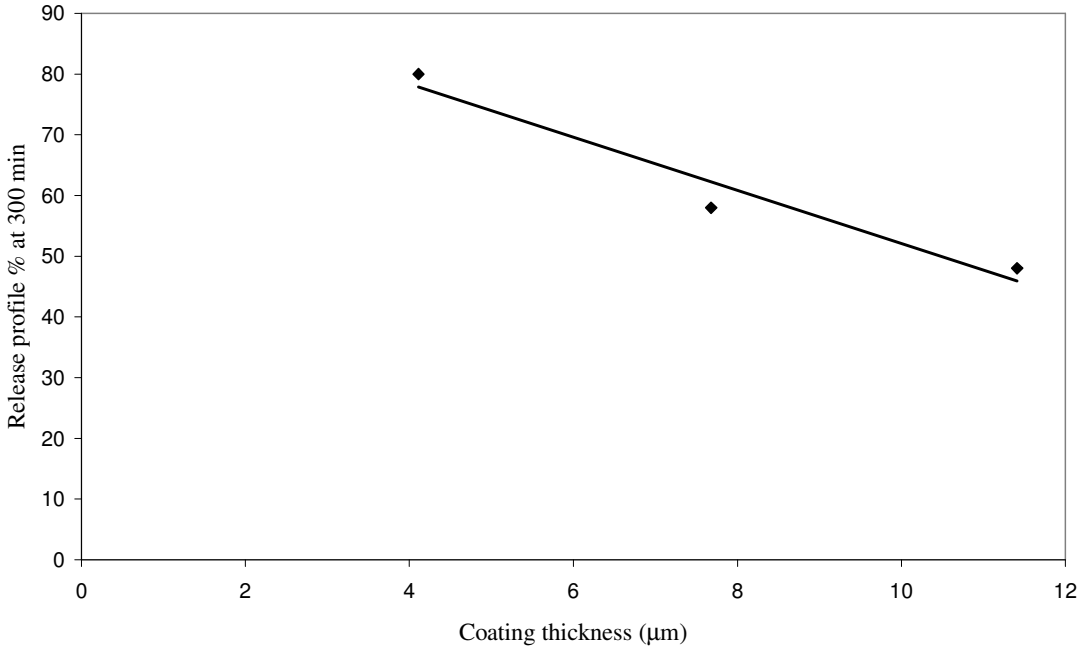


Figure 5.9 Release profile as a function of coating thickness of the tablet coated with crosslinked fibroin solution.

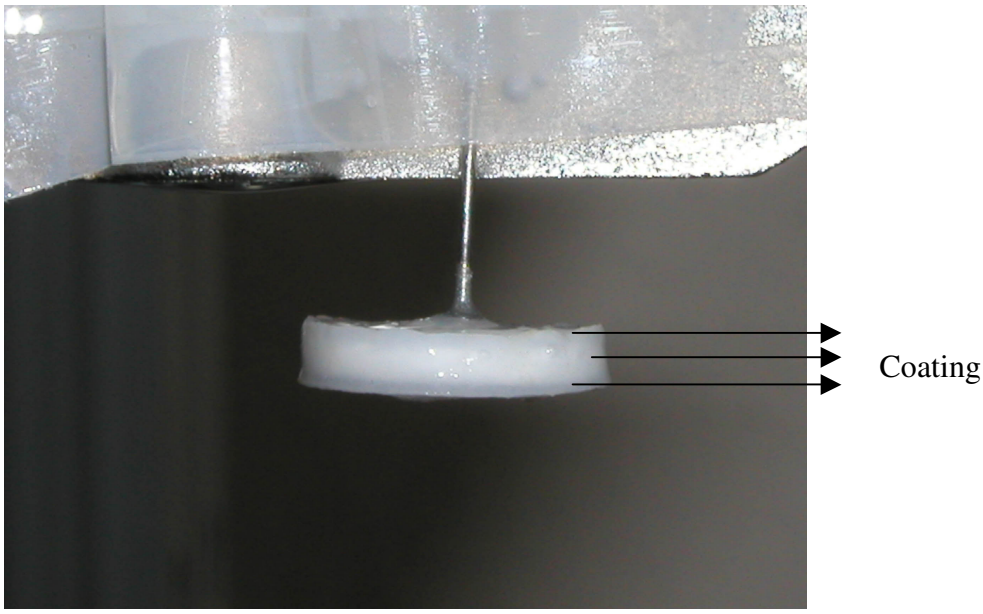


Figure 5.10. Photograph of the double coated tablet at the end of 300 min.

Figure 5.11 shows the comparison of the best release profiles of each groups that were investigated in this study. In this graph the release profile of the samples were examined up to 60% since the coated tablets had the same behaviours after 60% release. Although the sustained release profiles were obtained, the same result could not be observed for the uncoated tablet and the tablet coated with heat treated silk fibroin solution. In this study the plasticizing effect of PEG at the 17% concentration and the stabilizing effect of crosslinking silk fibroin with EDC allow to reach sustained release profile as shown in Figure 5.11.

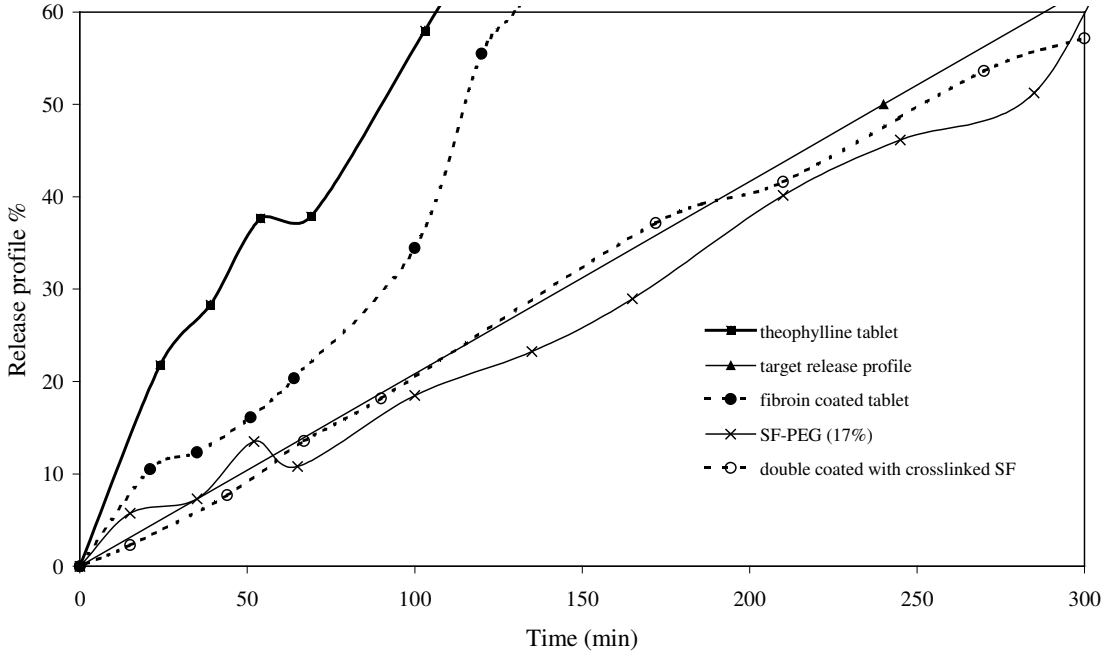


Figure 5.11. Comparison of release profile of name those shown in legend of the graph

CHAPTER 6

CONCLUSIONS AND RECOMENDATIONS

There have been many attempts to model the process of the drug release in polymeric materials. While the coating of the drugs with organic polymeric solutions is still widespread, the pharmaceutical industry has been exploring the alternatives to organic based tablet-coating formulations due to potential environmental safety and toxicity problems associated with some organic solvents. In this project aqueous silk fibroin solution was used as a coating material to achieve controlled drug release mechanism. and to overcome the problems that caused by organic solvents. The theophylline was used a model drug. In this project, the effects of crosslinked fibroin with EDC coating, the addition of plasticizer to the silk fibroin on drug release of theophylline were investigated. Also the effect of PEG concentration, thickness of the coating were examined. The drug release profile of all the samples were obtained by dissolution tests by using UV-spectrophometer... It was observed that the plasticizing effect of PEG and incorporation of fibroin with EDC enhanced the constant steady-state release rate of theophylline. The tablets coated with silk fibroin having 17% PEG solution required a longer dissolving time, 345 min. for 70% release based on target profile. A correlation between drug release rate and the thickness of the coating was evaluated, and it was found that lower rates of drug release from EDC cross-linked coated tablets was obtained by increasing the thickness of the coating. By adjusting the film thickness to 7.68 μm, the sustained release based on target profile was achieved. Additionally, the comparison of the best release profiles up to 60% of each groups was investigated. It was observed the tablets coated with fibroin solution having 17% PEG and double coated tablets by crosslinked fibroin solution allowed to reach sustained release profile. The morphology and film thickness values were determined by using scanning electron microscopy. The porous structure on the coating of the tablets. That allowed the diffusion of the drug was observed.

In this project, dip coating device was used to coat the tablets. However spray coating could be used in stead of dip coating in order to achieve homogeneous coating thickness.

Furthermore, different amounts of EDC/fibroin solution could be tested to obtain better sustain release profiles. In further studies, matrix system consists of theophylline and fibroin can be applied at high pressures.

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APPENDIX A

0.05 g of theophylline anhydrate was dissolved in 25 ml bidistilled water. From this solution, 25, 37.5, 50, 75, 100, 125, 150, 175, 200 µl of samplaes were taken and diluted with bidistilled water to 25 ml. Same procedures were applied to prepare buffer solutions with pH 3 and pH7.4, respectively.

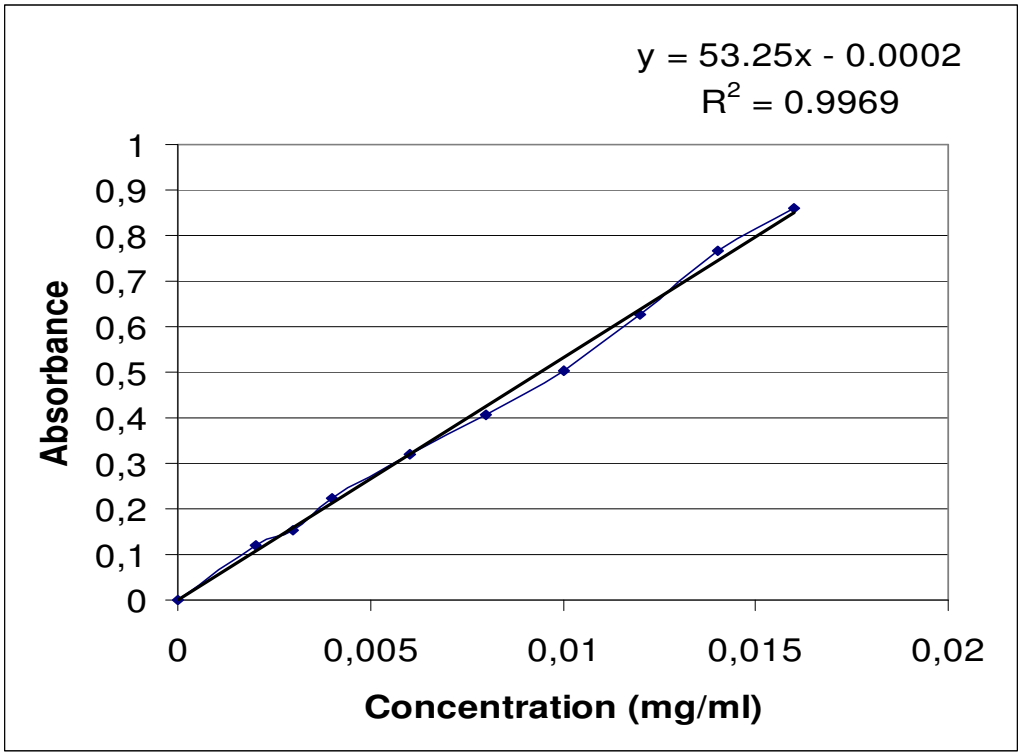

Figure A.1. Calibration curve of theophylline in pH 3 phosphate buffer solution..

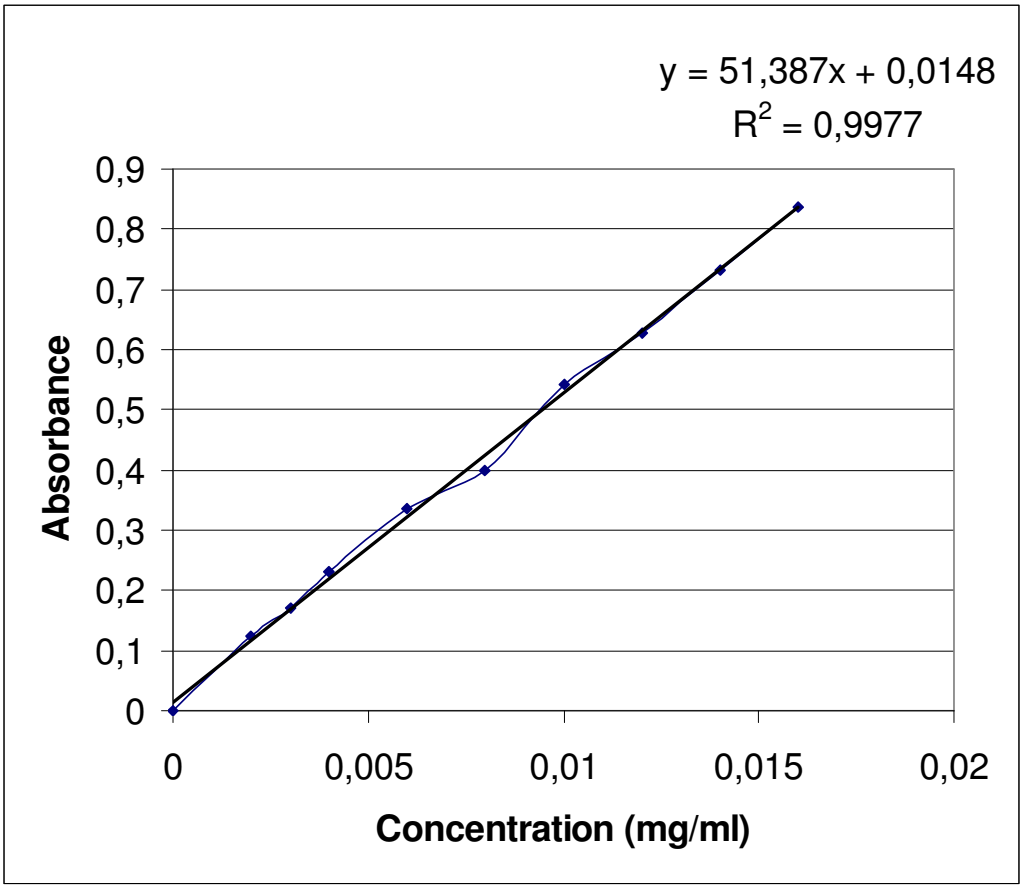

Figure A.2. Calibration curve of theophylline in pH 7.4 phosphate buffer solution.