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ของไคตินและโปรตีนจากเส้นไหม

Preparation and Characterization of Hydrogel from Chitin Derivative
and Silk Fibroin

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ABSTRACT

Natural polymer blend films composed of chitosan and silk fibroin were prepared by solution casting technique with various ratios of chitosan to silk fibroin, using glutaraldehyde as crosslinking agent. The effects of the ratio of chitosan to silk fibroin and crosslinking agent on mechanical properties, swelling behavior and drug releasing property of the blend films were studied. For the swelling behavior, the blend films exhibited a dramatic change in the degree of swelling when immersed in acidic solutions. The blend film with 80% chitosan content had the maximum degree of swelling. It appeared that crosslinking occurred in the blend films helped the films retain their three dimensional structure. In addition, FTIR spectra of the films showed evidence of hydrogen bonding interaction between chitosan and silk fibroin. Drug release characteristics of the blend films with various blend compositions were investigated using theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid as model drugs. It was found that the blend film with 80% chitosan content showed the maximum amount of drug release at pH 2.0 for all types of drugs. The maximum amount of salicylic acid, theophylline, diclofenac sodium and amoxicillin release from blend films with 80% chitosan content at pH 2.0 were 92.7%, 81.1%, 76.6%, and 37.2%, respectively. Drug release properties of the films with various blend compositions were also investigated using a modified Franz Diffusion Cell and pig skin was used as material representing human skin.

Keywords : Chitosan, silk fibroin, blend film, mechanical properties, drug release

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บทคัดย่อ

งานวิจัยนี้ได้ศึกษาการเตรียมแผ่นฟิล์มของพอลิเมอร์ผสมระหว่างไคโตซานและไฟโบรอินจากเส้นใยไหมที่มีอัตราส่วนของไคโตซานและไฟโบรอินต่างกันโดยการขึ้นรูปจากสารละลายกอลลูราตดีไฮด์เป็นสารที่ก่อให้เกิดการเชื่อมโยงที่เติมลงในพอลิเมอร์ผสมเพื่อกำหนดโครงสร้างแบบร่างแหภายในแผ่นฟิล์ม โดยศึกษาผลของอัตราส่วนในการผสมของไคโตซานและไฟโบรอินที่มีต่อสมบัติทางกล สมบัติการบวมตัวในสารละลาย และสมบัติในการควบคุมการปลดปล่อยยาออกจากแผ่นฟิล์ม จากผลการวิเคราะห์สเปกตรัมที่ได้จากฟูเรียร์ทรานสฟอร์มสเปกโตรโฟโตมิเตอร์พบว่าพันธะไฮโดรเจนเกิดขึ้นระหว่างไคโตซานและไฟโบรอิน สำหรับสมบัติการบวมตัวของแผ่นฟิล์มของพอลิเมอร์ผสม พบว่า ค่าการบวมตัวของแผ่นฟิล์มของพอลิเมอร์ผสมมีค่าสูงสุดเมื่อกำหนดปริมาณไคโตซานในแผ่นฟิล์มร้อยละ 80 นอกจากนี้การเติมสารก่อการเชื่อมโยงในแผ่นฟิล์มพอลิเมอร์ผสมทำให้แผ่นฟิล์มสามารถคงรูปร่างไว้ได้เมื่ออยู่ในสารละลายที่มีค่าพีเอชเป็นกรด ในการศึกษาการปลดปล่อยของยาจากแผ่นฟิล์มของพอลิเมอร์ผสม ตัวอย่างยาที่ใช้ได้แก่ ทีโอโพลิน กรดซาลิไซลิก ไคโคลฟีแนคโซเดียม และอะมอกซิซิลิน พบว่า ปริมาณยาที่ปลดปล่อยออกจากแผ่นฟิล์มพอลิเมอร์ผสมมีปริมาณสูงสุดเมื่อมีปริมาณไคโตซานเป็นองค์ประกอบในฟิล์มอยู่ร้อยละ 80 เมื่อเปรียบเทียบปริมาณยาแต่ละชนิดที่ปลดปล่อยออกจากฟิล์มพอลิเมอร์ผสมที่มีปริมาณไคโตซานเป็นองค์ประกอบร้อยละ 80 พบว่า ปริมาณของซาลิไซลิกที่ปลดปล่อยออกจากแผ่นฟิล์มมีมากกว่าทีโอโพลิน ไคโคลฟีแนคโซเดียมและอะมอกซิซิลิน ตามลำดับ ปริมาณสูงสุดของกรดซาลิไซลิก ทีโอโพลิน ไคโคลฟีแนคโซเดียม และอะมอกซิซิลินที่ถูกปลดปล่อยออกจากฟิล์มที่มีปริมาณไคโตซานอยู่ร้อยละ 80 ที่พีเอช 2 คิดเป็นอัตราร้อยละ 92.7 81.1 76.6 และ 37.2 ตามลำดับ นอกจากนี้ได้ศึกษาการปลดปล่อยยาออกมาจากแผ่นฟิล์มโดยใช้ Modified Franz Diffusion cell และใช้หนังหมูแทนผิวหนังของคนในการศึกษาการซึมผ่านของยา

คำหลัก : Chitosan, silk fibroin, blend film, mechanical properties, drug release

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CONTENTS

Title Page	i
Abstract (in English)	ii
Abstract (in Thai)	iii
Acknowledgements	iv
Contents	v
Chapter 1 : Preparation and Characterization of Cross-linked Chitosan/Silk Fibroin Blend Films	1
Chapter 2 : Drug Releasing Property of Cross-linked Chitosan/Silk Fibroin Blend Films	37
Chapter 3 : Study on Drug Release Characteristic of Cross-linked Chitosan/Silk Fibroin Blend Films by Using Modified Franz Diffusion Cell	61
Project Output	95
Appendices	96
Publications in international journals	
Publications in proceedings	

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER 1
PREPARATION AND CHARACTERIZATION OF CROSS-LINKED
CHITOSAN/SILK FIBROIN BLEND FILMS

ABSTRACT

Natural polymer blend films composed of chitosan and silk fibroin were prepared with various ratios of chitosan to silk fibroin, with and without glutaraldehyde as a crosslinking agent. The effects of the ratio of chitosan to silk fibroin and crosslinking agent on swelling behavior and mechanical properties of the blend films were studied. For the swelling behavior, the blend films exhibited a dramatic change in the degree of swelling when immersed in acidic solutions. The degree of swelling of the films increased as the chitosan content increased; the blend film with 80% chitosan content had the maximum degree of swelling. It appeared that crosslinking had occurred in the blend films which helped the films retain their three dimensional structure. In addition, FTIR spectra of the films showed evidence of hydrogen bonding interaction between chitosan and silk fibroin. For the effect of salt type, the films were immersed in various types of aqueous salt solutions, viz NaCl, LiCl, CaCl₂, AlCl₃, and FeCl₃. The films immersed in AlCl₃ and FeCl₃ aqueous solutions gave the maximum degree of swelling. The effects of AlCl₃ and FeCl₃ concentrations on swelling behavior were also investigated. It was found that the maximum degree of swelling of the films occurred at 1.0×10^{-2} M of AlCl₃ and FeCl₃ aqueous solutions. In addition, the tensile strength of the films increased by crosslinking in both dry and wet states whereas the elongation at break decreased.

INTRODUCTION

Increasingly, natural polymers are becoming more important because they are renewable resources and have low costs. Due to their interesting properties, including nontoxicity, biocompatibility, and biodegradability, natural polymers have been investigated with a view to expanding their utilization. Chitin is the most abundant naturally occurring biopolymer and is found in crustacean shells and lower plants

such as mushroom and cell wall of bacteria. Chitin is mainly poly (β -(1-4)-2-acetamido-D-glucose), which is structurally similar to cellulose except that a secondary hydroxyl group on the second carbon atom of the hexose repeat unit is replaced by an acetamide group (Andrady and Xu, 1997; Brack *et al.*, 1997). Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of poly (β -(1-4)-2-acetamido-D-glucose) unit and (β -(1-4)-2-amino-D-glucose) unit with the latter usually greater than 75% (Li *et al.*, 1997). Chitosan is a white, odorless, and biodegradable substance. Because of highly crystallinity structure, chitosan is insoluble in common organic solvents, acids, and alkalis. However, at ambient temperature it dissolves readily in weak inorganic acids over a wide range of concentrations because of salt formation (Li *et al.*, 1992). Viscous solutions of chitosan in acetic and formic acids can be used to cast films. Its film forming properties have been investigated. Chitosan can be blended with synthetic polymers, such as poly(vinyl alcohol) (Chandy and Sharma, 1992; Nakatsuka and Andrady, 1992), poly(acrylic acid) (Wang *et al.*, 1997), and poly(vinyl pyrrolidone) (Quarashi *et al.*, 1992) to yield products whose physical and chemical properties have some potential applications. In addition, chitosan can be blended with biomaterials, like cellulose (Hasegawa *et al.*, 1992, 1994), silk fibroin (Chen *et al.*, 1997), pectin (Yao *et al.*, 1996), collagen (Fang *et al.*, 1991), algenate (Kim *et al.*, 1992) etc. A blend of chitosan with poly(vinyl alcohol) was studied as a carrier for riboflavin and insulin for medical application (Shen *et al.*, 1998). In addition, a blend of chitosan/poly(vinyl alcohol) was investigated to improve a blood compatibility (Fang *et al.*, 1991). Chen *et al.* (1997) investigated a conformational transition of silk fibroin induced by blending with chitosan. The rigid chain of chitosan can induce transformation of the random coil conformation of silk fibroin to the β -sheet conformation because of the occurrence of hydrogen bonding between chitosan and silk fibroin. In addition, chitosan can be prepared as a hydrogel that is very in the medical area. Genpeng *et al.* (1991) developed a collagen-chitosan composite hydrogel for contact lens application.

Bombyx mori silk fibroin, a naturally occurring high-molecular weight fibrous protein, has recently been received a considerable interest. The majority of silk fibroin has highly periodic regions containing various types of amino acids. The basic, highly repetitive sections consist mainly of glycine, alanine, and serine (Shen *et al.*, 1998).

The sum of these amino acids is greater than 80 mol% of the total amino acid composition. Silk fibroin has been considered for application as a biomedical material because of its microbial resistance, biocompatibility, and nontoxicity (Mathur and Narang, 1990). It can be prepared in the forms of powder, gel and film. Silk fibroin films often lose their flexibility and elasticity, so in the dry state, they are very brittle and unsuitable for practical uses (Freddi *et al.*, 1995). To solve this problem, silk fibroin has to be blended with other synthetic or natural polymers to improve the mechanical properties. Among the great number of polymeric materials potentially suitable for blending with silk fibroin, natural polymers are preferred due to the favorable impact of naturally based polymer blends in various applications, especially in the medical field. Blends of silk fibroin with chitosan, an aminopolysaccharide, are of great interest. It was reported that chitosan can induce a conformational transition of silk fibroin from random coil to β -sheet structure (Chen *et al.*, 1997) and these two biopolymers can also form a hydrogel having a semi-interpenetrating polymer network morphology that is sensitive to pH and ion concentration changes. Chen, *et al.* (1997) studied the swelling behavior of chitosan/silk fibroin blend film in pH buffer solutions, showing that the blend films were remarkably swollen in acidic solutions. In addition, the degree of swelling of the blend films in various concentrations of $AlCl_3$ solution was also reported. However, the effect of concentrations of other salt types on the swelling behavior of chitosan/silk fibroin blend film has not been widely investigated.

In this study, chitosan/silk fibroin blend films were prepared with varying chitosan content. The effect of blend composition on the physical properties, mechanical properties, and swelling behavior of films were studied. The swelling behavior of the blend films was determined in pH buffer solutions and various types of salt solutions. The effect of varying concentration of various salt types on the swelling property of the blend films was determined. Furthermore, the effect of crosslinking agent on the mechanical properties, physical property, and swelling property was also investigated.

EXPERIMENT

Materials

Chitin was prepared from shrimp shells kindly supplied by the Suraphol Food Public Co., Ltd. Silk fibroin was obtained by degumming of raw silk fiber. Glacial acetic acid purchased from J. T. Baker was analytical grade of 99.9% w/w, and 50% w/w glutaraldehyde was purchased from Fluka Co., Ltd.

Equipment

Restch Seiving Machine

The chitosan powder with the size of 38 to 75 μm was sieved and collected separately by using Restch Sieving Machine type Vibro.

Capillary Viscometer

The viscosity-average molecular weight of chitosan was determined by using Cannon Ubbelohde-type number 50 of capillary viscometer.

FTIR Spectrophotometer

The FTIR spectrum of chitosan/silk fibroin blend films were recorded with Vector 3.0 Bruker FTIR Spectrophotometer with 16 scans at a resolution of 4 cm^{-1} . A frequency of 4000-400 cm^{-1} was observed by using deuterated triglycinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9 \text{ cm.Hz}^{1.2}.\text{w}^{-1}$.

Wide-Angle X-Ray Diffractometer (WAXD)

The wide-angle X-ray diffractometer used in this study was D/MAX-2000 series of Rigaku X-ray Diffractometer system. X-ray of Cu k-alpha at 40 kV/30 mA were used as a source. The k-beta filter was used to eliminate interference peak. Divergence slit and scattering slit at 1 deg together with 0.3 kV/30 mA were used as a source. The k-beta filter was used to eliminate interference peak. Divergence slit and scattering slit at 1 deg together with 0.3 mm of receiving slit were set on the instrument. The experiment was performed in the range of 5-30 degree with scan speed 5 deg/min and 0.02 deg of scan step.

Thermogravimetric Analyzer (TGA)

The thermogravimetric analyzer used to evaluate the thermal stability of the blend films was TGA 5.1 Dupont Instrument model 2950.

Lloyd Tensile Tester

The strength of the blend films was characterized by Lloyd Instrument LRX series of Lloyd tensile tester with the maximum load of 2500 N.

Gas Permeability Tester

The Brugger gas permeability tester type GDP/E as shown in Figure 1.1 was used to detect the permeability of oxygen gas through the blend films. The flow rate of oxygen was controlled at 100 cm³/min at ambient temperature.

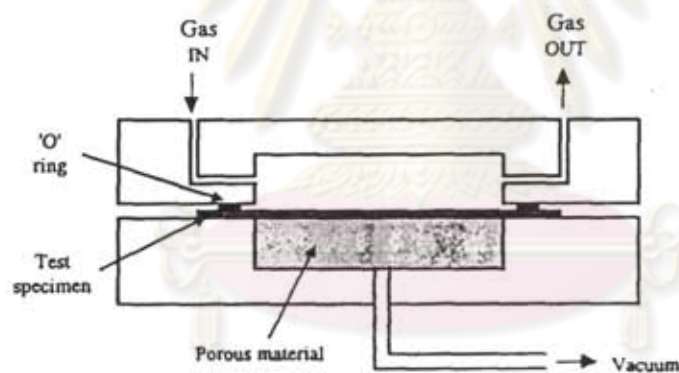


Figure 1.1 Schematic drawing of a Brugger gas permeability tester.

Methodology

Preparation of Chitin

Chitin was prepared by the method of Shimahara *et al.* (1988). The shrimp shell was cleaned and dried before grinding into small pieces. The decalcification of shrimp shell was performed by immersing in 1 N HCl solution for 2 days with occasional stirring, and the decalcified product was washed until neutral. Protein removal was performed in 4% w/w of NaOH solution by boiling further at 80-90°C for 4 h. The deproteinized portion was washed with deionized water until neutral. The product obtained was chitin.

Preparation of Chitosan

For chitosan preparation, chitin flakes were deacetylated by heating in 50% by weight of NaOH solution containing 0.5% by weight of NaBH₄ added based on the weight of chitin to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustively with deionized water until neutral. The resulting chitosan flakes were dried in an oven at 60°C for 24 h.

Degree of Deacetylation of Chitosan

The method used to determine the degree of deacetylation of chitosan is based on infrared spectroscopic measurement by Sannan (1978). About 3 mg of chitosan powder, passed through a 200-mesh sieve, was mechanically mixed with 400 mg of potassium bromide powder to prepare a KBr disk. An infrared spectrum was recorded in a range from 4000 cm⁻¹ to 400 cm⁻¹. The absorbances at 2878 cm⁻¹ (the C-H band) and 1550 cm⁻¹ (the amide II band) were evaluated by the baseline method. The degree of deacetylation was calculated from equation 3.1.

$$D = 98.03 - 34.68(A_{1550}/A_{2878}) \quad (1)$$

where D = degree of deacetylation (%)

A_{1550} = absorbance at 1550 cm⁻¹ (the C-H band)

A_{2878} = absorbance at 2878 cm⁻¹ (the amide II band).

Viscosity-Average Molecular Weight of Chitosan

Different concentration solutions (0.00, 0.00625, 0.0125, 0.025, 0.05, and 0.1 g/ 100 mL) of chitosan in 0.2 M acetic acid-0.1 M NaCl-4.0 M urea were prepared. An Ubbelohde viscometer was filled with 10 mL of sample and then equilibrated in water bath, which maintained the temperature at 25°C. The sample was pass through the capillary once before the running time was measured. Each sample was measured 3 times. The running times of solvent and solutions were used to calculate the relative viscosity, specific viscosity, and reduced viscosity. The reduced viscosity was plotted against the concentration with the intercept being the intrinsic viscosity.

$$\text{Relative viscosity } (\eta_{rel}) = t/t_s \quad (2)$$

$$\text{Specific viscosity } (\eta_{sp}) = (t/t_s) - 1 \quad (3)$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/C \quad (4)$$

$$\text{Intrinsic viscosity } [\eta] = (\eta_{red})_c \quad (5)$$

When t is the running time of chitosan solution, t_s is the running time of solvent and C is the concentration in g/100mL

The viscosity-average molecular weight of chitosan was determined based on the Mark-Houwink equation following

$$[\eta] = 8.93 \times 10^{-4} M^{0.71} \quad (6)$$

where $[\eta]$ is the intrinsic viscosity, M is viscosity-average molecular weight.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1 % by weight acetic acid solution. The chitosan solution was allowed to stand overnight at 4°C in a refrigerator to get rid of air bubbles before use.

Preparation of Silk Fibroin Solution

To obtain silk fibroin, raw silk fiber was degummed by heating in 0.5% Na₂CO₃ solution at 100°C for 1 h followed by washing with boiling water and drying at 60°C for 24 h in an oven. Silk fibroin 6 g was then dissolved in 94 g of 2:2:8 by mole of CaCl₂: EtOH: H₂O solvent system at 100°C for 15 minutes (Chen *et al.*, 1994). The resulting silk fibroin solution was filtered through the sintered glass filter and subsequently dialyzed against distilled water for 7 days. The dialyzed silk fibroin solution was filtered and diluted to achieve a concentration of 1% w/w.

Preparation of Blend Films

The blend films of chitosan and silk fibroin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of chitosan solution. The blend solution was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. These films were allowed to dry at ambient temperature for 72 h. When the films were dried completely, they were soaked in 0.5% by weight of NaOH in MeOH for 24 h to neutralize the acid and then washed exhaustively with MeOH. The blend films were dried at ambient temperature and stored over silica in a desiccator before use. For the crosslinked chitosan/silk fibroin blend films, glutaraldehyde used as crosslinking agent was added into the blend solution at the amount of 0.01 mole/glucosamine unit of chitosan.

FTIR Spectra of Chitosan/Silk Fibroin Blend Films

FTIR spectra of chitosan/silk fibroin were recorded on the Bruker Fourier transform infrared spectrophotometer, Model Vector 3.0 with 16 scans at a resolution of 4 cm^{-1} . The samples with the thickness of $10\text{ }\mu\text{m}$ were attached to the sample frames and scanned from frequency of 4000 to 400 cm^{-1} using a deuterated triglycinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9\text{ cm}\cdot\text{Hz}^{1/2}\cdot\text{w}^{-1}$.

Thermogravimetric Analysis of Chitosan/Silk Fibroin Blend Films

Thermogravimetric analysis of the blend films was carried out under N_2 atmosphere at heating rate of $10^\circ\text{C}/\text{min}$ from 40°C to 700°C . About 8 mg of sample was used for each measurement.

Crystallinity Determination

The wide-angle X-ray diffractograms of the blend films were recorded at room temperature using Rigaku X-ray Diffractometer system, Model D/Max-2000. The X-ray source was Cu k-alpha ($40\text{ kV}/30\text{ mA}$). The k-beta filter was used to eliminate interference peak. The dried films with thickness of $25\text{ }\mu\text{m}$ were attached to the sample holders and 2θ scan range was from 5 to 30° at a speed of $5^\circ/\text{min}$ and 0.02 degree for scan step.

Swelling Behavior Determination

The swelling samples were cut into the disk form with diameter of 16 mm and 25-30 μm in thickness. The samples were immersed in pH buffer solutions at various pH values and in various types of salt solutions. The degree of swelling was calculated from equation 7.

$$\text{Degree of swelling (\%)} = \frac{W - W_0}{W_0} \quad (7)$$

where W_0 is the weight of dry film and W is the weight of swollen film.

Mechanical Testing

Tensile strength and elongation at break of the blend films were performed with a Lloyd Tensile Tester according to the standard ASTM D882, at a gauge length of 50 mm and 20 mm/min of strain rate. The dimension of samples was 25 mm x 150 mm and the thickness was 35-40 μm . These mechanical properties were determined in both dry and wet states. For the dry state, the blend films were dried at 60°C for 24 h before measurement. For the wet state, the blend films were soaked in distilled water for 2 days to reach equilibrium before testing.

Oxygen Permeability Testing

The measurement of oxygen permeability of the blend films was performed with Brugger gas permeability tester. The blend films were cut into the circular form with the diameter of 110 mm and about 10 μm in thickness. The films were sealed completely with grease on the top of the porous material and the two halves of the permeability cell were clamped together. The 'O' ring ensured an air-tight seal between two halves. The oxygen gas was circulated through the top half of the permeation cell and vacuum applied below the specimen until all the air had been removed from the specimen. Vacuum was then turned off and the rate of oxygen gas permeation through the films was recorded with times. The oxygen permeability rate could be calculated from equation 3.8.

$$G = \frac{1.47 \times 10^9}{KN} \quad (8)$$

where G = permeability rate ($\text{cm}^3/\text{m}^2 \cdot \text{d} \cdot \text{bar}$)

K = temperature (Kelvin)

N = reciprocal of slope from the plot of the change of
the vacuum pressure versus time.

RESULTS AND DISCUSSION

Characterization of Chitosan

Shrimp shells generally compose of three major components, which are chitin, calcium carbonate, and protein. By solvent extraction, calcium carbonate and protein can be removed and leaving behind chitin as a white flaky portion.

In this research, chitin was prepared from shells of *Penaeus merguensis* shrimp by decalcification with hydrochloric acid solution and deproteinization with aqueous sodium hydroxide solution in order to remove calcium carbonate and protein, respectively. Because of the difficulty to dissolve chitin in common solvents, chitin is preferably derived further to chitosan in order to achieve the ease of dissolution and chemical modification. Chitin was deacetylated in 50% w/w of sodium hydroxide solution and chitosan with mainly reactive amino groups would be obtained.

The FTIR spectrum of chitosan is shown in Figure 1.1 and the absorption frequencies of characteristic bands of chitosan are summarized in Table 1.1.

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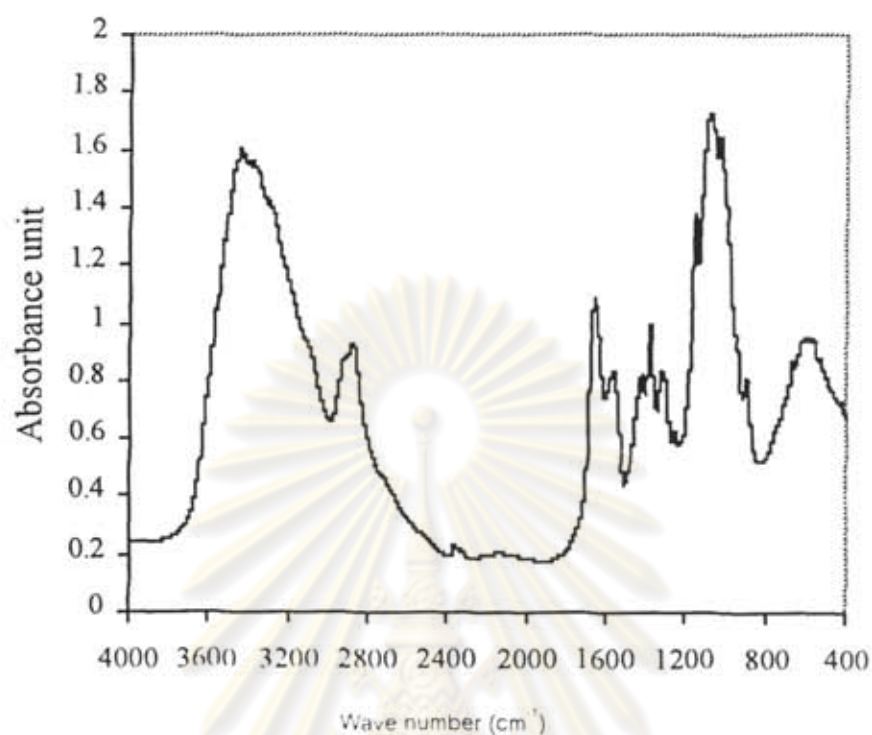


Figure 1.1 FTIR spectrum of chitosan.

Table 1.1 FTIR characteristic Absorption bands of chitosan.

Frequencies (cm ⁻¹)	Assignment and remarks	Reference(s)
1659	C=O stretching	Muzzarelli, 1977;
1561	NH deformation	Kurita <i>et al.</i> , 1993;
1317	CN band and CH ₂ wagging	Kim, <i>et al.</i> , 1996
1080 and 1031	C-O stretching vibration	Kim, <i>et al.</i> , 1994

Chitosan normally has greater extent of amino groups than acetamide groups at C₂ position of N-acetyl glucosamine units. The degree of deacetylation of chitosan depends on the severity of the deacetylated condition. According to the method of Sannan (1978), the degree of deacetylation of chitin can be calculated from FTIR to obtain to value of 80%.

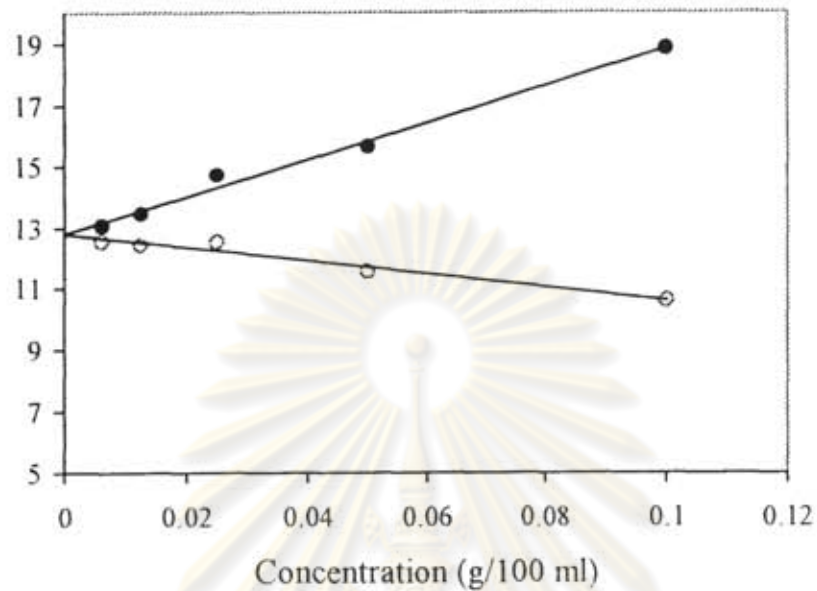


Figure 1.2 The plot of reduced viscosity $\left(\frac{\eta_{sp}}{C}\right)$ and $\left(\ln \frac{(\eta_{rel})}{C}\right)$ versus concentration of chitosan solution.

$\frac{\eta_{sp}}{C}$

 $\ln \frac{(\eta_{rel})}{C}$

Chitosan is a biopolymer. The molecular weight yield a wide range of chitosan varies from 1.0×10^5 to 1.2×10^6 depending on the nature of chitosan resources and the severity of the deacetylation process. In this study, chitosan was prepared from chitin, which was obtained from *Penaeus merguensis* shrimp shells. The molecular weight of chitosan was determined by viscometric method. By following the method of Lee (1978), the molecular weight of chitosan was derived from its intrinsic viscosity. The plot of reduced viscosity $\left(\frac{\eta_{sp}}{C}\right)$ and $\ln \frac{(\eta_{rel})}{C}$ versus concentration of chitosan solution is shown in Figure 4.2. This plot shows the extrapolated value of each line reaches the same position and this value was referred to intrinsic viscosity of chitosan. From Mark-Houwink equation, when a is 0.71 and k is 8.93×10^{-4} , the viscosity-average molecular weight of chitosan obtained from the calculation was 7.12×10^5 .

Characterization of Chitosan/Silk Fibroin Blend Films

FTIR Spectra Characterization

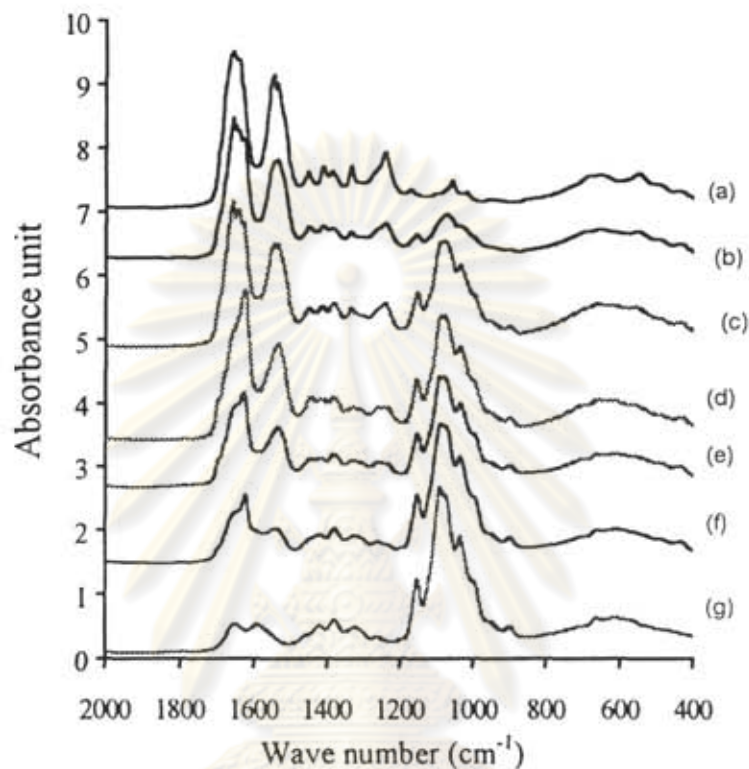


Figure 1.3 FTIR spectra of pure and blend films at various compositions of chitosan to silk fibroin with the addition of 0.01 mole of glutaraldehyde /glucosamine unit of chitosan. Chitosan:silk fibroin, (a) 0/100; (b) 20/80; (c) 40/60; (d) 50/50; (e) 60/40; (f) 80/20; (g) 100/0.

The molecular character of chitosan/silk fibroin blend films was examined by infrared spectroscopy. The FTIR spectrum of pure silk fibroin film is shown in Figure 1.3(a). The characteristic peaks of silk fibroin at 1663 cm^{-1} , 1547 cm^{-1} and 1242 cm^{-1} were attributed to amide I, amide II, and amide III of random coil conformation. While the characteristic absorption bands of chitosan at 1589 cm^{-1} belonged to amide I band and the others occurred at 1153 cm^{-1} and 898 cm^{-1} were the characteristic peaks of polysaccharide. For the spectrum of the blend film with 80 % silk fibroin content, the amide I band of silk fibroin showed another peak at 1625 cm^{-1} that was assigned to the β -sheet structure of silk fibroin (Yoshimizu and Asakara, 1990). As seen in Figure 1.3, the absorption band at 1625 cm^{-1} became stronger with increase of chitosan content. This agrees with the results reported by Chen *et al.* (1997) who concluded

that the increasing of β -sheet conformation of silk fibroin was caused by the hydrogen bond formation between amino groups of chitosan and amide groups of silk fibroin. These results revealed that in the blend films some originally random coil of silk fibroin chains changes to β -sheet structure during blending with chitosan. The occurrence of β -sheet structure leads to more regular structure of silk fibroin chains.

Crystallinity

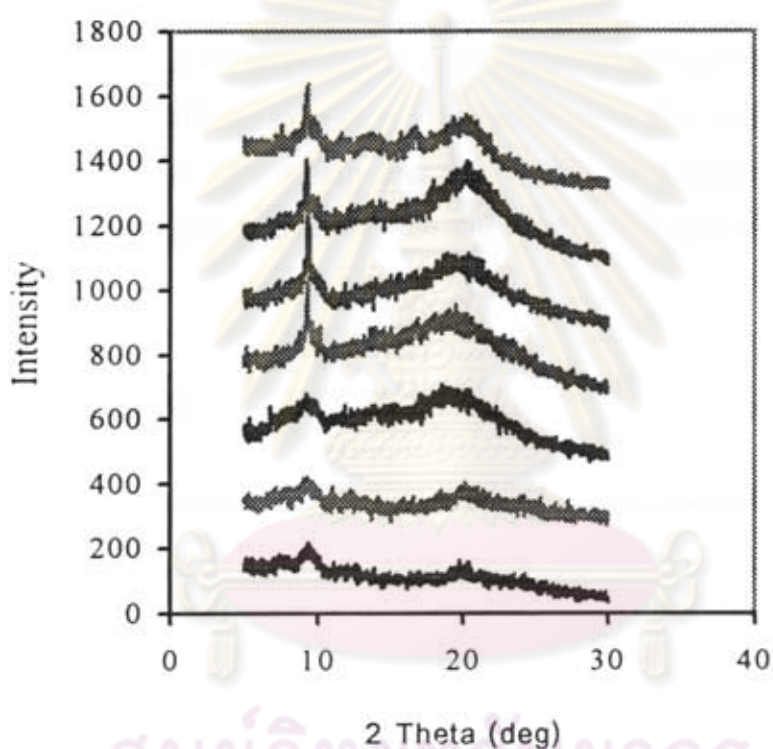


Figure 1.4 Wide-angle X-ray diffraction profile of pure and blend films. Chitosan : silk fibroin (a) 100/0: (b)80/20: (c) 60/40: (d) 50/50: (e) 40/60: (f) 20/80: (g) 0/100

The X-ray diffraction patterns of pure and the blend films are shown in Figure 1.4. The pure chitosan film exhibited the diffraction pattern of partial crystalline structure with 2θ peaks occurring at 9.35° and 20° . The silk fibroin film showed a non-crystalline structure. According to Freddi *et al.*, (1995), the dissolution of silk fibroin was caused by reagent penetrating into the adjacent chains and breaking hydrogen bonds between them. This led to the decrease in crystallinity of silk fibroin

films as compared to the original silk fibroin fiber. The pattern of the blend films exhibited a gradual transformation from characteristic crystalline peaks of chitosan to the completely amorphous pattern of silk fibroin with diffraction pattern increase of silk fibroin content in the blend film. Chen *et al.* (1995) studied the crystallinity of pure silk fibroin membrane without further treatment. The diffraction pattern of pure silk fibroin membrane showed no clear 2θ peak. This includes a mainly random coil conformation that is similar to present results. According to Hasegawa *et al.* (1992), the chitosan film showed the crystalline peaks at around 10° and 20° and the blend film of chitosan /cellulose showed gradual transformation between chitosan and cellulose. The analysis of Hasegawa *et al.* corresponds to our results. However, these all diffraction patterns did not give clear information about the crystallinity because the crystalline structures of chitosan and silk fibroin were remarkably frustrated during dissolution process. Therefore, the chitosan/silk fibroin blend films were mainly amorphous.

Thermal Stability

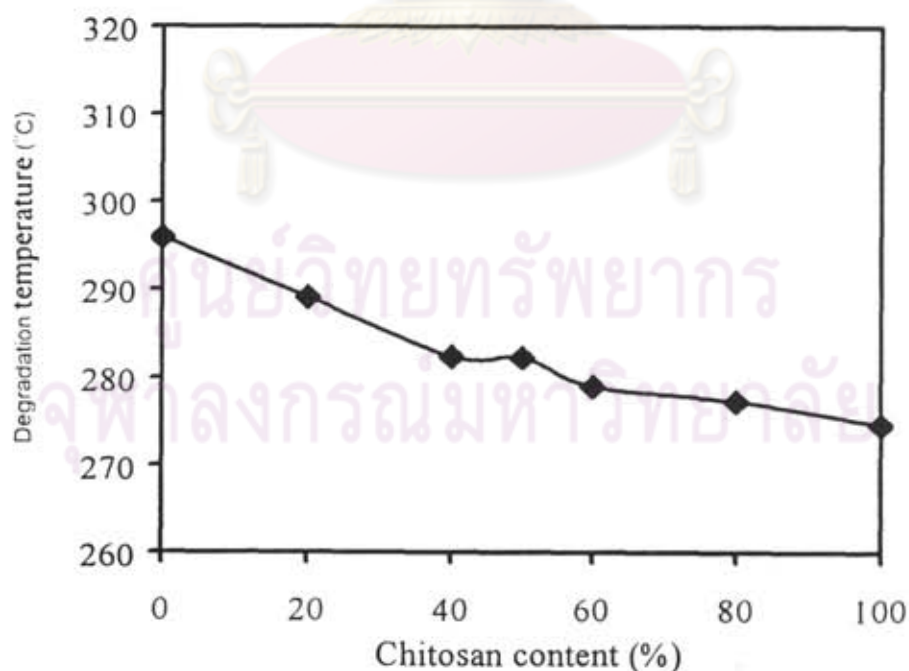


Figure 1.5 Thermal decomposition temperature of pure and blend films.

The thermal stability is an important functional property, which can definitively determine the performance of a material for practical uses. The degradation temperature of pure and the blend films as a function of chitosan content is shown in Figure 1.5. Pure chitosan and silk fibroin films were stable until at 274.68 °C and 295.87 °C, respectively. Then their weights dropped sharply resulting from the thermal decomposition of pure chitosan and pure silk fibroin. The thermal stability of the blend films falls between pure chitosan and pure silk fibroin films. The decomposition temperature of the blend films increased with increase of silk fibroin content. From this thermal behavior, the thermal stability of pure silk fibroin films was better than pure chitosan film. By blending with silk fibroin, the thermal stability of the blend film could be improved as compared to pure chitosan film. These results agree with the work of Tsukada *et al.* (1994) who carried out thermogravimetric analysis of *Antheraea pernyi Bombyx mori* silk fibroin blend films. The thermal behavior of *Antheraea pernyi Bombyx mori* silk fibroin blend films was between the two pure components. However, the decomposition temperature of *bombyx mori* silk fibroin films studied by Tsukada *et al.* (1994) was slightly different from the results in this study. This may be due to the difference in solvent system for dissolution and films preparation.

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Swelling Study

Equilibrium Water Content

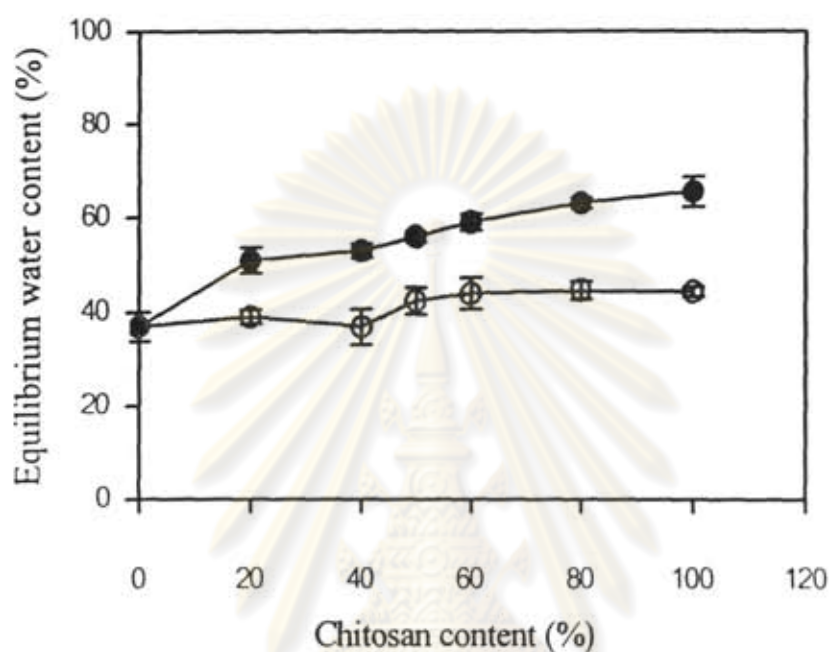


Figure 1.6 Equilibrium water content of chitosan/silk fibroin blend films.

● blend films without crosslinking; ○ crosslinked blend films.

The equilibrium water contents of the blend films with and without crosslinking are shown in Figure 1.6. The equilibrium water content increased as chitosan content increased. This may be due to the hydrophilicity of chitosan. In the case of the crosslinked blend films, they exhibited the lower in equilibrium water content as compared to the blend films without crosslinking. The reason for this is that the crosslinks restrict the swelling of the blend films because chitosan chains are held together by crosslinks. This decreases the amount of water molecules that can penetrate inside the blend films. Pure silk fibroin film showed the lowest equilibrium water content owing to its molecular structure. The major amino acid components of *bombyx mori* silk fibroin are glycine and alanine. These amino acids have hydrophobic side chains (glycine-H, alanine-CH₃) (Shen *et al.* 1998). Therefore, the absorbability of pure silk fibroin film was low. Liang *et al.* (1992) suggested that the water absorption of pure silk fibroin film was very low and it could be improved by blending with sodium alginate. This is consistent with our results that water

absorption of silk fibroin film was low and that chitosan could improve the water absorbability of silk fibroin.

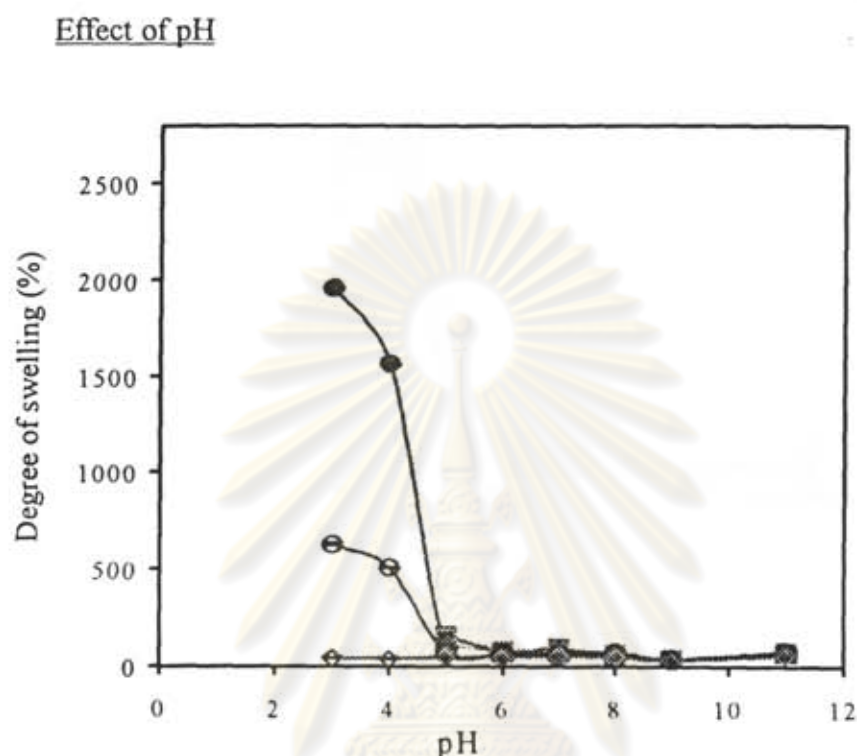


Figure 1.7 The degree of swelling of pure and the blend films without crosslinking in pH buffer solutions. Chitosan : silk fibroin, ■ 100/0; □ 80/20; ▲ 60/40; △ 50/50; ● 40/60; ○ 20/80; ◇ silk fibroin.

The effect of pH on the degree of swelling of pure and the blend films without crosslinking is shown in Figure 1.7. There was no change in the degree of swelling of pure silk fibroin over the whole pH range from pH 3 to pH 11. Likewise, the degree of swelling of pure chitosan film and the blend films was nearly constant when pH is higher than 5. However, in the range of pH less than 5, the degree of swelling of the blend films with less than 50 % chitosan content increased sharply proportional to the chitosan content. This can be explained that in the acidic pH range, the amino groups of chitosan are protonated leading to the destruction of hydrogen bonding between amino group of chitosan and amide group in the main chain of silk fibroin (Chen *et al.* 1997). The disappearance of hydrogen bonds between the polymer chains results in swelling of the blend films. It can be said that chitosan caused the blend films to respond to pH change and dissolved. For the blend films with higher than 50 % chitosan content swell rapidly in the acidic medium.

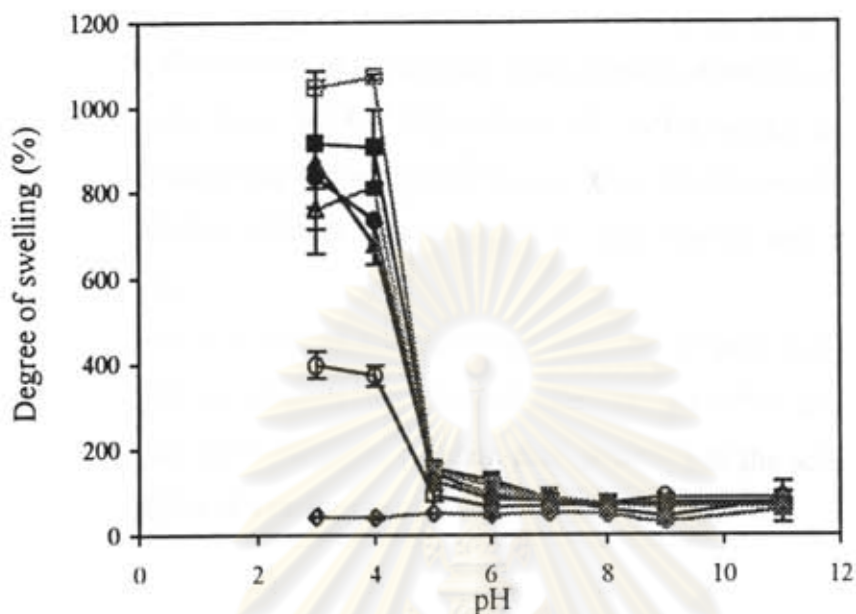


Figure 1.8 Degree of swelling of pure and blend films of chitosan and silk fibroin with crosslinking in various pH buffer solutions. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan.

■ Chitosan; □ 80/20; ▲ 60/40; △ 50/50; ● 40/60; ○ 20/80;
◇ silk fibroin.

The effect of pH on the degree of swelling of pure and the blend films with crosslinking is shown in Figure 1.8. When pH was less than 5, the degree of swelling of the crosslinked blend films increased with increasing chitosan content. The maximum degree of swelling was observed for the blend film with 80 % chitosan content. This may be due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan. Unlike that of the blend films without crosslinking, the crosslinked blend films with higher than 50% chitosan content could maintain their structures in acidic pH range. However, the degree of swelling of the crosslinked blend films was lower than the blend films without crosslinking. Wang *et al.* (1997) reported that the crosslinked chitosan network could prevent the semi-interpenetrating polymer network of chitosan and poly(acrylic acid) from collapse at low pH but crosslinking could limit swelling of the films. It can be explained that the crosslinking makes chitosan chains covalently link together and

form network that can prevent the dissolution of the films even though it restricts the swelling of the films. In contrast, the degree of swelling of the blend films did not exhibit any difference when pH was higher than 5. It can be explained by the fact that, at alkaline pH range, the number of protonated amino groups of chitosan is very low. The pKa of chitosan is about 6.3-6.5 (Hugerth *et al.*, 1997), which indicates that chitosan tends to be protonated in the acidic solution. Thus the degree of swelling of the blend films in alkaline solution was very low as compared to that of the blend films in acidic solution.

From the above, it is evident that the blend films of chitosan and silk fibroin are pH-responsive. This agrees with the study of Chen *et al.* (1997). Moreover, the crosslinking enabled the blend films to maintain their structure in the acidic medium but caused a lower degree of swelling.

Effect of Salt Type

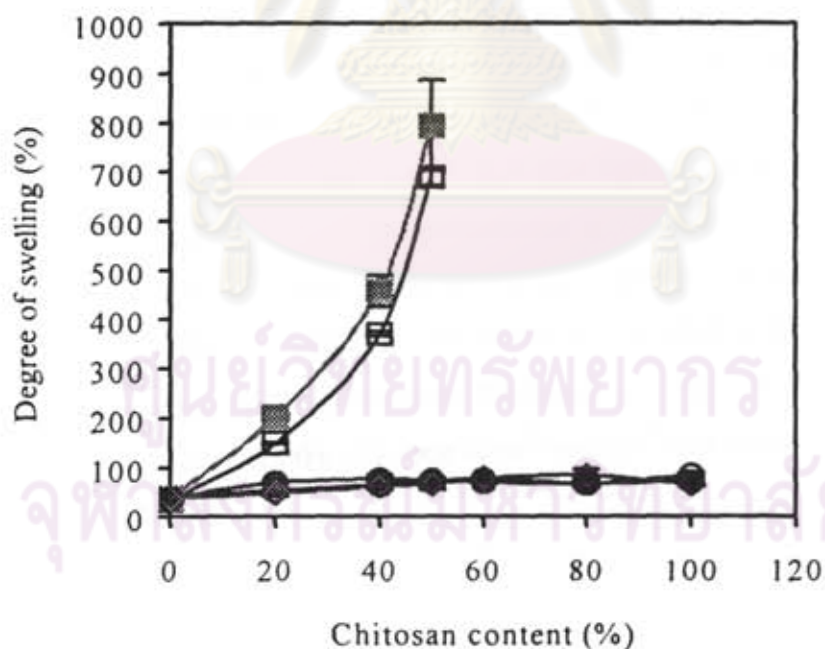


Figure 1.9 Degree of swelling of pure and blend films without crosslinking in various types of salt solutions. □ 0.25 M FeCl₃ solution; ■ 0.25 M AlCl₃ solution; ○ 0.25 M CaCl₂ solution; ● 0.25 M NaCl solution; ◇ 0.25 M LiCl solution.

The degree of swelling of pure and the blend films without crosslinking in various types of salt solutions is shown in Figure 1.9. It can be seen that the films exhibited a significant increase in the degree of swelling in 0.25 M AlCl_3 and 0.25 M FeCl_3 solutions as chitosan content increased. This is believed to be due to the formation of coordinated covalent bond between nitrogen at amino group of chitosan and Al^{3+} or Fe^{3+} . In solution, Al^{3+} is surrounded by six molecules of water while Cl^- acts as a counter ion (Gillespie *et al.*, 1989). This Al^{3+} hydrated still has empty orbital that is electron deficient. While nitrogen atom at amino group of chitosan has a lone pair electron that can coordinate to Al^{3+} . So Al^{3+} can form a coordinated covalent bond with amino group of chitosan resulting in greater degree of swelling at higher chitosan content. The transition metal ion, like Fe^{3+} , can also form a coordinate covalent bond with the amino group of chitosan. The covalent binding of Al^{3+} or Fe^{3+} has the effect of increasing the net positive charge on the chitosan. Therefore, increasing of chitosan content could enhance the degree of swelling of the blend films in both AlCl_3 and FeCl_3 solutions. At chitosan content greater than 50 %, the degree of swelling could not be reported because of overswelling leads to the disintegration of the films.

The swelling of the films in 0.25 M NaCl , 0.25 M LiCl , and 0.25 M CaCl_2 solutions were nearly the same for all blend compositions. The reason is that chitosan does not bind or bind weakly to alkali and alkali earth metal ions such as Na^+ , Li^+ , and Ca^{2+} resulting in lower degree of swelling (Wang *et al.*, 1997).

From Figure 1.9, it was observed that pure silk fibroin film had the lowest degree of swelling. By the addition of chitosan to silk fibroin film, the degree of swelling of silk fibroin could be enhanced. Moreover, the blend films of chitosan and silk fibroin were sensitive to the presence of Al^{3+} and Fe^{3+} ions in the solutions.

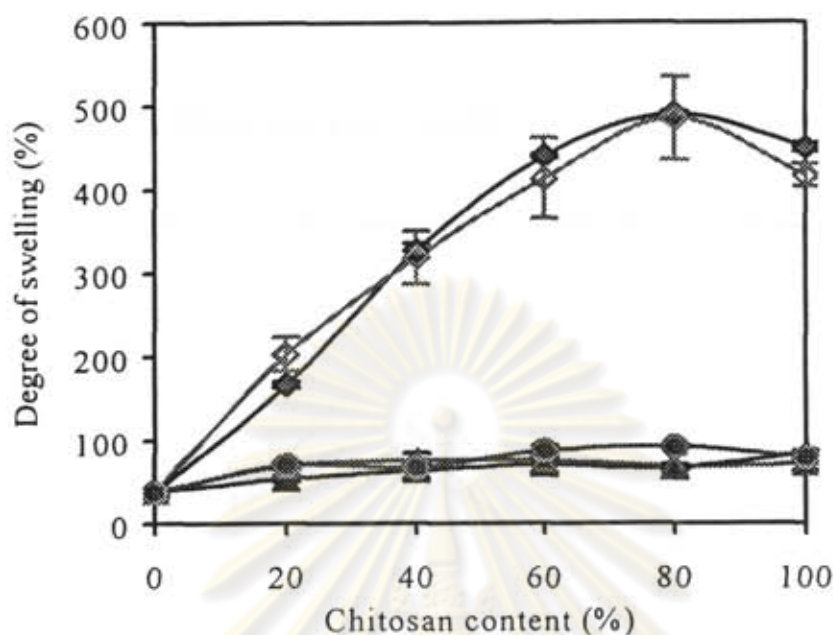


Figure 1.10 Degree of swelling of pure and blend films of chitosan and silk fibroin with crosslinking in various types of salt solutions. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan. ◆ 0.25 M FeCl₃ solution; ◇ 0.25 M AlCl₃ solution; ▲ 0.25 M CaCl₂ solution; △ 0.25 M NaCl solution; ● 0.25 M LiCl solution.

The degree of swelling of pure and the blend films with crosslinking in various types of salt solutions is shown in Figure 1.10. As chitosan content increases, the degree of swelling of pure and the blend films with crosslinking increases significantly in the case of immersing the films in AlCl₃ and FeCl₃ solutions. The reason has been mentioned already with respect to the results of Figure 1.9. For the blend films with crosslinking, the degree of swelling of blend films with greater than 50 % chitosan content can be observed and the highest degree of swelling is observed at 80 % chitosan content. This indicates that the presence of crosslinks can prevent the disintegration of the films by the formation of network between the chitosan chains. However, the crosslinks limit the swelling ability of the films. The degree of swelling of the crosslinked blend films is lower than that of the non-crosslinking blend films at the corresponding blend compositions. The degree of swelling of the crosslinked

blend films is very low in 0.25 NaCl, 0.25 M LiCl, and 0.25 M CaCl₂ solutions for a whole range of chitosan content.

Effect of AlCl₃ and FeCl₃ Concentrations

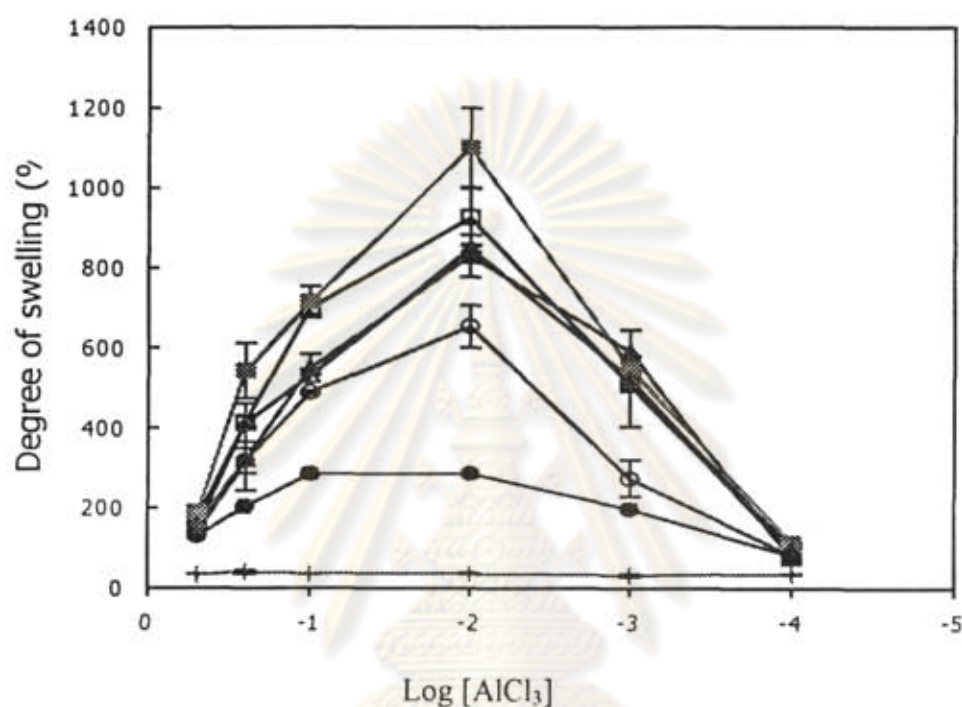


Figure 1.11 Degree of swelling of pure and blend films with crosslinking in various concentrations of AlCl₃ solution. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan. Chitosan : silk fibroin, ■ chitosan; □ 80/20; △ 60/40; ▲ 50/50; ○ 40/60; ● 20/80; ◇ silk fibroin.

From previous results, chitosan and blend films with crosslinking showed strong swelling in AlCl₃ and FeCl₃ solutions. Therefore, further investigations were carried out to study the effect of AlCl₃ and FeCl₃ concentrations on the swelling behavior of pure and the blend films with crosslinking.

The effect of AlCl₃ concentration on the degree of swelling of pure and the blend films with crosslinking is shown in Figure 1.11. The AlCl₃ concentration did not affect the degree of swelling of pure silk fibroin film at all salt concentrations because of the very low interaction between Al³⁺ and silk fibroin. However, chitosan and the blend films showed a strong variation in the degree of swelling relational to the changes in salt concentration. The degree of swelling increased significantly as

AlCl_3 concentration increased. When AlCl_3 concentration was 1.0×10^{-2} M, the degree of swelling of pure chitosan and the blend films showed the maximum value. The maximum degree of swelling belonged to the blend film with 80 % chitosan content. The occurrence of the highest degree of swelling at AlCl_3 concentration of 1.0×10^{-2} M can be explained by Donnan equilibrium effect (Yao *et al.*, 1993). Donnan equilibrium effect arises from the ionic osmotic pressure generated from mobile counterions, which accompany the bound ions on the network strands (Park & Hoffman, 1992). The high counterion concentration causes a large swelling pressure in the absence of excess mobile salt. At the AlCl_3 concentration of 1.0×10^{-2} M, the difference of ion concentration between the interior and exterior of the films is maximal, leading to a large imbalance in osmotic pressure, which causes the maximum degree of swelling of the films. The decrease in the degree of swelling when the AlCl_3 concentration exceeded 1.0×10^{-2} M occurred because of there was now an excess mobile ion concentration in the external solution. The concentration of amino groups of chitosan in the film available to bind Al^{3+} is depleted, no additional Al^{3+} becomes bound to the network strands, the excess mobile ions can penetrate into the film and screen the bound charges, and the degree of swelling decreases.

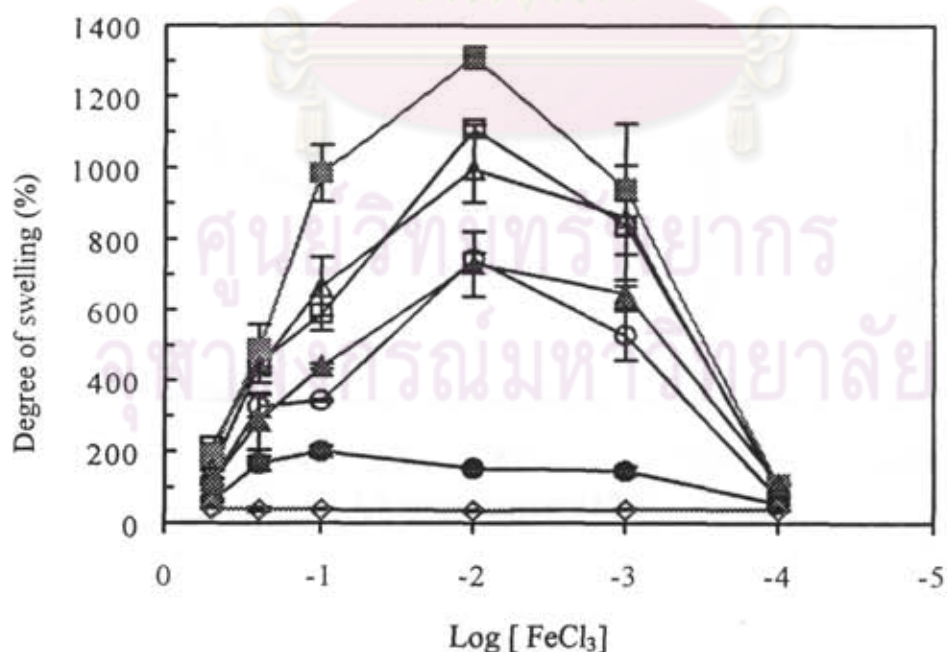


Figure 1.12 Degree of swelling of pure and blend films of chitosan and silk fibroin with crosslinking in various concentrations of FeCl_3 solution. Chitosan : silk fibroin, ■ Chitosan; □ 80/20; △ 60/40; ▲ 50/50; ○ 40/60; ● 20/80; ◇ silk fibroin.

The degree of swelling of pure and blend films with crosslinking in various concentrations of FeCl_3 solution is shown in Figure 12. The effect of FeCl_3 concentration on the degree of swelling of pure and the blend films with crosslinking was similar to that of the films immersed in AlCl_3 solutions. The FeCl_3 concentration that caused the maximum degree of swelling is 1.0×10^{-2} M. The blend film with 80 % chitosan content had the highest degree of swelling. The difference in the degree of swelling of the films immersed in different concentrations of FeCl_3 solution was also caused by Donnan equilibrium effect, as in the case of AlCl_3 solution. The degree of swelling of pure silk fibroin films was not affected by the FeCl_3 concentrations.

Mechanical Properties

Tensile Strength

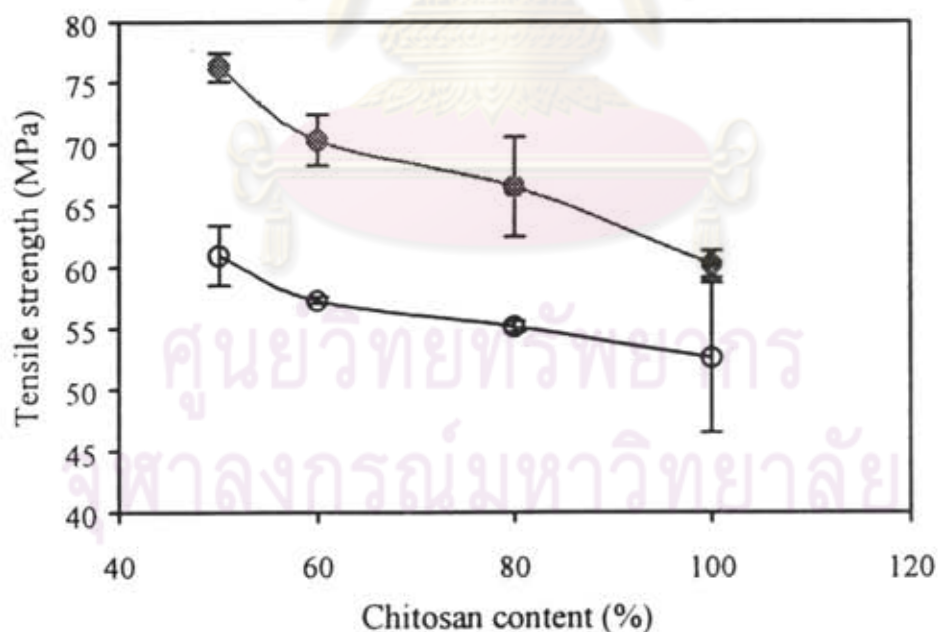


Figure 1.13 The tensile strength in the dry state of pure and blend films.

○ Films without crosslinking; ● Films with crosslinking

The tensile strength of the films as a function of chitosan content is shown in Figure 1.13. The tensile strength of pure silk fibroin and the blend films with less than 50 % chitosan content could not be monitored by Lloyd tensile tester because of their brittleness. Beyond these compositions, the tensile strength of the blend films containing from 50 % chitosan content could be measured. These results indicated that chitosan imparted the flexibility to the films by lowering the brittleness of silk fibroin when an appropriate amount of chitosan is added to the blend films. Chitosan has hygroscopic property that makes it absorb moisture easily. This absorbed moisture has a plasticized effect to the blend films resulting in an increase in flexibility. As chitosan content increased, the tensile strength slightly decreased in both with and without crosslinking. It is known that silk fibroin film in the dry state is very brittle. Freddi, *et al.* (1995) found that the addition of cellulose to silk fibroin could enhance the mechanical properties due to the hygroscopicity of cellulose. In Figure 1.13, the crosslinked blend films shows a slightly higher tensile strength than the non-crosslinked blend films in the dry state because the crosslinks play a key role as a bridge that links chitosan chain together. The network structure made the films stronger, and hence they exhibited a slightly higher tensile strength in the dry state. However, the increase in tensile strength also depended on the amount of crosslinking agent added. In this research, the amount of glutaraldehyde added was low resulting in a small increase in tensile strength.

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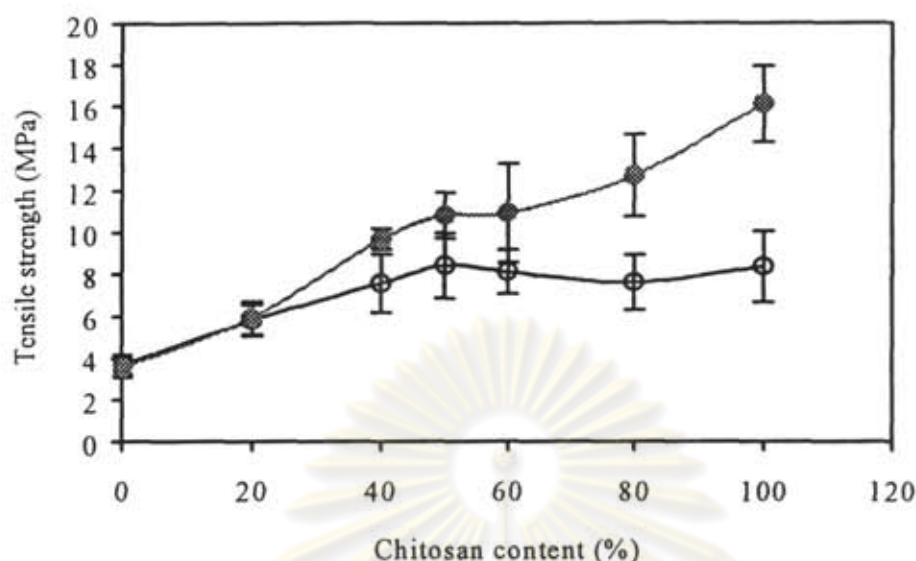


Figure 1.14 The tensile strength in the wet state of pure and blend films.

○ Films without crosslinking; ● Films with crosslinking

Because many end-uses of films involve the contact with water, the strength of films in the wet state is also important. The tensile strength in the wet state of pure and the blend films is shown in Figure 1.14. The tensile strength in the wet state remarkably decreased in comparison with that of the dry state because, in the wet state, the hydrogen bonding between polymer chains within the films is diminished by the water molecules. Hosogawa *et al.* (1991) found that the tensile strength in the wet state of chitosan and homogenized cellulose blend films was lower than that of the dry state. Figure 1.14 indicates that the tensile strength of the blend films without crosslinking tended to increase with up to 50 % chitosan content. For the blend films with chitosan content higher than 50 %, the tensile strength became rather constant as shown in Figure 4.14. It can be explained that, in the wet state, silk fibroin film is soft and very weak. The presence of chitosan in the blend films could improve the strength of the blend films because of the rigidity of chitosan chain. The crosslinked blend films exhibited a much higher tensile strength in the wet state with an increasing content of chitosan. Since the amount of crosslinking agent added was related to chitosan content, the tensile strength of the crosslinked blend films increased in accordance with chitosan content. It can be indicated that, in the dry state, chitosan

could improve the strength of pure silk fibroin film by imparting the flexibility to the blend films. Moreover, the tensile strength of the blend films in the wet state was also improved by the presence of chitosan in the blend films. The rigid chains of chitosan and the existence of crosslinks can enhance the strength of the blend films.

Elongation at Break

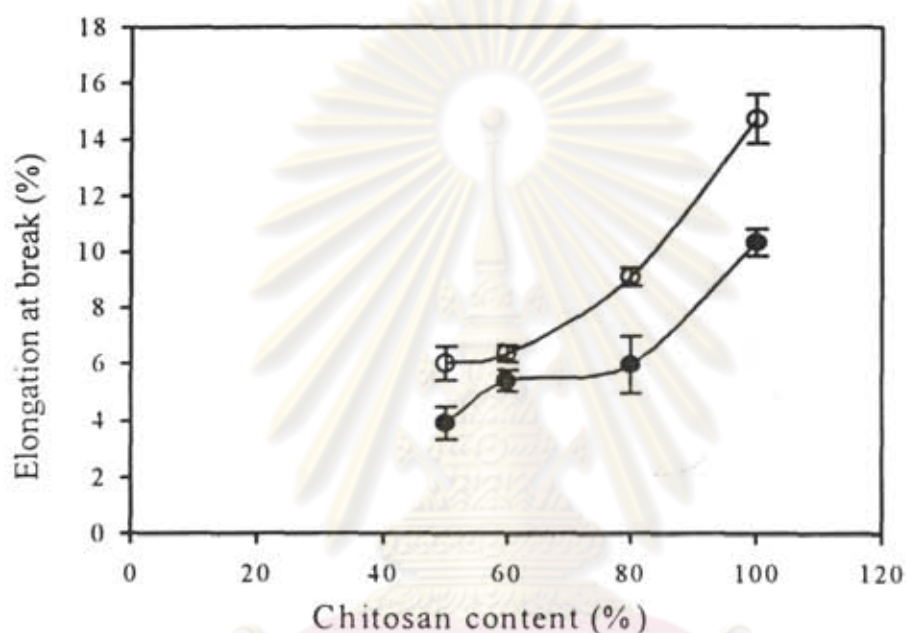


Figure 1.15 Elongation at break of pure and blend films in the dry state.

○ Films without crosslinking; ● Films with crosslinking

Elongation at break is another mechanical property that is important for determining the application of polymeric films. Figure 1.15 shows the elongation at break of pure and the blend films with and without crosslinking in the dry state. The elongation at break of pure and the blend films depended on the chitosan content. This figure does not show the values of the blend films with less than 50 % chitosan content because of the brittleness of the films, similar to the case of tensile strength in the dry state. However, when chitosan content was increased, the elongation at break increased. It is found that the presence of at least 50% chitosan content in the blend films can improve the flexibility of the blend films. The reason is that chitosan has a rigid chains which can withstand the tensile force as compared to silk fibroin that is deficient in tensile property. Moreover, this improvement may be derived from hydrogen bonding interaction between chitosan and silk fibroin within the blend films

(Chen *et al.*, 1997). Hydrogen bonds act as bridges between polymer chains. When the tensile force is applied, these hydrogen bonds help to relieve the stress concentration between polymer chains slowly leading to higher elongation at break. It was considered that the crosslinked blend films had a lower elongation at break since chitosan chains were held together by crosslinking agent. In addition, the presence of crosslinks limited the extensibility of chitosan chains resulting in decrease of elongation at break.

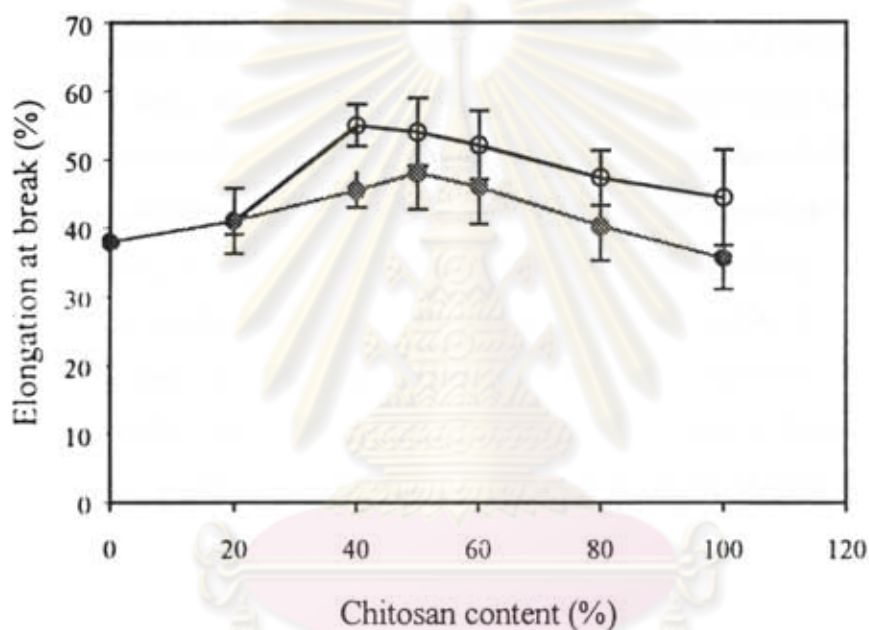


Figure 1.16 Elongation at break of pure and blend films at wet state.

○ Films without crosslinking; ● Films with crosslinking

The elongation at break in the wet state of pure and the blend films was determined. Figure 1.16 exhibited the elongation at break of pure and the blend films as a function of chitosan content. In the wet state, the elongation at break considerably increased as compared to that of the films in the dry state. At chitosan content between 40-60 %, the blend films had the highest values of elongation at break. When chitosan content is outside this range, the elongation at break of the films dropped. It can be interpreted that in the dry state, the hydrogen bonding between polymer chains is present as confirmed by the FTIR spectra of the films. However, when the films are immersed in water, the water molecules would penetrate inside the films and disrupt

the hydrogen bonding between polymer chains. These water molecules have a plasticized effect to the films by allowing the polymer chains segments to move easier (Freddi *et al.*, 1995). When tensile force is applied, the stress relaxation of polymer chains can occur faster than in the dry state where the chain mobility is restricted. Liang *et al.* (1992), suggested that the ductility of silk fibroin/sodium alginate blend films could be enhanced by the affect of absorbed and free water, which facilitates the chain mobility. Therefore, the blend films in the wet state had higher elongation break than that in the dry state. Similar to the films in the dry state, the crosslinked blend films showed lower elongation at break in the wet state than the blend films without crosslinking. This results from the presence of crosslinks within the films. These crosslinks limit the stretching of the films. Remunan-Lopez and Bodmeier (1997) concluded that the higher the degree of crosslinking, the smaller the water uptake and the elongation of the wet films. Consequently, the crosslinked blend films had lower elongation at break than the blend films without crosslinking.

From these results, in the dry state, the flexibility of silk fibroin film could be enhanced by blending with chitosan. While in the wet state, the elongation at break increased considerably due to the loss of interaction between polymer chains. In addition, the water molecules acted as a plasticizer to facilitate the chain mobility of polymers leading to higher elongation at break.

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Oxygen Permeability

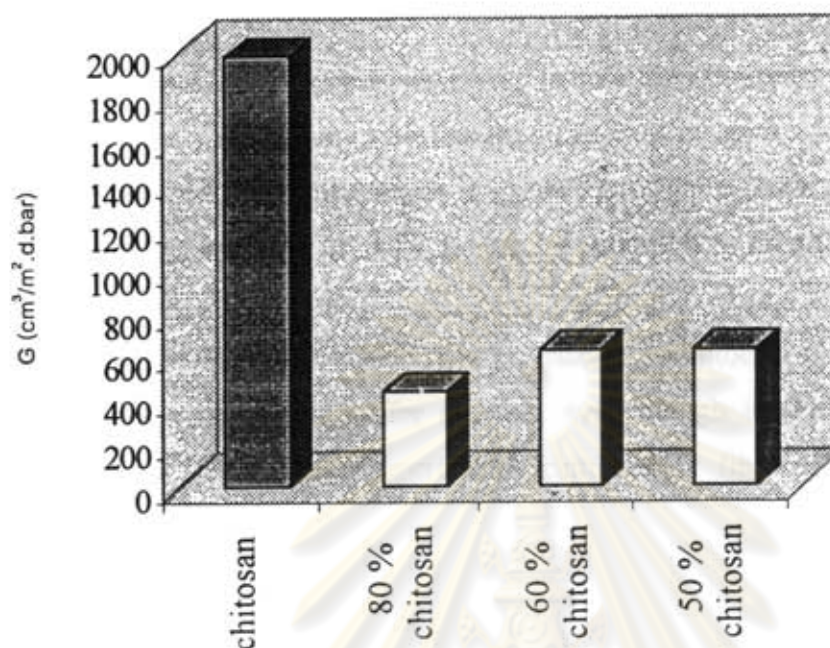


Figure 1.17 The oxygen permeability (G) of pure and the blend films.

Oxygen permeability of pure and the blend films is shown in Figure 1.17. Pure chitosan film showed high oxygen permeability as compared to the blend films. The addition of silk fibroin to the blend films led to the significant decreasing of the oxygen permeability. It may be due to the change in morphology of the blend films. By blending silk fibroin with chitosan, conformation of silk fibroin chains changed from random coil to β -sheet structure, which was the more compact and regular structure (Yashimizu and Asakara, 1990). The more compact structure restricted the oxygen to permeate through the films resulting in the lower oxygen permeability. The oxygen permeability of pure silk fibroin film and the blend films with up to 40 % chitosan content could not be determined due to the brittleness of the films. Hosokawa *et al.* (1992) found that oxygen permeability of chitosan films with 80 μm in thickness was lower than that of polyethylene but comparable to that of nylon and PET films. In these results, the addition of silk fibroin reduced the oxygen permeability to the blend films.

CONCLUSION

The composition of chitosan/silk fibroin blend films had a large effect on the mechanical properties, physical properties, and swelling behavior of the blend films. Blending silk fibroin with chitosan resulted in an improvement in tensile strength and elongation at break, and an increase in crystallinity. On the other hand, silk fibroin enhanced the thermal stability of chitosan. The addition of crosslinking agent to the blend films enhanced the mechanical properties. Furthermore, crosslinking was very important for the swelling behavior since it enabled retention of structural integrity of the films in the acidic pH buffer solution, even though it reduced the degree of swelling of the films. The properties of chitosan/silk fibroin blend films varied strongly with respect to changes in pH, salt type, and salt concentration. Therefore, these chitosan/silk fibroin blend films had pH and salt-responsive properties.



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CHAPTER 2

DRUG RELEASING PROPERTY OF CROSS-LINKED CHITOSAN/SILK FIBROIN BLEND FILMS

ABSTRACT

Crosslinked chitosan/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. Drug release characteristics of the blend films with various blend compositions were investigated. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid were used as model drugs. The release studies were carried out at 37°C in buffer solutions at pH 2.0, 5.5 and 7.2. It was found that the blend film with 80% chitosan content showed the maximum amount of drug release at pH 2.0 for all types of drugs. From swelling study, the maximum degree of swelling of the drug-loaded blend films was also obtained at pH 2.0 and 80 % chitosan content. The amounts of drugs released from films with 80% chitosan content were in the order: salicylic acid > theophylline > diclofenac sodium > amoxicillin. The maximum amount of salicylic acid, theophylline, diclofenac sodium and amoxicillin release from blend films with 80% chitosan content at pH 2.0 were 92.7%, 81.1%, 76.6%, and 37.2%, respectively.

INTRODUCTION

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

Silk fibroin is a fibrous protein that is composed of 17 amino acids, of which the main components are nonpolar species such as glycine, alanine, and serine. Silk fibroin can exist in 2 molecular conformations, random coil and β -sheet form. The conformational transition of silk fibroin from random coil to β -sheet structure can be induced by treatments such as heating, stretching or immersion in polar solvents. This

transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β -sheet structure is resistant to water and has good mechanical properties (Park *et al.*, 1999). Silk fibroin is considered a potential precursor to new materials and devices for biotechnological and biomedical utilizations. It has been reported that silk fibroin film has good oxygen permeability in the wet state, which suggests promising applications as a wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures, in biocompatible devices with controlled drug release (Tsukada *et al.*, 1994) and for bone binding functions. However, silk fibroin in the dry state is very brittle and unsuitable for practical use (Freddi *et al.*, 1995). To overcome this limitation, silk fibroin has been blended with other synthetic polymers, such as polyacrylamide (Freddi *et al.*, 1999) and poly(vinyl alcohol) (Yamaura *et al.*, 1990), or natural polymers, such as cellulose (Freddi *et al.*, 1995) and sodium alginate (Liang *et al.*, 1992), to improve mechanical and physical properties.

Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of $\beta(1\rightarrow4)$ -linked 2-acetamido-D-glucose unit and $\beta(1\rightarrow4)$ -linked 2-amino-D-glucose unit with the latter usually greater than 75% (Li *et al.*, 1997). Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-dimensional crosslinked network with the ability to absorb significant amount of water. Crosslinked chitosan hydrogels can swell extensively due to the positive charges on the network and respond to changes in the pH of the medium. Due to the benefits of being nontoxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for sustained drug delivery. For example, it was used for the delivery of drugs such as, prednisolone (Kofuji *et al.*, 2001) and diclofenac sodium (Gupta *et al.*, 2000). Furthermore, it has been reported that chitosan can induce a conformational transition of silk fibroin from random coil to β -sheet structure and that, using glutaraldehyde as a crosslinking agent, a polymer blend of these biopolymers can also form a hydrogel, having a semi-interpenetrating network structure (Chen *et al.*, 1997).

To our best knowledge, there has been no report on using chitosan/silk fibroin blend film as a drug delivery vehicle. This research is a preliminary study on using a

glutaraldehyde cross-linked chitosan/silk fibroin blend film as a matrix for a drug delivery system. The model drugs used were theophylline, diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effect of blend composition, degree of crosslinking, and pHs of the external swelling media on drug release from the blend films was investigated.

EXPERIMENT

Materials

Shrimp shell was kindly provided by Surapon Food Public Co., Ltd. Silk fiber (*Bombyx mori*) was degummed by treatment with 0.5% Na₂CO₃ at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk fibroin was dissolved in a triad solvent CaCl₂: EtOH: H₂O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was next filtered through the sintered glass filter, and subsequently diluted to achieve a concentration of 1% w/w.

Sodium hydroxide 50% w/w solution was kindly supplied by KPT Cooperation (Thailand). Glacial acetic acid 99.9% w/w purchased from J.T. Baker was analytical grade. Glutaraldehyde 50% w/w was purchased from Fluka.

Theophylline was purchased from Shanghai Wandai Pharmaceuticals, China. Diclofenac sodium was purchased from Tangyin Yongqi Chemical Industry Co., Ltd., China. Salicylic acid was purchased from Ajax Chemicals, Australia. Amoxicillin trihydrate was purchased from Antibiotics Co., Ltd., Spain.

Equipment

Restch Seiving Machine

The chitosan powder with the size of 38 to 75 μm was sieved and collected separately by using Retsch Seiving Machine type Vibro.

Capillary Viscometer

The viscosity-average molecular weight of chitosan was determined by using Cannon Ubbelohde-type number 50 of capillary viscometer.

FTIR Spectrophotometer

The FTIR spectrum of chitosan was recorded with Vector 3.0 Bruker FTIR Spectrophotometer with 16 scans at a resolution of 4cm^{-1} . A frequency of $4000\text{-}400\text{ cm}^{-1}$ was observed by using deuterated triglycerinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9\text{ cm.Hz}^{1/2}.\text{w}^{-1}$.

UV/Visible Spectrophotometer

The amount of drug release from chitosan films and blend films at pH 2.0, 5.5, and 7.2 was determined by using UV/Visible Spectrophotometer Lambda10, Perkin Elmer.

Methodology

Preparation of Chitin

Chitin was prepared from shrimp shell by decalcification and deproteinization to remove calcium carbonate and protein, respectively. The shrimp shells were cleaned and dried under sunlight before grinding into small pieces. Shrimp shell chips were treated by immersion in 1 N HCl solution for 2 days with occasional stirring. The decalcified product was washed with distilled water until neutral. Deproteinization was followed by boiling in 4% w/w of NaOH solution at $80\text{-}90^\circ\text{C}$ for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C in a convective oven for 24 h.

Preparation of Chitosan

Chitin was deacetylated by heating in 50% w/w NaOH solution containing 0.5% w/w sodium borohydride (NaBH_4) to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustively with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined, based on an IR spectroscopic method reported by Sannan (1978). About 3 mg of chitosan powder, passed through a 200-mesh sieve, was mechanically mixed with 400 mg of potassium bromide to prepare a KBr disk. An infrared spectrum was recorded in a range from 4000 to 400 cm^{-1} . The absorbances at 2878 cm^{-1} (the C-H band) and 1550 (the amideII band) were used to quantitate the degree of deacetylation. The degree of deacetylation was calculated from the equation 1.

$$D = 98.03 - 34.68(A_{1550}/A_{2878}) \quad (1)$$

where

D = degree of deacetylation (%)

A_{1550} = absorbance at 1550 cm^{-1}

A_{2878} = absorbance at 2878 cm^{-1} .

Viscosity-Average Molecular Weight of Chitosan

Chitosan solutions of different concentrations (0.00, 0.00625, 0.0125, 0.025, 0.05, and 0.1g/100mL) in 0.2 M acetic acid: 0.1M NaCl: 4.0 M urea were prepared. An Ubbelohde viscometer was filled with 10 mL of sample, which maintained the temperature at 25°C. The sample was passed through the capillary once before the running times were measured. Each sample was measured 3 times. The running times of solvent and solutions were used to calculate the relative viscosity, specific viscosity, and reduced viscosity. The reduced viscosity was plotted against the concentration and the intrinsic viscosity determined from the intercept. The corresponding equations are:

$$\text{Relative viscosity } (\eta_{rel}) = t/t_s \quad (2)$$

$$\text{Specific viscosity } (\eta_{sp}) = (t/t_s) - 1 \quad (3)$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/C \quad (4)$$

$$\text{Intrinsic viscosity } [\eta] = (\eta_{red})_{c \rightarrow 0} \quad (5)$$

where t is the flow time in seconds of chitosan solution, t_s is the flow time in seconds of solvent and C is the concentration of chitosan solution in g/100 mL.

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$$\text{Intrinsic viscosity } [\eta] = (\eta_{red})_{C \rightarrow 0} \quad (5)$$

where t is the flow time in seconds of chitosan solution, t_s is the flow time in seconds of solvent and C is the concentration of chitosan solution in g/100 mL.

The viscosity average molecular weight of chitosan was determined based on the Mark-Houwink equation (Lee *et al.*, 1974)

$$[\eta] = 8.93 \times 10^{-4} M^{0.71} \quad (6)$$

where $[\eta]$ is the intrinsic viscosity and M is viscosity average molecular weight.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1% w/w acetic acid. The chitosan solution was allowed to stand overnight at room temperature to get rid of air bubbles before preparation of films.

Preparation of Crosslinked Drug-Loaded Blend Films

Solutions containing chitosan and silk fibroin were prepared by mixing various ratios of 1% w/w of silk fibroin solution and 1% w/w of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added to the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added to the blend solutions to reach a concentration of 0.1% w/w. The blend solution containing a model drug was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto clean dry petri dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use.

Drug Release Studies

To study the release characteristics of the model drugs from the films, drug-loaded blend films were immersed in buffer solutions at pH 2.0, pH 5.5 and pH 7.2. At time intervals, 1-mL aliquots were withdrawn and assayed for the amount of drug released. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid released in the solutions were determined by a UV-Visible spectrophotometer (Perkin Elmer, Lambda 10) at 272, 275, 272, 299 nm, respectively, using calibration curves for each drug. The experiments were performed in triplicate. The percentages of released drugs were from average value of repeated three times.

RESULTS AND DISCUSSION

Effect of Blend Composition on Drug Release

The effect of blend composition on drug release is shown in Figure 2.1-2.4. Silk fibroin contents of 0, 20, 40, 50 and 60% in drug-loaded blend films were used in this study. The blend films with silk fibroin contents higher than 60% were not reported because the films were brittle and difficult to handle without cracking.

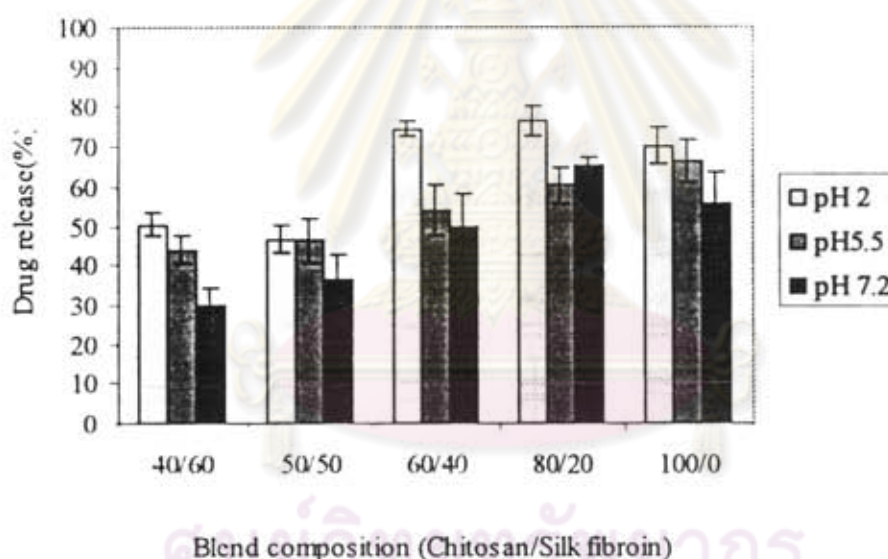


Figure 2.1 Effect of blend composition at various pH on releasing of diclofenac sodium

It was found that the maximum release of drug was observed for the blend film with 80% chitosan content for all types of model drugs. This could be explained by the term of swelling behavior of the blend films. It was found that the blend film with 80% chitosan content showed the maximum degree of swelling (Table 2.1).

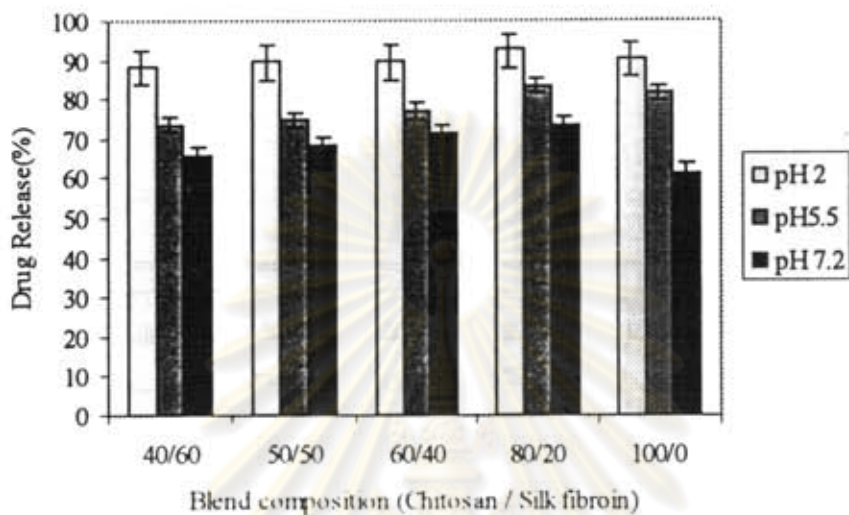


Figure 2.2 Effect of blend composition at various pH on releasing of salicylic acid.

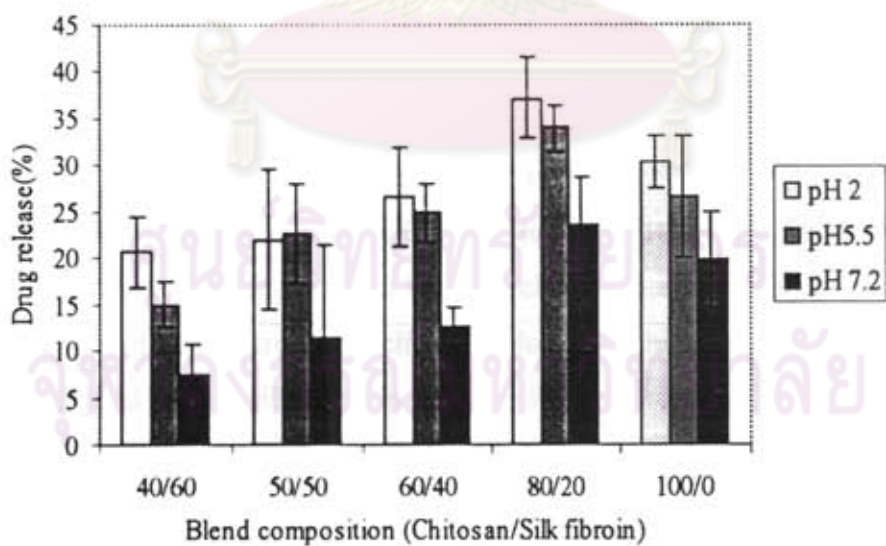


Figure 2.3 Effect of blend composition at various pH on releasing of amoxicillin.

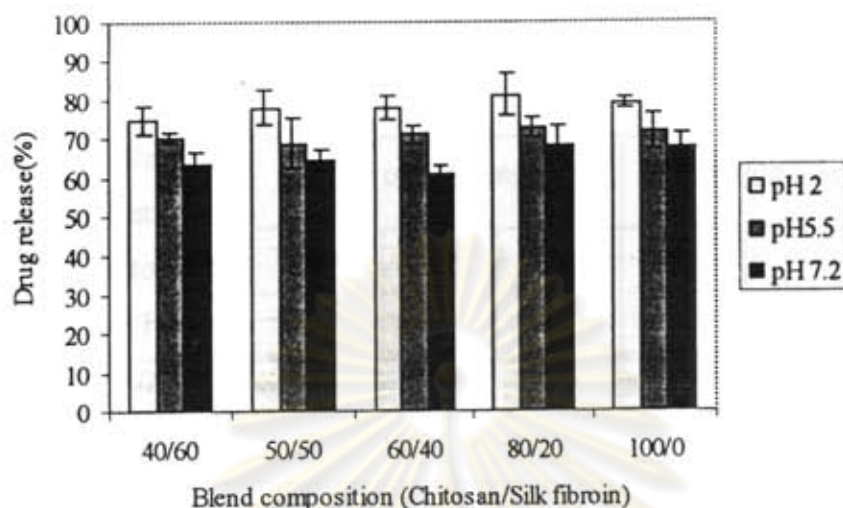


Figure 2.4 Effect of blend composition at various pH on releasing of theophylline.

Peppas *et al.* (1983) suggested that hydrogel delivery system was controlled by swelling behavior of hydrogel. Risbud *et al.* (2000) concluded that the release of amoxicillin from the air-dried and freeze-dried chitosan/poly(vinyl pyrrolidone) hydrogels was related to the degree of swelling of the hydrogels. Furthermore, Yao *et al.* (1993 and 1994) studied the release of chlorhexidini acetate and cimetidine from chitosan/polyether semi-interpenetrating hydrogel. They found that the higher degrees of swelling, the higher amounts of drug released. Chen *et al.* (1997) reported that the maximum degree of swelling of the blend films was observed for chitosan/silk fibroin blend film with 80% chitosan content. The swelling of chitosan/silk fibroin blend films may be occurred due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan. However, the lower amounts of released drug were obtained when silk fibroin content in the drug-loaded blend films was increased. Suesat *et al.* (2000) reported that there was no change in the degree of swelling of pure silk fibroin film immersed in buffer solutions for the whole pH range from pH 3 to pH 11. Therefore, swelling ability of the blend films depended on the amounts of chitosan content in the blend films.

Table 2.1 Degree of swelling and percent weight loss of drug- loaded crosslinked chitosan and blend films

Drug	Weight Ratio of Chitosan to Silk Fibroin	Degree of Swelling (%) ^a			Weight Loss (%) ^a		
		pH 2	pH 5.5	pH 7.2	pH 2	pH 5.5	pH 7.2
Salicylic acid	100:0	840	662	185	34.20	24.27	17.60
	80 : 20	1152	974	199	39.50	27.81	24.32
	60 : 40	763	535	179	35.20	24.21	15.4
	50 : 50	539	495	173	28.10	20.04	14.2
	40 : 60	582	471	169	23.70	19.86	13.8
Amoxicillin	100:0	643	492	194	20.38	19.98	17.73
	80 : 20	736	587	197	28.25	27.24	18.67
	60 : 40	639	472	168	22.30	20.75	18.25
	50 : 50	553	431	145	19.93	18.50	19.10
	40 : 60	504	409	129	18.10	17.63	17.98
Diclofenac sodium	100:0	753	653	167	35.43	30.07	28.6
	80 : 20	809	721	173	37.85	35.18	28.8
	60 : 40	783	600	125	30.53	30.01	23.4
	50 : 50	722	534	108	29.93	28.56	20.6
	40 : 60	687	497	102	21.20	20.01	17.5
Theophylline	100:0	306	252	167	38.87	35.54	26.14
	80 : 20	376	325	175	40.05	37.75	27.22
	60 : 40	360	289	159	36.67	32.23	23.44
	50 : 50	303	277	151	33.32	28.87	20.5
	40 : 60	232	221	142	30.01	25.34	18.7

^a the average value from three experiments.

Effect of pH on Drug Release

The effect of pH on drug released from chitosan and blend films is shown in Figure 2.1-2.4. Drug release profile was studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the amount of drug released from the systems was highest at pH 2.0 for all types of model drugs. This is in good agreement with the result of swelling as shown in Table 2.1. It appeared that the degree of swelling was the highest at pH 2.0 and tended to decrease when pH of swelling solution was increased. This result corresponded to the previous works, which also reported that the degree of swelling of the crosslinked chitosan/silk fibroin blend films was maximum at pH 2.0 and decreased when pH of the swelling solution was increased. It can be explained by the fact that in an acidic medium the amino groups of chitosan were protonized, resulting that the hydrogen bonds between chitosan and silk fibroin were broken and the network was dissociated. The blend films exhibited lower degree of swelling when pH was higher than 5. This may be due to the number of protonated amino groups of chitosan become lower at neutral and alkaline pH. The pK_a of chitosan is 6.3-6.5, which indicates that chitosan tends to protonate in acidic solution. Therefore, the degree of swelling of the blend films in alkaline solution was very low as compared to that of the blend films in acidic solution. Risbud *et al.* (2000) reported that the degrees of swelling of chitosan/poly(vinyl pyrrolidone) hydrogels were high in acidic solutions (pH 1.0, pH 2.0 and pH 3.0) and became lower in neutral and alkaline solutions (pH 7.2 and pH 9.2). The release of amoxicillin was found to be maximum at pH 1.0. Besides the release of drug is controlled by swelling condition of the carrier, drug release may be concerned with the erosion process. This process is associated with macroscopic changes in the appearance of the device, changes in the physicochemical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions. Table 2.1 shows the weight loss of chitosan and blend films. It was found that the weight loss of the films was highest at pH 2. This indicated that drug release by erosion process could be occurred in this system.

Effect of Drug Types on Drug Release

The effect of drug molecules on drug release is shown in Figure 2.5-2.7. The releases of model drugs, theophylline, salicylic acid, diclofenac sodium and amoxicillin, were studied at pH 2.0, pH 5.5 and pH 7.2.

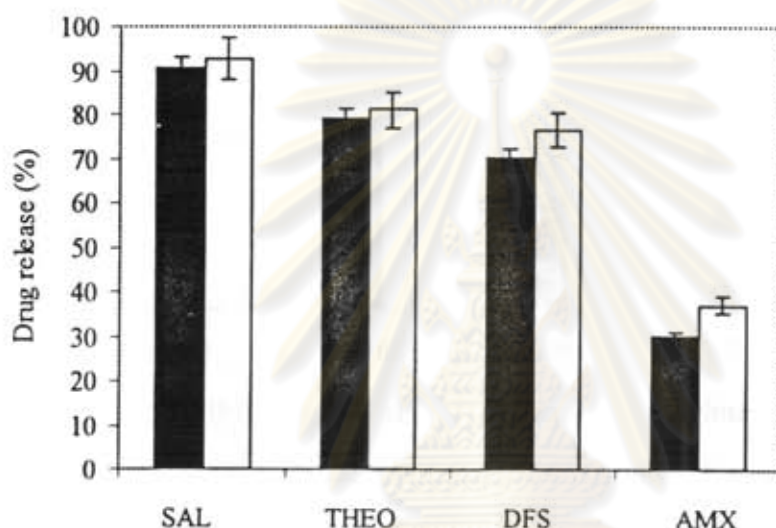


Figure 2.5 Comparison of the amounts of drugs released from chitosan and the blend film with 80% chitosan content at pH 2.0. ■ chitosan film □ blend film with 80% chitosan content.

It was found that the blend film at 80% chitosan content gave the highest amount of released drugs. The amounts of released salicylic acid at pH 2.0, pH 5.5 and pH 7.2 from blend film with 80% chitosan content were 92.7%, 83.4% and 73.5%, respectively. The amounts of theophylline released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan were 81.1%, 73.6% and 69.0%, respectively. The maximum amount of released salicylic acid at equilibrium was higher than that of theophylline. One factor that can affect the penetration of a drug from a polymer matrix is the molecular size of the drug. The molecule of salicylic acid was smaller than theophylline. Thus, the penetration of salicylic acid from the matrix was easier than theophylline. Diclofenac

sodium released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 76.6%, 66.1% and 65.1%, respectively.

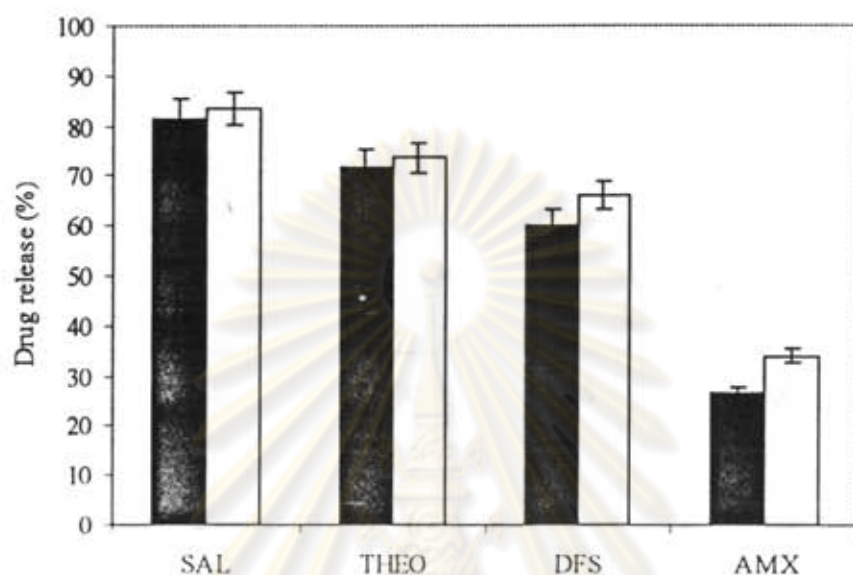


Figure 2.6 Comparison of the amounts of drugs released from chitosan and the blend film with 80% chitosan content at pH 5.5. ■ chitosan film □ blend film with 80% chitosan content.

The amount of diclofenac sodium released was less than those of theophylline and salicylic acid because diclofenac sodium did not dissolve in the blend solutions and appeared in the blend films as solid particles. Therefore, the diffusion of diclofenac sodium to the solution took longer time than salicylic acid and theophylline.

Among the drugs investigated in this study, the amounts of amoxicillin released from the blend films was the least values for all pH studied. It was found that the amount of amoxicillin released at pH 2.0, pH 5.5 and pH 7.2 were 37.2%, 34.0% and 23.5%, respectively. This may be due to the interaction between the drug molecule and polymer matrix. Risbud *et al.* (2000) reported the amoxicillin released from crosslinked chitosan-poly(vinyl pyrrolidone) air-dried hydrogel was about 31.68% and 27% at pH 1.0 and pH 2.0, respectively. They explained that the low amounts of drug released might be due to non-porous nature of the air-dried films.

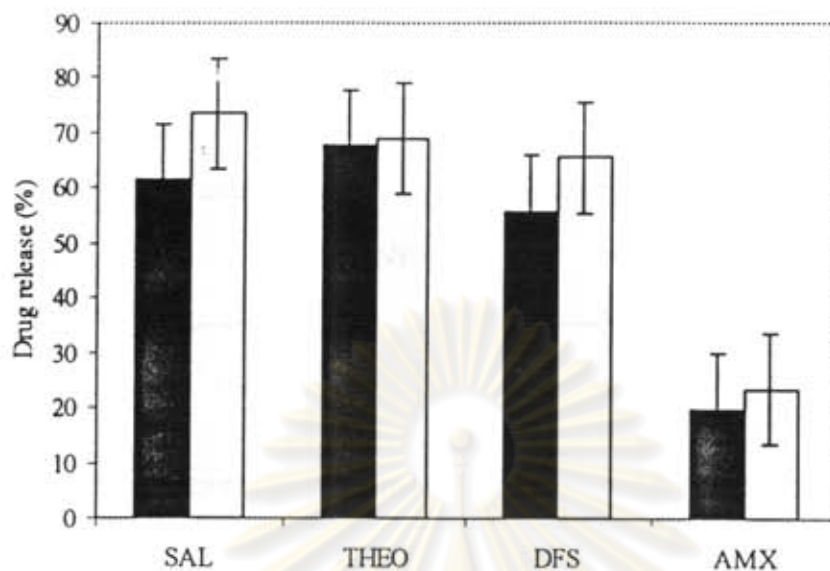


Figure 2.7 Comparison of the amounts of drugs released from chitosan and the blend film with 80% chitosan content at pH 7.2. ■ chitosan film □ blend film with 80% chitosan content.

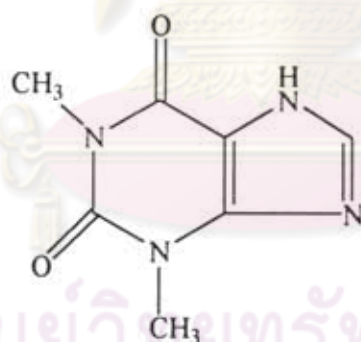


Figure 2.8 Chemical structure of anhydrous theophylline.

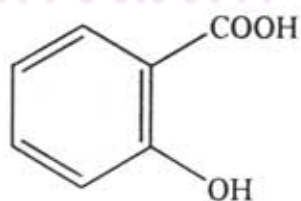


Figure 2.9 Chemical structure of salicylic acid.

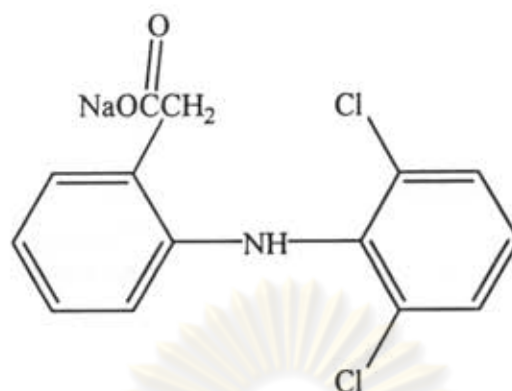


Figure 2.10 Chemical Structure of diclofenac sodium.

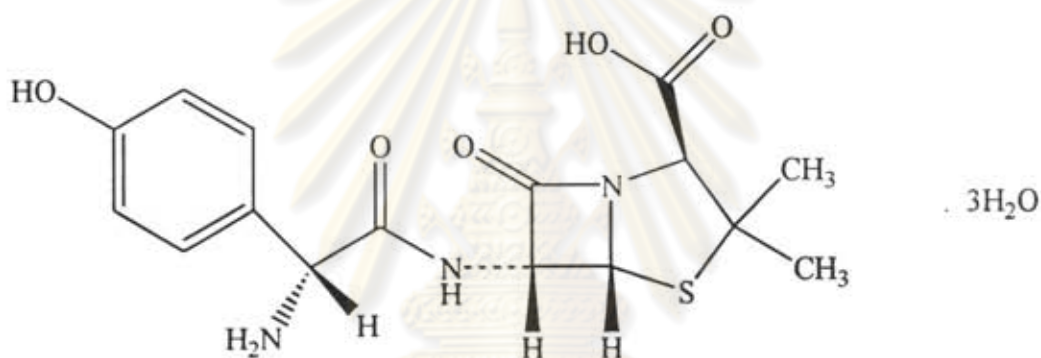


Figure 2.11 Chemical structure of amoxicillin trihydrate.

Effect of Immersion Time on Drug Release

The release profiles of each type of drug in buffer solutions at pH 2.0, 5.5, and 7.2 from the blend films with 80 % chitosan content as a function of immersion time are illustrated in Figure 2.12-2.14. The initial rates of drug releases were then calculated and the results are shown in Table 2.2. It was found that the release of theophylline from the blend films with 80% chitosan content was faster than the releases of other model drugs at all pH studied. Puttipipatkachorn (2001) studied the drug-polymer interaction between theophylline and chitosan by Fourier Transform Infrared Spectroscopy and solid state ^{13}C NMR spectroscopy. It was concluded that there was no interaction between

theophylline and chitosan. Therefore, theophylline was the fastest released drug in this study because there was no interaction between theophylline and chitosan.

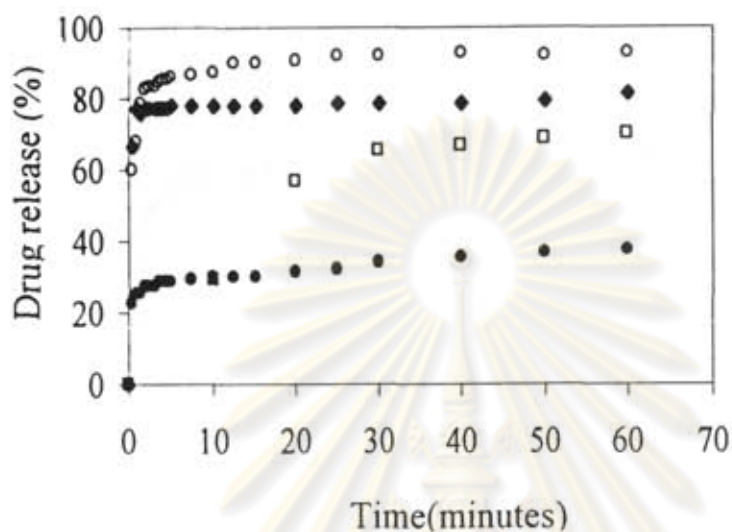


Figure 2.12 Drug release profiles from the blend films with 80% chitosan content at pH 2.0. ○ salicylic acid, ◆ theophylline, □ diclofenac sodium, ● amoxicillin trihydrate.

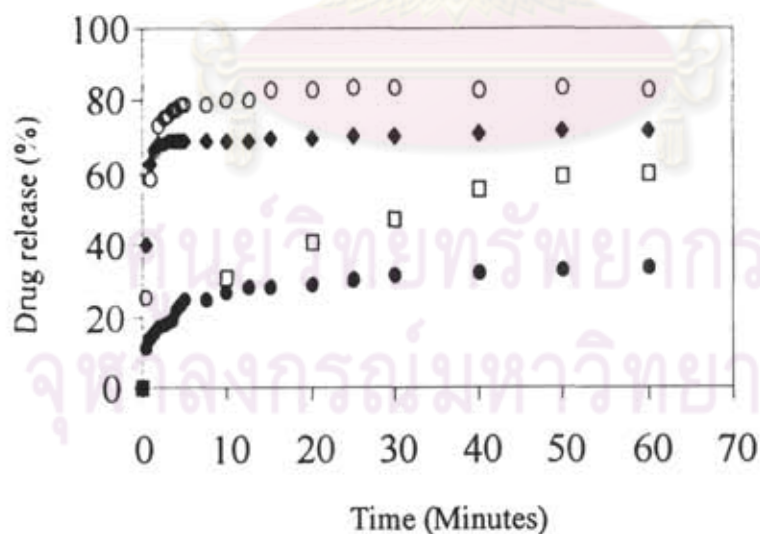


Figure 2.13 Drug release profiles from the blend films with 80% chitosan content at pH 5.5. ○ salicylic acid, ◆ theophylline, □ diclofenac sodium, ● amoxicillin trihydrate.

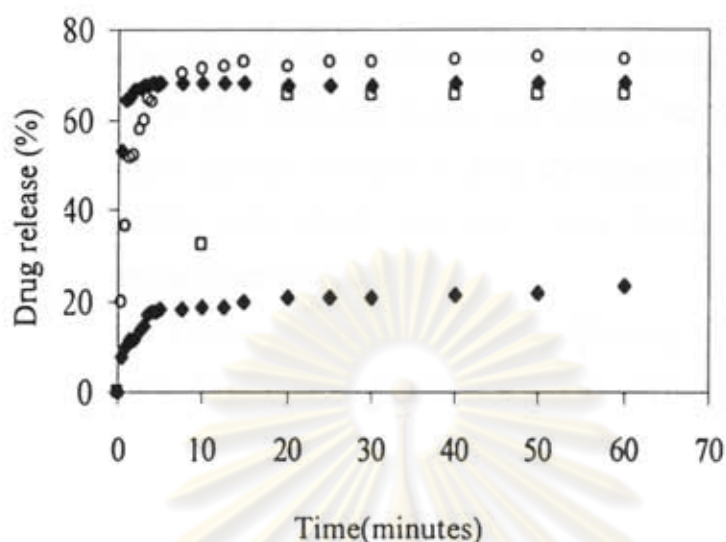


Figure 2.14 Drug release profiles from the blend films with 80% chitosan content at pH 7.2. ○ salicylic acid, ◆ theophylline, □ diclofenac sodium, ● amoxicillin trihydrate.

Table 2.2 Initial rate of drug release from the blend films with 80% chitosan content

Model drugs	Drug release rate ($10^{-5}/\text{sec}$)		
	pH 2.0	pH 5.5	pH 7.2
Salicylic acid	1.83	0.80	0.55
Theophylline	2.28	1.33	1.07
Diclofenac sodium	0.76	0.37	0.25
Amoxicillin	0.05	0.05	0.04

Moreover, the molecular size of theophylline was rather small compared with the other model drugs used. Then, theophylline could penetrate from the film easily than the others did.

The salicylic acid released from the blend films with 80% chitosan content was slower than theophylline. It could be noticed that the amount of salicylic acid released was greater than that of theophylline. However, the initial releasing rate of salicylic acid

was slower than that of theophylline. Puttipatkhachorn (2001) was also studied the interaction between salicylic acid and chitosan by using Fourier Transform Spectroscopy. It was found that there was the salicylate formation, which was occurred by the interaction between carboxylic groups of salicylic acid and amino groups of chitosan. Therefore, the rate of salicylic acid release was slower than theophylline due to the interaction between salicylic acid and chitosan.

Diclofenac sodium release was slower than theophylline and salicylic acid release. It can be explained that because the molecular size of diclofenac sodium is bigger than theophylline and salicylic acid. Therefore, diclofenac sodium released slower due to its difficulties of diclofenac sodium for penetrating to the external swelling media.

Among the model drugs used, amoxicillin release was the slowest released drug in all pH studies. Risbud (2000) investigated the surface morphology of the amoxicillin-loaded air-dried hydrogel of crosslinked chitosan and poly(vinyl pyrrolidone) by using glutaraldehyde as crosslinking agent. It was revealed that the surface morphology of the air-dried hydrogel showed non-porous nature and non-open channel structure. Accordingly, the amoxicillin was released from the polymer matrix very slowly. In addition, the molecular size of amoxicillin is the biggest compared with the other model drugs used. Therefore, the penetration of amoxicillin through the polymer matrix was slow.

Effects of Concentration of Crosslinking Agent on Drug Release

The effect of crosslinking agent concentration on drug release from the blend films is shown in Figure 2.15.

To study the effect of concentration of crosslinking agent on drug release, the salicylic acid-loaded blend films with 80% chitosan content containing glutaraldehyde concentrations of 0.001, 0.01, and 0.5 mole/glucosamine unit were used. It was found that the amount of salicylic acid released was decreased with the increasing concentration of glutaraldehyde at all pHs studies. It could be possibly explained by the term of degree of swelling as shown in Table 2.3. It indicated that the degree of swelling of the salicylic acid-loaded blend films was decreased with the increasing glutaraldehyde concentration.

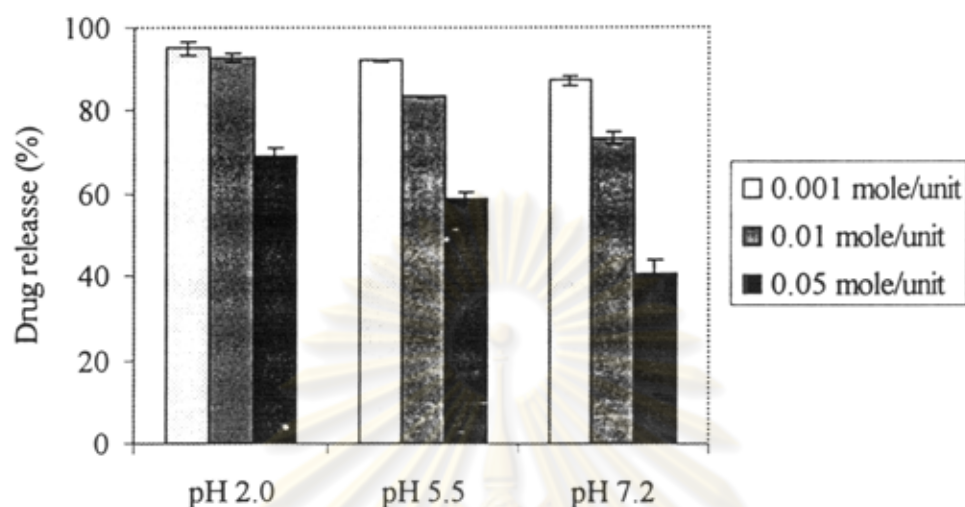


Figure 2.15 Salicylic acid released from the blend films with 80% chitosan content containing glutaraldehyde concentration of 0.001, 0.01, and 0.5 mole/glucosamine unit at pH 2.0, 5.5 and 7.2.

Table 2.3 Degree of swelling and percent weight loss of salicylic acid-loaded blend films with 80% chitosan containing various glutaraldehyde concentrations

pH	Degree of Swelling (%) ^a			Weight Loss (%) ^a		
	0.001 mole/unit	0.01 mole/unit	0.05 mole/unit	0.001 mole/unit	0.01 mole/unit	0.05 mole/unit
2.0	1453	1152	825	42.21	39.50	32.13
5.5	1213	974	672	33.16	27.81	22.81
7.2	320	199	123	25.19	24.32	19.25

^a the average from three experiments.

In 1990 Peppas and Korsmeyer investigated the effect of crosslinking concentration and the release properties of poly(vinyl alcohol) on diffusion of theophylline by using glutaraldehyde as crosslinking agent. They discovered that at low concentration, the

effect of crosslinking agent on the release of drug was very small, while at high crosslinking concentration a much larger effect on the drug diffusion was observed. This was attributed to the swelling behavior of the crosslink network. At low crosslinking concentration of crosslinking agent, the density of crosslinking was low that make hydrogel swell extensively. While the mesh size of the network was then big resulting in high penetration of drug particle to external environment. But at high crosslinking concentration, the degree of swelling was limited. Therefore, the mesh size of the network is closer to the size of drug, and the drug is difficult to penetrate to the external environment.

CONCLUSIONS

Drug release characteristics from crosslinked chitosan/silk fibroin blend films by using glutaraldehyde as crosslinking agent were studied using theophylline, salicylic acid, diclofenac sodium and amoxicillin as model drugs. The blend films with 80% chitosan content gave the maximum amounts of drug releases due to the maximum degree of swelling at this blend ratio. At chitosan content less than 80%, the amount of drug release from the blend films increased with increasing in chitosan content in the blend films. The amount of drug released at pH 2.0 was higher than at pH 5.5 and 7.2 because of the protonation of amino groups on chitosan at pH 2.0 resulting in the dissociation of hydrogen bond between chitosan and silk fibroin network. The maximum and the minimum of drug release were salicylic acid and amoxicillin, respectively. The order of the amount of released drugs from the minimum to the maximum was amoxicillin < diclofenac sodium < theophylline < salicylic acid. The sequence of initial rate of drug release from the slowest to the fastest release was amoxicillin < diclofenac sodium < salicylic acid < theophylline.

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CHAPTER 3
STUDY ON DRUG RELEASE CHARACTERISTICS OF CROSS-LINKED
CHITOSAN/SILK FIBROIN BLEND FILMS BY USING MODIFIED FRANZ
DIFFUSION CELL

ABSTRACT

Chitosan/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. Drug release properties of chitosan and blend films of various blend compositions were investigated *in vitro* using a modified Franz Diffusion Cell at 37°C and pH 5.5. Pig skin was used as material representing human skin. Theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate were used as model drugs. The order of drugs from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. For all model drugs, the blend films with 80% chitosan gave the maximum drug release. In addition, an increase in thickness of the films resulted in a decrease in the amount of drug released. All model drug release data could be fitted to either zero order or Higuchi's model indicating that the releases of model drugs from chitosan and the blend films were either rate-controlling or diffusion-controlled releases. It was expected that the chitosan/silk fibroin blend films could be used as matrix for sustained release of a drug for a transdermal drug delivery system.

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 acidic 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.

EXPERIMENT

Materials

The shell of *Penaeus merguensis* shrimps was kindly provided by Surapon Food Public Co., Ltd. Silk fibre (*Bombyx Mori*) was degummed by treatment with 0.5% Na₂CO₃ at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk

fibroin was dissolved in a triad solvent CaCl_2 : EtOH: H_2O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was next filtered through the sintered glass filter and subsequently diluted to achieve a concentration of 1% w/w.

Sodium hydroxide 50% w/w solution was kindly supplied by KPT Cooperation (Thailand). Glacial acetic acid 99.9% w/w purchased from J.T. Baker was analytical grade. Glutaraldehyde 50% w/w was purchased from Fluka.

Salicylic acid was purchased from Ajax Chemicals, Australia. Theophylline was purchased from Shanghai Wandai Pharmaceutical Co., Ltd., China. Diclofenac sodium was purchased from Tangyin Yongqi Chemical Industry Co., Ltd., China. Amoxicillin trihydrate was purchased from Anibiotics Co., Ltd., Spain. Other reagents are analytical grade and used without further purification.

Equipments

Capillary Viscometer

The viscosity-average molecular weight of chitosan was determined by using Cannon Ubbelohde-type number 50 of capillary viscometer.

FTIR Spectrophotometer

The FTIR spectrum of chitosan was recorded with Vector 3.2 Bruker FTIR Spectrophotometer with 16 scans at a resolution of 4 cm^{-1} . A frequency of $4000\text{-}400\text{ cm}^{-1}$ was observed by using deuterated triglycerinesulfate detector (DTGS) with specific detectivity of $1 \times 10^9\text{ cm}\cdot\text{Hz}^{1/2}\cdot\text{w}^{-1}$.

UV/Visible Spectrophotometer

The amount of drug releasing from chitosan films and the blend films at pH 5.5 was determined by using Perkin Elmer UV/visible Spectrophotometer model Lambda10.

Methodology

Chitin Preparation

Chitin was prepared from shrimp shell by decalcification and deproteinization to remove calcium carbonate and protein, respectively. The shrimp shells were cleaned and dried under sunlight before grinding into small pieces. Shrimp shell chips were treated by immersion in 1 N HCl solution for 2 days with occasional stirring. The decalcified product was washed with distilled water until neutral. Deproteinization was followed by boiling in 4% w/w of NaOH solution at 80-90°C for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C in a convective oven for 24 h.

Chitosan Preparation

Chitin was deacetylated by heating in 50% w/w NaOH solution containing 0.5% w/w sodium borohydride (NaBH₄) to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustively with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Degree of Deacetylation of Chitosan

The degree of deacetylation of chitosan was determined, based on an IR spectroscopic method reported by Sannan (1978). About 3 mg of chitosan powder, passed through a 200-mesh sieve, was mechanically mixed with 400 mg of potassium bromide to prepare a KBr disk. An infrared spectrum was recorded in a range from 4000 to 400 cm⁻¹. The absorbances at 2878 cm⁻¹ (the C-H band) and 1550 (the amideII band) were used to determine the degree of deacetylation. The degree of deacetylation was calculated from the equation 1.

$$D = 98.03 - 34.68(A_{1550}/A_{2878}) \quad (1)$$

where D = degree of deacetylation (%)

A_{1550} = absorbance at 1550 cm^{-1}

A_{2878} = absorbance at 2878 cm^{-1} .

Viscosity-Average Molecular Weight of Chitosan

Chitosan solutions of different concentrations (0.00, 0.0125, 0.025, 0.050, 0.075 and 0.1g/100ml) in 0.2 M acetic acid: 0.1 M sodium acetate were prepared. An Ubbelohde viscometer was filled with 10 ml of sample, which maintained the temperature at 30°C . The sample was passed through the capillary once before the running times were measured. Each sample was measured 3 times. The running times of solvent and solutions were used to calculate the relative viscosity, specific viscosity, and reduced viscosity. The reduced viscosity was plotted against the concentration and the intrinsic viscosity determined from the intercept. The corresponding equations are:

$$\text{Relative viscosity } (\eta_{rel}) = t/t_s \quad (2)$$

$$\text{Specific viscosity } (\eta_{sp}) = (t/t_s) - 1 \quad (3)$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/C \quad (4)$$

$$\text{Intrinsic viscosity } [\eta] = (\eta_{red})_{C \rightarrow 0} \quad (5)$$

where t is the flow time in seconds of chitosan solution, t_s is the flow time in seconds of solvent and C is the concentration of chitosan solution in g/100 ml.

The viscosity average molecular weight of chitosan was determined based on the Mark-Houwink equation (Lee *et al.*, 1974)

$$[\eta] = 7.52 \times 10^{-4} M^{1.0016} \quad (6)$$

where $[\eta]$ is the intrinsic viscosity and M is viscosity average molecular weight.

Chitosan Solution Preparation

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1% w/w acetic acid. The chitosan solution was allowed to stand overnight at room temperature to get rid of air bubbles before preparation of films.

Crosslinked Drug-Contained Blend Films Preparation

Solutions containing chitosan and silk fibroin were prepared by mixing various ratios of 1% w/w of silk fibroin solution and 1% w/w of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added to the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added to the blend solutions to reach a concentration of 1.0% w/w. The blend solution containing a model drug was stirred slowly for 12 h, residues some amounts of drug that over the solubility of drug, and left overnight to get rid of air bubbles before casting onto clean dry petri dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use.

Pig Skin Preparation

Permeation experiments were performed with full-thickness pig skin which were excised from a side of pigs. The whole pig skins were surgically removed and cleaned with sterile normal saline. The subcutaneous fat, tissue, blood vessel and epidermal hair were carefully removed by blunt section. The skin was free of obvious holes or defects. The full thickness skin was cleaned with normal saline and finally with distilled water, blotted dry, wrapped with aluminium foil and stored frozen before use. To perform in-vitro skin permeation experiment, full thickness skin was thawed at room temperature and cut into pieces (peripheral of circumference cell cap area) and a unit of drug-loaded blend films was applied onto the stratum corneum surface of the skin and then mounted individually between the half-cells.

Spectrophotometric Analysis of Model Drug

UV/visible Spectrophotometer model Lambda10 (Perkin Elmer) was employed to determine the maximum spectra of model drugs (theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate). It was performed by scanning the UV absorption in a wavelength range of 350-200 nm. Model drugs in aqueous solution was prepared for scanning the maximum absorption wavelength. The procedure was done at an ambient condition with a scan speed of 240 nm/min. The characteristic peaks were observed for theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate at maximum wavelength of 272, 299, 275 and 272 nm, respectively. The absorbance values at the maximum wavelength of model drugs were read and the correspondent model drug concentrations were calculated from the calibration curve. The calibration curves were plotted between the concentrations of drugs and the absorbance. The various concentrations of drug were in range 0.1-1 mg/100 ml.

In vitro Skin Permeation of Drug

The *in vitro* skin permeation of drug from prepared membrane was studied using a modified Franz diffusion cell. The full-thickness pig skin was mounted onto the receptor compartment with the stratum corneum side facing upward into the donor compartment and the dermal side facing downward into the receptor compartment. A unit of drug-contained blend film was placed over the skin and the whole assembly was clamped together with the donor cap on the top. The receptor compartment was then filled with the acetate buffer solution pH 5.5 constantly stirred using a magnetic stirrer and maintained at 37°C by a circulating waterbath. A portion (0.5 ml each) of buffer solutions were withdrawn from the receptor compartment at predetermined time intervals of ¼, ½, ¾, 1, 1½, 2, 2½, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours; the samples were replaced with an equal volume of freshly prepared pH 5.5 acetate buffer solutions (drug-free). The drug concentrations in these samples were determined by the UV/visible spectrophotometer method.

RESULTS AND DISCUSSION

Effect of Blend Composition on Drug Release

Drug releases from the drug-loaded chitosan/silk fibroin blend films containing chitosan contents of 100, 80, 60, 50 and 40% were studied at 37°C and pH 5.5 using modified Franz diffusion cells. However, drug release from the blend films with chitosan content less than 40% are not reported because the films were very brittle and difficult to handle without cracking. The results of drug release study are shown in Table 3.1. For all model drugs, the maximum drug releases were found for the blend film with 80% chitosan content. The amounts of theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate released from the blend films with 80% chitosan content were 77.44%, 92.14%, 73.09% and 32.00%, respectively. Compared to pure chitosan films, the amounts of model drug released from the blend films with 80% chitosan content were higher than that released from pure chitosan film (Figure 3.1). It is known that the drug release from hydrogels is mainly controlled by swelling-controlled release mechanism (Peppas *et al.*, 1983). According to this, the degrees of swelling of chitosan and the blend films after releasing of drugs were investigated and the results are shown in Table 3.1. It was found that the maximum degrees of swelling of the drug-loaded films were obtained for the blend film with 80% chitosan content. This result is in good agreement with the study of Chen *et al.* (1997b). Chen *et al.* (1997b) studied on swelling behavior of glutaraldehyde-crosslinked chitosan and silk fibroin blend films and reported that the maximum degree of swelling was observed for the blend film with 80% chitosan content. For chitosan/silk fibroin blend film, hydrogen bonding between amino group of chitosan and amide group of silk fibroin can be formed at pH higher than pKa of amino groups of chitosan (pKa = 6.3-6.5). However, at pH less than the pKa, the protonation of amino group of chitosan occur and hydrogen bonding disappear, resulting in swelling state of the films. The dissociation of the hydrogen bonding between amino group of chitosan and the amide group of silk fibroin was shown in Figure 3.2 (Chen *et al.*, 1997b).

Table 3.1 Drug release and degree of swelling of chitosan and the blend films of chitosan and silk fibroin

Model drug	Weight ratio of chitosan to silk fibroin	Drug release ^(a) (%)	Degree of swelling ^(a) (%)
Theophylline	100 : 0	66.26	708.82
	80 : 20	77.44	958.69
	60 : 40	58.37	389.45
	50 : 50	48.77	358.78
	40 : 60	40.04	313.41
Salicylic acid	100 : 0	87.31	951.23
	80 : 20	92.14	1056.87
	60 : 40	68.51	488.70
	50 : 50	65.20	441.29
	40 : 60	49.74	404.89
Diclofenac sodium	100 : 0	63.25	554.82
	80 : 20	73.09	663.04
	60 : 40	50.74	553.25
	50 : 50	27.00	502.68
	40 : 60	18.58	493.30
Amoxicillin	100 : 0	28.83	698.95
	80 : 20	32.00	940.48
	60 : 40	21.57	448.60
	50 : 50	18.83	398.95
	40 : 60	14.09	352.89

(a) The values at equilibrium state

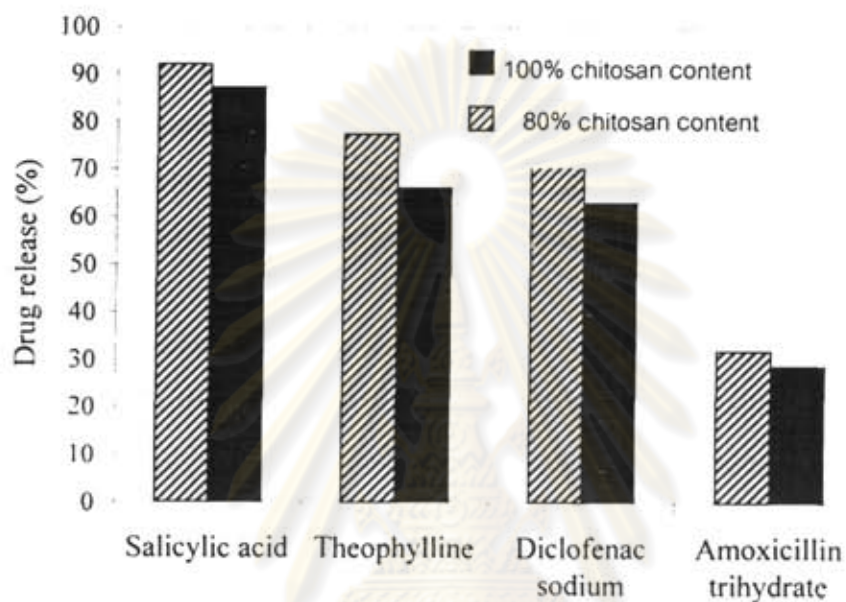


Figure 3.1 Comparison of percentages of drug released from chitosan and the blend film with 80% chitosan content. (■) 100% chitosan content and (▨) 80% chitosan content.

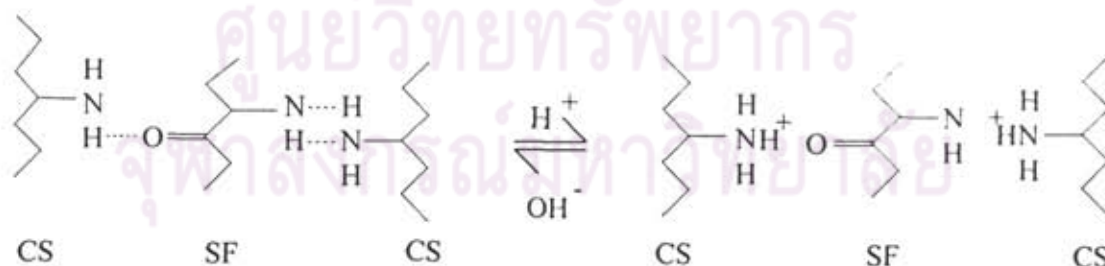


Figure 3.2 Formation of hydrogen bond between chitosan (CS) and silk fibroin (SF) in the semi-interpenetrating network and the dissociation of chitosan and silk fibroin by breaking down the hydrogen bond in the acidic medium, Chen *et al.* (1997b).

Furthermore, it was found that as chitosan content in the blend films decreased from 80% to 40%, the degrees of swelling of the blend films as well as the releasing amounts of drug decreased. Since drug release property correlated to swelling behavior of the blend films and swelling ability of the blend films depended on the blend composition, it might be said that drug release from the blend films was influenced by the blend composition of the films. In addition to swelling-controlled release mechanism, drug release can be occurred by erosion process. According to this, the weight losses of the films after releasing of drug were also determined (data not shown). Since significant weight losses of the films could be observed, the release of drugs from the films might be occurred by erosion process as well.

Effect of Drug Nature on Drug Release

Since all model drugs had the maximum drug releases for the blend film with 80% chitosan content, Figure 3.1 shows only the percentages of drug released from the blend film with this blend composition compared to those released from pure chitosan film. It was found that the order of drug from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. The same trends were obtained for the other blend compositions. The main factors that can affect the penetration of drug from a polymer matrix are molecular weight of drug, drug-polymer interaction and solubility of drug in the blend solution. When consider the molecular weights of model drugs that are 138, 180, 318 and 387 for salicylic acid, theophylline, diclofenac sodium and amoxicillin trihydrate, respectively, it can be seen that the order of drug above arranged from the model drugs with the lowest molecular weight to that with the highest molecular weight. It might be explained that the drug with lower molecular weight could penetrate from the network of the films easier than the drug with higher molecular weight.

Furthermore, the interaction between polymer matrix and drug also affect the drug release property of the films. The structures of model drugs are shown in Figure 3.3-3.6. For salicylic acid (Figure 3.3), there is carboxylic group in its molecule. Puttipipatkachorn *et al.* (2001) studied on drug-polymer interaction of

chitosan and salicylic acid. It has been reported that carboxylic group of salicylic acid could interact with amino group of chitosan by the formation of salicylate anion, as shown in Figure 3.7. Therefore, there might be some interaction between salicylic acid and chitosan in the blend films. For theophylline (Figure 3.4), there is no functional group that can react with either chitosan or silk fibroin in the blend films. In addition, Puttipatkhachorn *et al.* (2001) reported that there is no drug-polymer interaction between theophylline and chitosan. In this study, the interactions between chitosan and the model drugs were determined by FTIR technique and the results are shown in Figure 3.8-3.11. The FTIR spectrum of chitosan (Figure 3.8(a)) shows the symmetric carboxylate anion stretching at around 1411 cm^{-1} , indicating that chitosan in the film was in the form of chitosonium acetate (Yao *et al.*, 1994). The characteristic peaks of NH group in chitosan appear at around 1561 cm^{-1} (amino group of chitosan and amide II of chitin) and 1257 cm^{-1} (amide III of chitin) (Figure 3.8(a)). Moreover, the characteristic peaks assigned to saccharide structure appear at around 898 cm^{-1} and 1153 cm^{-1} (Chen *et al.*, 1997a). For the silk fibroin film (Figure 3.8(b)), the absorption band at around 1650 cm^{-1} (amide I) and 1542 cm^{-1} (amide II) which are attributed to the random coil conformation were observed (Chen *et al.*, 1997a and Yoshimizu *et al.*, 1990). In case of salicylic acid (Figure 3.9(e)), the carbonyl stretching peak was observed at around 1656 cm^{-1} (Moffat, 1986). For salicylic acid-loaded blend film, a new peak at around 1628 cm^{-1} assigned to an asymmetric NH_3^+ bending was observed (Figure 3.9(d)) (Silverstein *et al.*, 1991). A new peak was also observed at around 1384 cm^{-1} , which was assigned to the symmetric carboxylate anion stretching of salicylate anion (Figure 3.9(d)). It might be indicated that salicylic acid could interact with chitosan at the position of amino group to form salicylate salt. This results were correlated to the results of Puttipatkhachorn *et al.* (2001) who studied the drug-polymer interaction of salicylic acid and theophylline in chitosan films by FTIR and solid state ^{13}C NMR spectroscopy. In case of theophylline (Figure 3.8(e)), the absorption bands at around 1720 cm^{-1} (C=O stretching), 1676 cm^{-1} , 1567 cm^{-1} (C=C stretching), 1485 cm^{-1} (C=N stretching), 1314 cm^{-1} and 1242 cm^{-1} (C-N, C-O vibration) were observed. For theophylline-loaded blend film (Figure 3.8(d)), the FTIR spectrum of theophylline-

loaded blend film did not show any new peak or peak shift indicating that no drug-polymer interaction was observed in theophylline-loaded blend films.

In case of diclofenac sodium, although diclofenac sodium has carboxylic group in its structure (Figure 3.5), it is difficult to interact with amino group of chitosan because of its bulky group. In this study, characteristic bands of diclofenac sodium (Figure 3.10(e)) were in 3350-3310 cm^{-1} region (NH stretching vibration), 3100-3000 cm^{-1} region (aromatic CH stretching vibration), 1600-1550 cm^{-1} region (asymmetrical carboxyl stretching vibration) and around 1400 cm^{-1} (symmetrical carboxyl stretching vibration) (Nasir *et al.*, 1996). The shift of peaks in the drug-loaded blend films was not observed in FTIR spectrum. It could be suggested that there was no interaction between polymer matrix and diclofenac sodium.

For amoxicillin trihydrate, similar to diclofenac sodium although the presence of carboxylic group in its molecule (Figure 3.6), it might not be able to interact with amino group of chitosan because its bulky group made it difficult to interact with chitosan. The FTIR spectrum of amoxicillin trihydrate is shown in Figure 3.11(e). Characteristic peaks were observed at around 1250 cm^{-1} (phenol CO combination band), 1396 cm^{-1} (dimethyl CH deformation and phenol OH combination band), 1482 cm^{-1} (amide II, NH bending, CN stretching combination band and NH_3^+ symmetric deformation), 1686 cm^{-1} (amide I, CO stretch) and 1775 cm^{-1} (β -lactam CO stretching) (Brittain, 1994). The FTIR peaks of amoxicillin trihydrate did not change when amoxicillin trihydrate was loaded into chitosan/silk fibroin blend film. Therefore, it might be suggested that there was no interaction between chitosan and amoxicillin trihydrate.

Therefore, among four model drugs, salicylic acid was the only one that possibly had interaction with chitosan in the blend films. The effect of this drug-polymer interaction on drug release could be observed in drug release profile of salicylic acid as shown in Figure 3.12(a). It was found that the release of salicylic acid had an initial lag time that the other model drugs did not have. It might be suggested that the initial lag time was spent for dissociation of the interaction between salicylic acid and chitosan before the release of drug occurred. In addition, the solubility of drug in the blend solution is another factor that can affect the drug release. The solubility in water of salicylic acid, theophylline, diclofenac sodium and

amoxicillin trihydrate are 2.17 mg/ml, 8.3 mg/ml, 21 mg/ml and 1-10 mg/ml, respectively (Brittain, 1994 and Florey, 1975). From our results, although the solubility of diclofenac was higher than that of salicylic acid, the releasing amounts of diclofenac were less than those of salicylic acid. It might be explained that drug release property resulted from a combination of various factors, e.g. molecular weight of drug, interaction between drug and polymer matrix and solubility of drug. However, in this study, molecular weights of model drugs seem to be a dominant factor that influenced drug release property of chitosan/silk fibroin blend films.

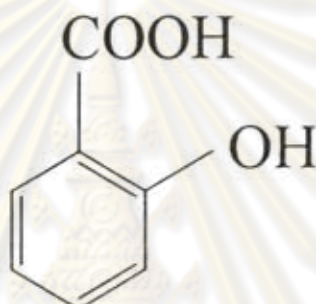


Figure 3.3 Structure of salicylic acid.

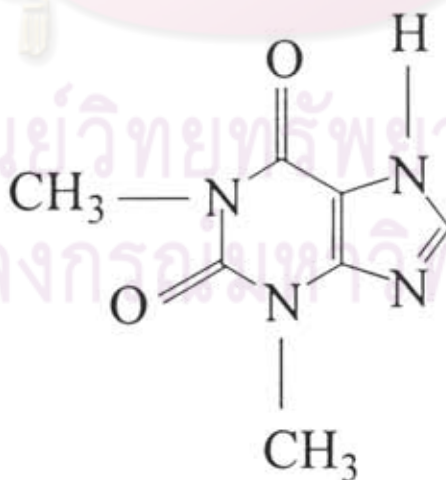


Figure 3.4 Structure of theophylline.

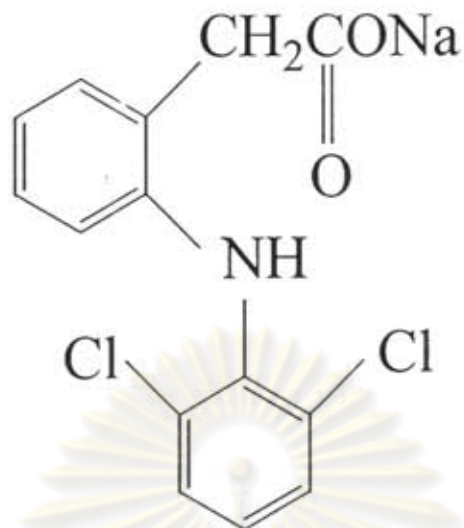


Figure 3.5 Structure of diclofenac sodium.

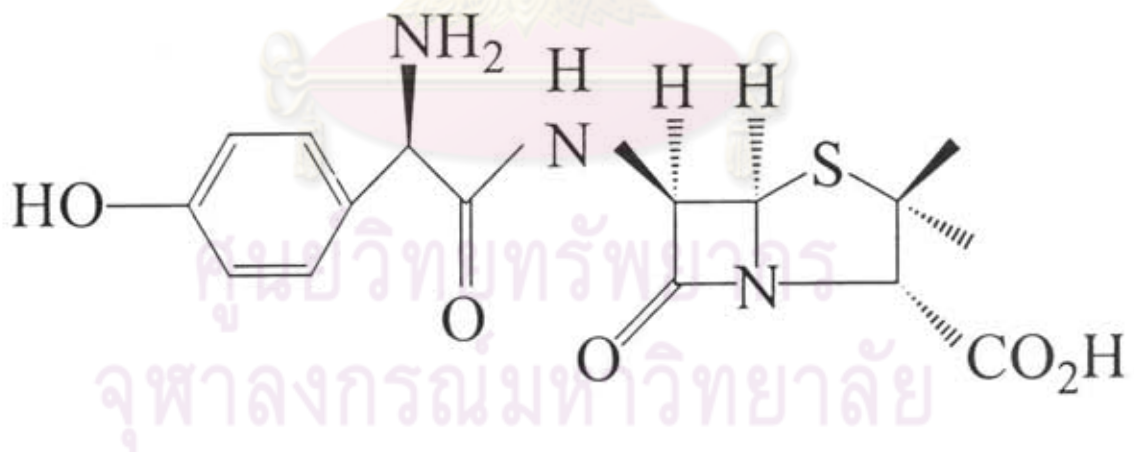


Figure 3.6 Structure of amoxicillin trihydrate.

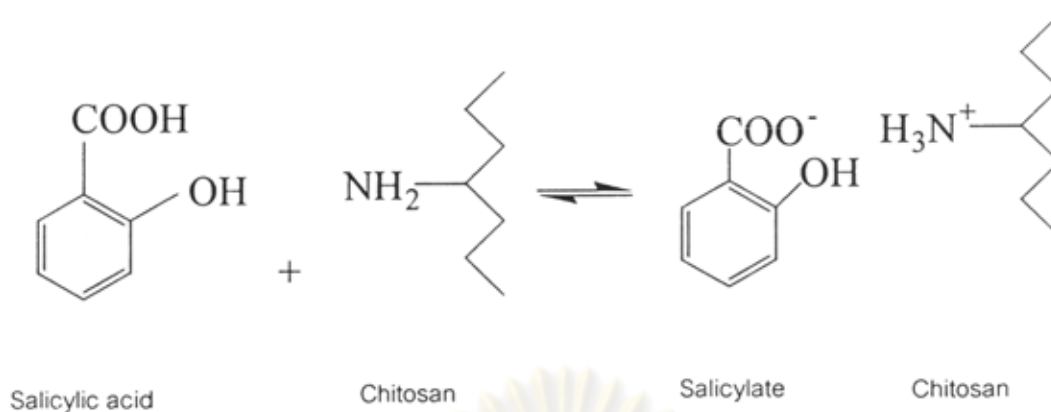


Figure 3.7 Interaction between salicylic acid and chitosan.

Table 3.2 FTIR characteristic absorption bands of chitosan

Frequencies (cm^{-1})	Assignment and remarks
1411	Symmetric COO^- stretch
1561	Amino group of chitosan and amide II of chitin
1257	Amide III of chitin
1153, 898	Saccharide structure

Table 3.3 FTIR characteristic absorption bands of silk fibroin

Frequencies (cm^{-1})	Assignment and remarks
1650	Amide I, C=O stretching
1542	Amide II, N-H bending and C-N stretching

Table 3.4 FTIR characteristic absorption bands of theophylline

Frequencies (cm^{-1})	Assignment and remarks
1720	C=O stretching
1676, 1567	C=C stretching
1485	C=N stretching

1446	C-H bending
1314, 1242	C-N, C-O vibration

Table 3.5 FTIR characteristic absorption bands of salicylic acid

Frequencies (cm^{-1})	Assignment and remarks
1656	C=O stretching
1628	Asymmetric NH_3^+
1440	C=C stretching
1384	Asymmetric COO^-
690,760	Aromatic C-H bending

Table 3.6 FTIR characteristic absorption bands of diclofenac sodium

Frequencies (cm^{-1})	Assignment and remarks
3350-3310	N-H stretching
3100-3000	Aromatic C-H stretching vibration
1600-1550	Asymmetrical C=O stretching vibration
1400	Symmetrical C=O stretching vibration

Table 3.7 FTIR characteristic absorption bands of amoxicillin trihydrate

Frequencies (cm^{-1})	Assignment and remarks
1775	β -lactam C=O stretching
1686	amide I, C=O stretching
1482	amide II, N-H bending and C-N stretching
1396	dimethyl C-H deformation and phenol -OH
1250	phenol C=O

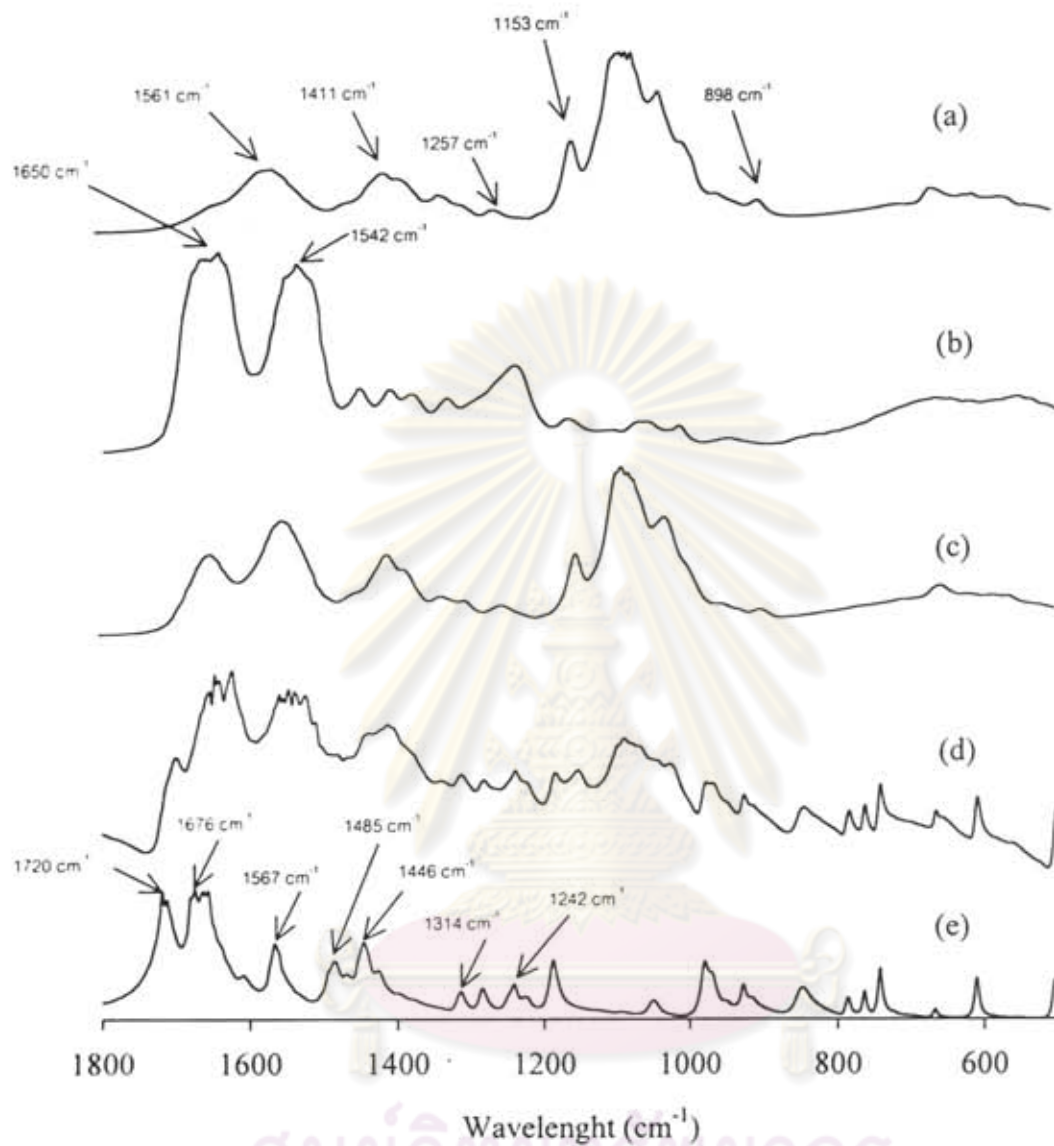


Figure 3.8 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) theophylline-loaded blend films with 80% chitosan content and (e) theophylline.

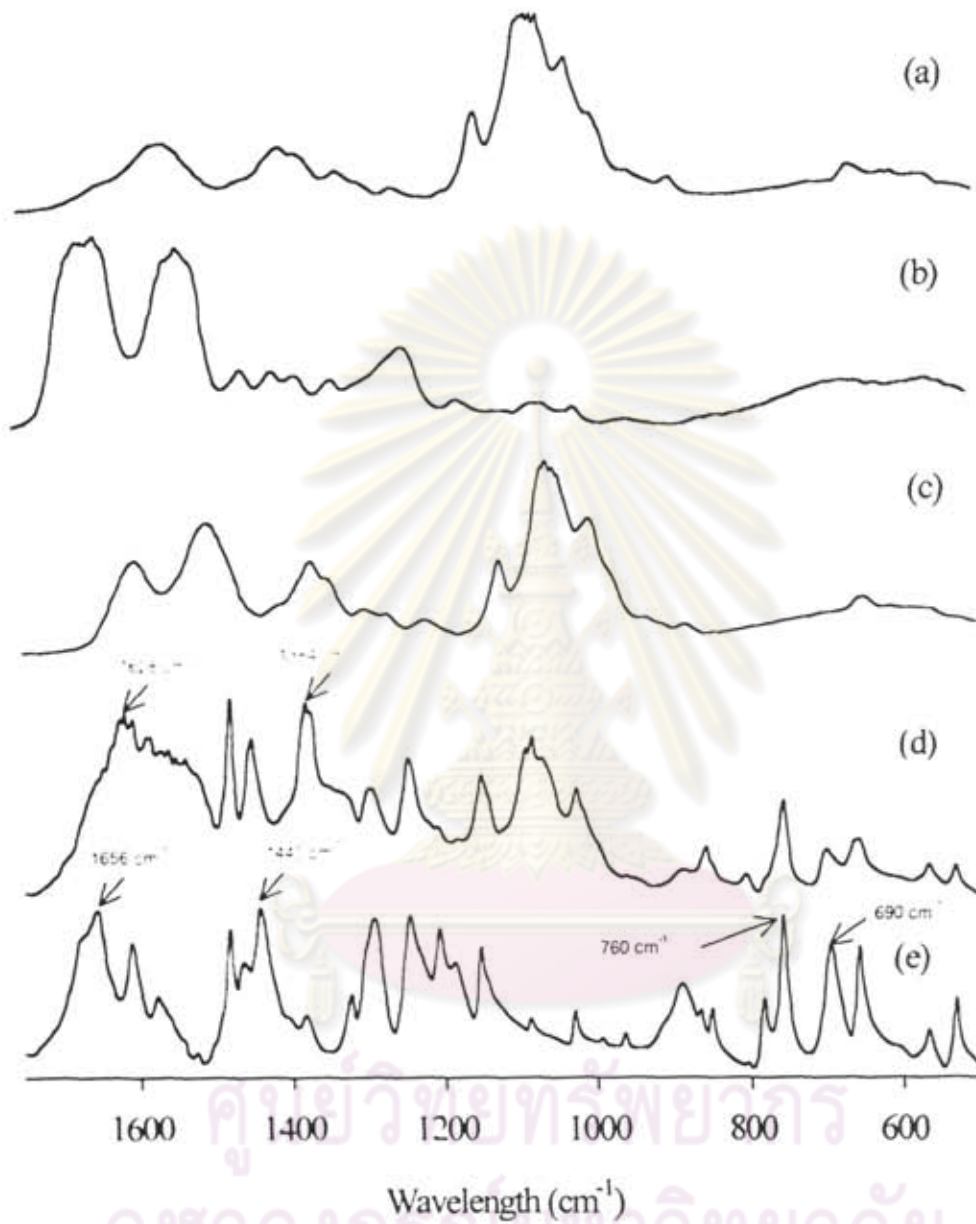


Figure 3.9 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) salicylic acid-loaded blend films with 80% chitosan content and (e) salicylic acid.

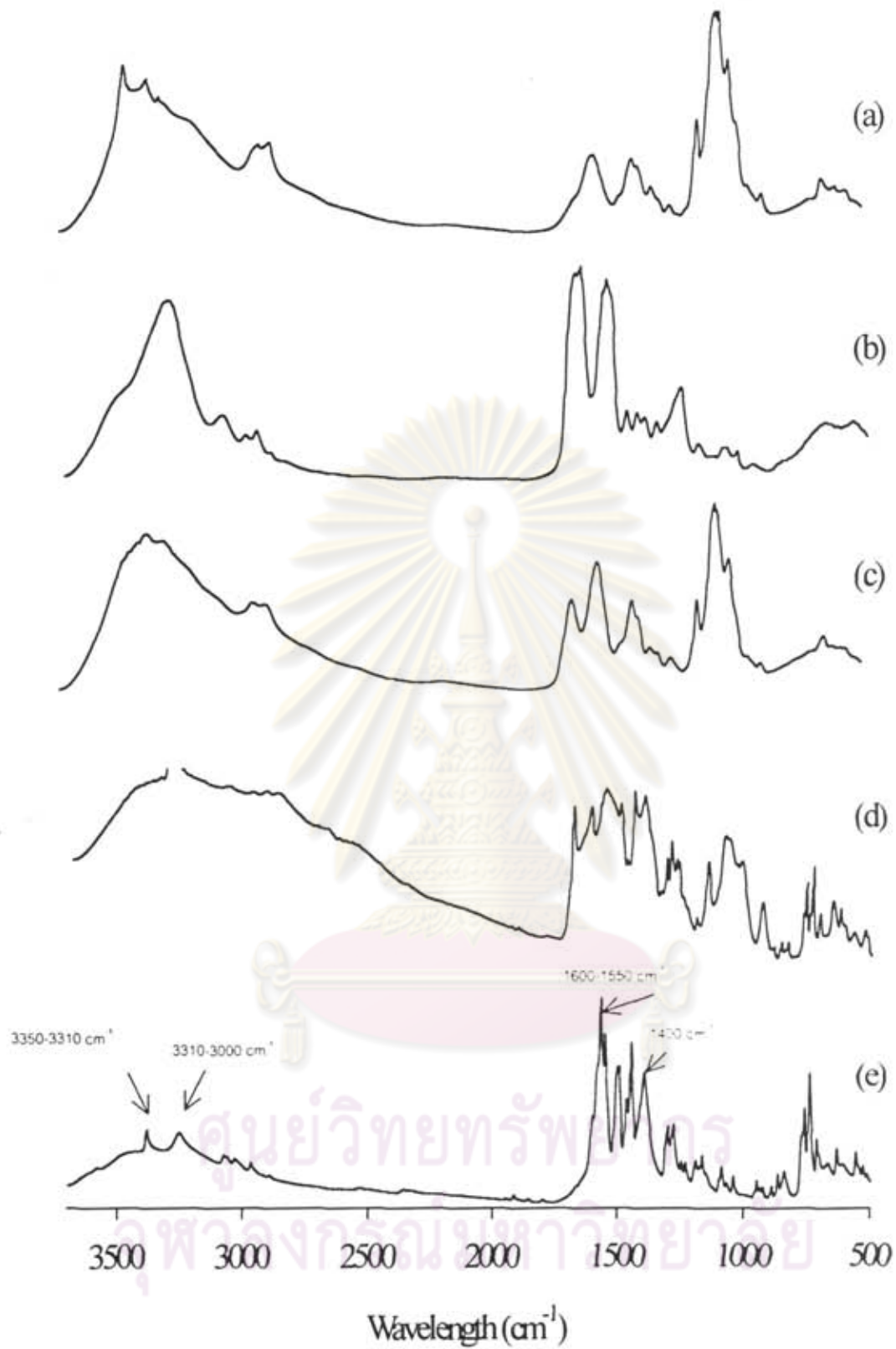


Figure 3.10 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) diclofenac sodium-loaded blend films with 80% chitosan content and (e) diclofenac sodium.

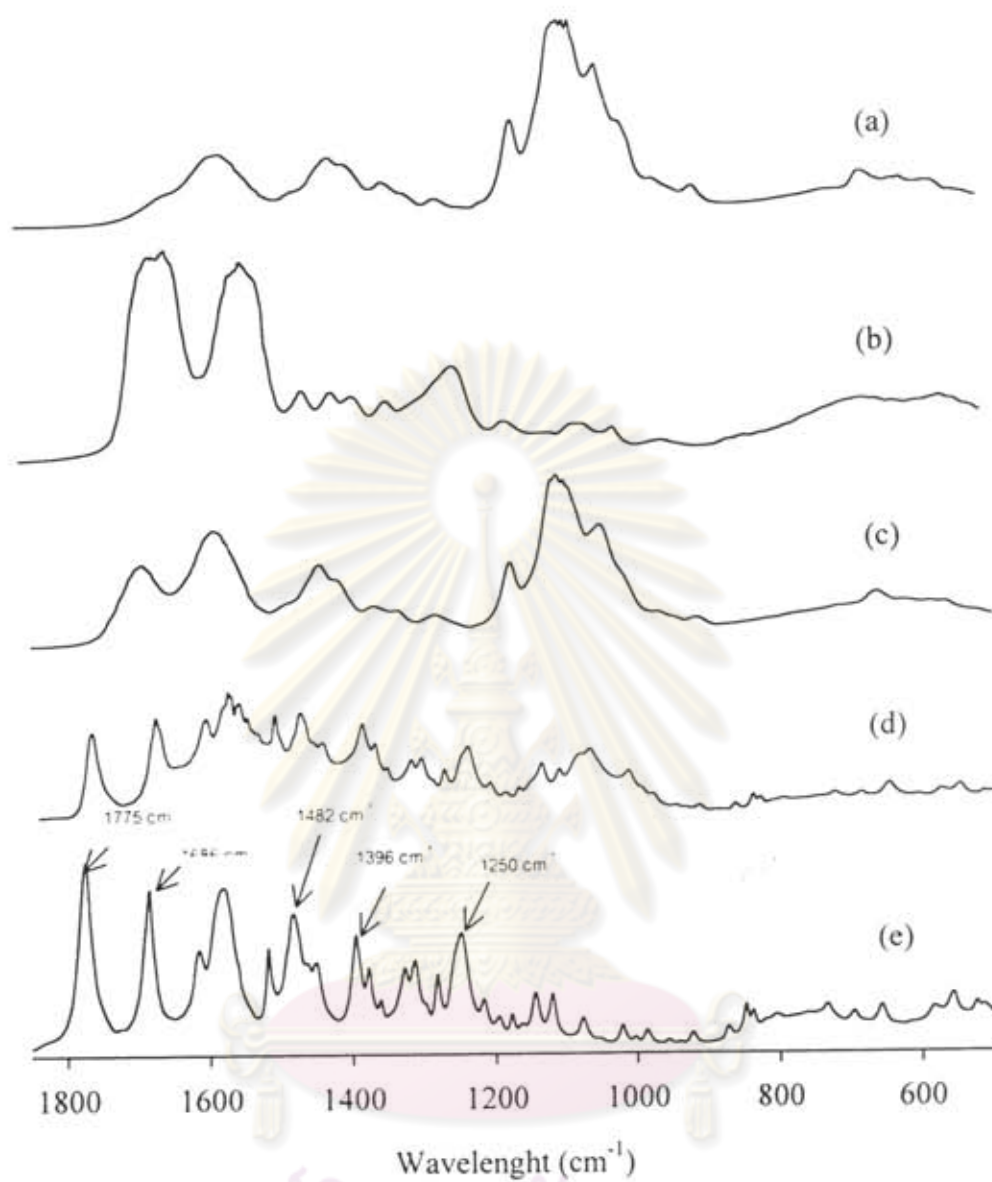


Figure 3.11 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) amoxicillin trihydrate-loaded blend films with 80% chitosan content and (e) amoxicillin trihydrate.

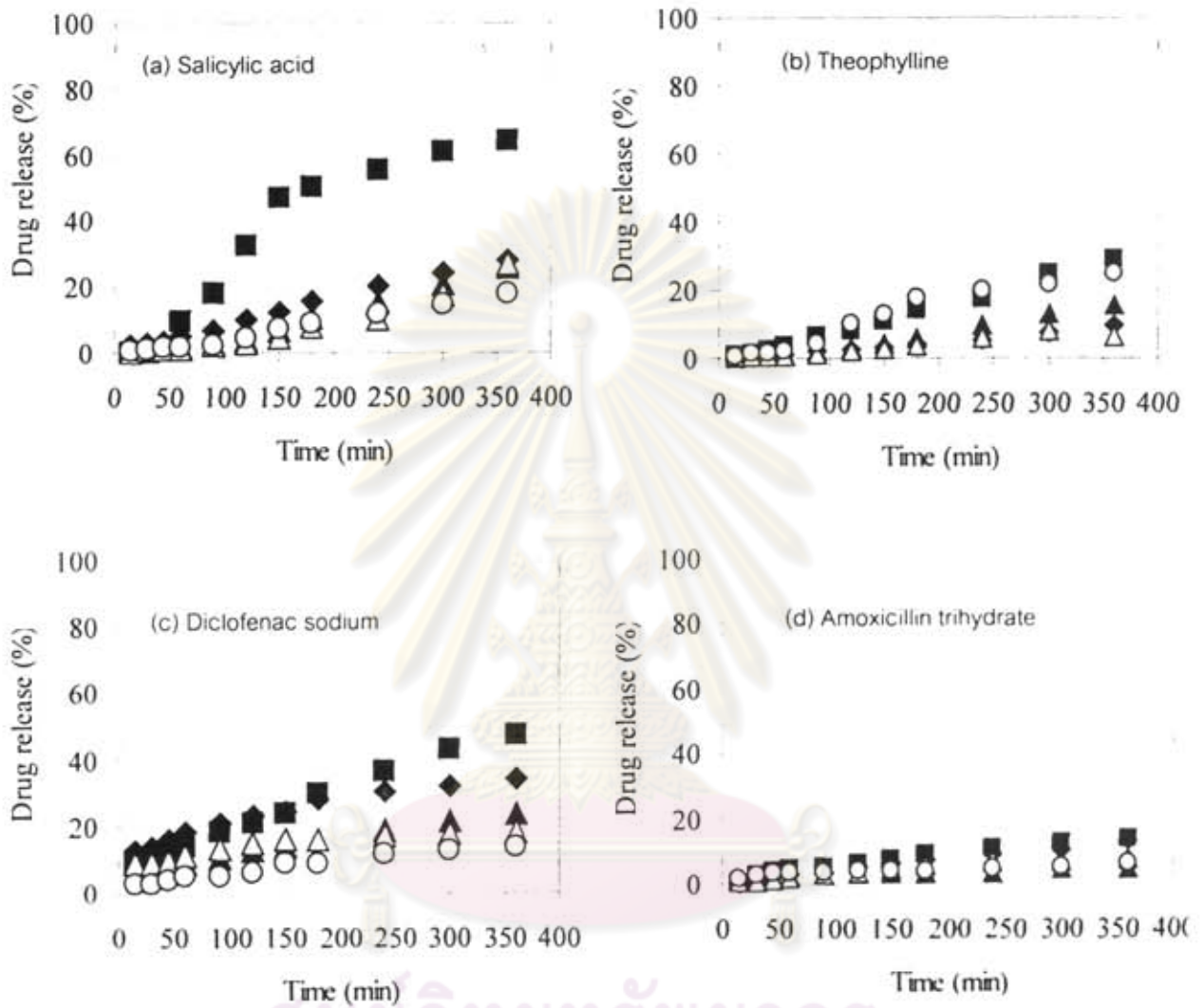


Figure 3.12 Drug release profile for pure chitosan and the blend films.

(◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

Effect of Releasing Time on Drug Release

The release profiles of each model drug for chitosan and the blend films are illustrated in Figure 3.13-3.16. The releasing amounts of drug from the films increased as releasing time increased until reached the equilibrium. It is known that the release of drug from hydrogel is controlled by swelling-controlled mechanism. According to this, swelling behavior of chitosan and the blend films as a function of time were also investigated using the diffusion cell. The results on swelling behavior of chitosan and the blend films at 37°C and pH 5.5 are shown in Figure 3.17. The degree of swelling of chitosan and the blend films remarkably increased at the initial stage and finally reached the equilibrium. At the initial stage when the dry films contacted with the pig skin saturated with pH 5.5 at 37°C, the solution from the pig skin diffused into the films leading to swollen stage of hydrogel. At this stage, when the films became swollen, the drug inside the films would penetrate out of the films. The diffusion of water into the films and the diffusion of drugs from the films occurred until the films reached the equilibrium state.



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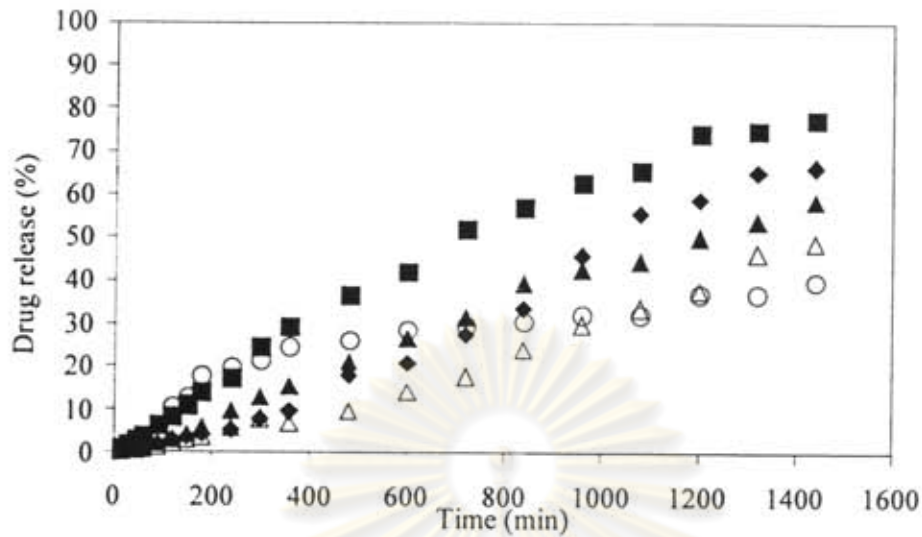


Figure 3.13 Effect of releasing time on releasing of theophylline. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

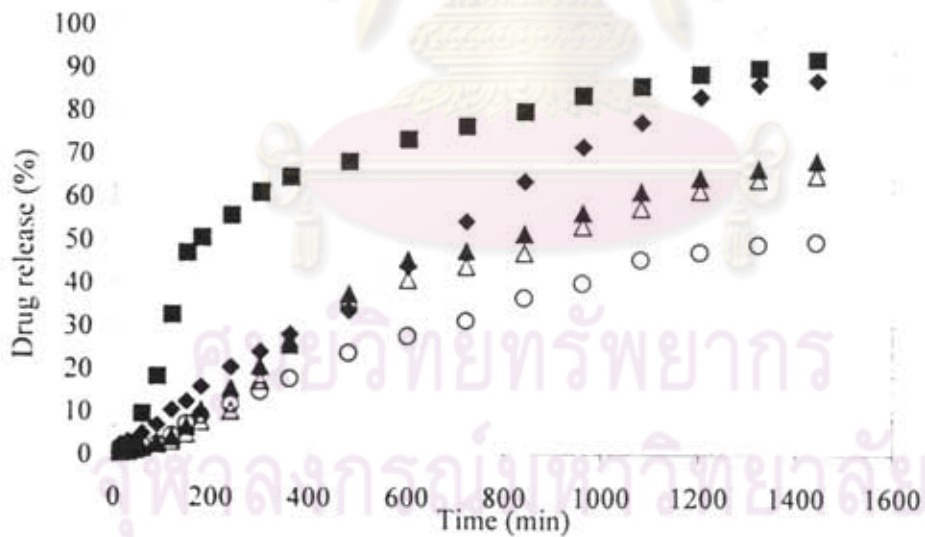


Figure 3.14 Effect of releasing time on releasing of salicylic acid. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

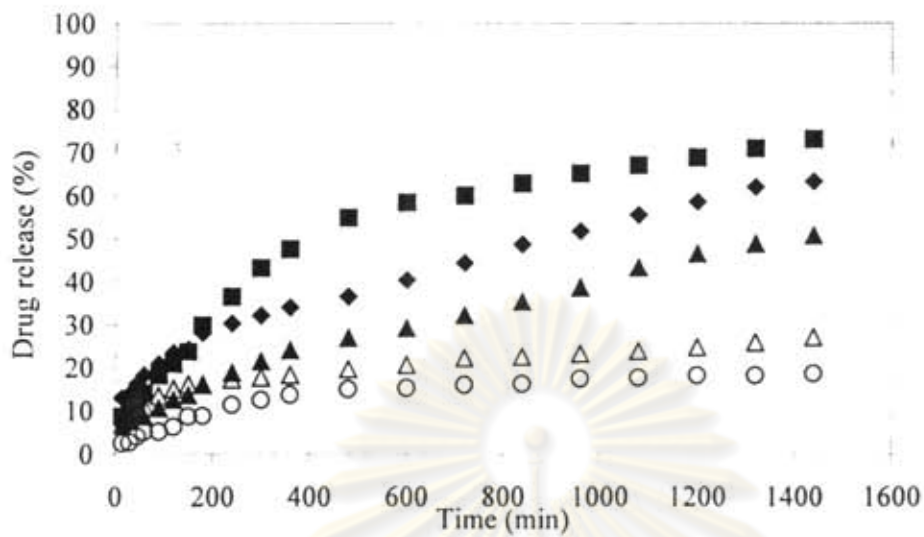


Figure 3.15 Effect of releasing time on releasing of diclofenac sodium. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

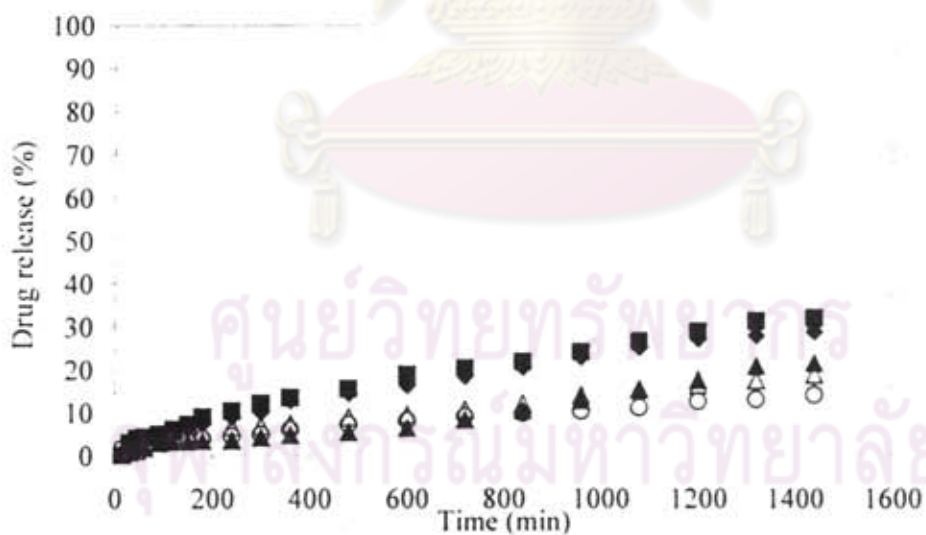


Figure 3.16 Effect of releasing time on releasing of amoxicillin trihydrate. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

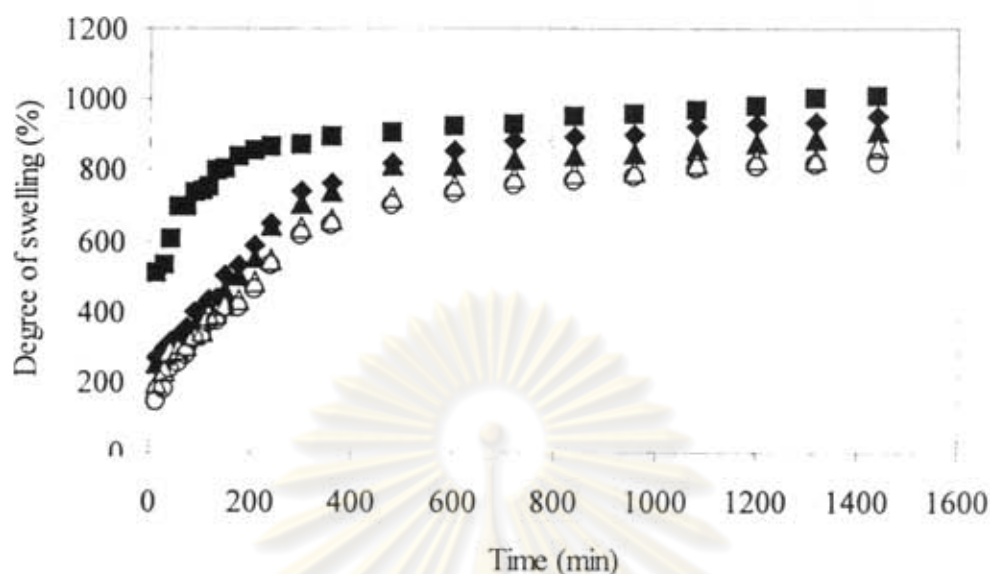


Figure 3.17 Degree of swelling of chitosan/silk fibroin blend films as a function of time (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content.

Effect of Thickness on Drug Release

Since the length of diffusion distance concerns the thickness of films, the effect of the film thickness on drug release was investigated and the results are shown in Figure 3.18. The experiment was done for the blend film with 80% chitosan content and the model drug used was theophylline. The three ranges of thickness studied were 20-30 μm , 50-60 μm and 100-120 μm . It was found that the amount of theophylline released from the films with the thickness of 20-30 μm , 50-60 μm and 100-120 μm were 77.44%, 14.92% and 10.41%, respectively. The more thickness of the film, the longer diffusion path. This resulted in the lower amounts of drug released. Therefore, the thin film is recommended to obtain high amount of released drug.

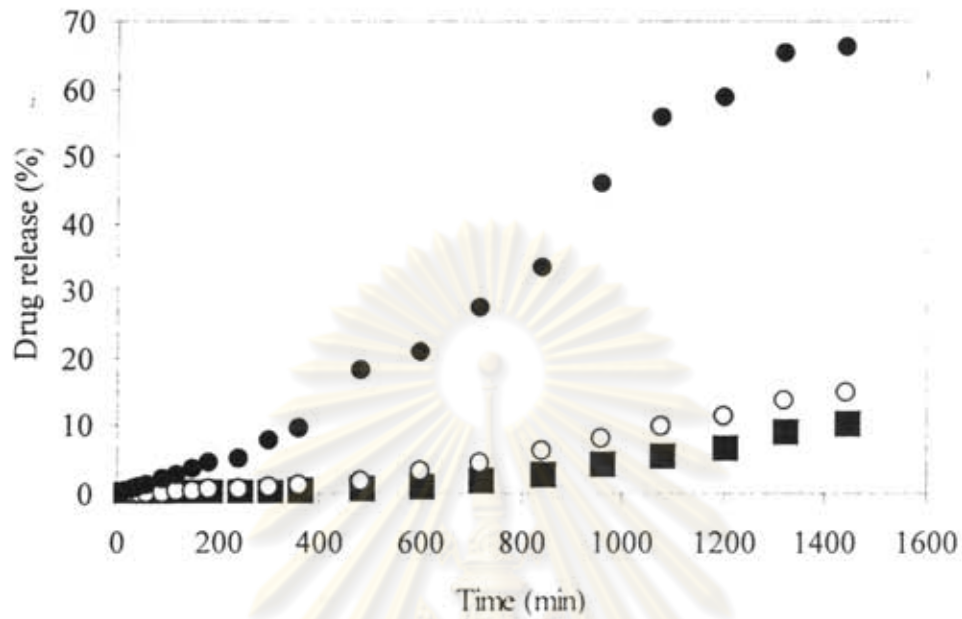


Figure 3.18 Drug release profile for theophylline-loaded blend films with 80% chitosan content. The thickness of the films were (●) 20-30 μm , (○) 50-60 μm and (■) 100-120 μm .

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CONCLUSION

The releases of theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate from crosslinked chitosan/silk fibroin blend films were investigated using modified Franz diffusion cell. The blend composition (chitosan and silk fibroin) could affect the degree of swelling and the releases of model drug from chitosan and the blend films. For all model drugs studied, the maximum drug releases were obtained for the blend films with 80% chitosan content. The results of drug releases correlated to the swelling behavior of the blend films. The higher the degrees of swelling, the higher the amounts of drug released. This might be said that the releases of model drugs from the blend films were mainly occurred due to swelling-controlled release mechanism. However, the release of model drugs occurred due to erosion process as well. The orders of drugs from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. Although there are several factors, such as molecular weight of drug, interaction between drug and polymer matrix and solubility of drug, affecting the drug release characteristics, it seemed that molecular weight of drug played an important rule on drug release in this study. In addition, the thickness of the films was another factor that influenced on the amount of drug released. The increase in the thickness of the films resulted in the decreases in the amounts of drug released. In term of kinetics, all the drug release data were either fitted to zero order or Higuchi's model. It could be said that the drug permeation was either rate-controlled or diffusion-controlled release. From this study, it might be concluded that the crosslinked chitosan/silk fibroin blend films were possibly used as the matrix of the transdermal drug delivery system.

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PROJECT OUTPUT

I. International Journal

Rujiravanit, R.*, Kruaykitanon, S., Jameison, A.M., and Tokura, S. "Preparation of crosslinked chitosan/silk fibroin blend films for drug delivery system", *Macromolecular Bioscience*, in submission.

Peesan, M., **Rujiravanit, R.***, and Supaphol, P. (2003) "Characterization of beta-chitin/poly(vinyl alcohol) blend films", *Polymer Testing*, 22(4), 381-387.

II. International Conference

Rujiravanit, R., Kuratchatchaval, K., Jamieson, A.M., and Tokura, S. (2003) "Preparation and characterization of CM-chitin/PVA blend films", *in* *Advanced in Chitin Science*, Vol. VI, Proceedings from the 5th International Conference of the European Chitin Society, K.M. Varum, A. Domard, and O. Smidsrod (Eds.), NTNU Trondheim, Trondheim, Norway, 26-28 June 2002, 275-276.

Kruaykitanon, S., **Rujiravanit, R.**, Jameison, A.M., and Tokura, S. (2002) "Drug-release characteristics of cross-linked chitosan/silk fibroin blend films", *in* *Advanced in Chitin Science*, Vol. V, Proceedings of the 5th Asia Pacific Chitin and Chitosan Symposium & Exhibition, K. Suchiva, S. Chandkrachang, P. Methacanon, and M.G. Peter (Eds.), National Metal and Materials Technology Center, Bangkok, Thailand, 13-15 March 2002, 678-684.

Meanjai, K., **Rujiravanit, R.**, and Tokura, S. (2002) "Preparation and characterization of CM-chitin/silk fibroin blend films", *in* *Advanced in Chitin Science*, Vol. V, Proceedings of the 5th Asia Pacific Chitin and Chitosan Symposium & Exhibition, K. Suchiva, S. Chandkrachang, P. Methacanon, and M.G. Peter (Eds.), National Metal and Materials Technology Center, Bangkok, Thailand, 13-15 March 2002, 317-323.

In future plan

Rujiravanit, R., Limpapat, S., Jamieson, A.M., and Tokura, S. "Sustained release of drug from chitosan and silk fibroin blend films", *The 9th International Chitin-Chitosan Conference*, Montreal, Canada, 27-30 August 2003.

Rujiravanit, R., Rotchanarak, T., and Tokura, S. "Drug release characteristics of CM-chitin/silk fibroin blend films", *The 9th International Chitin-Chitosan Conference*, Montreal, Canada, 27-30 August 2003.



APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



Publications

International journals

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



Characterisation of beta-chitin/poly(vinyl alcohol) blend films

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Abstract

Blend films of β -chitin (derived from squid pens) and poly(vinyl alcohol) (PVA) were prepared by a solution casting technique from corresponding solutions of β -chitin and PVA in concentrated formic acid. Upon evaporation of the solvent, films prepared from pure β -chitin and pure PVA were found to be transparent, while the film having 50/50 composition was found to be cloudy. Miscibility of the polymers in the amorphous phase of the films at various compositions was assessed using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) techniques. The glass transition temperature of the blend films was found to increase slightly with an increase in the β -chitin content. The effect of blend compositions on apparent degree of crystallinity, mechanical properties, and swelling behavior of the as-prepared blend films was also investigated.

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Keywords: β -chitin; Poly(vinyl alcohol); Blend film

1. Introduction

Natural polymers as biotechnological or biomedical resources have been widely investigated because of their unique properties, which include, for example, nontoxicity, degradability, and biological compatibility. Chitin or poly(*N*-acetyl-D-glucosamine) is a polysaccharide which is abundantly available in nature as a component in cell walls of various fungi, as well as in shells of various insects and crustaceans. Chitin is predominantly present as a fibrillar crystalline material. Based on infrared spectroscopy and X-ray diffraction data, chitin can be found in one of the three crystalline forms [1] α -chitin, β -chitin and γ -chitin, respectively. The molecules in orthorhombic α -chitin are arranged very tightly in an anti-parallel fashion. α -Chitin is mainly present in shells of crabs, lobsters and shrimps. β -Chitin, obtained from

squid pens, takes the monoclinic form in which the chains are arranged in a parallel fashion, while γ -chitin is the form in which the molecules are arranged in both parallel and anti-parallel manner. As a result of the molecular packing, intermolecular interactions in β -chitin are weaker than those in α -chitin, making β -chitin being more susceptible to dissolution in a number of solvents. This finally results in β -chitin being more reactive and versatile.

Studies related to film formation of chitin (i.e., α -chitin) have not been so popular as those of its deacetylated derivatives, i.e., chitosan. This is because chitin is insoluble in most common organic solvents, a direct result of the strong intra- and inter-molecular hydrogen bonding [2,3], while chitosan can even be dissolved in dilute organic acids. In certain applications, especially in the biomedical fields, chitin is more favorable than chitosan. This is due to the fact that the acetamide group present in chitin is similar to the amide linkage of protein in living tissues [4], making chitin more biocompatible than chitosan.

Blending is an especially important process for

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developing industrial applications of polymeric materials and compatibility among components has a marked influence on the resulting physical properties of polymer blends [5]. Through a suitable choice of polymer pairs, blends of polymers can often be tailor-made to exhibit specific and desirable properties. Blending a natural polymer with a synthetic one seems to be an alternative way of preparing polymeric alloys to meet specific applications. Studies related to blends of α -chitin with a synthetic polymer, e.g., polycaprolactone [6,7], poly(3-hydroxybutyric acid) [8], and polyamide-6 [3], are available in the open literature.

Due to its good solubility, β -chitin can be solution-cast into films, but, because of its molecular rigidity and high overall apparent degree of crystallinity, the films obtained show rigid character. Blending β -chitin with another flexible, synthetic polymer seems to be an attractive way for improving properties of the films. Poly(vinyl alcohol) (PVA) is a nontoxic, water-soluble, synthetic polymer that is widely used in biomedical applications. With its excellent film-forming ability, PVA is a good candidate for use as membranes and hydrogels [9,10].

In this present contribution, β -chitin/PVA blend films were prepared by solution-casting from solutions of β -chitin and PVA in concentrated formic acid at various compositional ratios. The effect of blend compositions on physical properties, thermal properties, mechanical properties, morphology, and swelling behavior was studied and compared with those of pure components.

2. Experimental details

2.1. Materials

β -Chitin was prepared from squid pens by acid and alkali treatment. β -Chitin was pulverized prior to use into powder, the size of which ranged from 71 to 75 μm . PVA, purchased from Fluka, has the degree of polymerization of ca. 1600 and the degree of hydrolysis of ca. 99.5%. Formic acid (reagent grade, BDH Laboratory) and ethylene glycol (J. T. Baker) were used as received.

2.2. Preparation of blend films

PVA was first dissolved thoroughly in concentrated formic acid (99%) to prepare 1% by weight (wt%) solution. Later, a known amount of β -chitin powder was suspended in concentrated formic acid (99%) at room temperature to prepare 1 wt% solution and the suspension was frozen overnight at 0 °C. After thawing at room temperature, the solution was filtered with a glass filter. A series of β -chitin/PVA blend films with different blend compositions were then prepared by solution-casting technique. The films obtained were allowed to dry at 60

°C for 12 h. The final thickness of the dried films was in the range of 30–50 μm . All of the as-prepared films were kept under dry conditions before further use.

2.3. Measurements

Infrared spectra of the as-prepared films were recorded using a Bruker vector 3.0 FTIR spectrophotometer (FTIR). A Mettler DSC 822e/400 (DSC) was used to investigate thermal behavior of the films. To set the thermal history for all samples, each sample was first heated to 190 °C and then cooled to 0 °C at the scanning rate of 10 °C \times min⁻¹. The thermal properties of the films were measured in the second heating scan at the heating rate of 10 °C \times min⁻¹. The glass transition temperature (T_g) and the melting temperature (T_m) were determined as the inflection point of the specific heat increment and the onset of the endothermic melting peak of DSC traces, respectively. Thermal stability of the films was evaluated by a Perkin Elmer TGA7 (TGA) operated under nitrogen atmosphere and at a heating rate of 10 °C \times min⁻¹ from 30 to 750 °C. A Rigaku D/MAX-2000 wide-angle X-ray diffractometer (WAXD) equipped with a $\text{CuK}\alpha$ X-ray source operating at 40 kV and 30 mA was used to obtain diffractograms on the as-prepared films over the 2 θ range of 5–40° and the scanning speed of 5 degree \times min⁻¹. Morphology of the etched surface of selected samples was observed on a JEOL 5200-2AE scanning electron microscope (SEM). A Lloyd tensile tester was used to assess the mechanical properties of the as-prepared films. The gauge length was 125 mm, and the crosshead speed used was 12.5 mm \times min⁻¹.

The swelling behavior of the as-prepared films was carried out by measuring the weight of the films after immersion in distilled water and various salt solutions (i.e., 0.25 M solutions of NaCl, CaCl_2 , and FeCl_3) for 0–8 h in comparison with the dry weight of the films prior to the immersion. The degree of swelling was determined according to the following relationship:

$$\text{Degree of swelling (\%)} = [(W_s - W_d) / W_d] \times 100, \quad (1)$$

where W_s and W_d represent the weight of the films after and prior to immersion. It is important to note that all the experiments were carried at room temperature. Finally, the equilibrium degree of swelling of the as-prepared films was also determined after immersion in water or corresponding solutions for 4 days.

3. Results and discussion

Solutions of pure β -chitin, pure PVA, and their blends appeared to be homogeneous and transparent. The color of the solutions varied from colorless of pure PVA solution to yellowish with increasing β -chitin content. After

evaporation of the solvent, the as-prepared films of pure β -chitin and pure PVA were found to be transparent, while the 50/50 β -chi.in/PVA blend film was found to be cloudy. In addition, it was found that the blend films became more brittle with increasing β -chitin content.

3.1. Characteristics of β -Chitin/PVA blend films

Since the molecules of both β -chitin and PVA are capable of forming hydrogen bonds, it is expected that some specific interactions could be formed between the molecules of different species. In this work, observation of the as-prepared blend films using FTIR did not indicate the presence of such intermolecular interactions (results not shown). However, Lee et al. [11] reported, based on their FTIR results, that intermolecular interactions between the molecules of β -chitin and PVA could in fact exist, because they found shifting of both hydroxyl and carbonyl stretching bands upon blending β -chitin with PVA. The difference between our results and their may be due to the difference in the molecular weight characteristics of the constituents studied.

Miscibility of β -chitin and PVA at various weight compositions was investigated by observing the T_g values of the as-prepared pure and blend films. Fig. 1 shows the second heating thermograms for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films. For pure PVA film, the T_g value was found to be ca. 35 °C, which was ca. 25 °C lower than that observed for the neat resin. This could be a result of the oxidative degradation upon dissolution in concentrated formic acid or the plasticizing effect due to the presence of residual solvent molecules in the as-prepared films or both. Based on DSC results, the T_g value for pure β -chitin film could not be observed in this work. However, Kim et al. [12] used a more sensitive DMTA technique to measure the T_g value for pure β -chitin and they reported it to be ca. 170 °C. For as-prepared blend films, single T_g shoulder peak was clearly observed for each blend composition

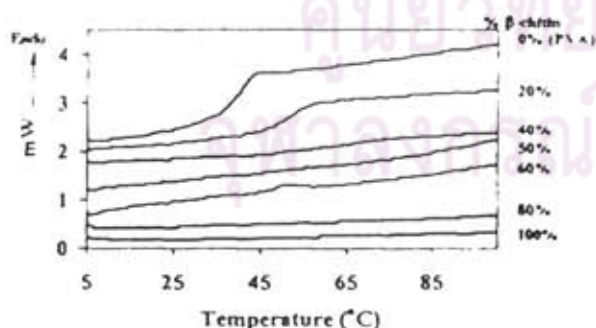


Fig. 1. The second heating thermograms for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films (recorded at 10 °C/min⁻¹) in the temperature range where a glass transition should be observed.

The facts that only single T_g peak was observed for each blend composition and that the resulting T_g value was found to increase slightly with increasing β -chitin content indicated partial miscibility of β -chitin and PVA in the amorphous phase at the molecular level for any given compositional ratio.

The melting endotherms for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films are shown in Fig. 2. Clearly, the T_m value for pure PVA film was found to be ca. 180 °C, and the position of the melting endotherms for β -chitin/PVA blend films at various compositions tended to shift to a lower temperature with increasing β -chitin content. For pure β -chitin film, it is surprising to observe a broad endothermic peak, the onset of which was observed at ca. 120 °C. This could be a result of the relaxation of the acetamide groups attached to the C2 position in β -chitin chains [12]. It is worthy to note that the T_m value for pure β -chitin could not be observed, a direct result of the rigid-rod nature of the β -chitin molecular backbones making them being susceptible to degradation before melting. This phenomenon is, in fact, typical for many other polysaccharides.

Thermal stability of the as-prepared films can be observed by TGA technique. Fig. 3 shows the TGA curves for pure β -chitin, pure PVA, and 50/50 β -chitin/PVA blend films. All of the samples tested showed initial weight loss at ca. 50 °C, likely a result of moisture evaporation upon heating. The amount of moisture content in all of the samples tested was almost similar. According to the derivative TGA curves, pure PVA film was found to degrade at ca. 270 °C (see Fig. 3; curve (a)), while pure β -chitin film showed two degradation peaks at ca. 262 and 349 °C, respectively (see Fig. 3; curve (c)). Apparently, the 50/50 β -chitin/PVA blend film exhibited degradation behavior intermediate to those of the pure components, exhibiting two degradation peaks at ca. 269 and 342 °C, respectively (see Fig. 3; curve (b)). Table 1 lists the degradation peak values

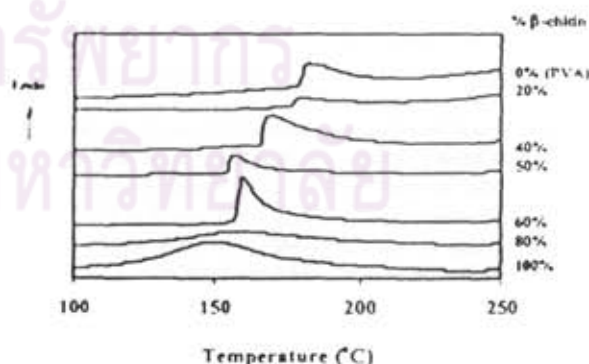


Fig. 2. The second heating thermograms for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films (recorded at 10 °C/min⁻¹) in the temperature range where a melting endotherm should be observed.

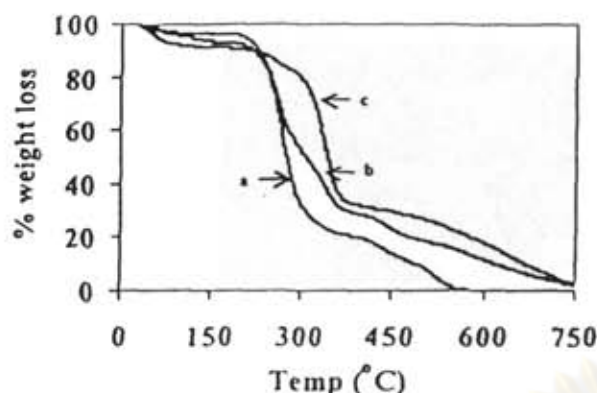


Fig. 3. TGA curves for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films (recorded at $10^\circ\text{C}\times\text{min}^{-1}$).

Table 1
Degradation temperature(s) of pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films

Type of film	1st T_d ($^\circ\text{C}$)	2nd T_d ($^\circ\text{C}$)
β -chitin	262 ± 0	349 ± 1
80/20 β -chitin/PVA	264 ± 2	348 ± 1
60/40 β -chitin/PVA	265 ± 1	346 ± 2
50/50 β -chitin/PVA	269 ± 1	342 ± 2
40/60 β -chitin/PVA	271 ± 2	335 ± 2
20/80 β -chitin/PVA	280 ± 2	-
PVA	270 ± 2	-

(denoted T_d) observed for all of the as-prepared films studied. For most blend compositions, the degradation behavior of the blend films was found to be intermediate to those of the pure components. Interestingly, only 20/80 β -chitin/PVA blend film exhibited only single degradation peak, with the T_d value being much greater than those of the pure components. The reason for such peculiarity will be the matter for further investigation.

Fig. 4 illustrates WAXD patterns for pure β -chitin,

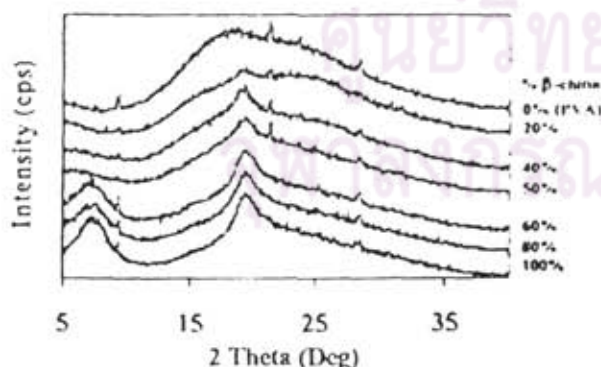


Fig. 4. WAXD patterns for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films.

pure PVA, and a series of β -chitin/PVA blend films. Obviously, the WAXD pattern for pure β -chitin film exhibited two crystalline peaks at the 2θ angles of ca. 7.4 and 19.4° , respectively. This observation is in general accordance with the finding by Ren and Tokura [13], who reported that the two characteristic crystalline peaks of β -chitin were found at 8.50 and 19.98° , corresponding to the (010) and (020) and (110) reflection planes, respectively. When PVA crystallized in a monoclinic unit cell (with the cell characteristics: $a = 0.781$ nm, $b = 0.252$ nm, and $c = 0.511$ nm, $\alpha = \beta = 90^\circ$, $\gamma = 97.1^\circ$) [14], the main peaks in the WAXD pattern should appear at the 2θ angles of 11.3 , 19.7 , 22.9 , 28 , 32.5 , and 40.9° [15]. According to Fig. 4, the WAXD pattern for pure as-prepared PVA film only showed a broad crystalline peak at the 2θ angle of ca. 18.7° . For β -chitin/PVA blend films, the diffractograms appeared to be intermediate to those of the pure components. It is evident that, as β -chitin increased, not only did the intensity of β -chitin characteristic crystalline peaks become less pronounced, especially when β -chitin content was lower than 50 wt%, but the crystalline peaks became broader as well, suggesting a decrease in the size of β -chitin crystals as well as in the apparent degree of crystallinity. This might be a result of a dilution effect when PVA was blended with β -chitin.

Even though not shown in this paper, surface morphology of β -chitin/PVA blend films was also observed by scanning electron microscopy. After drying the films at room temperature for 48 h, the films with porous structure were obtained for all of the blend compositions. On the contrary, when the films were instead dried in an oven at 60°C for 12 h, shrinkage in the films was observed. In order to observe the level of compatibility between β -chitin and PVA in as-prepared β -chitin/PVA blend films which were earlier dried at 60°C , the films were etched in hot ethylene glycol which is a good solution to PVA and the resulting SEM micrographs are shown in Fig. 5. According to Fig. 5, certain level of phase separation in the micrometer scale is obvious in all of the blend compositions studied.

3.2. Tensile properties

The mechanical properties, in terms of tensile strength and percentage of elongation at break, were determined for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films and the results are reported as a function of β -chitin content in Figs. 6 and 7, respectively. For pure β -chitin film, the tensile strength and the percentage of elongation at break were found to be ca. 5.1 MPa and 2.4% , respectively. This agreed particularly well with the results obtained by Kim et al. [12], who reported that the tensile strength and the percentage of elongation at break for pure β -chitin film, which was solution-casted from its solution in formic acid, were ca.

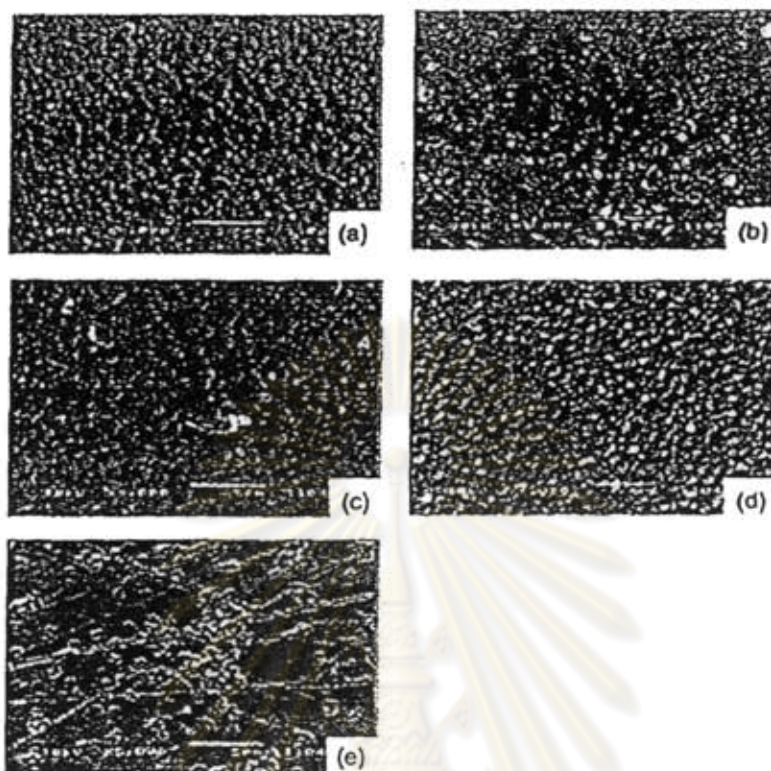


Fig. 5. Scanning electron micrographs of ethylene glycol-etched β -chitin/PVA blend films for (a) 80/20, (b) 60/40, (c) 50/50, (d) 40/60, and (e) 20/80 blend compositions, respectively.

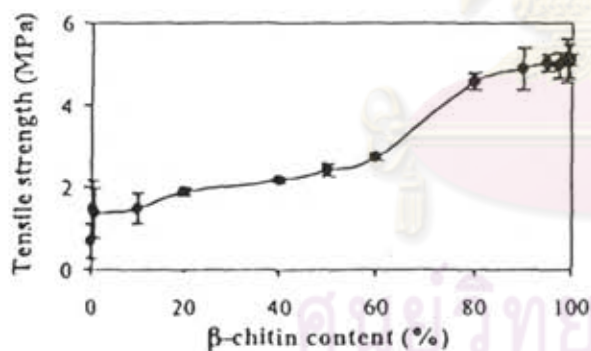


Fig. 6. Tensile strength for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films

5.2 MPa and 5%, respectively. On the other hand, PVA exhibited a much softer character, with its tensile strength and the percentage of elongation at break being ca. 0.7 MPa and 165.2%, respectively. For β -chitin/PVA blend films, the tensile strength was found to increase, with increasing β -chitin content, from ca. 0.7 to 5.1 MPa, at the expense of the percentage of elongation at break, which was found to decrease from ca. 165.2 to 2.9%. Physically, the blend films appeared to be more brittle as β -chitin content increased.

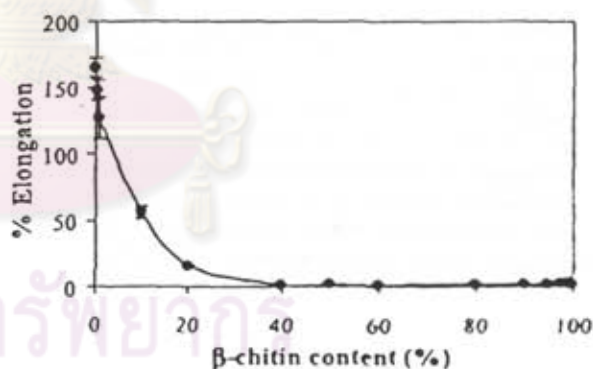


Fig. 7. Percentage of elongation at break for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films

3.3. Swelling behavior

The degree of swelling of pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films with different blend compositions is shown in Fig. 8 as a function of immersion time in distilled water. For a given blend composition, the degree of swelling increased with increasing immersion time. After 8 h of immersion time, it is interesting to note that the ultimate degree of swell-

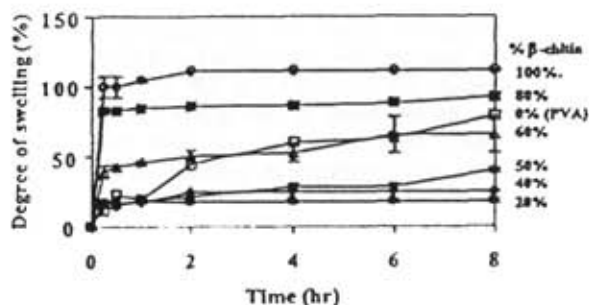


Fig. 8. Dynamic degree of swelling in distilled water for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films as a function of immersion time.

ing of pure β -chitin film was greater than that of pure PVA film (i.e., ca. 100% versus ca. 80%). With increasing β -chitin content, the ultimate degree of swelling after 8 hours of immersion time of β -chitin/PVA blend films was found to increase from ca. 15 to ca. 90%, when β -chitin content increased from 20 to 80 wt%. This behavior is in general agreement with results obtained for IPN hydrogel composed of β -chitin and PEG macromer [16].

The equilibrium degree of swelling (after 4 days of immersion time) for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films as a function of β -chitin content is showed in Fig. 9. Interestingly, the equilibrium degree of swelling of pure PVA film was now greater than that of pure β -chitin film (i.e., ca. 190% versus ca. 110%). Comparison of the results shown in Fig. 8 suggests that pure β -chitin film reached the equilibrium much faster than pure PVA film. For β -chitin/PVA blend films, the equilibrium degree of swelling was found to increase from ca. 30 to ca. 95%, when β -chitin content increased from 20 to 80 wt%. This is in accord with the ultimate degree of swelling after 8 h of immersion time observed earlier. It is rather surprising, however, that both the ultimate and equilibrium degrees of swelling of

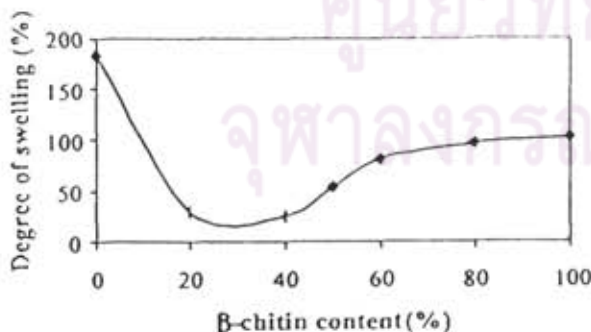


Fig. 9. Equilibrium degree of swelling in distilled water for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films.

the 20/80 β -chitin/PVA blend film were found to be the lowest among the films studied, despite the high level of PVA content.

The equilibrium degree of swelling in various media (i.e., water, NaCl, CaCl₂, and FeCl₃ solutions at the concentration of 0.25 M) of pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films was investigated and the results are shown in Fig. 10. Evidently among of the media studied, FeCl₃ solution was the best medium to swell the as-prepared films. According to a known fact that, when being present in water, ferric ion, Fe³⁺, can exist in an hydrated form, e.g., Fe(H₂O)₆³⁺ [17], and the bulky size of the hydrated ferric ion can thus be responsible for the high degree of swelling of the as-prepared films studied. It could be further deduced from the results obtained that the blend films swell more substantially in trivalent ion solutions than in monovalent and bivalent ion solutions.

4. Conclusions

In this contribution, β -chitin/PVA blend films were prepared by solution-casting from solutions of β -chitin and PVA in concentrated formic acid at various compositional ratios. The effect of blend compositions on physical properties, thermal properties, mechanical properties, morphology, and swelling behavior was investigated and the results were compared with those of pure components. DSC measurements showed that the glass transition temperatures of the blend films increased with increasing β -chitin content, while melting temperatures tended to shift to a lower temperature. Thermal stability of the blend films was found to be intermediate to those of the pure components. WAXD patterns indicated a reduction in the apparent degree of crystallinity of β -chitin with increasing PVA content. Surface morphology of ethylene glycol-etched β -chitin/PVA blend films suggested that a certain level of phase separation in a micrometer scale was found for blend films of all

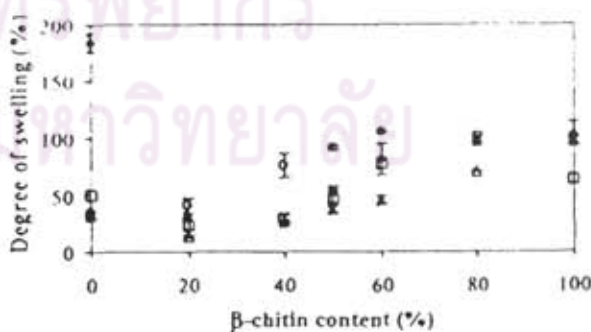


Fig. 10. Equilibrium degree of swelling in various media for pure β -chitin, pure PVA, and a series of β -chitin/PVA blend films. Keys: (\blacklozenge) water, (\square) NaCl, (\triangle) CaCl₂, and (\circ) FeCl₃.

blend compositions. The tensile strength of the blend films was found to increase, with increasing β -chitin content, from ca. 0.7 to 5.1 MPa, at the expense of the percentage of elongation at break which was found to decrease from ca. 165.2 to 2.9%. The equilibrium degrees of swelling in distilled water of β -chitin/PVA blend films of all blend compositions were found to be lower than those of the pure constituents, with that of the 20/80 β -chitin/PVA blend film being the lowest. Lastly, 0.25 M FeCl₃ solution, among the various swelling media investigated, was the best to swell most of the films studied.

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Preparation of Crosslinked Chitosan/Silk Fibroin Blend Films for Drug Delivery System

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Summary

Crosslinked chitosan/silk fibroin blend films were prepared by solution casting technique using glutaraldehyde as a crosslinking agent. Drug-released characteristics of the blend films with various blend compositions were investigated. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid were used as model drugs. The release studies were done at 37°C in buffer solutions pH 2.0, pH 5.5 and pH 7.2. It was found that the blend films with 80% chitosan content showed the maximum amounts of model drugs released at pH 2.0 for all types of drugs. This result corresponded to swelling ability of the blend films. From swelling study, the maximum degrees of swelling of the drug-loaded blend films were obtained at this pH and this blend composition. The amounts of drugs released from the films with 80% chitosan content from the highest to the lowest values obtained in the following sequence: salicylic acid > theophylline > diclofenac sodium > amoxicillin. This could be due to the effects of molecular sizes of drugs, solubility of drugs in the blend solutions and interaction between drugs and polymer matrix.

Keyword: chitosan; drug delivery system; silk fibroin

Introduction

A drug delivery system may be a matrix of polymer incorporating a drug. Polymeric drug carrier systems have several advantages in optimizing patient treatment regimes. In particular, swelling-controlled release systems are capable of delivering drugs at constant rates over an extended period of time. In these systems, the rate of drug delivery is controlled by the balance between drug (solute) diffusion across a concentration gradient, the polymer relaxation occurring as the crosslinked polymer imbibes water, and the osmotic pressure occurring during the swelling process. Furthermore, a binary polymer matrix constituted of two polymers of different hydrophilic character is another possibility for controlling the degree of swelling of the system and the solute diffusion rate from the matrix.

Chitosan, poly[β -(1-4)-linked-2-amino-2-deoxy-D-glucose], is an aminopolysaccharide derived from N-deacetylation of chitin. Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-dimensional crosslinked polymeric material with the ability to absorb significant amount of water. Crosslinked chitosan hydrogel can swell extensively due to the positive charges on the network and response to change in pH of medium. Due to the benefits of being non-toxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for the sustained delivery of drug. For example, it was investigated for the delivery of drugs such as prednisolone^[1] and diclofenac sodium^[2]. There have been many studies on the blends of chitosan with various kinds of polymers^[3-7] in order to obtain some improved properties. It

is worth to investigate drug release properties of these chitosan-based blends in order to develop more efficient drug delivery devices.

Silk fibroin is a fibrous protein that is composed of 17 amino acids and its main components are nonpolar ones such as glycine, alanine, and serine. Silk fibroin can exist in two general conformations, random coil and β -sheet form. The conformation transition of silk fibroin can be induced to change from random coil to β -sheet structure by treatments such as heating^[8], stretching or immersion in polar solvents^[9]. This transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β -sheet structure is resistant to water and has good mechanical properties^[10]. Silk fibroin is considered to be an interesting starting material for developing new materials and devices for biotechnological and biomedical utilization. It has been reported that silk fibroin film has good oxygen permeability in wet state^[11], which suggests promising applications of silk fibroin as wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures^[12] and biocompatible devices with controlled drug release^[13]. However, silk fibroin in dry state is very brittle and unsuitable for practical uses^[14]. To overcome this limitation, silk fibroin has been reported to blend with other synthetic polymers, such as polyacrylamide^[15] and poly(vinyl alcohol)^[16], or natural polymers, such as cellulose^[14] and sodium alginate^[17], to improve mechanical and physical properties. Among these, the blend of silk fibroin and chitosan has been interesting. It has been reported that chitosan could induce the conformational transition of silk fibroin from random coil to β -sheet structure^[10] and a polymer blend of these biopolymers could also form a hydrogel having a semi-interpenetrating polymer network by using glutaraldehyde as a crosslinking agent^[18].

This research is a preliminary study on using crosslinked chitosan/silk fibroin blend film as a matrix for drug delivery system. The model drugs used were theophylline,

diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effects of blend composition, drug nature, swelling time, degree of crosslinking, and pH of the external swelling media on drugs released from the blend films were investigated.

Experimental Part

Materials

Shrimp shell was kindly provided by Suraphol Food Public Co., Ltd., Thailand. Silk fiber (*Bombyx mori*) was degummed by treatment with 0.5% Na_2CO_3 at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk fibroin was dissolved in triad solvent CaCl_2 : EtOH: H_2O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was filtered through the sintered glass filter and subsequently diluted to achieve a concentration of 1 wt.-%.

Theophylline was purchased from Shanghai Wandai Pharmaceuticals, China. Diclofenac sodium was purchased from Tangyin Yongqi Chemical Industry Co., Ltd., China. Salicylic acid was purchased from Ajax Chemicals, Australia. Amoxicillin trihydrate was purchased from Antibiotics Co., Ltd., Spain. The chemical structures of these model drugs are shown in Figure 1. All other chemicals and solvents were of analytical grade and were used without further purification.

Preparation of Chitin

Chitin was prepared from shrimp shell by decalcification and deprotenization to remove calcium carbonate and protein, respectively. The shrimp shell was cleaned and dried under sunlight before grinding into small pieces. The shrimp shell chips were treated by immersing in 1 N HCl solution for 2 days with occasional stirring. The decalcified

product was washed with distilled water until neutral. Deprotonization was followed by boiling in 4 wt.-% of NaOH solution at 80-90°C for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C in a convective oven for 24 h.

Preparation of Chitosan

Chitin was deacetylated by heating in NaOH 50 wt.-% solution with sodium borohydride (NaBH_4) 0.5 wt.-% based on the weight of chitin to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustively with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1 wt.-% of acetic acid solution. The chitosan solution was allowed to stand overnight at room temperature to reduce of air bubbles before preparation of films.

Preparation of Crosslinked Drug-loaded Blend Films.

The blend solutions of chitosan and silk fibroin were prepared by mixing various ratios of 1 wt.-% of silk fibroin solution and 1 wt.-% of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added into the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added into the blend solutions to achieve a

concentration of 0.1 wt.-%. The blend solution containing a model drug was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use. The thickness of the films were kept between 25-30 μm (measured by Peacock digital thickness gauge model PDN12N)

Drug Release Studies

To study the release characteristics of the model drugs from the films, drug-loaded blend films were immersed in buffer solutions pH 2.0, pH 5.5 and pH 7.2 at 37°C. At a time interval, 1-mL aliquots were withdrawn and assayed for the amount of drug released. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid released in the solutions were determined by a UV-Visible spectrophotometer (Perkin Elmer, Lambda 10) at 272, 275, 272, 299 nm, respectively. The experiments were done in triplicate. The percentages of released drugs were calculated from calibration curves of each model drug.

Results and Discussion

Effect of Blend Composition on Drug Release

The effect of blend composition on drug release is shown in Figure 2-5. Silk fibroin contents of 0, 20, 40, 50 and 60% in drug-loaded blend films were used in this study. The blend films with silk fibroin contents higher than 60% were not reported because the films were brittle and difficult to handle without cracking. It was found that the maximum release of drug was observed for the blend film with 80% chitosan content for all model drugs. This could be explained by the term of swelling behavior of the blend films. Table 1 shows the degrees of swelling of the drug-loaded blend films of different blend

compositions. It was found that the blend film with 80% chitosan content showed the maximum degree of swelling. It is known that for hydrogel delivery system the releasing of drug was controlled by swelling behavior of hydrogel. The swelling of carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment. Several chitosan-based hydrogels have been investigated for a potential application as drug delivery devices. Risbud *et al.* (2000)^[20] indicated that the releases of amoxicillin from the air-dried and freeze-dried chitosan/poly(vinyl pyrrolidone) hydrogels were related to the degree of swelling of the hydrogels. Yao *et al.* (1993 and 1994)^[21-22] studied the release of chlorhexidini acetate and cimetidine from chitosan/polyether semi-interpenetrating hydrogel. They found that the higher the degrees of swelling, the higher the amounts of drug released. In this study, the swelling of chitosan/silk fibroin blend films may be occurred due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan^[19].

Effect of pH on Drug Release

Since the swelling of polymeric gels can be triggered by a change in the environmental surrounding such as pH, the effect of pH on drug released from chitosan and the blend films was investigated and the result is shown in Figure 2-5. Drug release properties of the films were studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the highest amounts of drugs released from the systems were observed at pH 2.0 for all model drugs. This is in good agreement with the results of swelling of the films shown in Table 1. It appeared that the highest values of the degrees of swelling were obtained at pH 2.0 and the degrees of swelling of the films tended to decrease as pH of swelling solution was increased. It can be explained by the fact that in an acidic medium the amino groups of

chitosan are protonized, resulting that the hydrogen bonds between chitosan and silk fibroin are broken and the network is dissociated.^[20] The blend films exhibited lower degree of swelling in neutral medium. This may be due to the decrease in the number of protonated amino groups of chitosan at this pH. The pK_a of chitosan is 6.3-6.5, indicating that chitosan tends to protonate in acidic solution. Therefore, the degrees of swelling of the films at pH 7.2 were lower than those of the films in acidic solution. Risbud *et al.* (2000)^[16] reported that the degrees of swelling of chitosan/poly(vinyl pyrrolidone) hydrogels were high in acidic solutions (pH 1.0, pH 2.0 and pH 3.0) and became lower in neutral and alkaline solutions (pH 7.2 and pH 9.2). The release of amoxicillin from the films was found to be maximum at pH 1.0. Besides the release of drug being controlled by swelling behavior of the carrier, drug release may be concerned with the erosion process. This process is associated with macroscopic changes in the appearance of the device, including changes in the physicochemical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions. Table 1 shows the weight losses of chitosan and the blend films in the conditions studied. It was found that the weight losses of the films at pH 2 were higher than the values at pH 5.5 and pH 7.2. This indicated that drugs released by erosion process could also be occurred in this system.

Effect of Model Drug Nature on Drug Release

Comparison of the amounts of model drug released from chitosan and the blend film with 80% chitosan content is shown in Figure 6-8. The amounts of drugs released from the blend film with 80% chitosan content were higher than those released from pure chitosan films for all model drugs. The amounts of salicylic acid released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 92.7%, 83.4% and 73.5%, respectively. The amounts of theophylline released at pH 2.0, pH 5.5 and pH 7.2 from the

blend film with 80% chitosan were 81.1%, 73.6% and 69.0%, respectively. The releasing amounts of salicylic acid at equilibrium were higher than those of theophylline at all pH studied. In addition to drug solubility, another factor that can affect the penetration of a drug from a polymer matrix is the molecular size of drug. The molecule of salicylic acid (MW = 138.12) was smaller than theophylline (MW = 180.16). Due to the better solubility and smaller size, salicylic acid could diffuse from the matrix to the medium outside easier than theophylline. The amounts of diclofenac sodium released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 76.6%, 66.1% and 65.1%, respectively. Since diclofenac sodium did not completely dissolve in the blend solutions and remained in the blend films as solid particles. Therefore, the amounts of diclofenac sodium released to the solution were less as compared to salicylic acid and theophylline.

Among the model drugs investigated in this study, the amounts of amoxicillin released from the blend films with 80% chitosan content were the lowest values at all pH studied. It was found that the amount of amoxicillin released at pH 2.0, pH 5.5 and pH 7.2 were 37.2%, 34.0% and 23.5%, respectively. Risbud *et al.* (2000)^[20] has also reported that the releasing of amoxicillin from crosslinked chitosan-poly(vinyl pyrrolidone) air-dried hydrogel was rather low, about 31.68% and 27% at pH 1.0 and pH 2.0, respectively. This may be due to the interaction between the drug molecule and polymer matrix. Amoxicillin has carboxylic group that can interact with amino group of chitosan. In addition, among the model drugs used in this study, the molecular size of amoxicillin (MW = 381.45) is the biggest. Accordingly, the diffusion of amoxicillin through the polymer matrix was low.

Effect of Swelling Time on Drug Release

The release profiles of each model drug in buffer solutions at pH 2.0, 5.5, and 7.2 from the blend films with 80% chitosan content are illustrated in Figure 9-11. It was found

that the release of theophylline from the blend films with 80% chitosan content was very fast at all pH studied. Puttipipatkachorn (2001)^[23] investigated the drug-polymer interaction between theophylline and chitosan by FTIR and solid state ¹³C NMR spectroscopy. It was concluded that there was no interaction between theophylline and chitosan. Moreover, the molecular size of theophylline was rather small as compared with the other model drugs used. Then, theophylline could easily penetrate from the blend film to the medium.

The release of salicylic acid from the blend films with 80% chitosan content was also fast but a little slower than theophylline at pH 5.5 and pH 7.2. It was known that the salicylate formation can occur by the interaction between carboxylic group of salicylic acid and amino group of chitosan.^[23] Therefore, the releases of salicylic acid at pH 5.5 and pH 7.2 were faster than at pH 2.0 due to more ionized carboxylic groups that can interact with amino groups of chitosan.

Due to the poor solubility of diclofenac sodium in the blend solution, some diclofenac sodium remained as solid particles in the blend film. Accordingly, at initial stage, a time was needed for dissolving and penetrating of the drug from the blend film to the external medium. Therefore, the release of diclofenac sodium was slower and took longer time to reach the equilibrium as compared with the other model drugs.

Since amoxicillin has several polar groups, including hydroxyl group, amino group and carboxylic group, which can interact with the polymer matrix, the interaction between the drug and the polymer matrix can be formed. As a result, the amount of amoxicillin released from the blend films was only about 20-30%. However the rate of drug release occurred within 10 minutes. This may be due to the sufficient swell of the blend film resulting in the fast release of unbound drug.

Effect of Concentration of Crosslinking Agent on Drug Release

The effect of crosslinking agent concentration on drug release from the blend films is shown in Figure 12. To study the effect of concentration of crosslinking agent on drug release, the salicylic acid-loaded blend films with 80% chitosan content containing glutaraldehyde concentrations of 0.001, 0.01, and 0.5 mole/glucosamine unit were used. It was found that the amount of salicylic acid released from the blend film decreased with the increasing of glutaraldehyde concentration at all pH studied. It could possibly be explained by the term of degree of swelling (Table 2). The result revealed that the degree of swelling of the salicylic acid-loaded blend films decreased with the increasing of glutaraldehyde concentration. This is attributed to the swelling behavior of the crosslink network. At low concentration of crosslinking agent, the density of crosslinking is low that make hydrogel swell extensively. While the mesh size of the network become big resulting in high penetration of drug molecules to external environment. On the other hand, at high concentration of crosslinking agent, the degree of swelling is limited. Therefore, the mesh size of the network is closer to the size of drug, and the drug is more difficult to penetrate to the external environment.

Conclusion

Drug-released characteristics of crosslinked chitosan and its blend films with silk fibroin using glutaraldehyde as a crosslinking agent were studied. Theophylline, salicylic acid, diclofenac sodium and amoxicillin were used as model drugs. The maximum amounts of drug released were obtained from the blend film with 80% chitosan content. This corresponded to the highest swelling ability of the blend film with this blend ratio. The drugs releases were high in acidic medium due to the protonation of the amino groups on chitosan at acidic pH, resulting in the dissociation of hydrogen bonds between chitosan

and silk fibroin. The maximum and the minimum releases of model drugs were salicylic acid and amoxicillin, respectively. The differences in the rates and the amounts of drug released from the blend films may be due to the solubility of model drugs in the blend solutions, the molecular sizes of drug molecules and interaction between drugs and polymer matrix. Crosslinking is necessary to retain the gel structures after swelling. However, it will limit swelling ability of gels, resulting in the decreasing of the amounts of drug released. These preliminary results suggested the possibility of using crosslinked chitosan/silk fibroin blend films as a drug delivery carrier. Further investigations are now in progress to evaluate a potential application of crosslinked chitosan/silk fibroin blend films as a transdermal drug delivery device.

Acknowledgement

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List of Figure

Figure 1. Chemical structures of model drugs.

Figure 2. Effect of blend composition on releasing of diclofenac sodium. ▽ pH 2.0, ◻ pH 5.5, ■ pH 7.2

Figure 3. Effect of blend composition on releasing of salicylic acid. ▽ pH 2.0, ◻ pH 5.5, ■ pH 7.2

Figure 4. Effect of blend composition on releasing of amoxicillin. ▽ pH 2.0, ◻ pH 5.5, ■ pH 7.2

Figure 5. Effect of blend composition on releasing of theophylline. ▽ pH 2.0, ◻ pH 5.5, ■ pH 7.2

Figure 6. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 2.0. ■ chitosan film ▽ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 7. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 5.5. ■ chitosan film ▽ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 8. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 7.2. ■ chitosan film ▽ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 9. Drug release profiles from the blend films with 80% chitosan content at pH 2.0. (○) Salicylic acid, (◆) theophylline, (□) diclofenac sodium, (▲) amoxicillin trihydrate

Figure 10. Drug release profiles from the blend films with 80% chitosan content at pH 5.5. (○) Salicylic acid, (◆) theophylline, (□) diclofenac sodium, (▲) amoxicillin trihydrate

Figure 11. Drug release profiles from the blend films with 80% chitosan content at pH 7.2. (○) Salicylic acid, (◆) theophylline, (□) diclofenac sodium, (▲) amoxicillin trihydrate

Figure 12. Salicylic acid released from the blend films with 80% chitosan content containing various glutaraldehyde concentrations at pH 2.0, 5.5 and 7.2. ▽ 0.001 mole/glucosamine unit, ◻ 0.01 mole/glucosamine unit, ■ 0.05 mole/glucosamine unit

List of table

Table 1. Degree of swelling and percent weight loss of drug-loaded crosslinked chitosan and blend films

Table 2. Degree of swelling and percent weight loss of salicylic acid-loaded blend films with 80% chitosan containing various glutaraldehyde concentrations



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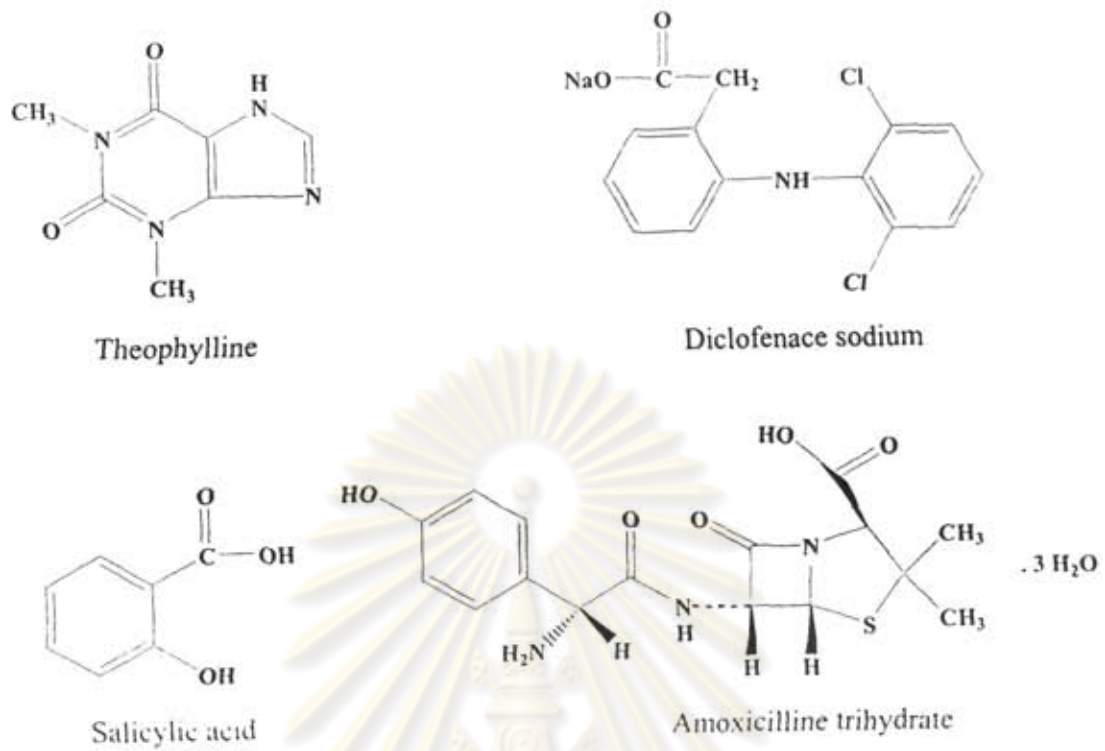


Figure 1

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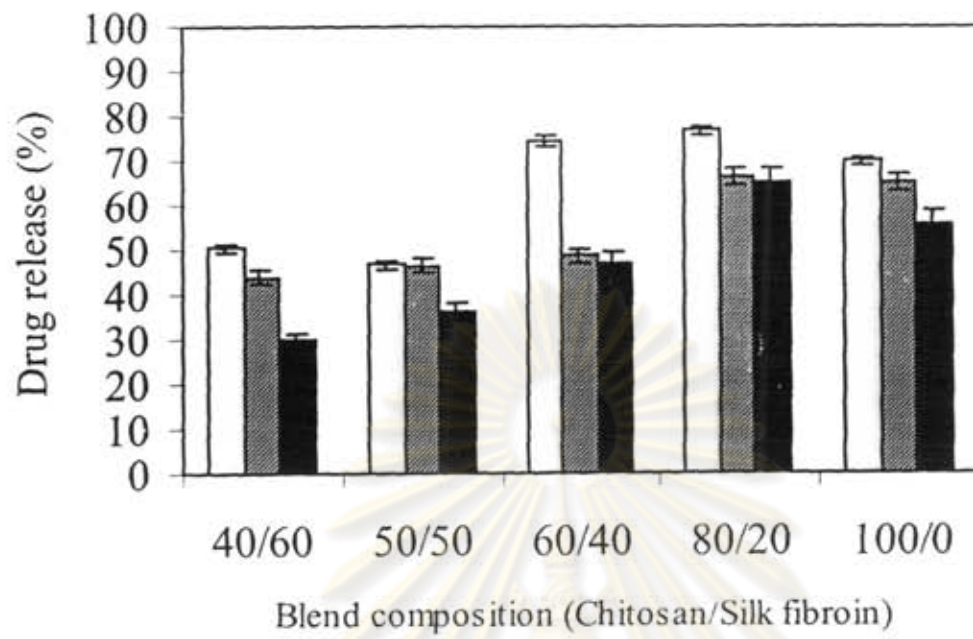


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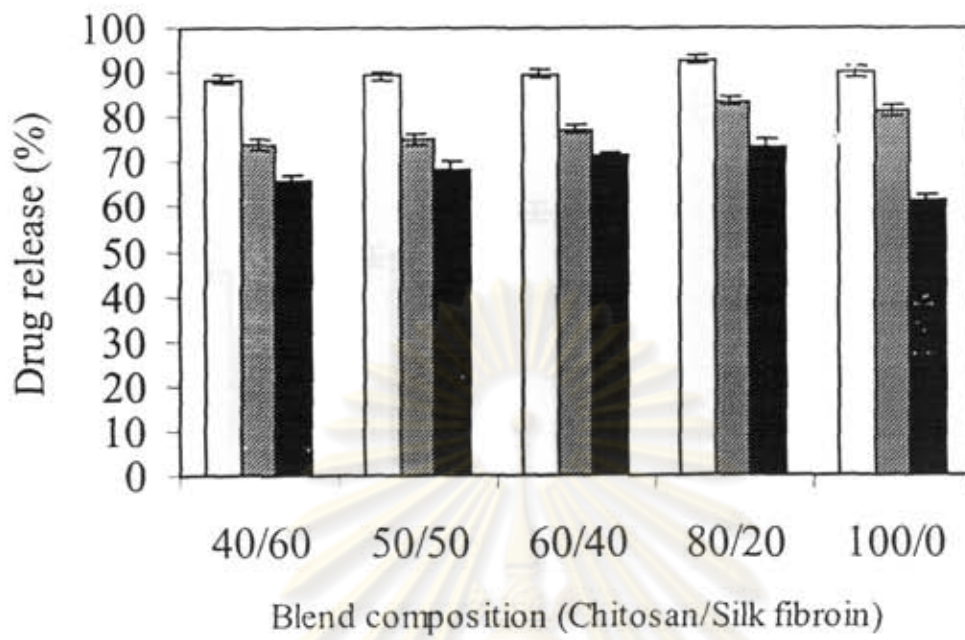


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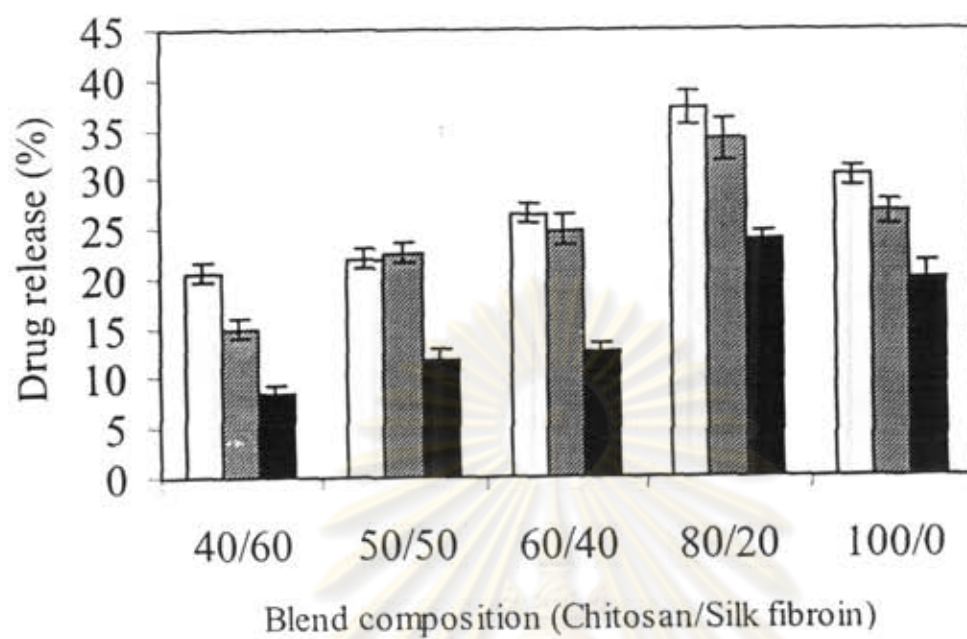


Figure 4

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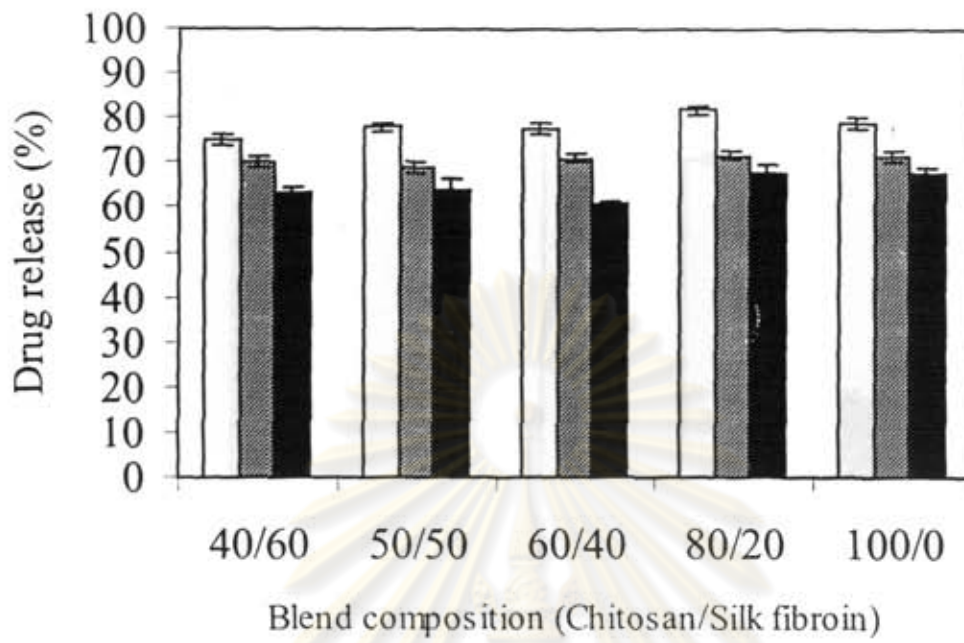


Figure 5

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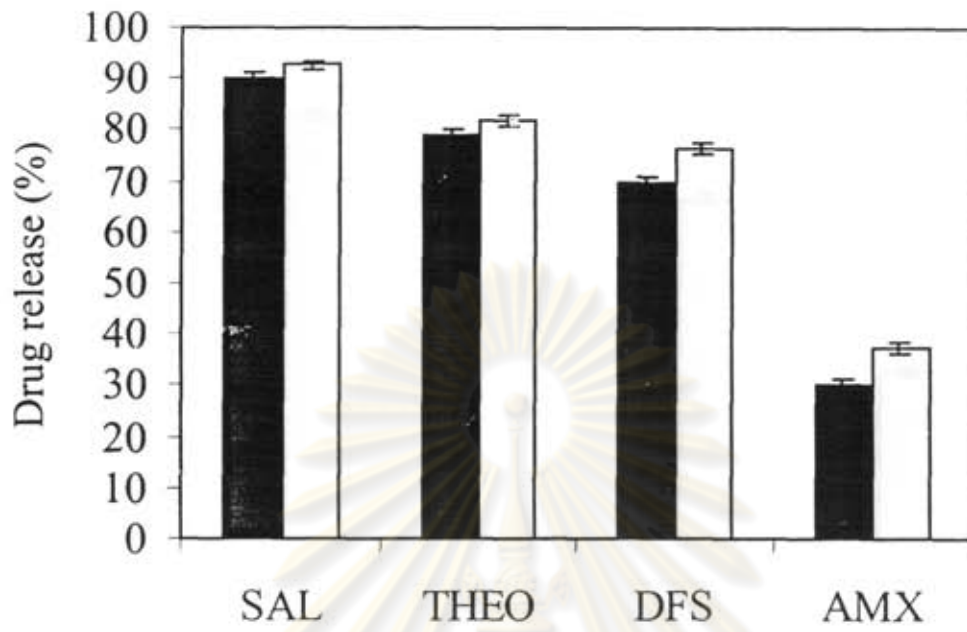
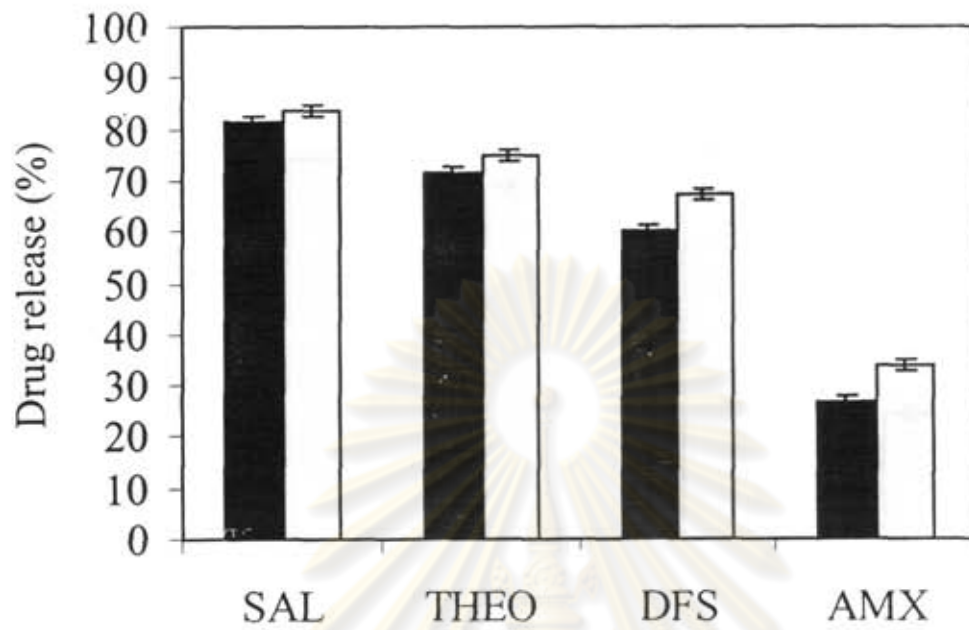


Figure 6

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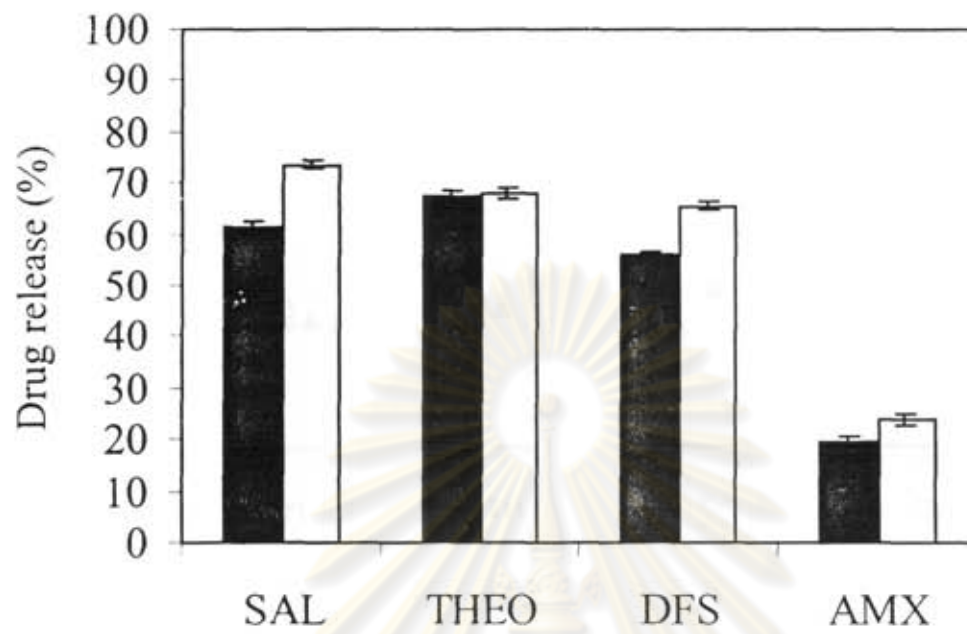


Figure 8

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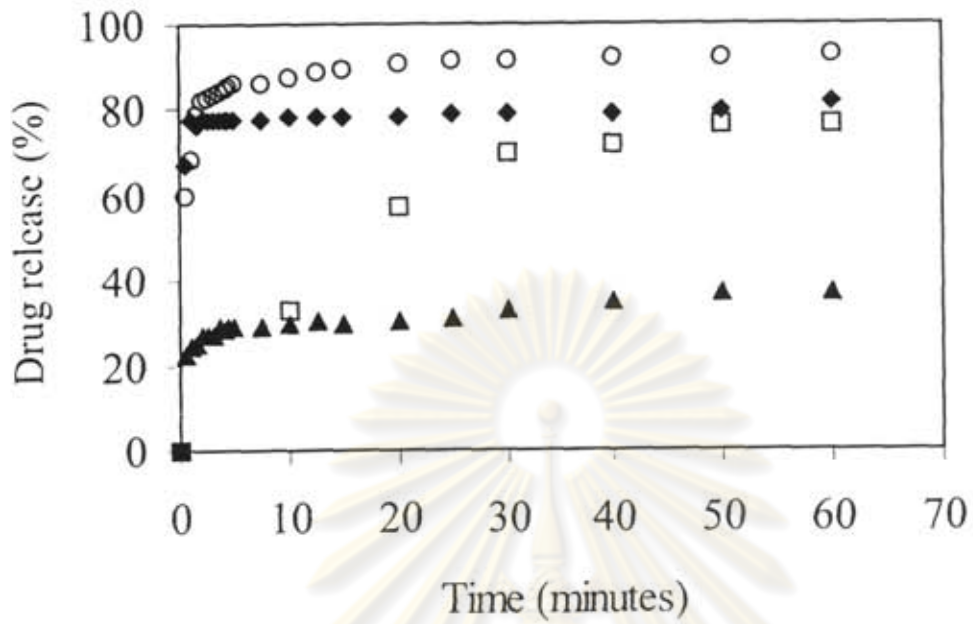


Figure 9

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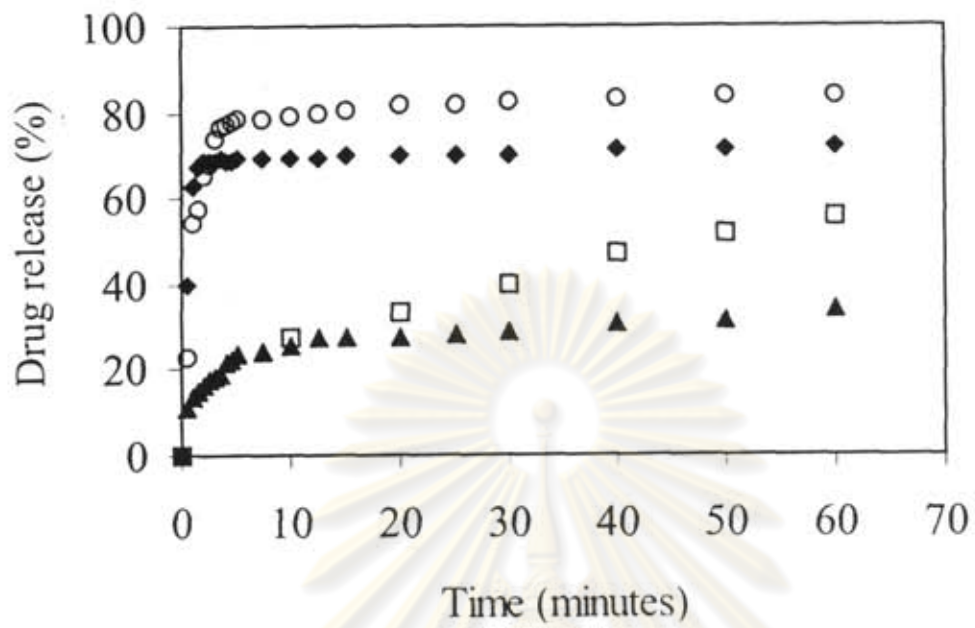


Figure 10

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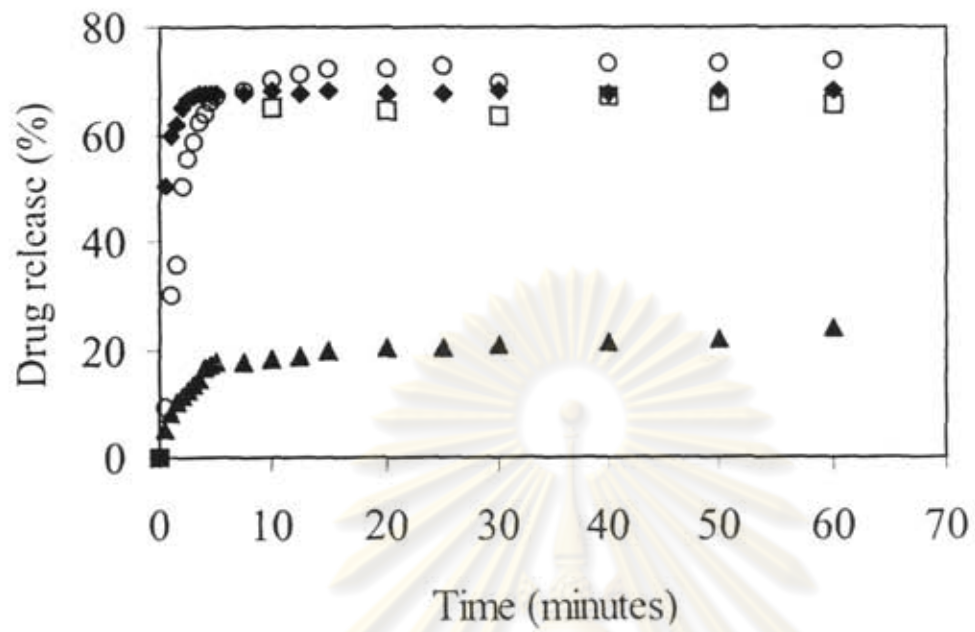


Figure 11

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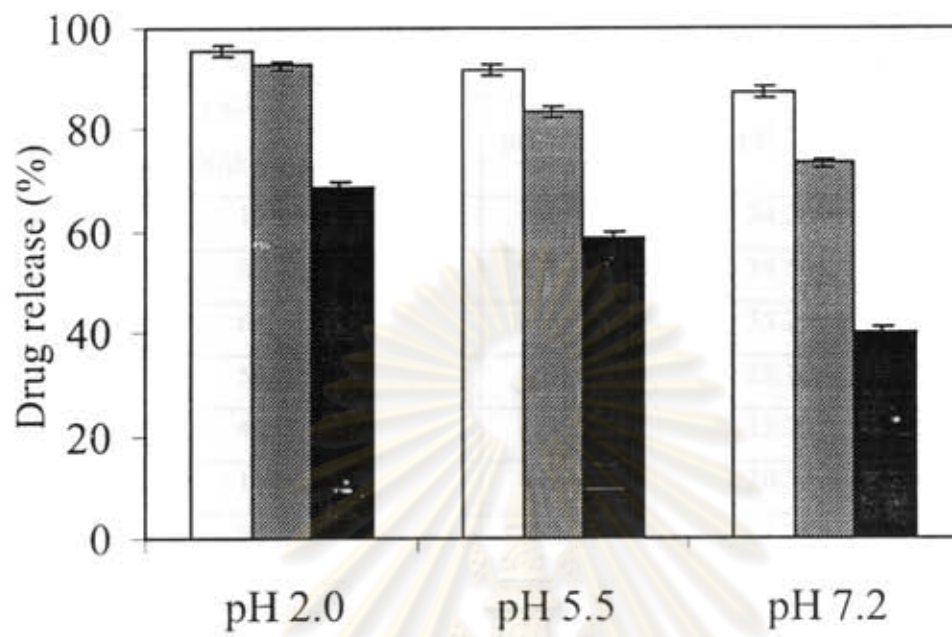


Figure 12

ศูนย์วิทยทรัพยากร
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Table 1

Drug	Weight Ratio of Chitosan to Silk Fibroin	Degree of Swelling (%) ^a			Weight Loss (%) ^a		
		pH 2.0	pH 5.5	pH 7.2	pH 2.0	pH 5.5	pH 7.2
Salicylic acid	100:0	840	662	185	34.20	24.27	17.60
	80:20	1152	974	199	39.50	27.81	24.32
	60:40	763	535	179	35.20	24.21	15.40
	50:50	539	465	173	28.10	20.04	14.20
	40:60	582	471	169	23.70	19.86	13.80
Amoxicillin	100:0	643	492	194	20.38	19.98	17.73
	80:20	736	587	197	28.25	27.24	18.67
	60:40	639	472	168	22.30	20.75	18.25
	50:50	553	431	145	19.93	18.50	19.10
	40:60	504	409	129	18.10	17.63	17.98
Diclofenac sodium	100:0	753	653	167	35.43	30.07	28.60
	80:20	809	721	173	37.85	35.18	28.80
	60:40	783	600	125	30.53	30.01	23.40
	50:50	722	534	108	29.93	28.56	20.60
	40:60	687	497	102	21.20	20.01	17.50
Theophylline	100:0	306	252	167	38.87	35.54	26.14
	80:20	376	325	175	40.05	37.75	27.22
	60:40	360	289	159	36.67	32.23	23.44
	50:50	303	277	151	33.32	28.87	20.50
	40:60	232	221	142	30.01	25.34	18.70

^a the average value from three experiments

Table 2

pH	Degree of Swelling (%) ^a			Weight Loss (%) ^a		
	Glutaraldehyde concentration (mole/glucosamine unit)			Glutaraldehyde concentration (mole/glucosamine unit)		
	0.001	0.01	0.05	0.001	0.01	0.05
2.0	1453	1152	825	42.21	39.50	32.13
5.5	1213	974	672	33.16	27.81	22.81
7.2	320	199	123	25.19	24.32	19.25

^a the average value from three experiments



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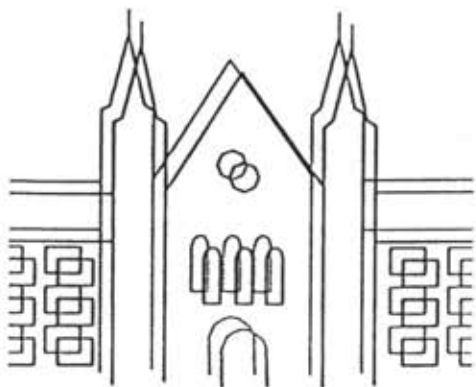
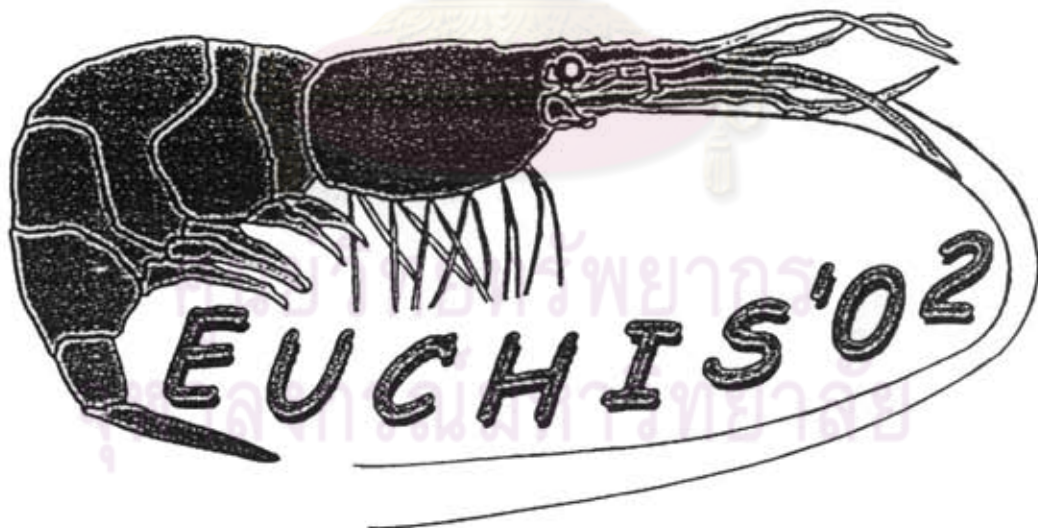
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ADVANCES IN CHITIN SCIENCE

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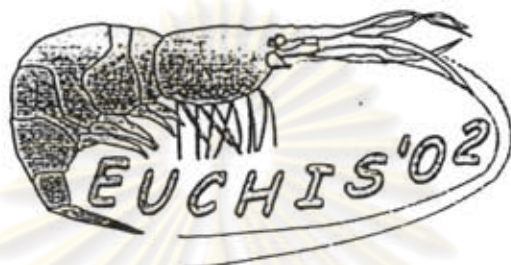
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PREPARATION AND CHARACTERIZATION OF CM-CHITIN/PVA BLEND FILMS

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Keywords: CM-chitin, Poly(vinyl alcohol), Swelling behavior

INTRODUCTION

Carboxymethyl-chitin (CM-chitin) is a water-soluble derivative of chitin. Poly(vinyl alcohol) (PVA) is a nontoxic water-soluble synthetic polymer. In this study, CM-chitin/PVA blend films were prepared by varying blend compositions of CM-chitin and PVA. The effect of blend composition, pH and salt type on the degree of swelling of the blend films were investigated.

EXPERIMENTAL

The blend films of PVA and CM-chitin were prepared by mixing various ratios of 1% by weight of PVA solution and 1% by weight of CM-chitin solution. For crosslinked CM-chitin/PVA blend films, glutaraldehyde was added into the solution to achieve the concentration of 0.01% before casting onto the clean dry plastic plates. The films were allowed to dry at 40°C in an oven for 24 h.

The blend films were cut into the disk form with diameter of 16 mm and 25-30 μm in thickness. The weights of the completely dried samples were measured, and the samples were dipped into a vial filled with different pH buffer solutions and different salt solutions (LiCl, NaCl, CaCl₂ and FeCl₃) with the concentration of 0.25 M at room temperature. The degrees of swelling of these samples were calculated.

RESULTS AND DISCUSSION

Effect of pH on Swelling Behavior of the Blend Films

The effect of pH on the degree of swelling of CM-chitin/PVA blend films with various blend compositions is shown in Figure 1. The degrees of swelling of PVA films were rather constant for the whole pH range from pH 3 to 11 due to pH stability of PVA. On the other hand, the degree of swelling of CM-chitin films and the blend films increased substantially in both acidic and alkaline pH ranges. The pK_a of the carboxymethyl group and amino group are 3.4 and 6.4, respectively [1]. The reason to explain the effect of pH on the degree of swelling of CM-chitin films and the blend films is that in acidic pH solutions, the amine groups of CM-chitin are ionized leading to the dissociation of the adjacent chains [2]. For alkaline pH solutions, the effect of pH on the degree of swelling of CM-chitin films increased because of the presence of the carboxymethyl groups that are ionizable functional groups of CM-chitin. It could say that the CM-chitin/PVA blend films showed the pH sensitive property.

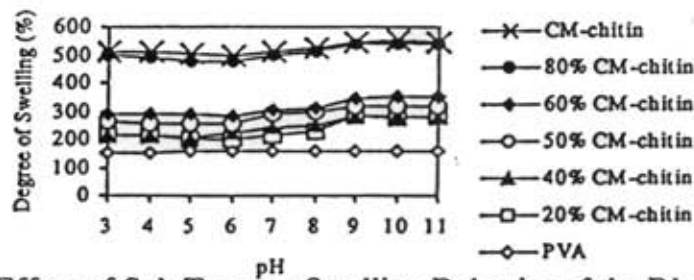


Figure 1
Degree of swelling of CM-chitin/PVA blend films with addition of 0.01% glutaraldehyde as a function of pH.

Effect of Salt Type on Swelling Behavior of the Blend Films

The degrees of swelling of pure and the blend films in various types of salt solutions are shown in Figure 2. It was found that, for all salt solutions, the degrees of swelling of the blend films increased as CM-chitin content increased, especially, when CM-chitin content were higher than 40%. However, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solutions (NaCl and LiCl). For CaCl₂ solution, Tokura and coworkers (1983) found that CM-chitin can bind calcium ions even in the presence of monovalent cations [1]. Watanabe and coworkers (1992) reported that the addition of iron (III) chloride into CM-chitin solution induces gel formation. This indicated that CM-chitin can also bind with Fe³⁺ [3].

For PVA films and the blend films with CM-chitin content less than 40%, the effect of different salt types on the change in degree of swelling of the blend films was very small. From Figure 2, it was observed that pure PVA films had the lowest degree of swelling. By the addition of CM-chitin to PVA films, the degree of swelling of PVA in salt solutions could be enhanced.

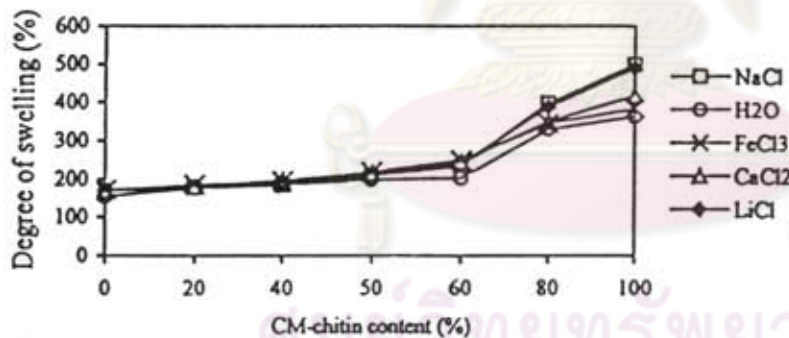


Figure 2
Effect of salt type on degree of swelling of chitin/PVA blend films with the addition of 0.01% glutaraldehyde function of CM-chitin content.

CONCLUSION

The blend compositions of CM-chitin/PVA blend films had a large effect on the swelling behavior of the blend films. The swelling behavior of CM-chitin/PVA blend films varied with the respect to changes in pH and salt type, indicating that the blend films had pH- and salt-responsive properties.

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DRUG-RELEASED CHARACTERISTICS OF CROSS-LINKED CHITOSAN/SILK FIBROIN BLEND FILMS

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ABSTRACT

Crosslinked chitosan/silk fibroin blend films were prepared by solution casting technique using glutaraldehyde as a crosslinking agent. Drug-released characteristics of the blend films with various blend compositions were investigated. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid were used as model drugs. The release studies were carried out at 37°C in buffer solutions pH 2.0, pH 5.5 and pH 7.2. It was found that the blend films with 80% chitosan content showed the maximum amounts of model drugs released at pH 2.0 for all types of drugs. This result corresponded to swelling ability of the blend films. From swelling study, the maximum degrees of swelling of the drug-loaded blend films were obtained at this pH and this blend composition. The amounts of drugs released from the films with 80% chitosan content from the highest to the lowest values obtained in the following sequence: salicylic acid > theophylline > diclofenac sodium > amoxicillin. This could be due to the effects of molecular sizes of drugs, solubility of drugs in the blend solutions and interaction between drugs and polymer matrix.

INTRODUCTION

Nowadays, natural polymers such as protein and polysaccharide have become more and more important for their rich resources and low cost. Especially, they are useful materials in biomedical areas due to their non-toxicity, biodegradability, and biocompatibility. Silk fibroin is a fibrous protein that is composed of 17 amino acids and its main components are nonpolar ones such as glycine, alanine, and serine [1]. Silk fibroin can exist in two general conformations, random coil and β -sheet form. The conformation transition of silk fibroin can be induced to be changed from random coil to β -sheet structure by treatments such as heating, stretching or immersion in polar solvents [2]. This transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β -sheet structure is resistant to water and has good mechanical properties [3]. Silk fibroin is considered to be an interesting starting material for developing new materials and devices for biotechnological and biomedical utilizations. It has been reported that silk fibroin film has good oxygen permeability in wet state, which suggests promising applications of silk fibroin as a wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures [4], biocompatible devices with controlled drug release [5] and bone binding functions [6]. However, silk fibroin in dry state is very brittle and unsuitable for practical uses [7]. To overcome this limitation, silk fibroin has been reported to be blended with other synthetic polymers, such as polyacrylamide [8] and poly(vinyl alcohol) [9], or natural polymers, such as cellulose [7] and sodium alginate [10], to improve mechanical and physical properties.

Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of $\beta(1\rightarrow4)$ -linked 2-acetamido-D-glucose unit and

$\beta(1\rightarrow4)$ -linked 2-amino-D-glucose unit with the latter usually greater than 75%. Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-dimensional crosslinked polymeric material with the ability to absorb significant amount of water. Crosslinked chitosan hydrogels can swell extensively due to the positive charges on the network and response to changes in pH of medium. Due to the benefits of being non-toxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for the sustained delivery of drug. For example, it was investigated for the delivery of drugs such as prednisolone [12] and diclofenac sodium [13]. Furthermore, it has been reported that chitosan could induce a conformational transition of silk fibroin from random coil to β -sheet structure [3] and a polymer blend of these biopolymers could also form a hydrogel having a semi-interpenetrating polymer network by using glutaraldehyde as a crosslinking agent [14]. Up to now, there are no reports on using crosslinked chitosan/silk fibroin blend film as drug delivery device. This research is a preliminary study on using cross-linked chitosan/silk fibroin blend film as a matrix for drug delivery system. The model drugs used were theophylline, diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effects of blend composition, degree of crosslinking, and pHs of the external swelling media on model drugs released from the blend films were investigated.

EXPERIMENTAL

Materials

Shrimp shell was kindly provided by Suraphol Food Public Co., Ltd. Silk fiber (*Bombyx mori*) was degummed by treatment with 0.5% Na_2CO_3 at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk fibroin was dissolved in triad solvent CaCl_2 : EtOH: H_2O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was filtered through the sintered glass filter and subsequently diluted to achieve a concentration of 1% w/w.

Preparation of chitin

Chitin was prepared from shrimp shell by decalcification and deprotenization to remove calcium carbonate and protein, respectively. The shrimp shell was cleaned and dried under sunlight before grinding into small pieces. The shrimp shell chips were treated by immersing in 1 N HCl solution for 2 days with occasional stirring. The decalcified product was washed with distilled water until neutral. Deprotenization was followed by boiling in 4% w/w of NaOH solution at $80\text{--}90^\circ\text{C}$ for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C for 24 h.

Preparation of chitosan

Chitin was deacetylated by heating in NaOH 50% w/w solution with sodium borohydride (NaBH_4) 0.5% w/w based on the weight of chitin to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustively with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1% w/w of acetic acid solution. The chitosan solution was allowed to stand overnight at room temperature to reduce of air bubbles before preparation of films.

Preparation of crosslinked drug-loaded blend films.

The blend solutions of chitosan and silk fibroin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added into the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added into the blend solutions to achieve a concentration of 0.1% w/w. The blend solution containing a model drug was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use.

Drug Release Studies

To study the release characteristics of the model drugs from the films, drug-loaded blend films were immersed in buffer solutions at pH 2.0, pH 5.5 and pH 7.2. At a time interval, 1-mL aliquots were withdrawn and assayed for the amount of drug released. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid released in the solutions were determined using an UV-Visible spectrophotometer (Perkin Elmer, Lambda 10) at 272, 275, 272, and 299 nm, respectively. The experiments were done in triplicate. The percentages of released drugs were calculated from calibration curves.

RESULTS AND DISCUSSION

Effect of blend composition on drug release

The effect of blend composition on drug release is shown in Figure 1. Silk fibroin contents of 0, 20, 40, 50 and 60% in drug-loaded blend films were used in this study. The blend films with silk fibroin contents higher than 60% were not reported because the films were brittle and difficult to handle without cracking. It was found that the maximum release of drug was observed for the blend film with 80% chitosan content for all types of model drugs. This could be explained by the term of swelling behavior of the blend films. It was found that the blend film with 80% chitosan content showed the maximum degree of swelling (Table 1). Peppas *et al.* [15] suggested that hydrogel delivery system was controlled by swelling behavior of hydrogel. Risbud *et al.* [16] concluded that the release of amoxicillin from the air-dried and freeze-dried chitosan/poly(vinyl pyrrolidone) hydrogels was related to the degree of swelling of the hydrogels. Furthermore, Yao *et al.* [17,18] studied the release of chlorhexidini acetate and cimetidine from chitosan/polyether semi-interpenetrating hydrogel. They found that the higher degrees of swelling, the higher amounts of drug released. Chen *et al.* [14] reported that the maximum degree of swelling of the blend films was observed for chitosan/silk fibroin blend film with 80% chitosan content. The swelling of chitosan/silk fibroin blend films may be occurred due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan. However, the lower amounts of released drug were obtained when silk fibroin content in the drug-loaded blend films was increased. Suesat *et al.* [19] reported that there was no change in the degree of swelling of pure silk fibroin film immersed in buffer solutions for the whole pH range from pH 3 to pH 11. Therefore, swelling ability of the blend films depended on the amounts of chitosan content in the blend films.

Effect of pH on drug release

The effect of pH on drug released from chitosan and blend films is shown in Figure 1. Drug release profile was studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the amount of drug released from the systems was highest at pH 2.0 for all types of model drugs. This is in good agreement with the result of swelling as shown in Table 1. It appeared that the degree of swelling was the highest at pH 2.0 and tended to decrease when

pH of swelling solution was increased. This result corresponded to the previous works [14,19], which also reported that the degree of swelling of the crosslinked chitosan/silk fibroin blend films was maximum at pH 2.0 and decreased when pH of the swelling solution was increased. It can be explained by the fact that in an acidic medium the amino groups of chitosan were protonized, resulting that the hydrogen bonds between chitosan and silk fibroin were broken and the network was dissociated [14]. The blend films exhibited lower degree of swelling when pH was higher than 5. This may be due to the number of protonated amino groups of chitosan become lower at neutral and alkaline pH. The pK_a of chitosan is 6.3-6.5, which indicates that chitosan tends to protonate in acidic solution²⁰. Therefore, the degree of swelling of the blend films in alkaline solution was very low as compared to that of the blend films in acidic solution. Risbud *et al.* [16] reported that the degrees of swelling of chitosan/poly(vinyl pyrrolidone) hydrogels were high in acidic solutions (pH 1.0, pH 2.0 and pH 3.0) and became lower in neutral and alkaline solutions (pH 7.2 and pH 9.2). The release of amoxicillin was found to be maximum at pH 1.0. Besides the release of drug is controlled by swelling condition of the carrier, drug release may be concerned with the erosion process. This process is associated with macroscopic changes in the appearance of the device, changes in the physicomechanical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions [21]. Table 1 shows the weight loss of chitosan and blend films. It was found that the weight loss of the films was highest at pH 2. This indicated that drug release by erosion process could be occurred in this system.

Effect of drug types on drug release

The effect of drug molecules on drug release is shown in Figure 2. The releases of model drugs, theophylline, salicylic acid, diclofenac sodium and amoxicillin, were studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the blend film at 80% chitosan content gave the highest amount of released drugs. The amounts of released salicylic acid at pH 2.0, pH 5.5 and pH 7.2 from blend film with 80% chitosan content were 92.7%, 83.4% and 73.5%, respectively. The amounts of theophylline released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan were 81.1%, 73.6% and 69.0%, respectively. The maximum amount of released salicylic acid at equilibrium was higher than that of theophylline. One factor that can affect the penetration of a drug from a polymer matrix is the molecular size of the drug. The molecule of salicylic acid (MW = 138.12) was smaller than theophylline (MW = 180.16). Thus, the penetration of salicylic acid from the matrix was easier than theophylline. Diclofenac sodium released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 76.6%, 66.1% and 65.1%, respectively. The amount of diclofenac sodium released was less than those of theophylline and salicylic acid because diclofenac sodium did not dissolve in the blend solutions and appeared in the blend films as solid particles. Therefore, the diffusion of diclofenac sodium to the solution took longer time than salicylic acid and theophylline.

Among the drugs investigated in this study, the amounts of amoxicillin released from the blend films was the least values for all pH studied. It was found that the amount of amoxicillin released at pH 2.0, pH 5.5 and pH 7.2 were 37.2%, 34.0% and 23.5%, respectively. This may be due to the interaction between the drug molecule and polymer matrix. Risbud *et al.* [16] reported the amoxicillin released from crosslinked chitosan-poly (vinyl pyrrolidone) air-dried hydrogel was about 31.68% and 27% at pH 1.0 and pH 2.0, respectively. They explained that the low amounts of drug released might be due to non-porous nature of the air-dried films.

CONCLUSION

Drug -released characteristics from crosslinked chitosan and its blend films with silk fibroin by using glutaraldehyde as a crosslinking agent was studied. Theophylline, salicylic acid, diclofenac sodium and amoxicillin were used as model drugs. The amount of drug released from the blend film with 80% chitosan content was the highest value corresponding to the highest swelling ability of the blend film with this blend ratio. The drug released in acidic pH media was higher than neutral or alkaline pH media because the protonation of the amino groups on chitosan at acidic pH resulting in the dissociation of hydrogen bond between chitosan and silk fibroin network. The maximum and the minimum of released drug were salicylic acid and amoxicillin, respectively. The difference in the amounts of drug released may be due to the effect of the molecular size of drug molecules and interaction between drugs and polymer matrix. Finally, theophylline was the fastest release because no drug polymer interaction between theophylline and chitosan was observed.

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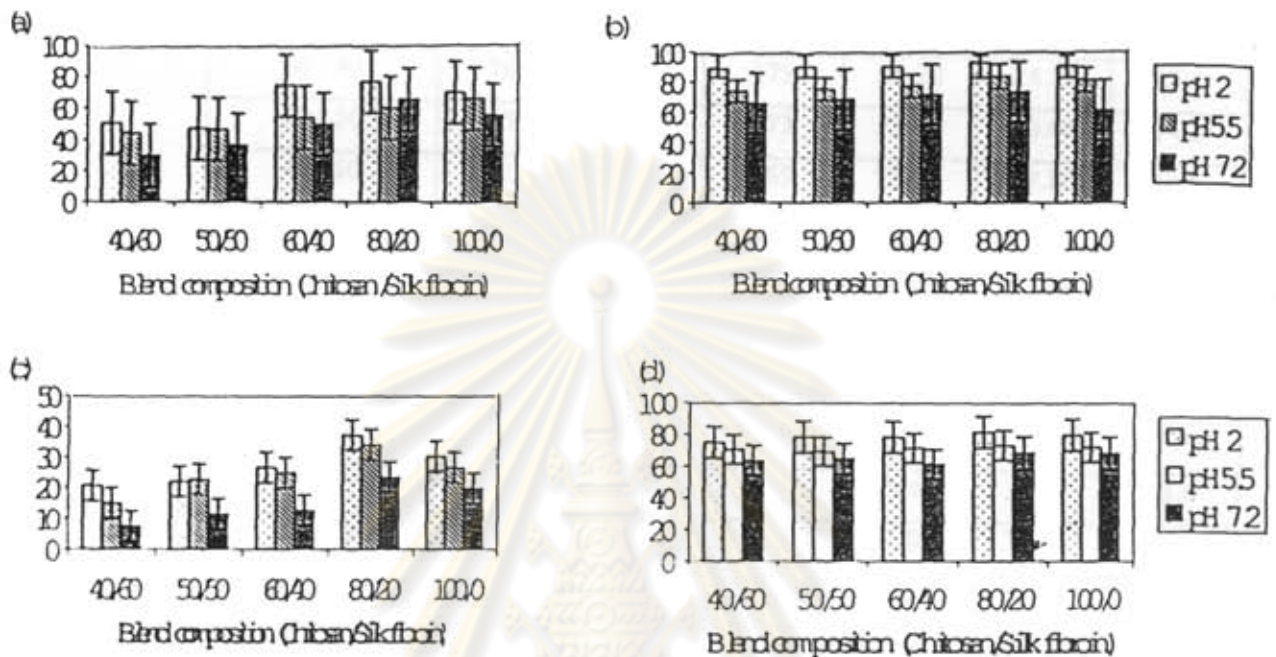


Figure 1 Effect of blend compositions on releasing of (a) diclofenac sodium, (b) salicylic acid, (c) amoxicillin and (d) theophylline at pH 2.0, pH 5.5 and pH 7.2 for 60 min.

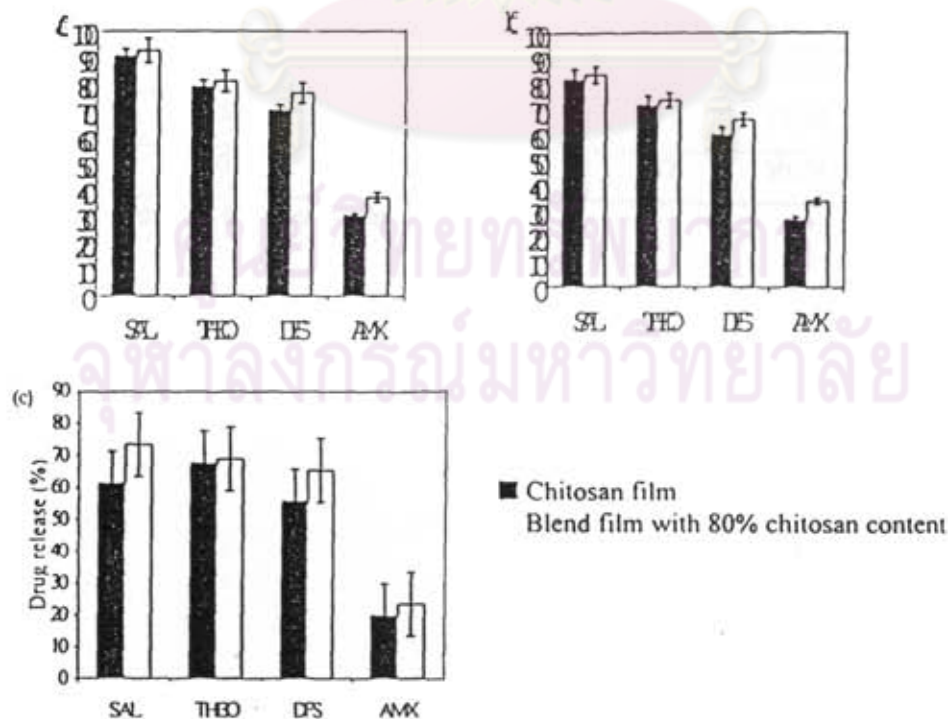


Figure 2 Comparison of the amounts of drugs released from chitosan and the blend film with 80% chitosan at (a) pH 2.0, (b) pH 5.5 and (c) pH 7.2. SAL= salicylic acid; THEO =theophylline; DFS = diclofenac sodium; and AMX = amoxicillin.

Table 1 Degree of swelling and % weight loss of drug- loaded crosslinked chitosan and blend films

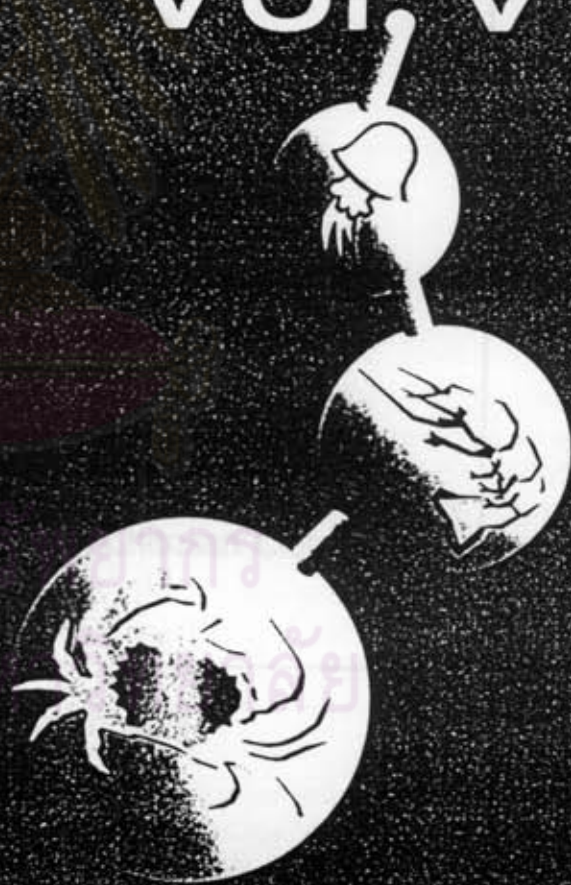
Drug	Weight Ratio of Chitosan to Silk Fibroin	Degree of Swelling (%) ^a			Weight Loss (%) ^a		
		pH 2	pH5.5	pH 7.2	pH 2	pH5.5	pH 7.2
Salicylic acid	100:0	840	662	185	34.20	24.27	17.60
	80 : 20	1152	974	199	39.50	27.81	24.32
	60 : 40	763	535	179	35.20	24.21	15.4
	50 : 50	539	495	173	28.10	20.04	14.2
	40 : 60	582	471	169	23.70	19.86	13.8
Amoxicillin	100:0	643	492	194	20.38	19.98	17.73
	80 : 20	736	587	197	28.25	27.24	18.67
	60 : 40	639	472	168	22.30	20.75	18.25
	50 : 50	553	431	145	19.93	18.50	19.10
	40 : 60	504	409	129	18.10	17.63	17.98
Diclofenac sodium	100:0	753	653	167	35.43	30.07	28.6
	80 : 20	809	721	173	37.85	35.18	28.8
	60 : 40	783	600	125	30.53	30.01	23.4
	50 : 50	722	534	108	29.93	28.56	20.6
	40 : 60	687	497	102	21.20	20.01	17.5
Theophylline	100:0	306	252	167	38.87	35.54	26.14
	80 : 20	376	325	175	40.05	37.75	27.22
	60 : 40	360	289	159	36.67	32.23	23.44
	50 : 50	303	277	151	33.32	28.87	20.5
	40 : 60	232	221	142	30.01	25.34	18.7

^a The average values from three experiments

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PREPARATION AND CHARACTERIZATION OF CM-CHITIN/SILK FIBROIN BLEND FILMS

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ABSTRACT

Films of CM-chitin and silk fibroin were prepared with various ratios of CM-chitin to silk fibroin, with and without glutaraldehyde as a cross-linking agent. The effects of the ratios of CM-chitin to silk fibroin and cross-linking agent on swelling behavior of the blend films were studied. The structures and properties of the blends were characterized by FT-IR, X-ray diffraction, differential thermal analysis, and thermogravimetric analysis.

INTRODUCTION

Silk fibroin is a fibrous protein that is composed of 17 amino acids. The highly repetitive sections consist mainly of glycine, alanine and serine [1]. The sum of these repetitive amino acids is greater than 80 mol% of the total amino acid composition. The primary structure arising from this characteristic amino acid composition contains many –(-Gly-Ala-)_n- repeating unit. Silk fibroin can be prepared in the form of powder, gel and film from either silk fiber, after dissolution with concentrated salt solution, or liquid silk taken directly from the nature silk gland. Silk fibroin has become more important because of its properties, such as non-toxicity, biodegradability and good biological compatibility. It has been investigated for biomaterials in biotechnological and biomedical fields. For example, the glucose sensor prepared by using silk fibroin as enzyme substrate to immobilize glucose oxydase showed high stability against pH and temperature changes [2]. Silk fibroin has been studied for biomedical applications such as surgical sutures [3], wound covering materials [4], wound-repairing, and bone binding function [5]. However, silk fibroin is very brittle and almost unsuitable for practical use in the dry state [6]. Some inferior physical and mechanical properties of silk fibroin membranes can be improved by blending with other natural or synthetic polymers. Water absorption, mechanical properties, and thermal stability of silk fibroin films were improved by blending with sodium alginate [7]. The addition of cellulose to silk fibroin permitted preparation of films with excellent elastic behavior [8]. Silk fibroin/PVA blend films showed increased permeability to neutral salts [9].

Chitin is a natural abundant polysaccharide that is widely distributed in crustaceans, insects, mushrooms and in the cell walls of bacteria. Chitin consists of 2-acetamido-2-deoxy-D-glucose through a β (1 \rightarrow 4) linkage. Chitin has become attractive because of its rich resources and some interesting properties such as biocompatibility, biodegradability and non-toxicity. Chitin has been found to be useful as a biodegradable pharmaceutical carrier [10], a blood anticoagulant [11], and a wound-healing accelerator [12]. Chitin has also proved to be a highly effective antigen [13]. However, chitin has a limitation. It is known that chitin is insoluble in most common solvents except for strong acids such as methanesulfonic, sulfuric and formic acids. The insolubility of chitin has been suggested to be due to its rigid crystalline structure through intra- and inter-molecular hydrogen bonds [14]. This property can be improved by chemical modification of chitin. Chitin could be modified by introducing carboxymethyl groups to enhance its solubility in water.

Carboxymethyl-chitin (CM-chitin) is a water soluble chitin derivative. CM-chitin is soluble not only in acidic media but at any pH. CM-chitin has been investigated as polymeric drug [15], wound healing [16], cosmetic ingredients for hair and skin cares [17], and chelating agent [18]. However, silk fibroin and CM-chitin blend films still have not been investigated. In this study the effect of blend compositions on physical properties, as well as swelling behavior, of the blend films were investigated.

EXPERIMENTAL

Materials: Shrimp shell was kindly supplied from Surapol Food Public Co., Ltd. Chitin was prepared by the method of Shimahara and Takigushi [19]. The degree of deacetylation of chitin, determined by infrared spectroscopic measurement according to the method of Sannan *et al.* [20], was 31.30%. Chitin was powdered to size in the range of 71-75 μm before use.

Preparation of CM-chitin: CM-chitin was prepared by the method of Hirano [21]. Alkaline chitin was prepared by suspending powdered chitin in 42% NaOH solution. After the suspension was allowed in desiccator for 30 min under reduced pressure, crushed ice was added and the mixture was mechanically stirred for 30 min in an ice bath to dissolve chitin. A viscous alkaline chitin solution was obtained. For successful synthesis of CM-chitin, the concentration of NaOH solution should not less than 14%. Monochloroacetic acid solution was prepared by dissolving in 14% NaOH solution in an ice bath and was added dropwise into the alkaline chitin solution with stirring over 30 min. After standing overnight at room temperature, the mixture was neutralized with acetic acid under cooling in an ice bath and dialyzed against running water for 2 days, followed by dialysis against distilled water for 1 day. The dialysate was centrifuged at 5000 rpm for 20 min, in order to remove insoluble material, and the supernatant was added to 3 volumes of acetone. After standing overnight, the precipitate was collected by centrifugation and washed with acetone. The product was resuspended in ethanol and collected by filtration. After drying at room temperature, CM-chitin Na salt was obtained. The degree of substitution of CM-chitin was 0.44 as estimated by elemental analysis (Perkin Elmer PE2400 Series II). The viscosity average molecular weight of CM-chitin was 7.70×10^4 .

Preparation of silk fibroin solution: Raw silk fiber (*Bombyx mori*) was degummed by heating in 0.5% Na_2CO_3 solution at 100°C for 1 h followed by washing with boiling water and drying at 60°C for 24 h in an oven. Degummed silk fibroin 6 g was then dissolved in 94 g of 1:2:8 by mole of CaCl_2 :EtOH: H_2O solvent system at 100°C for 15 min [22]. The resulting silk fibroin solution was filtered through the sintered glass filter and subsequently dialyzed against distilled water for 7 days. The dialyzed silk fibroin solution was filtered and diluted to achieve a concentration of 1% w/w.

Preparation of blend films: The blend films of silk fibroin and CM-chitin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of CM-chitin solution. The blend solution was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. For the crosslinked silk fibroin/CM-chitin blend films, glutaraldehyde used as crosslinking agent was added into the blend solution to achieve the concentration of 0.005%, 0.01%, and 0.05%.

Measurements: Infrared spectra of pure and blend films were measured by a Bruker FTIR spectrophotometer, model Vector 3.0, with 32 scans at a resolution of 4 cm^{-1} . Wide-angle X-ray diffraction patterns were recorded with an X-ray diffractometer (D/MAX-2000 series of Rigaku X-ray Diffractometer system). The X-ray source was Ni-filtered $\text{Cu K}\alpha$ radiation (40 kV/30 mA). The films were scanned from 5 to 30 degree 2θ at speed of 5 degree/min. Differential scanning calorimetry (DSC) measurements were performed on a

Perkin Elmer DSC 7. A 5 mg of cut film was compressed and sealed in an aluminum pan. DSC curves of each film were obtained from the second heating run at a rate of 10°C/min, after the first run of heating up to 190°C and cooling to 25°C at the same rate of 10°C/min, under nitrogen atmosphere. A thermogravimetric analyzer (Dupont, model TGA 2950) was used to investigate the thermal stability of the blends with a heating rate of 10°C/min from 30°C to 600°C. The swelling behavior of the blend films was carried out by measuring the weight of the films after immersion in distilled water, buffer pH solution, and various salt solutions (i.e., 0.25 M solutions of LiCl, NaCl, CaCl₂ and FeCl₃) for 24 hours in comparison with the dry weight of the film before immersion. The blend films samples were 16 mm in diameter and 30-40 µm in thickness. The degree of swelling was determined according to the following equation:

$$\text{Degree of swelling (\%)} = [(W_s - W_d)/W_s] \times 100,$$

where W_s and W_d denote the weights of swollen and dry films, respectively.

RESULTS AND DISCUSSION

Characterization of CM-chitin/silk fibroin blend films

FTIR Analysis: The conformation characterization of pure and blend films, as well as the study of specific interactions between CM-chitin and silk fibroin were carried out. The FTIR spectrum of silk fibroin [Fig.1(a)] showed the characteristic absorption bands at 1654 cm⁻¹ (amide I), 1542 cm⁻¹ (amide II), 1243 cm⁻¹ (amide III), and 669 cm⁻¹ (amide V), assigned to random coil form. The FTIR spectrum of CM-chitin [Fig.1(b)] showed the characteristic absorption bands at 1647 cm⁻¹ (amide I), 1559 cm⁻¹ (amide II). The spectrum of the blend film with 50% silk [Fig.1(c)] showed amide I band and amide II band at 1651 cm⁻¹ and 1558 cm⁻¹, respectively. The peak shift between amide I and amide II of silk fibroin and CM-chitin was observed. This might suggest that there was some intermolecular interactions between silk fibroin and CM-chitin.

X-ray Diffraction Patterns: Wide-angle X-ray diffraction (WAXD) patterns of the films are shown in Fig.2. CM-chitin exhibited crystalline peak at $2\theta = 9.4^\circ$ and $2\theta = 19.3^\circ$ [Fig.2 (a)]. The silk fibroin films showed a non-crystalline structure. According to Freddi *et al.* [8], the dissolution of silk fibroin was caused by reagent penetrating into the adjacent chains and breaking hydrogen bonds between polymer chains. This led to the decrease in crystallinity of silk fibroin films as compared to the original silk fibroin fiber. The pattern of the blend films exhibited a gradual transformation from characteristic crystalline peaks of CM-chitin to the completely amorphous pattern of silk fibroin with the increasing of silk fibroin content in the blend films. Chen *et al.* [22] studied the crystallinity of pure silk fibroin membrane and found that the diffraction pattern of pure silk fibroin membrane showed no clear 2θ peak. Our result showed that the diffraction patterns of pure and blend films did not give clear information about the crystallinity because the crystalline structure of CM-chitin and silk fibroin were remarkably frustrated during dissolution process. Therefore, the CM-chitin/silk fibroin blend films were mainly amorphous.

Thermal Property: Thermal property of pure and blend films were characterized by differential scanning calorimeter (DSC). The DSC thermograms of pure and blend films are shown in Fig.3. The pure silk fibroin film showed endothermic peak at 284.86°C [Fig.3(a)]. According to Tsukada *et al.* [9] who studied thermal property of silk fibroin/PVA blend films, it was demonstrated that silk fibroin with low degree of crystallinity thermally decomposed at around 280-290°C. Therefore, it suggested that the endotherms observed at 284.86°C should be attributed to the thermal decomposition of silk fibroin film. The pure CM-chitin film showed the glass transition temperature at 244.70°C [Fig.3(g)]. According to Sakurai *et al.* [23], the glass transition temperature of chitosan

was reported to be 203°C. The thermograms of blend films are showed in Fig.3(b-f). The DSC thermograms of the blend films with 40% and 50% CM-chitin content [Fig.3(c-d)] showed the broad peak of decomposition endotherm in lower temperature range and observed an upward shift of glass transition temperature of CM-chitin. The 60/40 and 80/20 CM-chitin/silk fibroin blends [Fig.3(e-f)] showed only the shift of glass transition temperature of CM-chitin. While at 20/80 CM-chitin/silk fibroin showed only the shift of decomposition endotherm. It could be suggested that there was some inter-molecular interactions occurred between CM-chitin/silk fibroin films.

Thermal stability: The decomposition temperatures of pure and blend films (Fig.4) were characterized by thermogravimetric analysis (TGA). The TGA curves of pure silk fibroin and CM-chitin films showed the decomposition temperatures at 299.34°C and 265.70°C, respectively. The decomposition temperatures of the blend films were in the range of the decomposition temperatures of pure silk fibroin and pure CM-chitin. The result showed that the decomposition temperatures of the blend films decreased with increasing of CM-chitin content.

Swelling Study

Equilibrium Water Content (EWC)

The effect of immersion time on the water content of the blend films is shown in Fig.5. All samples in water reached an equilibrium state within 3 h. Fig.6 shows EWC of the films as a function of CM-chitin content. The EWC of pure CM-chitin film was approximately 545%. Pure silk fibroin could not be detected because it was very brittle and cracked after water was blotted out from the film surface. The EWC of blend films increased with increasing CM-chitin content. Khor *et al.* [24] suggested that the ability to absorb water of CM-chitin films is attributed to the introduction of carboxymethyl group on the glucose residue. The presence of carboxymethyl groups distributing along chitin chains disrupts the H-bonding interactions between adjacent chitin chains.

Effect of pH

The effect of pH on the degree of swelling of CM-chitin and the blend films is shown in Fig.7. It was found that pure CM-chitin and blend films could swell in both acidic and alkaline pH. Tokura *et al.* [25] reported the pK_a values of the carboxyl group and amino group of CM-chitin are 3.40 and 6.40, respectively. At pH less than 7 (acidic pH solution), the degrees of swelling of CM-chitin and blend films slightly increased, suggesting that the amino groups of CM-chitin molecules ionized leading to the dissociation of the adjacent chains. At pH higher than 7 (alkaline pH solution), the degrees of swelling of CM-chitin and blend films increased, suggesting that the carboxymethyl groups ionized leading to the dissociation of the adjacent chains. The effect of glutaraldehyde concentration on the swelling property of the blend film at 50/50 blend ratio as a function of pH is shown in Fig.8. The degree of swelling decreased with increasing of glutaraldehyde concentration in the films. When the concentrations of cross-linking agent increased more hydroxyl groups in CM-chitin was consumed due to the cross-linking reaction. The response of the blend films with 50% CM-chitin content to a step change in pH is shown in Fig.9. In this experiment, the films were brought into a buffer solution at pH 6 and then transferred to a buffer solution at pH 10 so that an abrupt swelling was ensured. Later, the films were placed back into a buffer solution at pH 6. The result showed that swelling behavior of the blend films was reversible when the environmental pH changed. The blend films showed a pH-sensitive swelling characteristic that may be applicable to a controlled-release system.

Effect of salt type

The degree of swelling of CM-chitin/silk fibroin blend films in various types of salt solutions is shown in Fig.10. The salt solutions used in this study were NaCl, LiCl, CaCl₂

and FeCl₃ solution. The concentration of salt solution was 0.25M. It was found that the blend film with 60% CM-chitin content showed the highest degree of swelling in NaCl, LiCl, and CaCl₂ solution. However, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solution (NaCl and LiCl) and divalent salt solution (CaCl₂). For pure CM-chitin, the degrees of swelling in salt solutions from the highest to the lowest were in the following order: NaCl > LiCl > CaCl₂ > FeCl₃. Tokura *et al.* (1983b) studied the specific binding site of calcium ions on CM-chitin (insoluble water typed, DS=0.28). It was found that CM-chitin could bind with calcium ions even in the presence of monovalent cations such as sodium or potassium. The IR spectrum between the CM-chitin and Ca²⁺-loaded CM-chitin appeared that the tetrahedral chelation of CM-chitin toward Ca²⁺ is assisted by the acetamide and hydroxyl groups in addition to carboxyl groups.

CONCLUSIONS

Silk fibroin could enhance the thermal stability of CM-chitin. When CM-chitin content in the blend films increased, equilibrium water content and degree of swelling of the blend films in pH buffer and salt solution, especially in NaCl and LiCl, increased. The cross-linking was very important for the swelling behavior. The cross-linking agent concentration had influence to the swelling behavior of the blend film. When the concentrations of cross-linking increased, the degrees of swelling of the blend films decreased.

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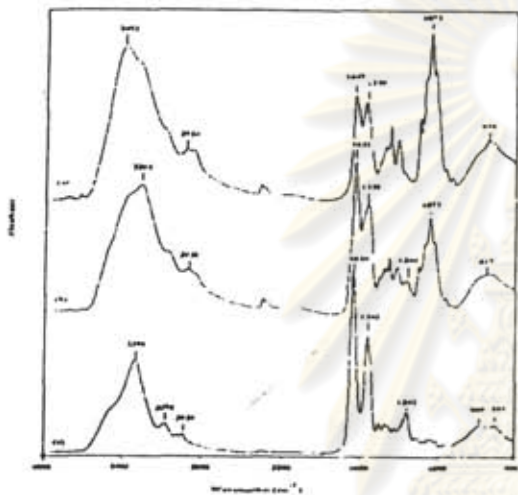


Fig.1. FTIR spectra of pure and blend films of CM-chitin/silk fibroin: (a) 100/0 (CM-chitin); (b) 50/50; and (c) 0/100 (silk fibroin).

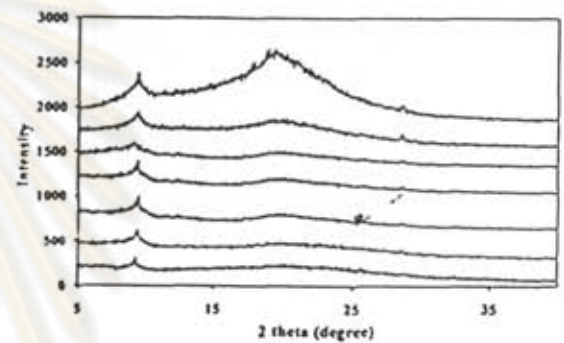


Fig.2. WAXD patterns of CM-chitin/silk fibroin blend films.

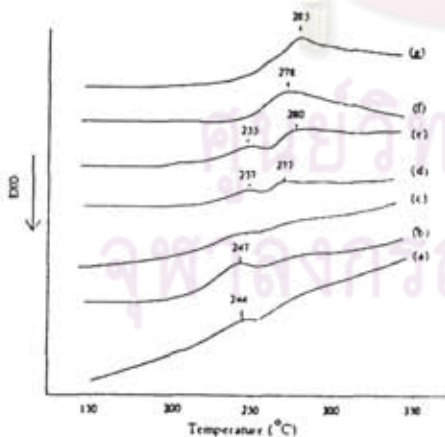


Fig.3. DSC thermograms of CM-chitin/silk fibroin blend films: (a) 100/0 (CM-chitin); (b) 80/20; (c) 60/40; (d) 50/50; (e) 40/60; (f) 20/80; and (g) 0/100 (silk fibroin).

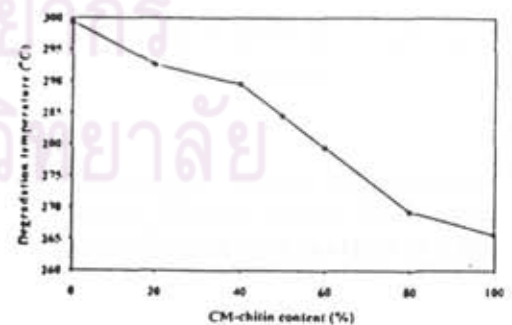


Fig.4. Decomposition temperature of CM-chitin/silk fibroin blend films.

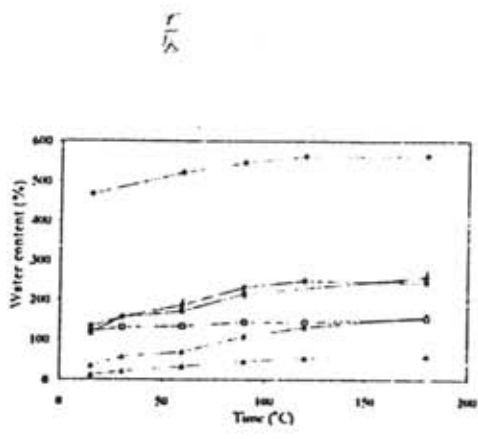


Fig.5. Effect of immersion time on water content of CM-chitin/silk fibroin blend films (containing 0.01% glutaraldehyde): ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; △ 20/80. Decomposition temperature of CM-chitin/silk fibroin blend films.

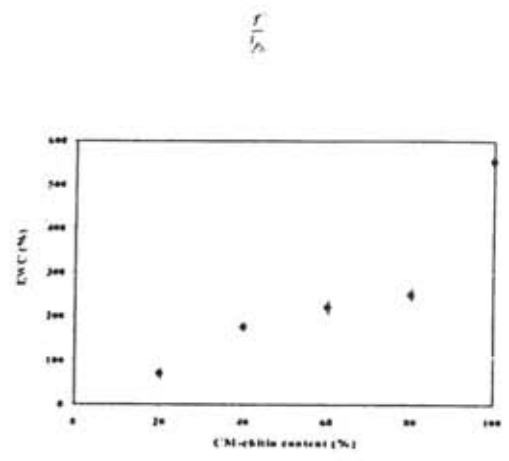


Fig.6. Equilibrium water content of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde.

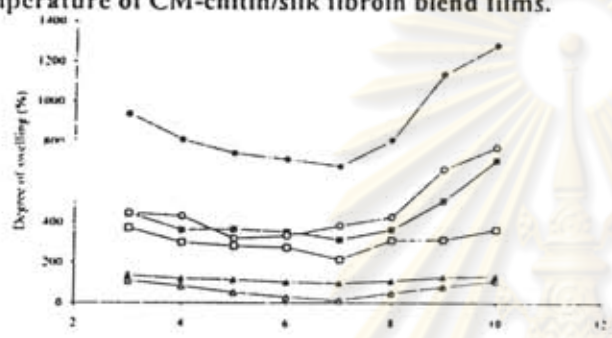


Fig.7. Degree of swelling of CM-chitin/silk fibroin blend films (containing 0.01% glutaraldehyde) as a function of pH: ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; △ 20/80.

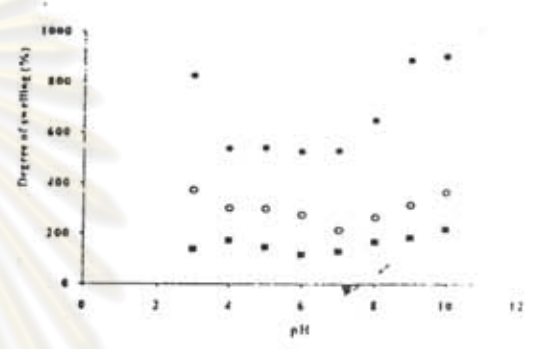


Fig.8. Effect of glutaraldehyde concentration on degree of swelling of CM-chitin/silk fibroin blends films as a function of pH. ● 0.005% glutaraldehyde; ○ 0.01% glutaraldehyde; ■ 0.05% glutaraldehyde.

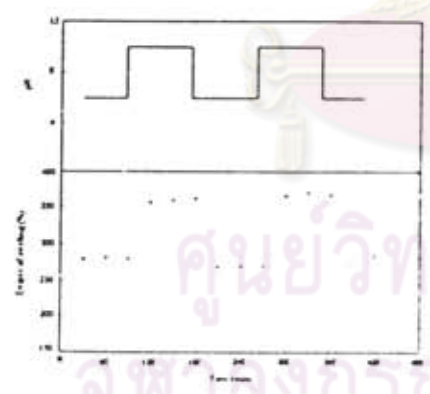


Fig.9. Degree of swelling of CM-chitin/silk fibroin blend films with 50/50 blend ratio containing 0.01% glutaraldehyde on a step change in pH.

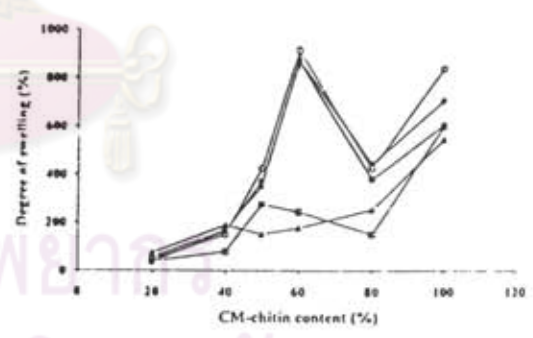


Fig.10. Effect of salt types on degree of swelling of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde as a function of CM-chitin content. ● LiCl; ○ NaCl; ■ CaCl₂; □ FeCl₃; ▲ H₂O.

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Sustained Release of Drug from Chitosan and Silk Fibroin Blend Films

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Chitosan/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. Drug release properties of chitosan and blend films of various blend compositions were investigated *in vitro* using a modified Franz Diffusion Cell at 37°C and pH 5.5. Pig skin was used as material representing human skin. Theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate were used as model drugs. The order of drugs from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. For all model drugs, the blend films with 80% chitosan gave the higher drug release. In addition, an increase in thickness of the films resulted in a decrease in amount of drug released. All model drug release data were fitted to zero order or Higuchi's model indicating that the releases of model drug from chitosan and blend films were either rate-controlling or diffusion-controlled releases. It was expected that the chitosan/silk fibroin blend films could be used as the matrix for sustained release of a drug for a transdermal drug delivery system.

Key words: Chitosan; Silk Fibroin; Blend Films; Sustained Release; Drug Delivery System
(Oral presentation; Application section)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Drug Release Characteristics of CM-Chitin/Silk Fibroin Blend Films

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CM-chitin/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. The effects of pH and blend composition on swelling behavior of the blend films were investigated. CM-chitin and the blend films exhibited the minimum degree of swelling at pH 4 and showed pH-sensitive character for every blend composition studied. The degree of swelling of the blend films increased as the CM-chitin content increased. Drug release characteristics of CM-chitin and the blend films at 37 °C and simulated physiological pHs, i.e. pH 2.0, 5.5 and 7.2, were investigated using theophylline, diclofenac sodium, amoxicillin and salicylic acid as model drugs. It was found that the releases of all model drugs from CM-chitin and the blend films at pH 7.2 were higher than at pH 2.0 and pH 5.5. The amounts of model drugs released from the films from the highest to the lowest were in the order: salicylic acid > theophylline > diclofenac sodium > amoxicillin. The drug releasing property of CM-chitin/silk fibroin blend films was compared to that of CM-chitin/PVA blend films using salicylic acid as model drug. Both of them showed similar drug release characteristic. However, the percentages of salicylic acid released from CM-chitin/silk fibroin blend films were a little bit lower than those obtained from CM-chitin/PVA blend films.

Key words: CM-Chitin; Silk Fibroin; Blend Films; Drug Release
(Poster presentation; Application section)

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