FULL REPORT

Preparation of Biodegradable Blend Films from Cellulose Derivatives and Silk-protein

การเตรียมแผ่นฟิล์มผสมที่ย่อยสลายได้ตามธรรมชาติจากอนุพันธ์ของเซลลูโลสและโปรตีนไหม

By

Dr. Patchara Punyamoonwongsa
Dr. Suwanna Deachathai
School of Science
Mae Fah Luang University

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Mae Fah Luang University
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Dr. Patchara Punyamoonwongsa
Dr. Suwanna Deachathai
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Executive Summary

Membranes made of cellulose acetate butyrate (CAB) have been widely used in both biotechnology and pharmaceutical fields to purity and separate macromolecules. They are considered as one of the good membranes due to their good resistance to chemicals, ease of fabrication, good mechanical strength and low-protein binding. Despite these advantages, the uses of these membranes are still limited by their non-uniform morphology, non-biodegradability and lack of specific binding site. In order to improve their performance, CAB was blended with a natural polymer, silk fibroin (SF), derived from the domesticated *Bombyx mori* cocoons. The blend films were prepared by mixing various mass percentages of SF to CAB in a formic/acetone mixture solvent, prior casting onto a glass mold. The structural and physical properties of the blend films were investigated by using UV-Vis spectrophotometry, Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC), Fourier-transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Tensile testing and X-ray diffraction. Results indicated that SF is partially compatible with CAB and the protein could also acts as a plasticizer in the cellulose matrix. These are possibly due to the preferential interactions of the two components in the system. Surprisingly, the regenerated SF solution exhibits antibacterial activity against *S. marcescens, M. luteus, E. Coli* and *E. aerogenes*, thus highlighting its potential use as antimicrobial peptides to combat against infectious diseases for a wider range of applications. Blending the silk protein with CAB was proven to be able to create new hybrid materials with a wide range of property. This is also expected to impart biodegradability together with, biological activity to allow further chemical modification and specific site binding for membrane and other high performance applications.

Abstract

In this study, a new source of esterified cellulose membranes was developed by blending cellulose acetate butyrate (CAB) with the *Bombyx mori* silk fibroin (SF) using a solvent casting technique. The blend solutions at various mass percentages of SF were initially prepared in a formic acid/acetone mixture solvent, prior to casting onto a glass petri-dish. Different parameters were optimized to achieve homogeneous blend films with good physicochemical properties. Results indicated that SF/CAB films with good properties were obtained when casting in a mixture solvent containing 15-25% formic acid contents, using high molecular weight CAB (e.g., 50,000 g/mol), lower SF contents (e.g., 1-2% w/w) and higher casting temperature of 50°C. The presence of up to 2% (w/w) SF in the blends does not significantly affect the film transparency. However, SF lowers thermal and mechanical properties of the neat CAB film, particularly in the high molecular weight CAB systems. Data analysis of UV-Vis Spectrophotometry, X-ray diffraction (XRD), Fourier-transformed Infrared (FTIR) spectroscopy, Thermal analysis, Tensile testing and Scanning Electron Microscopy (SEM) proved that SF is partially compatible with CAB and the protein could also acts as a plasticizer in the cellulose matrix. These are possibly due to the preferential interactions of the two components in both amorphous and crystalline microdomains, possibly through both hydrogen bonding and hydrophobic association. Interestingly, an aqueous SF solution displayed antibacterial activity against four pathogens, including *S. marcescens, M. luteus, E. Coli* and *E. aerogenes*, as evidenced by the presence of zone of inhibition. This makes a new SF/CAB blend as one of promising candidates for a development of intelligent and biodegradable cellulose-based membranes with reasonable price over the existing source of materials.

บทคัดย่อ

การศึกษานี้ ได้พัฒนาเยื่อเมมเบรนเซลลูโลสเอสเทอร์ชนิดใหม่จากการผสมเซลลูโลส อะซิเตท บิวทิเรต (CAB) เข้ากับไฟโบรอิน (SF) จากไหมไทยสายพันธุ์ *Bombyx mori* โดยใช้เทคนิคหล่อขึ้นรูปแบบใช้ตัวทำละลาย ทั้งนี้ ได้ทำการเตรียมสารละลายผสมที่มีร้อยละโดยมวลของ SF แตกต่างกันในตัวทำละลายผสมของ กรดฟอร์มิก/อะซิโทน จากนั้นจึงทำการหล่อขึ้นรูปโดยการเทลงบนจานเพาะเชื้อแก้ว ตัวแปรต่าง ๆ ได้ถูกนำมาปรับเปลี่ยนเพื่อหาสภาวะที่เหมาะสม ที่ทำให้ได้แผ่นฟิล์มผสมเนื้อเดียวกัน และมีสมบัติเคมีเชิงกายภาพที่ดี ผลการทดลองพบว่าฟิล์ม SF/CAB ที่มีคุณสมบัติดี สามารถเตรียมได้จากการหล่อขึ้นรูปในตัวทำละลายผสมที่มีร้อยละโดยปริมาตรของกรดฟอร์มิก คือ 15-25% โดยการใช้ CAB ที่มีมวลโมเลกุลสูง (เช่น 50,000 กรัม/โมล) ใช้สัดส่วนของ SF ไม่สูงมากนัก (เช่น ร้อยละ 1-2 โดยมวล) และขึ้นรูปที่อุณหภูมิสูง 50 องศาเซลเซียส การมี SF ผสมในปริมาณที่น้อยกว่าร้อยละ 2 โดยมวล ไม่มีผลกระทบต่อค่าความใสของฟิล์ม แต่อย่างไรก็ดี SF ไปลดเสถียรภาพทางความร้อน และคุณสมบัติเชิงกลของแผ่นฟิล์มบริสุทธิ์ CAB โดยเฉพาะอย่างยิ่งในระบบที่ใช้ CAB ที่มีมวลโมเลกุลสูง การวิเคราะห์โดย UV-Vis Spectrophotometry, X-ray diffraction (XRD), Fourier-transformed Infrared (FTIR) spectroscopy, Thermal analysis, Tensile testing และ Scanning Electron Microscopy (SEM) พิสูจน์ได้ว่า SF สามารถเข้ากันได้บางส่วนกับ CAB อีกทั้งยังสามารถเป็นสารตัวเติมในตัวกลางเซลลูโลสได้อีกด้วย ซึ่งอาจเนื่องมาจากอันตรกิริยาระหว่างโมเลกุลของทั้งสององค์ประกอบ ทั้งในบริเวณอสัณฐานและบริเวณที่เป็นผลึก ซึ่งอาจเกิดได้ทั้งแบบพันธะไฮโดรเจนและแรงยึดเหนี่ยวที่ไม่ชอบน้ำ สิ่งที่น่าสนใจอีกประการหนึ่งของสารละลาย SF คือความสามารถในยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียที่ทำให้ก่อโรค 4 ชนิด ได้แก่ *S. marcescens, M. luteus, E. Coli* และ *E. aerogenes* ซึ่งถูกพิสูจน์ได้จากการเกิดบริเวณของการยับยั้ง คุณสมบัติดังกล่าวนี้ ทำให้ของผสม SF/CAB เป็นวัสดุที่มีความเป็นไปได้อย่างยิ่ง สำหรับการนำมาใช้เพื่อพัฒนาเมมเบรนเซลลูโลสที่สามารถย่อยสลายได้ตามธรรมชาติ มีความชาญฉลาด และมีราคาสมเหตุสมผลมากว่าแหล่งวัสดุที่มีอยู่แล้วในปัจจุบัน

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ABBREVIATIONS AND SYMBOLS

CE = Cellulose ester
CA = Cellulose acetate
CAB = Cellulose acetate butyrate
LCAB = Low molecular weight CAB
HCAB = High molecular weight CAB
SF = Silk fibroin
RSF = Regenerated silk fibroin
SFCAB = Blend of SF and CAB
FTIR = Fourier-transform infrared
UV-Vis = Ultraviolet-visible
DSC = Differential scanning calorimetry
TGA = Thermogravimetric analysis
SEM = Scanning electron microscopy
% = Percentage
% T = Percentage of transmittance
% v/v = Percentage of volume by volume
% w/v = Percentage of weight by volume
% w/w = Percentage of weight by weight
^{o}C = Degree Celsius
min = Minute
hr = Hour
mg = Milligram
mm = Millimeter
nm = Nanometer
mL = Milliliter
cm^{-1} = Wavenumber
$T_{d,max}$ = Degradation temperature for a maximum mass loss rate
T_m = Crystalline melting temperature
MPa = Mega Pascal
S.D = Standard deviation

CHAPTER 1
INTRODUCTION

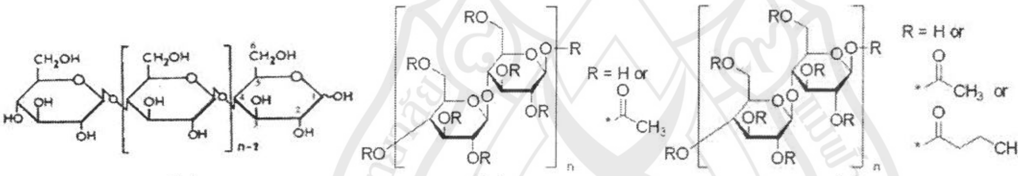
1.1 Background

Bio-macromolecules from various renewable biomass resources, such as polysaccharides and proteins have been considered as alternatives for petroleum-based polymers. **Cellulose** (Fig.1.1a), the most abundant and important bio-macromolecules in nature, have long been used in many applications due to its excellent properties such as inexpensive, high mechanical strength but light-weight, low density, optical transparency, ability to functionalize and biodegradability. Despite these advantages, the applicability of cellulose is still limited due to its poor solubility. Generally, cellulose is insoluble and infusible in water and in most of organic solvents. This is because of its strong inter- and intramolecular hydrogen bonds [1]. Chemical modification of cellulose, for example by etherification or esterification, is one of many ways to improve its performance. Numerous cellulose derivatives, obtained by different chemical reactions, are commercialized and used in many applications ranging from food packaging to parts of automobiles.

Cellulose esters (CE), such as cellulose acetate (**CA**) and cellulose acetate butyrate (**CAB**), chemical structures shown in Fig.1.1b-c, are the esterified derivatives of cellulose which have better mechanical strength, water absorbability and processability than cellulose. They widely used as fibers, plastics, adhesives and membranes in many applications [2]. Membranes made from CE have been used in both biotechnology and pharmaceutical fields to purify and separate macromolecules. They are also used for seawater desalination, waste water treatments and for filtering of suspended solids, methanol, ethanol and urea in reverse osmosis process [3]. The practical uses of CE membrane both in biotechnology and pharmaceutical fields are strongly associated with its morphology [4], permeability, surface functionality, physicochemical properties, stability and thermal degradation. Thermal degradation of CA usually yields toxic products that are harmful to human. This can be reduced by the addition of flame retardants or additives to improve its thermal stability [2]. Membrane separation efficacy of the CE was found to be dependent on the structure, size and density of the pores formed. These parameters are tunable and primarily determined by the casting and annealing conditions used to form the membranes [3]. *Although* the parameters can be experimentally optimized, but the morphological details of the resultant membranes, under the same processing conditions, are still varied from sample to sample. *Additionally*, typical CE substrates are rigid and provide only non-specific binding sites due to the unreactive functional groups on the polymer chain. This limits the applicability uses of CE membranes for other high performance applications where specific binding sites are essentially needed. *Furthermore*, CE membranes are known to be non-biodegradable under natural environments. **Thus**, there is a need to develop the new method to fabricate CE membrane with controllable mechanical properties, tunable porosity, great ability to undergo chemical modification and acceptable biodegradability.

Blending of CE with other polymers is one of many good ways to improve its performance. Generally, blending is convenient, economic and is possible to produce a range of materials with properties completely different from those of the blend constituents. The final properties of the blend depend on the properties of its polymeric components, composition and mainly, miscibility of the polymers. Among all available sources of materials, proteins, a macromolecule derived from renewable resources, have received much interest due to their abundance, renewability, low cost and good biodegradability. Their tremendous potential to blend with other macromolecules as well as to substitute for non-biodegradable plastics and high performance materials has previously been published in different systems by many researchers [1, 5-7].

In this project, cellulose acetate butyrate (CAB), one of the most commonly used CE, was physically blended with the *Bombyx mori* silk-fibroin **(SF)** using a *solvent casting* technique. A fibrous protein, SF, is an interesting material due to its controllable biodegradability and mechanical strength as well as its large scale-production. Moreover, proteins have long been reported as a naturally occurring plasticizer for some thermoplastic polymers. It is, thus, believed that this SF/CAB blend system may be an ideal candidate, but with reasonable price, for a wide range of applications including as the filtration membranes.



(a) (b) (c)

Fig.1.1 Chemical structures of; (a) cellulose, (b) cellulose acetate (CA) and (c) cellulose acetate butyrate (CAB).

1.2 Objectives

The following are objectives of this research.

- To prepare SF/CAB blend films by using the solvent film casting technique.
- To optimize the casting conditions by varying a number of variables.
- To characterize the blended films through the uses of different analytical techniques.

1.3 Scopes of Research

In this project, a thin film comprising of CAB and SF polymer was prepared by using a solvent film casting technique. To achieve the optimal casting conditions, a number of parameters such as the solvent casting system, the polymer blend composition and the drying condition, were studied using different analytical techniques. Various properties of the blended films including physical, chemical and thermal properties were also investigated in this study as a mean to explore the structural integrity, the polymer compatibility as well as to evaluate the blend for its potential uses as thin films or any other biodegradable materials.

This project has **three** phase plans to achieve its objectives. They are summarized as shown below.

Phase 1: Optimizing the casting conditions. The strategic plans are;

1.1 Specify the casting solvent system used.
1.2 Vary the total mass content of the blend in the solvent chosen.

Phase 2: Study the relevant factors. The strategic plans are;

2.1 Study the effects of SF content on the homogeneity of the blend films.
2.2 Study the effects of drying conditions on the optical transparency and homogeneity of the blend films.
2.3 Identify the optimal casting conditions for SF/CAB blend system.

Phase 3: Characterization of the SF/CAB blend films. The strategic plans are;

3.1 Explore the structural integrity of the blend films through the use of the Fourier Transform Infrared Spectroscopy (FTIR).
3.2 Investigate the polymer compatibility of the blend films by using the Differential Scanning Calorimetry (DSC) and the Thermogravimetric analysis (TGA).
3.3 Investigate the morphological details through the use of the Scanning Electron Microscopy (SEM).
3.4 Determine the mechanical strength of the blend films using the Universal tensile testing machine.

CHAPTER 2
LITERATURE REVIEW

Cellulose is the most abundant biodegradable plastic on earth with very high crystallinity. Mainly, it comes from wood and stalks of sugar cane bagasse. The main uses of cellulose are for paper, membranes, dietary fibers, explosives and textiles. Cellulose is generally insoluble and infusible in all organic solvent due to the strong hydrogen bond. Due to its low processibility, cellulose derivatives are widely used instead of pure cellulose. Chemical reactions such as etherification and esterifiation functionalize the free hydroxyl groups to improve its thermoplastic behavior. Numerous derivatives are commercialized such as cellulose acetate, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl cellulose, hydroxyl alkyl cellulose, carboxy methyl cellulose, fatty acid esters of cellulose. Owing to their rigidity, moisture vapor permeability, grease resistance, clarity, and appearance, cellulose esters (CE) are widespread used. Cellulose acetate (CA) is the most common used cellulose derivative that exhibit similar tensile strength to polystyrene, but it still has a problem with the thermal processing due to its high glass transition temperature [8]. Cellulose acetate butyrate (CAB) is the esterified cellulose which has better strength and low water absorbability than CA. CAB is a thermoplastic with gloss surface and good weatherability. They are applied widely ranging from food packaging to parts of automobiles [9]. Cellulose and its derivative are mineralized by micro-organisms due to the activity of the cellulose enzyme complex, which results in the formation of cellobiose and glucose, leading to mineralization in cellulose biochemical cycles. Several enzymes act synergistically in the breakdown of cellulose in a series of hydrolysis reactions. Those enzymes which can biodegrade via oxidative pathways have already been reported.

Membranes made from CE are widely used in many applications, including ultrafiltration for protein purification and concentration, waste water treatment as well as the biosensor. The most common commercial CE-based membranes are made of cellulose acetate (CA), cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB). They are considered as one of the good membrane materials because of their good resistance to fouling, chlorine tolerance, chemical stability and low-protein binding [10]. Despite these benefits, the uses of CE membrane are still limited by its non-uniform pore structure. No matter which method is employed, there will always be a range of pore sizes which deviation from the mean value [11]. It is then suggested to use a membrane whose stated molecular weight cut-off point is lower than the molecular weight of the protein interested.

Blending of different polymers is an attractive, effective and economic way of imparting new properties to a product. Natural based biodegradable polymer such as the fibroin protein, derived from *Bombyx mori* cocoons, were previously blended with cellulose-based materials [12, 13]. The structural and physical properties of the cellulose/SF blend films were investigated by using Thermogravimetric Analysis (TGA), Differential Scanning Calorimeter (DSC), Fourier Transform Infrared Spectroscopy (FTIR), Wide Angle X-ray Scattering (WAXD), Scanning Electron Microscope (SEM) and Dynamic Mechanical Thermal Analysis (DMTA).

The polymer blends, prepared by dissolving in a metal complex solution, coagulation and finally washing, were transparent in any proportion. The resultant membranes displayed a narrower pore distribution as proven by the SEM. The strong hydrogen bonds between the two polymers, as evidenced by the FTIR, SEM and DMTA, indicated a **miscible** SF/cellulose blend system. Interpretation of WAXD and FTIR spectra proved the crystalline arrangements of both SF (β-form) and cellulose (Cellulose II) in the blend films. Moisture regain of the blend films increased as a function of cellulose content. Interestingly, the mechanical strength of the blend films increased from 1.5 kg/mm^2 (of silk fibroin film) to 4 kg/mm^2 (of cellulose film). Moreover, the elongation at break of SF films significantly improved by blending with cellulose. The improved mechanical properties, once again, suggested strong intermolecular hydrogen bond and so, good compatibility between these two components.

Blending SF with cellulose or its derivatives is expected, not only to improve the performance of CE, but also to **introduce amino acid groups** into cellulosic materials, thus, allowing further functionalization. Furthermore, SF is sensitive to heat, mechanical forces, and various solvents. The change of structure and physico-chemical properties of SF can then be induced by stretching, compression, annealing and various treatments. This may allow tailoring of properties to meet specification for the manufacture of other high performance materials. Silk fibroin, a fibrous protein polymer that produced from silkworms, possesses excellent properties such as remarkable mechanical strength, environmental stability, biocompatibility, controllable biodegradability and morphological flexibility. These highlight it an ideal material for a wide range of applications [14]. In addition, protein has long been reported to act as a plasticizer in some thermoplastic polymers. It can interpose itself between the polymer chains and decrease the forces holding the chain together. In another word, it takes a role as a lubricant to facilitate the movements of the polymer over each other, disrupting the interactions between the chains (i.e., hydrogen bond, Van de Waals and ionic forces) and depressing the glass transition temperature by the increased polymer free volume [15]. This superior character makes it possible to improve flexibility and other thermoplastic behavior of the CE [16].

The aim of this study is to explore the potential of SF/CAB blend systems as a new feedstock for the manufacture of the high performance cellulose-based membranes. The most commonly used process, a solution casting, was chosen to prepare the SF/CAB blend films. A number of parameters such as the solvent casting system, the polymer blend composition and the drying condition, were studied to achieve the optimal casting conditions. To explore the structural integrity as well as the polymer compatibility, various properties of the blend films including physical, chemical and thermal properties were investigated through the combination uses of different analytical techniques. The casted films were finally evaluated for their practical uses based on their physicochemical properties.

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

Bombyx mori silk cocoons were received as a gift from the Silk Innovation Center, Mahasarakham University, Thailand. Cellulose acetate butyrate (CAB) was purchased from Sigma-aldrich. All other chemicals were of analytical grade and used without further purification.

3.2 Methods

3.2.1 Preparation of the Regenerated Silk Fibroin

Silk cocoons were cut into pieces, washed and dried. Completely dried silk cocoons were boiled twice in an aqueous solution of 0.5% (w/v) of sodium carbonate. The remaining silk fibroin residue was rinsed thoroughly, several times, with hot distilled water. The degummed silk fibroin was dissolved in a ternary solvent of calcium chloride/ethanol/water for 3 hours. The resultant solution was dialyzed at room temperature for 3 days against distilled water using a cellulose dialysis tube. The obtained solution was finally freeze dried at -40°C for 20 hours, yielding a pale yellow SF powder.

3.2.2 Optimizing of the Casting Process

The solution casting method was selected for the fabrication of SF/CAB blend films. Briefly, appropriate amount of the polymer blend was dissolved in the solvent prior to casting onto a mold. After the evaporation of solvent, the blend remains as a solid thin film. This method is the oldest but yet stills a common technique for the production of thin films. Its advantages include the ease of process, low-cost production, formation of a more uniform and transparent film as well as avoiding thermodegradation. Despite these advantages, the selection of solvent can, somehow, limit its applicability. Ideally, the polymer blend should be completely soluble and stable in a volatile solvent, providing a reasonable viscous blend solution. This allows the formation of a uniform casted film.

Many parameters affect the homogeneity and morphology of the films casted by this method. Such parameters are the cast substrate, blend composition and the solvent evaporation rate [17, 18]. To optimize the casting conditions for the SF/CAB film, a number of parameters were studied. These include the type of solvent, the blend composition, the SF content and the drying conditions (evaporation rate). The following are list of important parameters selected for this study. For all experiments, a glass petri-dish with diameter of 5 cm was used as a casting mold.

3.2.2.1 The solvent system

To obtain a homogeneous blend solution and so, a uniform blend film, both CAB and SF should be dissolved and maintain stable in the casting solvent. A range of available volatile solvents, as illustrated in Table 3.1, was chosen for the screening test. The ultimate choice was made based on its capability to dissolve both compartments. The blend solution was then prepared from the solvent chosen, casted onto a mold and final dried in a fume hood at room temperature for at least 3 days.

Table 3.1 A range of volatile solvents for the screening test.

Solvent	Polarity
Acetone	10.4
Chloroform	3.1
Dichloromethane	7.3
Diethyl ether	2.9
N,N-dimethyformamide	13.7
Dimethyl sulfoxide	16.4
Distilled water	16.0
Ethyl acetate	5.3
Formic acid	10.0
Tetrahydrofuran	5.7

3.2.2.2 The blend composition

The composition of the polymer blend influences the formation, structures, miscibility, and mechanical properties of the casted film. It is thus, a necessary step to investigate how the incorporation of SF affects the properties of the obtained films. In this study, the mass to volume ratio of the polymer blend in solvent was kept constant and the SF contents in the blend were varied from 0 to 50% (w/w). Films with different SF contents were prepared using a method previously described in Section 3.2.2.1The optical transparency of the films will also be investigated as a mean to evaluate the macroscopic phase separation in the films.

3.2.2.3 The drying condition

Typically, a system with high solvent evaporation rate forms a solid thin film within a short period of time. This affects the morphology, miscibility, crystallinity and mechanical properties of the films [17, 18]. In this project, the effect of solvent evaporation rate was examined by evaluating the properties of the films casted under different drying conditions. Two types of the blend films were prepared. The first type was the films casted at room temperature, as previously described in Section 3.2.2.1. The second one was those immediately casted in a hot-air dryer maintained at 50°C for at least 3 days (denoted as a heat treatment). It was assumed that the hot-drying process accelerates the solvent evaporation, and enhances a better film formation [19].

3.2.3 Characterization of the SF/CAB Blend Films

To explore both physical and chemical integrity of the SF/CAB blend films as well as to evaluate the film properties, various analytical techniques, will be utilized as shown below.

3.2.3.1 Film transparency

The UV-Vis spectrophotometer (Genesys 20; Thermo) was used to measure the optical transparency of the blend film. The percent transmittance (%T) was measured at a wavelength of 600 nm.

3.2.3.2 Fourier Transform Infrared spectroscopy (FTIR)

All infrared spectra were recorded in the range of 2400-4000 cm^{-1} using a Spectrum GX FTIR spectrometer (PerkinElmer). Each spectrum was acquired by accumulation of 32 scans with a resolution of 4 cm^{-1}. Background measurements were subtracted from the sample readings.

3.2.3.3 Thermal Properties

A DSC822E Differential Scanning Calorimeter (DSC) (Mettler Toledo) and a TGA/SDTA 851E thermogravimetric analyzer (Mettler Toledo) were used to measure the temperature transitions of CAB, SF and their blends. All measurements were performed under nitrogen atmosphere with a scanning rate of 10°C/min. Thermogravimetric Analysis (TGA) was carried out in the range from 25°C to 600°C. For differential scanning calorimetry (DSC) analysis, samples (3-5 mg) were sealed in aluminum crucibles and heated from 25°C to 220°C. The upper temperature limit for the DSC was selected based on the initial decomposition temperature as observed from the TGA.

3.2.3.4 Scanning Electron Microscopy (SEM)

Surface morphology and interior structure of the films was investigated by using a LEO 1450VP microscope. SEM images were acquired after gold sputtering at acceleration voltage of 15 kV and at 16 mm working distance.

3.2.3.5 Mechanical properties

The tensile test for the blend films was performed at room temperature on the tensile testing machine (INSTRON-5566). The method used was adapted from the ASTM D638-03. The crosshead speed was 10 mm/min and the initial gauge length was 30 mm.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of the Regenerated SF

Regenerated silk fibroin (RSF) was extracted from *Bombyx mori* silk cocoon and was further freeze-dried into powder to promote a longer shelf-life. A dried powder obtained is pale-yellow in color (Fig.4.1a) and it is well-dissolved in a formic acid/water-mixed solvent, producing a yellowish solution shown in Fig.4.1b. Furthermore, RSF powder shows good water absorbability during the air-exposure. These indicate a more favorable of SF to exist in the more hydrophilic conformations. The extraction yields of the freeze-dried protein are shown in Table 4.1. Results indicate a fairy high yield with good reproducibility. The average SF recovery is around 52.50%.

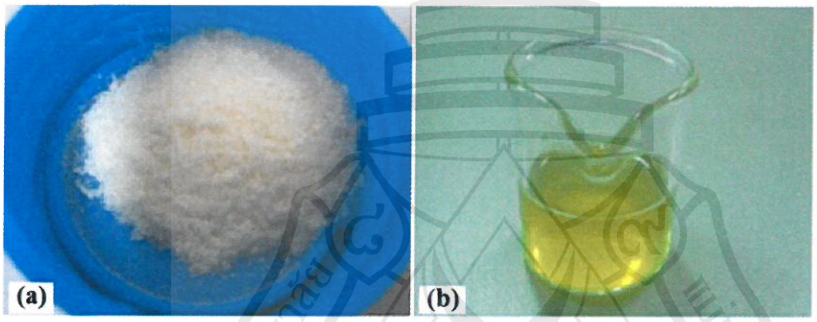


Fig.4.1 Pictures of; (a) RSF and (b) formic acid aqueous solution of RSF.

Table 4.1 Extraction yield of silk fibroin (SF) from the *Bombyx mori* silk cocoons

Batch No.	Weight of cocoon (g)	Yield of SF (g)	% Yield
1	2.0023	1.2632	63.09
2	1.0093	0.5031	49.85
3	2.0158	1.0119	50.20
4	2.0241	0.9379	46.34
5	3.1112	1.6494	53.01
		Average	52.50

4.2 Screening of Solvent System

The casting solvent is prominent parameter for solvent film casting as it influences polymer miscibility, morphology, structure, permeability, surface properties, mechanical properties and separation of blends films. Moreover, nature of casting solvent can dominate the polarity and volatility of blend films. In this study, a screening of casting solvent for the fabrication of SF/CAB blend film was initially performed. After that, a range of solvent composition was varied to optimize the casting conditions for the blended film.

In order to obtain a homogeneous blended film, both CAB and SF should be ideally soluble and stable in the casting solvent. Table 4.2 shows solubility of CAB and SF in common casting solvents.

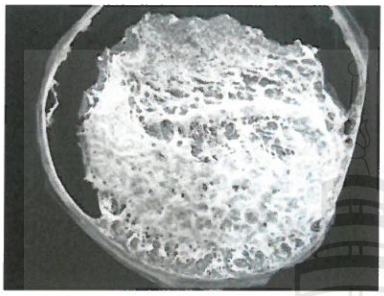
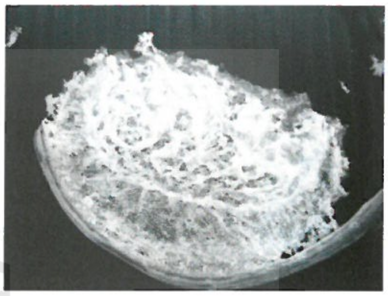
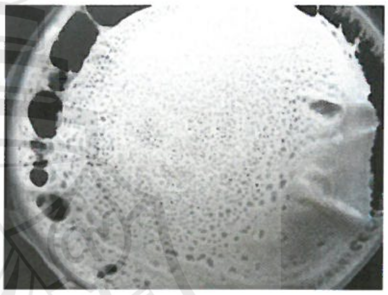
Table 4.2 Solubility of RSF and CAB in different casting solvents

Casting solvent	**Solubility of CAB**	**Solubility of SF**
Acetone	Soluble	Insoluble
Chloroform	Soluble	Insoluble
Dichloromethane (DCM)	Soluble	Partially soluble
Diethyl Ether	Insoluble	Slowly soluble
N,N-dimethylformamide (DMF)	Soluble	Insoluble
Dimethyl Sulfoxide (DMSO)	Insoluble	Soluble
Distilled Water	Insoluble	Soluble
Ethyl Acetate	Soluble	Insoluble
Formic Acid	Insoluble	Soluble
Tetrahydrofuran (THF)	Insoluble	Soluble

It is found that CAB dissolves in acetone, chloroform, DMF, DCM, and ethyl acetate only, while SF dissolves in diethyl ether, DMSO, distilled water, formic acid, and THF (see Table 4.2). Although DCM could dissolve both CAB and SF, but after a while, there was a phase separation. Furthermore, CAB cannot be dissolved in formic acid and SF cannot be either in acetone. Interestingly, a mixed solvent of formic acid and acetone shows ability to dissolve both of them. ***Therefore***, a formic acid/acetone solvent system was selected to fabricate CAB/SF blended films.

The next question that arises is the optimal volume ratio of formic acid to acetone. Additional experiment was conducted to clarify this matter and some of the results were shown in Table 4.3.

Table 4.3 Visual observation of the blend films (10% SF content) obtained from different casting conditions.

Total mass content (% w/v)	Formic acid/acetone ratio (% v/v)	
	15	40
5		
10		

It was found that the increased formic acid content decreases the rate of film formation (or another word, reduced evaporation rate). A comparison of photographs shown in Table 4.3 suggests that this effect (e.g. at 40% formic acid content) induces the morphological change of the blend film into porous structure. A formation of numerous pores usually weakens the materials' mechanical properties. Thus, there should be a certain limit for the acid content in the blend solution. Another interesting point is that, the increased mass content not only increases the film forming ability, but also enhances the mechanical strength of the films. This is obviously seen in Table 4.3 and Fig.4.2 (from top to bottom).

Fig.4.2 The SF/CAB films casted at room temperature. All films were casted in a 2 mL of a mixture solvent containing 15% (v/v) formic acid.

Although the increased mass content could enhance the structural integrity of the resultant films, however, it could also lead to a side-effect concerning a high viscosity. Under this undesired condition, the air-bubbles would be trapped during solidification, resulting in the formation of the pore-like structures within the blend film and so the weakening of the film properties. Results from the solvent screening test suggested that homogeneous SF/CAB films with good mechanical properties could be obtained when the following parameters are optimized.

1. **A volume ratio** (% v/v) of formic acid to acetone. A lower formic acid/acetone ratio (up to 25%) is recommended to avoid a phase separation.
2. A **total mass content** (% w/v). A range of 5-10% is recommended to enhance the film forming ability and to avoid the problem concerning too high viscosity.

4.3 Effects of the Blend Composition

In order to investigate how the increased SF content affects the properties of the blend film, the SF content in the blending system was varied between 0% and 40% (w/w). The more interesting point can be seen in the systems with higher SF contents (from left to right in Fig.4.2). It is found that the higher SF content, the more phase separation and the lesser thickness is the film. The photographs with higher magnification are also shown in Fig.4.3 to indicate the morphological change of the films with respect to the increased SF content. Nonetheless, the incorporation of 5-10% (w/w) SF improves the film homogeneity as compared to the pure CAB film. This indicates that when the blend composition is carefully optimized, there might be the preferential intermolecular interactions between the two components within this blend system.

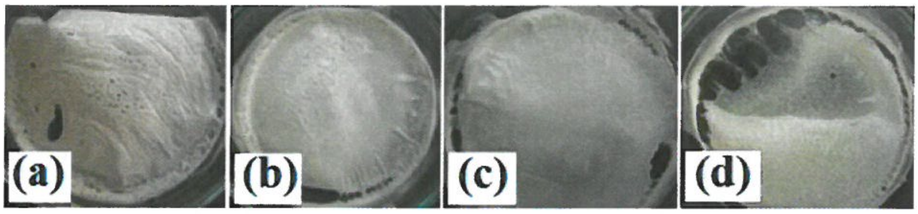


Fig.4.3 The CAB/SF films casted at room temperature containing; (a) 0%, (b) 5%, (c) 10% and (d) 40% SF contents. A total mass content for all films was at 10% (w/v) and the formic acid/acetone volume ratio was at 15% (v/v).

4.4 Effects of the Drying Condition

It is clearly found that the films casted at 50°C were transparent and homogeneous with little or no sign of phase separation (Fig.4.4, Fig.4.5), indicating a better interaction between the CAB and SF. The energy input is likely to speed up the evaporation rate of the solvent thus, prohibiting the polymeric chains to self-aggregate into their own crystalline regions. As a result, the intermolecular interactions between the two components, especially in amorphous domains, would become stronger and a phase separation within the film would then be diminished.

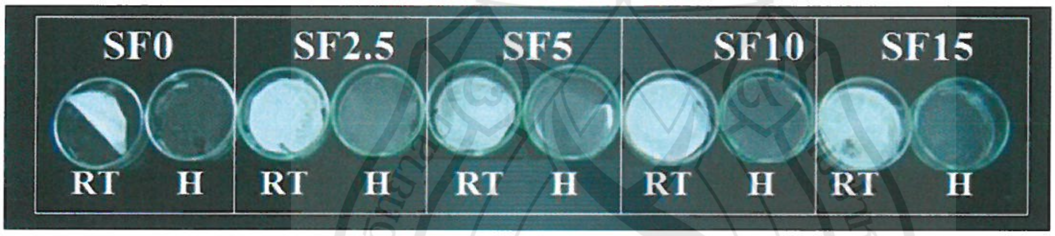


Fig.4.4 The SF/CAB blend films containing 0% (SF0), 2.5% (SF2.5), 5% (SF5), 10% (SF10) and 15% (SF15) (w/w) SF contents. All films (10% total mass content) were casted in a 15:85 (formic acid/acetone) mixture solvent. The drying conditions used were at room temperature (RT) and at elevated temperature (50°C) in the oven (H).

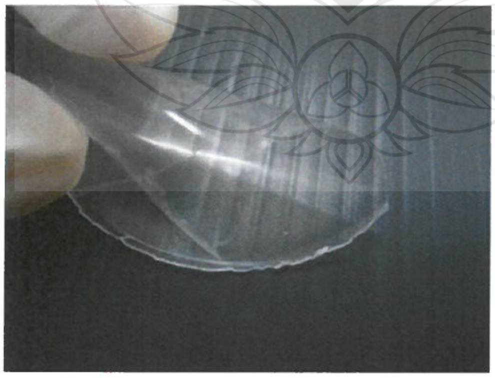


Fig.4.5 The SF/CAB blend films containing 10% SF content casted from a 15:85 (formic acid/acetone) mixture solvent and at 50°C.

The fact that the films with higher SF content become more opaque, thinner and weaker (Fig.4.4, left to right) implies that there might be a certain limit of which SF can be well-incorporated without causing a phase separation. However, more experiments, such as mechanical tests, should certainly be performed in the future to explain this correlation.

4.5 Film Transparency

Polymer compatibility of the blend systems typically depends on the structure, architecture and average molecular weight of their own parental constituents. It is thus, quite interesting to explore further how these affect the phase separation in the casted films. In this pilot study, the effect of CAB molecular weight on the film homogeneity (%T) was examined by casting two sets of the blend films; one from the low-molecular weight (LCAB) and another one from the high-molecular weight CAB (HCAB). Optical transparency (%T) of the resultant blend films at different SF contents (0%, 1% and 2% w/w) made from either LCAB or HCAB are shown in Fig.4.6.

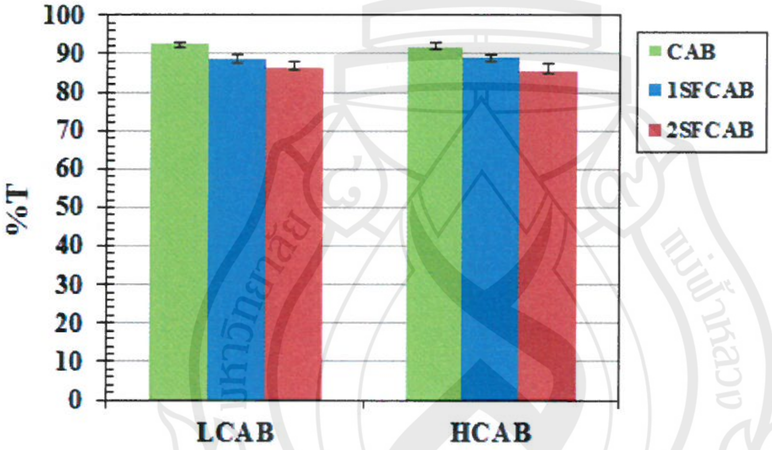


Fig.4.6 Optical transparency (%T) at 600 nm of SF/CAB blend films (5% mass content) containing 0% (CAB), 1% (1SFCAB) and 2% (2SFCAB), casted in a 15% (formic acid) mixture solvent and at 50°C. The films were made from either low- (LCAB) or high-molecular weight CAB (HCAB).

As seen in Fig.4.6, all films display the %T values of more than 85%, higher than that of commercial poly(ethylene) plastics (25-40%). The films based on LCAB and HCAB show similar %T values, indicating that at low SF contents (≤ 2%), the incorporation of the protein is independent of the polymer molecular weight. It is worth mentioning here that the optical transparence is one of the physicochemical properties that reflect only on a macroscopic level. In order to compliment this matter, additional experiments by advanced analytical techniques need to be performed. Nonetheless, the fact that the films from HCAB and LCAB display similar %T values proofs relatively strong preferential interactions between SF and CAB. As expected, there is a limit of which the protein could be incorporated without causing any macroscopic phase separation. This is evidenced by a slightly decreasing in the %T for both LCAB and HCAB-based systems, consistent with the results previously discussed in Fig.4.4.

Up until now, optimized casting conditions to achieve the blend films with good structural integrity, as shown in Fig.4.7, are given below.

1. A lower formic acid/acetone volume ratio (up to 25% v/v).
2. A lower total mass content (between 5-10% w/v).
3. A lower SF content (up to 5% w/w).
4. An elevated drying condition (e.g., 50°C)

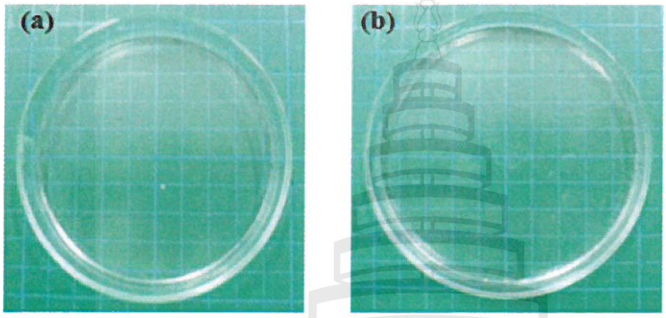


Fig.4.7 Visual observation of; (a) 2SFHCAB and (b) 2SFLCAB films casted at 50°C, in a 20% formic acid solvent mixture and at a total mass content of 5% (w/v).

4.6 Structural Analysis

FTIR spectroscopy was employed to characterize the structural aspect of the casted films. FTIR spectra of RSF, HCAB, LCAB and the blend films were presented in Fig.4.8.

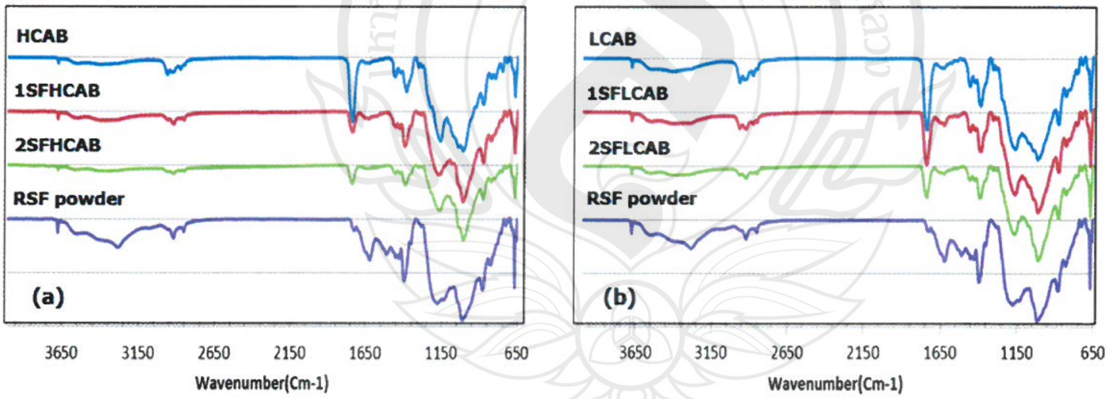


Fig.4.8 FTIR spectra of CAB/SF blend films containing; 0%, 1% and 2% SF contents. The blend films were made from either; (a) HCAB or (b) LCAB. Spectrum of pure SF film was also plot for comparison.

As seen in Fig.4.8, both pure HCAB and LCAB films show a sharp carbonyl (C=O) band at around 1735 cm^{-1} and a broad hydroxyl (–OH) stretching band between and 3050-3650 cm^{-1}, respectively. The regenerated SF (RSF) powder shows the absorption bands at 1637 cm^{-1} (Amide I, C=O stretching), 1549 cm^{-1} (Amide II, N–H deformation) and at 1243 cm^{-1} (Amide III). RSF sample also exhibits a broad peak between 3000 to 3700 cm^{-1}, attributed to the overlapped peaks of N-H and -OH vibrational modes. The spectra of all SF/CAB blend films are found to be no more than an overlapping spectrum of the CAB and SF. This proves the coexistence of both components in the casted films.

4.7 Thermal Properties

Fig.4.9 and Fig.4.10 show the first derivative plots of TGA thermograms of different casted films made by the LCAB (low-molecular weight) and HCAB (high-molecular weight), respectively. Their degradation temperatures ($T_{d,\,max}$) are summarized in Table 4.4.

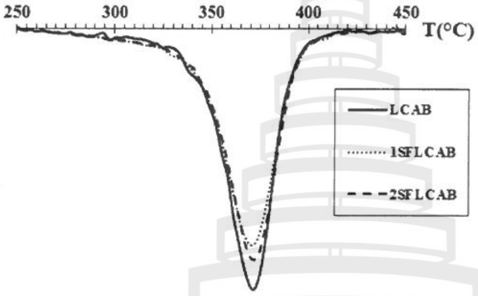


Fig.4.9 First derivative plots of thermogravimetric analysis (TGA) thermograms of the films based on LCAB containing 1% (1SFLCAB) and 2% (2SFLCAB). All films were casted in a formic acid/acetone (15:85) solvent and at a 5% mass content.

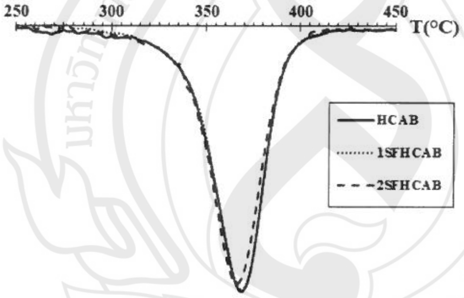


Fig.4.10 First derivative plots of thermogravimetric analysis (TGA) thermograms of the films based on HCAB containing 1% (1SFLCAB) and 2% (2SFLCAB). All films were casted in a formic acid/acetone (15:85) solvent and at a 5% mass content.

As may be noticed in Fig.4.9 and Table 4.4, the $T_{d,\,max}$ of LCAB (0% SF content) is around 372°C, while the 1SFLCAB film shows a value of around 371°C, slightly lower than that of the former one. The result suggests that at this stage, SF can disperse, interact and disrupt the crystallization of the CAB. This is more convinced by the disappearance of the crystalline melting temperature ($T_{m,\,CAB}$) of the 1SFLCAB (1% SF content) shown in Table 4.4. Different behavior is observed for the case of blend film with a 2% SF content (2SFLCAB). In this case, the $T_{d,\,max}$ value is identical to that of the neat LCAB, indicating that under this condition, the protein has little or no effect on the crystalline packing of CAB.

The effect of the increased molecular weight on the crystallization behavior of the CAB was evaluated by comparing the $T_{m, CAB}$ of the LCAB with the HCAB, see Table 4.4. It is found that the HCAB displays higher $T_{m, CAB}$ (150.47°C) value as compared to the LCAB (134.82°C). This is a typical behavior found in most of the polymeric systems and is associated with the fact that the higher polymer chain length offers stronger inter- and intramolecular interactions, leading to a well-packed crystalline domains and so the higher melting temperature.

Table 4.4 Temperature transition of various films casted at 50°C and in a mixture solvent (15% formic acid).

Film	Temperature transition (°C)	
	$T_{m, CAB}$	$T_{d, max}$
LCAB	134.82	371.92
1SFLCAB	-	370.74
2SFLCAB	135.79	371.94
HCAB	150.47	368.89
1SFHCAB	144.48	369.04
2SFHCAB	145.02	366.64

Data interpretation in Table 4.4 suggests that the incorporation of SF in the blends considerably reduces both $T_{m, CAB}$ and $T_{d, max}$ values of HCAB. It is worth noticing that this effect is less pronounced for the case of LCAB-based system, wherein the addition of 2% silk, somehow, could lower the values by no more than one degree. This implies that intersperse of SF may be more favorable in HCAB than in LCAB system. Supplemental data by other technique such as the Universal Tensile Testing is thus, required to shed some light on this uncertainty.

4.8 Mechanical Properties

Average tensile stress values of the film from either LCAB or HCAB at 0%, 1% and 2% SF contents with and without the addition of the 1% plasticizer (glycerol) are shown in Fig.4.11. Data analysis of the films is also summarized in Table 4.5.

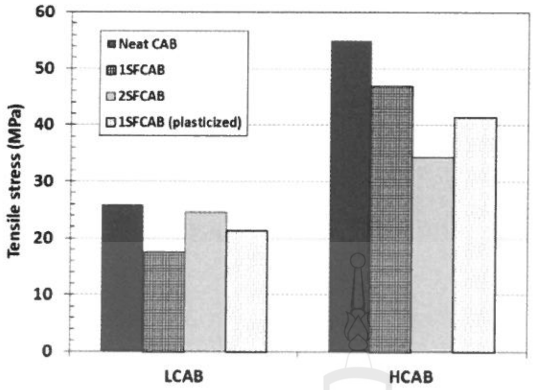


Fig.4.11 Average tensile stress values of the casted films from either LCAB or HCAB at different mass percentages of SF. All samples were tested in triplicate.

Table 4.5 Analysis of mechanical properties of the films at different SF content (% by weight) with and without the addition of the plasticizer.

Sample	Tensile stress (MPa)	Tensile strain (%)
LCAB (neat)	25.93 ± 0.05	1.82 ± 0.38
1SFLCAB	17.47 ± 0.22	1.44 ± 0.52
2SFLCAB	24.52 ± 2.46	1.79 ± 0.37
1SFLCAB (plasticized)	21.19 ± 3.40	1.59 ± 0.50
HCAB (neat)	55.03 ± 4.06	3.54 ± 0.30
1SFHCAB	**46.85 ± 2.39**	**3.87 ± 0.09**
2SFHCAB	34.27 ± 0.03	2.19 ± 0.10
1SFHCAB (plasticized)	41.28 ± 2.48	2.84 ± 0.17

Results confirm a huge impact of the polymer molecular weight on the physicochemical properties of the resultant films. Obviously, all the HCAB-based films exhibit higher tensile stress and % elongation at break as compared to those from the LCAB-based films. This finding supports the fact that the increased chain length offers stronger associative forces acted between the polymer chains and so a tighter molecular chain packing as proposed earlier. The addition of 1% SF reduces the tensile stress of the neat HCAB from around 55.0 MPa to around 46.9 MPa. However, this slightly raises the % elongation from 3.5% to 3.9% (Table 4.5), suggesting the favorable interactions of the two compartments in an amorphous region and the potential plasticizing effect of the protein in the HCAB system. A further addition of SF (2%) progressively weakens the mechanical properties of HCAB film. This time, SF polypeptide chains disperse and interfere the molecular packing of the CAB both in amorphous and crystalline domains.

4.9 Surface Morphology

Surface morphology of the SF/CAB blend films, containing 0%, 1% and 2% SF contents, was analyzed by SEM. Their SEM micrographs are shown in Fig.4.12 and Fig.4.13.

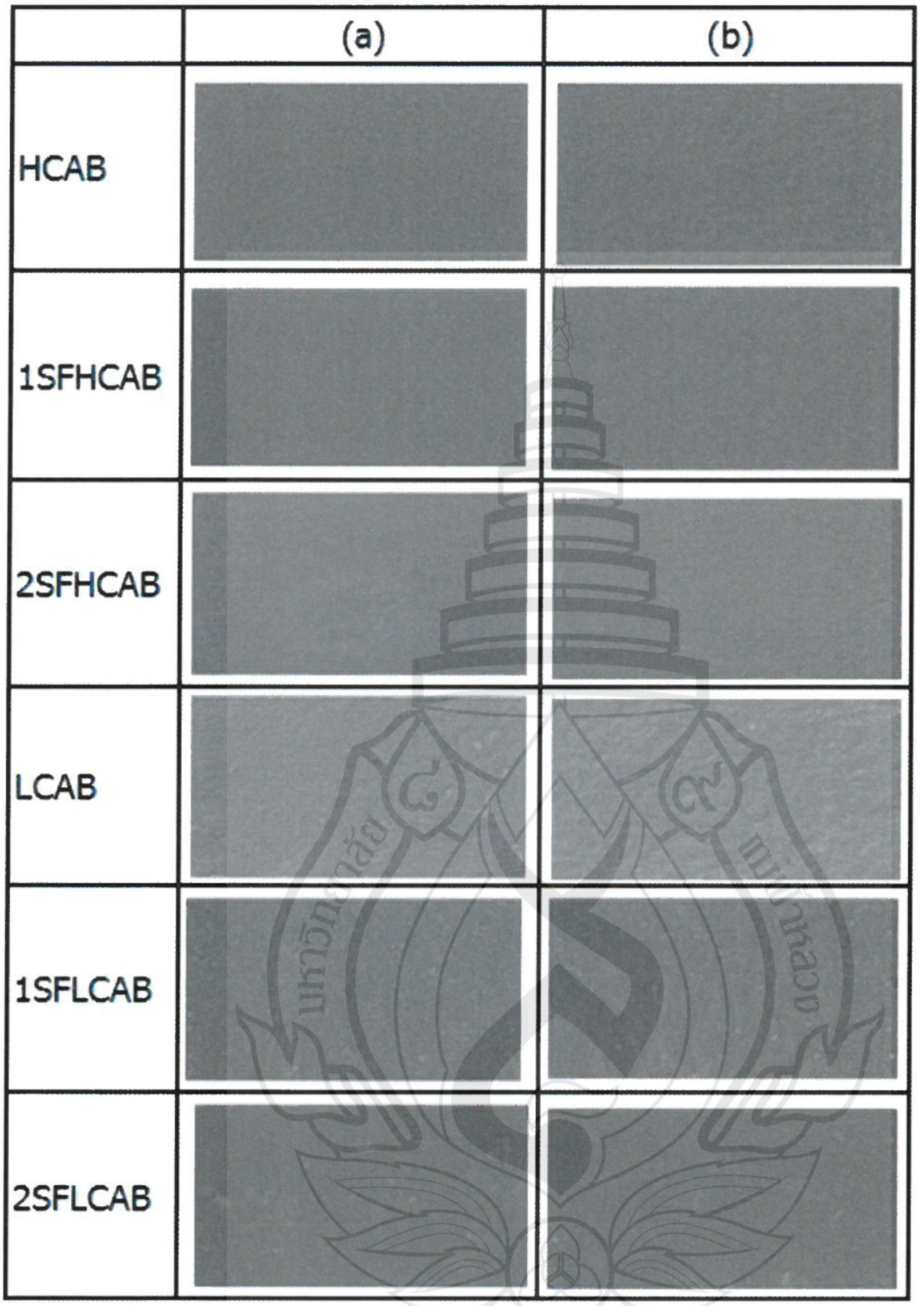


Fig.4.12 SEM images of the casted films containing 0% (HCAB, LCAB), 1% (1SFHCAB, 1SFLCAB) and 2% (2SFHCAB, 2SFLCAB) SF contents. All films were casted in a 20% formic acid and at 50°C. Images in Columns (a) and (b) were taken at x500 and x1000 Magnifications.

As seen in Fig.4.12, most of the blend films display a smooth surface morphology at x50, x500 and x1000 Magnifications. Furthermore, the cross-sectional images reveal the interspersed microfibers throughout the casted films (Fig.4.13).

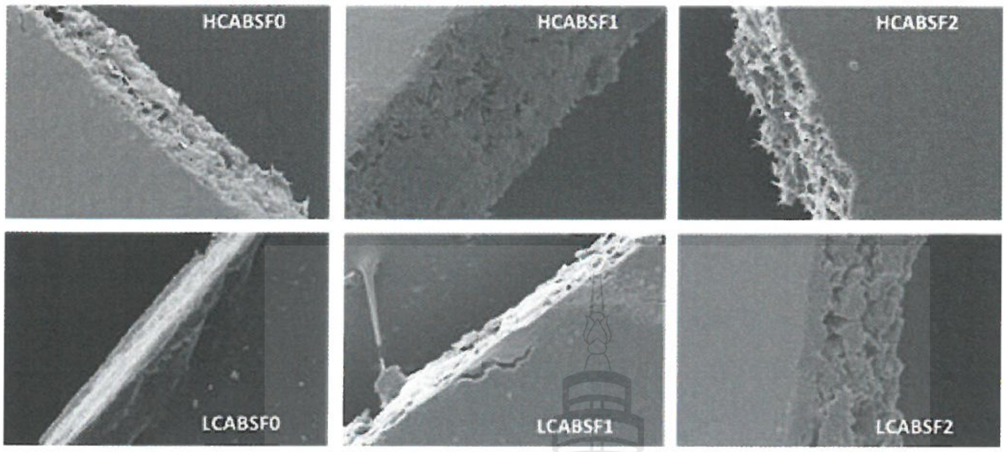


Fig.4.13 SEM images (cross-section) of either HCAB/SF or LCAB/SF blend films containing 0%, 1% and 2% SF contents. Films were casted in a 20% formic acid (x1000 Magnification) and at 50°C.

4.10 Antibacterial Activity

Antimicrobial peptide has gained increasing attention as a new strategy for combating infectious diseases without affecting the host cell. This pilot study was performed to additionally explore the underexploited "antimicrobial activity" of the regenerated silk fibroin (RSF) aqueous solution as a mean to highlight its unique functionality for a development of bioactive and/or smart materials. This study successfully demonstrated, *for the first time*, the antibacterial activity of SF against nine bacterial pathogens including *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Serratia marcescens*, by using a disc diffusion assay. It was hoped that the results would provide valuable details for therapeutic uses, biomedical and active packing applications.

Interpretation of data shown in Fig.4.14 and Table 4.6 suggests that the aqueous solution of RSF exhibit antibacterial activity against four bacterial species, which are ***S. marcescens***, ***M. luteus***, ***E. coli*** and ***E. aerogenes.*** Interestingly, an inhibitory effect of SF seems to be limited by its own diffusion controlled process within an agar media. Additional experiments are thus, needed to perform in the near future to clear up this uncertainty. The fact that RSF possesses this biological activity offers it one of the most promising candidates for a wider range of applications, such as in the fields of biomedical, pharmaceutical and biotechnology.

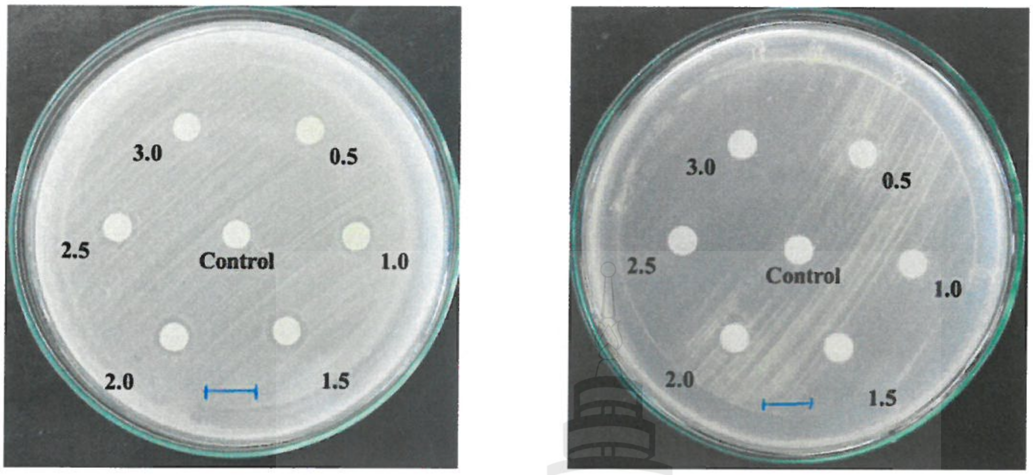


Fig.4.14 Antibacterial activity of regenerated silk fibroin against; *S. marcescens* (left) and *M. luteus* (right). Different concentrations of SF were used ranging from 0.5-3.0 mg/ml. Bar line indicates 1 cm.

Table 4.6 Inhibitory effect of regenerated silk fibroin

Concentration (mg/ml)	Zone of inhibition diameter (mm ± S.D)			
	S. marcescens	*M. luteus*	*E. coli*	*E. aerogenes*
0.5	8.69±0.08	11.10±0.00	9.70±0.09	8.59±0.08
1.0	8.70±0.31	11.61±1.28	9.62±0.23	9.26±0.14
1.5	8.66±0.18	10.10±0.00	9.70±0.11	8.99±0.02
2.0	8.69±0.29	10.60±0.00	9.53±0.47	9.10±0.47
2.5	9.00±0.08	9.00±0.00	9.62±0.31	9.03±0.38
3.0	8.91±0.25	10.05±0.00	10.18±0.14	9.18±0.38

CHAPTER 5
CONCLUSIONS AND FURTHER WORKS

In this research project, the homogeneous SF/CAB blend films were successfully prepared by the solvent casting technique using a mixed formic acid/acetone solvent system as a casting solvent. The film casting conditions were optimized by varying a number of parameters. The following are important parameters to achieve the blend films with good structural integrity and physicochemical properties.

1. A lower formic acid/acetone volume ratio (**15-25%** v/v).
2. A lower total mass content (**5-10%** w/v).
3. A lower SF content (**1-5%** w/w).
4. An elevated drying condition (**e.g., 50°**C)
5. A **high**-molecular weight CAB (e.g., 50,000 g/mol)

Data of Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Fourier-transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM) and the tensile testing proves the preferential interactions of the CAB and SF, possibly through the hydrogen bonding and hydrophobic association, in both amorphous and crystalline regions of the films. The presence of SF (1-2%) in the system does not significantly affect the film transparency. However, this lowers thermal and mechanical strength of the films. The plasticizing effect of fibroin was observed in the HCAB system with 2% SF addition. Surprisingly, the aqueous solution of regenerated SF exhibits antibacterial activity against *S. marcescens*, *M. luteus*, *E. coli* and *E. aerogenes*. This highlights one of the most interesting properties of the protein for a wider range of applications, particularly as bioactive and smart materials.

In summary, this project demonstrated a potential of SF/CAB blend system as an alternative material for the generation of a new biodegradable membrane with controllable properties, capacity to undergo functionalization, but reasonable price over the existing biodegradable sources of material. Although, some useful details were obtained from this study, however, many other properties of the blends are still needed to be examined to completely explore their practical uses as biodegradable materials. Such properties are biodegradability, water and gas permeability and processability by melt blending. Additional experiments to examine these properties, therefore, need to be conducted in the near future.

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ประวัติหัวหน้าโครงการวิจัย

1. ชื่อ- สกุล (ภาษาไทย/ ภาษาอังกฤษ)

(Thai) ดร. พัชรา ปัญญามูลวงษา

(English) Dr. Patchara Punyamoonwongsa

2. ตำแหน่งปัจจุบันหน่วยงานที่อยู่ที่ติดต่อได้สะดวก

ตำแหน่งทางวิชาการ: ดร.

ที่อยู่ที่สามารถติดต่อได้: สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง 333 หมู่ 1 ตำบลท่าสุด อำเภอเมือง จังหวัดเชียงราย 57100

โทรศัพท์: (ที่ทำงาน) 053-916778

โทรสาร: 053-916776

Email: patchara@mfu.ac.th หรือ p.punyamoonwongsa@sci.mfu.ac.th

3. ประวัติการศึกษา

ระดับการศึกษา	ระยะเวลา	มหาวิทยาลัย
วิทยาศาสตรบัณฑิต (เคมี) สาขาเคมีเชิงฟิสิกส์	2538-2541	มหาวิทยาลัยเชียงใหม่ จ. เชียงใหม่
วิทยาศาสตรมหาบัณฑิต (เคมี) สาขาเคมีเชิงฟิสิกส์	2542-2544	มหาวิทยาลัยเชียงใหม่ จ. เชียงใหม่
Doctor of Philosophy (Polymer Science)	2546-2550	Aston University, Birmingham, England
Post-doctoral Fellow	2553	University of New South Wales, Sydney, Australia

4. สาขาวิชาการที่มีความชำนาญพิเศษ(แตกต่างจากวุฒิการศึกษา)ระบุสาขาวิชาการ

Surface Chemistry / Biomedical Polymers / Responsive Polymers / Biopolymers

5. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัย

5.1 <u>หัวหน้าโครงการวิจัย</u>

<u>ชื่อโครงการวิจัย:</u>

- การศึกษาพฤติกรรมการรวมตัวของสารเชิงซ้อนสังเคราะห์โปรตีน-ลิปิดเลียนแบบธรรมชาติ (สัดส่วน 100%)
- การเตรียมแผ่นฟิล์มผสมที่ย่อยสลายตัวได้ตามธรรมชาติจากอนุพันธ์ของเซลลูโลสและโปรตีนไหม (สัดส่วน 90%)
- ซิลค์ไฮโดรเจลไฟโบรอินที่มีโครงสร้างแบบเชื่อมขวางสำหรับการควบคุมการปลดปล่อยตัวยาธรรมชาติ α-mangostin (สัดส่วน 60%)

5.2 งานวิจัยที่ทำเสร็จแล้ว

- การศึกษาพฤติกรรมการรวมตัวของสารเชิงซ้อนสังเคราะห์โปรตีน-ลิปิดเลียนแบบธรรมชาติ (แหล่งทุน: มหาวิทยาลัยแม่ฟ้าหลวง ปี 2552)

  การเผยแพร่:

  1) **P. Punyamoonwongsa** and B.J. Tighe. Association behaviour of the synthetic protein-lipid complex analogues. *Advanced Materials Research*. 2012; 506: 134-137.
  2) **P. Punyamoonwongsa.** Association behavior of the synthetic protein-lipid complex analogues. Proceeding and Oral Presentation at the "Chiang Mai International Conference on Biomaterials and Applications (CMICBA 2011)".

- การเตรียมแผ่นฟิล์มผสมที่ย่อยสลายตัวได้ตามธรรมชาติจากอนุพันธ์ของเซลลูโลสและโปรตีนไหม (แหล่งทุน: มหาวิทยาลัยแม่ฟ้าหลวง ปี 2554)

  การเผยแพร่:

  1) Hyejin Kwon and **P. Punyamoonwongsa**. Silk-protein and Cellulose Blend Films as Bioplastics. *KU Journal of Science (In Press)*.
  2) Patchara Punyamoonwongsa, **Parawut Kesornsunt** and Kamonchanok Thananukul. Preparation and Characterization of Biodegradable Cellulose Ester Blend Films. Poster presentation at "the 3rd Research Symposium on Petrochemical and Materials Technology and the 18th PPC Symposium on Petroleum, Petrochemicals, and Polymers".
  3) C. Witthayaprapakorn, K. Thananukul, N. Suttenun, **P. Punyamoonwongsa** and Robert Molloy. Preparation of Biodegradable Polymer Blended of Poly(*L*-lactic acid)/Cellulose acetate films in the Solvent Mixture. *Advanced Material Research*. 2013; 664: 702-706.
  4) **Patchara Punyamoonwongsa**, Kamonchanok Thananukul, Parawut Kesornsunt, Winita Punyodom. Plasticized Blends of Cellulose Acetate Butyrate and Poly(butylene succinate) as Biodegradable Plastics. *Burapha Science Journal*. (*Accepted*)
  5) Pimsuda Inwong and **Patchara Punyamoonwongsa**. Compatibility of silk fibroin and cellulose acetate butyrate blend system. Poster presentation at the "1st Mae Fah Luang International Conference (MFUIC)", Chiangrai (2012).

6) Nuchada Suttenun, Kunyanut Chomee, Sajee Praditsarn, Ekachai Chukeatirote and **Patchara Punyamoonwongsa**. Antibacterial activity of silk fibroin. Poster presentation at the "Pure and Applied Chemistry International Conference 2013 (PACCON2013)", Chon Buri (2013).

5.3 งานวิจัยที่กำลังทำ

1) <u>โครงการที่ได้รับการจัดสรรงบประมาณแล้ว</u>

- ชื่อโครงการ: ซิลค์ไฮโดรเจลไฟโบรอินที่มีโครงสร้างแบบเชื่อมขวางสำหรับการควบคุมการปลดปล่อยตัวยาธรรมชาติ α-mangostin (60%)
- แหล่งทุน: สำนักงานคณะกรรมการวิจัยแห่งชาติ ประจำปี 2556 ตามมติคณะรัฐมนตรี
- สถานภาพการทำวิจัย: ดำเนินการไปแล้ว 40%

การเผยแพร่

1) **Patchara Punyamoonwongsa** and Nuchada Suttenun. Synergetic effects of solvent system and the addition of polyethylene glycol on the silk gelation. Proceeding and oral presentation at "the 38th Congress on Science and Technology of Thailand (STT 38)", Chiang Mai (2012).

2) <u>โครงการที่กำลังรอจัดสรรงบประมาณ</u>

- ชื่อโครงการ: ซิลค์ไฮโดรเจลไฟโบรอินที่มีโครงสร้างแบบเชื่อมขวางสำหรับการควบคุมการปลดปล่อยตัวยาธรรมชาติ α-mangostin (60%)
- แหล่งทุน: สำนักงานคณะกรรมการวิจัยแห่งชาติ ประจำปี 2557 ตามมติคณะรัฐมนตรี
- สถานภาพการทำวิจัย: ยังไม่ได้ดำเนินการ