

**AN APPROACH FOR PREPARING A BIO-HYBRID MATERIAL  
WITH SPECIAL WATER AFFINITY FROM  
POLY(VINYL ALCOHOL), RICE STARCH  
AND SILK FIBROIN FOR WOUND  
DRESSING APPLICATION**

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<b>Thesis Title</b>	An Approach for Preparing a Bio-Hybrid Material with Special Water Affinity from Poly(vinyl alcohol), Rice Starch and Silk Fibroin for Wound Dressing Application	
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## **ABSTRACT**

A hybrid material composing of polyvinyl alcohol (PVA), rice starch (RS) and silk fibroin (SF) with opposite water affinity on each surface was successfully prepared. The hybrid material was prepared by placing PVA/RS/SF hydrogel onto glycerol-modified PVA/RS/SF film and leaving them to adhere. SF content in the hydrogels was optimized first by mixing various SF amounts to a mixture of PVA:RS (2:1 by weight). The 8.00 part per hundred of polymer (php) of SF was an appropriate concentration revealed by the maximum values of gel fraction and percent porosity. This SF content caused the blend to exhibit the highest water saturation and best mechanical properties. The glycerol-modified hydrophobic film with the same PVA/RS/SF composition was

prepared and found that the water contact angle of the film had increased approximately three times after water soaking. The hybrid material was then prepared and characterized. As evidenced by physical appearance and SEM images, the interfaces between layers became homogeneous after 30 h layering time. Water contact angle values pointed out the hydrophilic nature of the hydrogel surface, while the film surface was hydrophobic. Coupling of the hydrogel with the film improved overall mechanical properties as indicated by stress and % elongation at break.

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## LIST OF ABBREVIATIONS AND SYMBOLS

ATR	attenuated total reflectance
cm	centimeter
cm <sup>2</sup>	square centimeter
°C	degree Celsius
g	gram
g/L	gram per liter
g/mol	gram per mole
h	hour
K	Kelvin
L	liter
m <sup>2</sup>	square meter
min	minute
mL	milliliter
mm	millimeter
mm <sup>3</sup>	cubic millimeter
mol %	mole percentage
MPa	megapascals
MW	molecular weight
nm	nanometer

O <sub>2</sub>	oxygen gas
php	part per hundred of polymer
PVA	poly(vinyl alcohol)
RS	rice starch
SEM	scanning electron microscopy
SF	silk fibroin
% v/v	percent volume by volume
% w/v	percent weight by volume
% w/w	percent weight by weight
μL	micro liter
α	alpha
β	beta
θ	contact angle

# **CHAPTER 1**

## **INTRODUCTION**

A hybrid material is defined as a material that consists of two constituents at nano level. Research and development of bio-hybrid materials for temporary skin covers or as wound dressing is becoming a subject of great commercial interest [1]. Wound dressing hydrogels are three-dimensional polymeric networks and are available in sheet form or as a spreadable viscous gel with their uniquely interesting properties such as easy replacement, transparency to allow healing follow-up, oxygen permeability, and so on [2, 3].

### **1.1 Wound dressing**

Wound dressing is an artificial skin that can meet the requirements such as higher vapor or gas permeation and protection of wound from infection and dehydration [4]. A dressing is used by a doctor, caregiver and/or patient to help a wound heal and prevent further issues like infection or complications. Dressings are designed to be in direct contact with the wound, which is different from a bandage that holds the dressing in place. Wound dressings serve a variety of purposes depending on the type, severity and position of the wound. Aside from the major function of reducing the risk of infection, dressings are also important to help: stop bleeding and start clotting so the wound can heal, absorb any excess blood, plasma or other fluids, wound debridement and begin the healing process [5].



## Types of wound dressing [5]

*Hydrocolloid:* Hydrocolloid dressings are used on burns, light to moderately draining wounds, necrotic wounds, under compression wraps, pressure ulcers and venous ulcers.

*Hydrogel:* This type of dressing is for wounds with little to no excess fluid, painful wounds, necrotic wounds, pressure ulcers, donor sites, second degree or higher burns and infected wounds.

*Alginate:* Alginate dressings are used for moderate to high amounts of wound drainage, venous ulcers, packing wounds and pressure ulcers in stage III or IV.

*Collagen:* A collagen dressing can be used for chronic or stalled wounds, ulcers, bed sores, transplant sites, surgical wounds, second degree or higher burns and wounds with large surface areas.

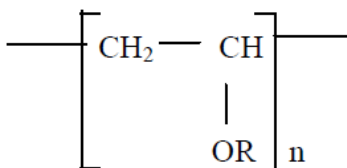
In addition, there are other wound dressings available such as foams and compression in addition to secondary and cover dressings like wraps, gauze and tape.



**Figure 1.1** Various types of commercial wound dressing sample

## 1.2 Poly (vinyl alcohol) [10, 11]

Poly(vinyl-alcohol) (PVA) was first prepared by Hermann and Haehnel in 1924 by hydrolyzing polyvinyl acetate in ethanol with potassium hydroxide. It is produced commercially from polyvinyl acetate, usually by a continuous process. The acetate groups are hydrolyzed by ester interchange with methanol in the presence of anhydrous sodium methyrate or aqueous sodium hydroxide. The physical characteristics and its specific functional uses depend on the degree of polymerization and the degree of hydrolysis. PVA is classified into two classes namely, partially hydrolyzed and fully hydrolyzed. Partially hydrolyzed PVA is used in the foods. PVA is an odorless and tasteless, translucent, white or cream colored granular powder. It is used as a moisture barrier film for food supplement tablets and for foods that contain inclusions or dry food with inclusions that need to be protected from moisture uptake. PVA is not known to occur as a natural product. The structure of PVA (partially hydrolyzed) is given below:



where R = H or COCH<sub>3</sub>

PVA has various applications in the food industries as a binding and coating agent. It is a film coating agent especially in applications where moisture barrier/protection properties are required. As a component of tablet coating formulations intended for products including food supplement tablets, PVA protects

the active ingredients from moisture, oxygen and other environmental components, while simultaneously masking their taste and odor. PVA has the advantages of excellent film forming and adhesive properties, together with high thermal stability.

PVA is a great interest because of its many desirable characteristics specifically for various pharmaceutical and biomedical applications. The crystalline nature of PVA has been of specific interest particularly for physically cross-linked hydrogels prepared by repeated cycles of freezing and thawing [12].

### **PVA hydrogel**

The hydrogels consist of a three dimensional network that swells in the presence of water or biological fluids maintaining its shape, they are soft and elastic [13]. The hydrogels can absorb an excess of wound exudates, protect a wound from secondary infection, and effectively promote the healing process by providing a moisturized wound healing environment [14].

PVA hydrogel is one of the well-known polymer gel that due to its good biocompatibility, hydrophilicity and capability of swell in water or biological fluids has been used in several biomedical and pharmaceutical applications. PVA has a capacity for high strength, biocompatibility, biodegradability, water sorption. PVA is a semicrystalline synthetic polymer that possesses the ability to form physically cross-linked hydrogels. PVA hydrogels prepared by repeatedly freezing and thawing PVA aqueous solutions have attracted much attention in the last years for their several potential applications, for instance, a biomedical material for drug delivery, as a biotechnology for bioseparation, and as a carrier for cell immobilization.

### *Freezing and thawing method*

The freezing and thawing method offers several advantages with respect to other methods such as chemical cross-linking and radiation-induced cross-linking, for example it is simple and doesn't require any additional chemicals or high temperature.

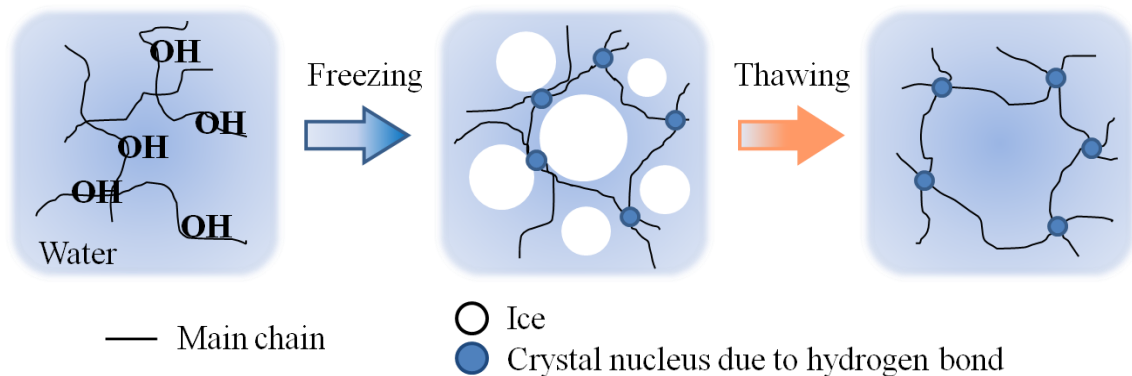
The gelation system is shown below:

(a) The cooling of the initial polymer solution to the point of the onset of crystallization

(b) The freezing of the sample when the main mass of low-molecular-weight liquid is crystallized and the polymer is concentrated in the so-called unfrozen liquid microphase

(c) The incubation of the system at given negative temperature

(d) The defrosting of the system. The three-dimensional network of gel is primarily formed; the role of physical junction knots in the gel is played by microcrystallites arising upon the interaction between neighbor polymer chains due to the hydrogen bonding of pendant hydroxyls groups of the polymer



**Figure 1.2** Scheme of gelation by repetition of freezing and thawing

### ***Gelation of PVA by freezing and thawing method***

The presence of PVA in a frozen system affects the processes of the crystallization and recrystallization of ice to form the gel and the gel final structure. This influence substantially depends on the viscosity of unfrozen liquid microphase and, hence, on the concentration of polymer, its molecular mass, chain tacticity and naturally, on the temperature of freezing–storage and the regime of defrosting. The faster the defrosting process, the weaker and less thermoresistant the formed gel species are and if the thawing rate exceeded approximately 10 °C/min, no PVA gel were obtained at all. The reason is that intermolecular polymer–polymer interactions take a fairly long time to exercise. The temperatures in the vicinity of -2 °C may be considered as the thermal conditions of the highest efficiency of PVA cryotropic gelation.

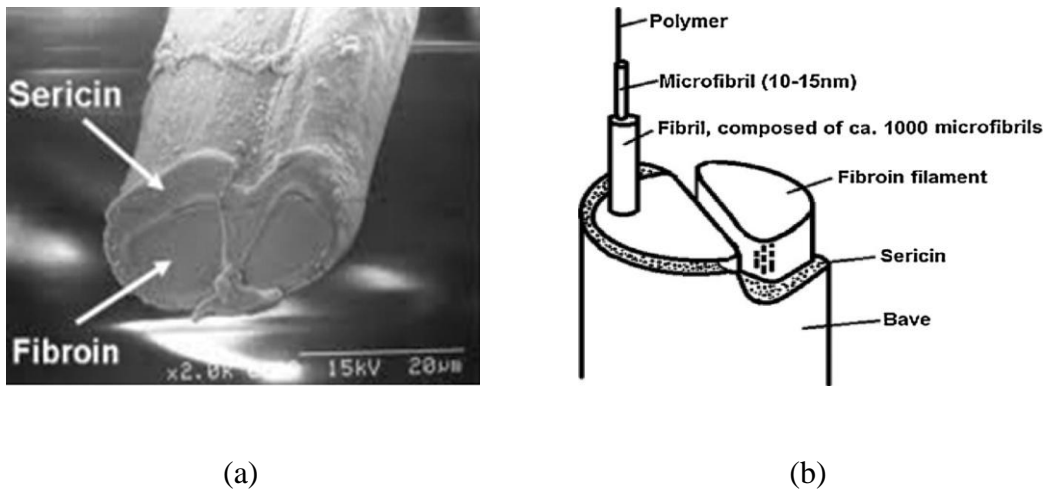
### ***Characteristic of PVA gel fabricated by freezing and thawing method***

In this system, it is known that negative effect of aging, including worse physical and mechanical properties may be prevented by using a drying/re-hydration protocol able to keep the physical properties of the primary prepared PVA hydrogels. Hydrogels obtained by repeatedly [15] freezing and thawing water/PVA solutions show a porous structure, rubber elasticity, and are mechanically stable over a large range of temperatures. These properties, along with lack of toxicity, biocompatibility, and large water content, make freeze/thaw PVA hydrogels attractive biological matrices.

However, the PVA gel degradation rate and oxygen gas permeability are not satisfactory for further applications. Thus, more improvement of these properties is required. One of the methods used to improving PVA properties is blending PVA with other materials. Natural polymers are usually preferred in biomaterials due to their excellent biological properties, including cell adhesion, mechanical properties similar to the natural tissue, biodegradability and biocompatibility [16]. Thus many researchers prepared PVA blends with various natural polymers such as alginate, chitosan and hyaluronic acid [17-19].

### **1.3 Silk [20-24]**

Silk, a well-known natural fiber, has been used commercially as a highly valued textile fiber for centuries and as biomedical sutures for decades. Its best qualities in terms of strength, elasticity, softness, absorbancy, affinity for dyes, and adaptability to various forms of twisting continue to satisfy various marketing demand. The silk fiber, derived from silkworm *Bombyx mori*, composed primarily of two protein components: fibroin and sericin. The core fibers (fibroin filament) are encased in sericin (a family of glue-like proteins) that holds the fibroin filaments together to form the composite fibers of the cocoon case to protect the growing worm. Generally in silk fiber, fibroin and sericin proteins are present at about 70 % and 30 % w, respectively. The combination of fibroin and sericin in raw silk is shown in **Figure 1.3**.



**Figure 1.3** The combination of fibroin and sericin in raw silk (a) SEM cross-sectional image of silk fiber and (b) schematics diagram of *Bombyx mori* silk [25]

### Structure of silk protein [26, 27]

In general, the structure of protein can be classified in four types: primary, secondary, tertiary and quaternary structures (**Figure 1.4**).

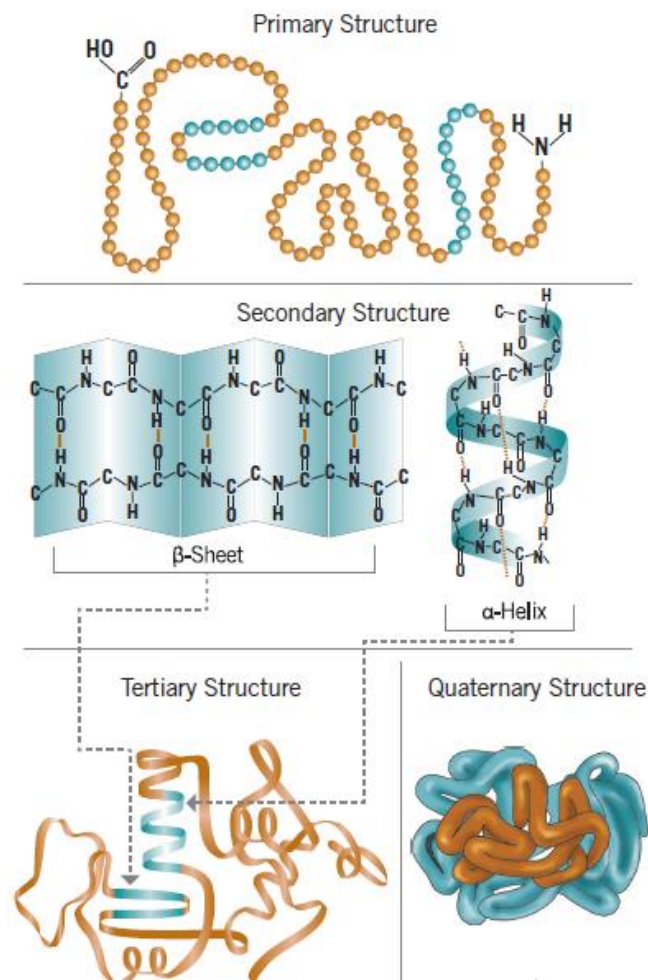
The primary structure of a protein refers to the linear sequence of amino acids in the polypeptide chain. Amino acids contain both a basic amino group and an acidic carboxyl group. This di-functionality allows the individual amino acids to join together in long chains by forming peptide bonds: amide bonds between the  $-NH_2$  of one amino acid and the  $-COOH$  of another. Sequences with fewer than 50 amino acids are generally referred to as peptides, while the terms protein or polypeptide are used for longer sequences. A protein can be made up of one or more polypeptide molecules.

The secondary structure of silk can be classified in two types, the  $\alpha$ -helix and  $\beta$ -sheet. The  $\alpha$ -helix form occurs via the hydrogen bonding between the hydrogen atom of amino groups and the oxygen atom of carboxyl groups in polypeptide chain. Whereas, the  $\beta$ -sheet is caused by hydrogen bonding between the hydrogen atoms (amino group) and the oxygen atoms (carboxyl group) of amino acids on two chains lying side-by-side.

The overall three-dimensional shape of an entire protein molecule is the tertiary structure. The protein molecule will bend and twist in such a way as to achieve maximum stability or lowest energy state. Although the three-dimensional shape of a protein may seem irregular and random, it is fashioned by many stabilizing forces due to bonding interactions between the side-chain groups of the amino acids.

Quaternary structure, many proteins are made up of multiple polypeptide chains, often referred to as protein subunits. These subunits may be the same (as in a homodimer) or different (as in a heterodimer). The quaternary structure refers to how these protein subunits interact with each other and arrange themselves to form a larger aggregate protein complex. The final shape of the protein complex is once again stabilized by various interactions, including hydrogen-bonding, disulfide-bridges and salt bridges.



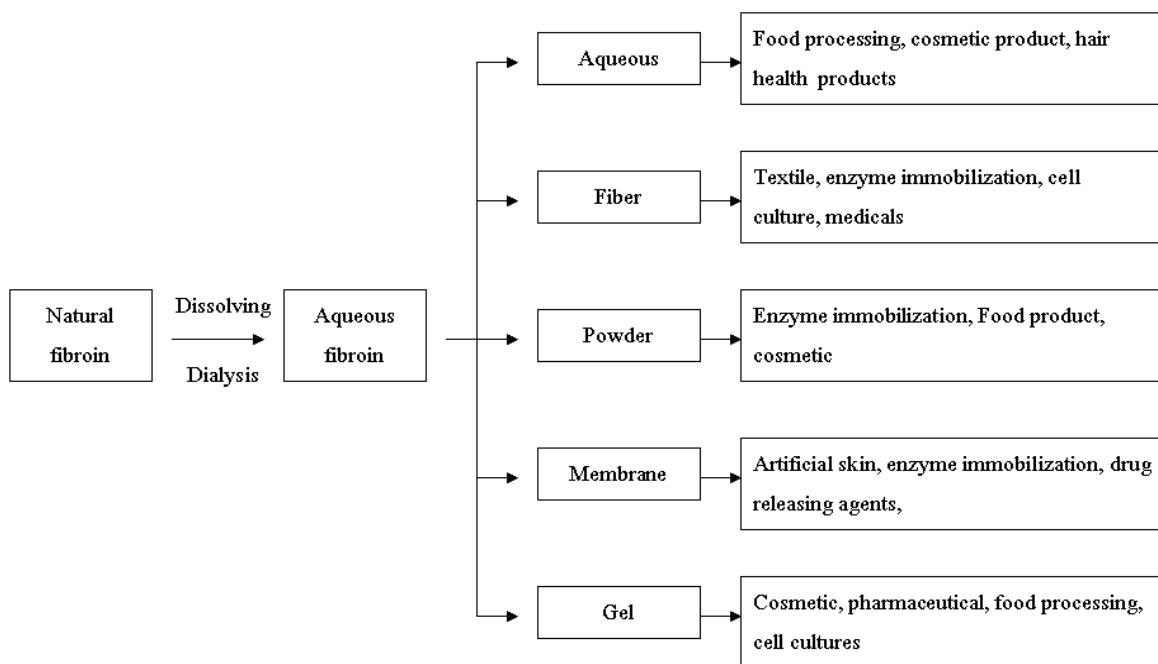


**Figure 1.4** Common structure of protein

### Silk fibroin

The silk fibroin (SF) is a fibrous protein. Its chemical composition is characterized by the presence of relatively few types of amino acid residues with small side chains, the sum of the three simplest amino acids (glycine, alanine, and serine) accounting for more than 80 mol %. The primary structure arising from this characteristic of amino acid sequence was proposed as  $[\text{Gly-Ala-Gly-Ala-Gly-Ser}]_n$ .

The molecular mass of SF is about 25-350 kDa [23]. It has been an important textile stock for a long time due to its unique tensile strength and elasticity, good thermal stability, hygroscopicity and microbial resistance. Recently, natural SF has been developed and utilized for other purposes. An aqueous SF exhibits good characteristics which can be used as the starting material for the preparation of different kinds of SF-based biomaterial, such as powder, gel, homogeneous membranes, and porous membranes. The modern utilizations of the SF in various forms depend on the applications (**Figure 1.5**).



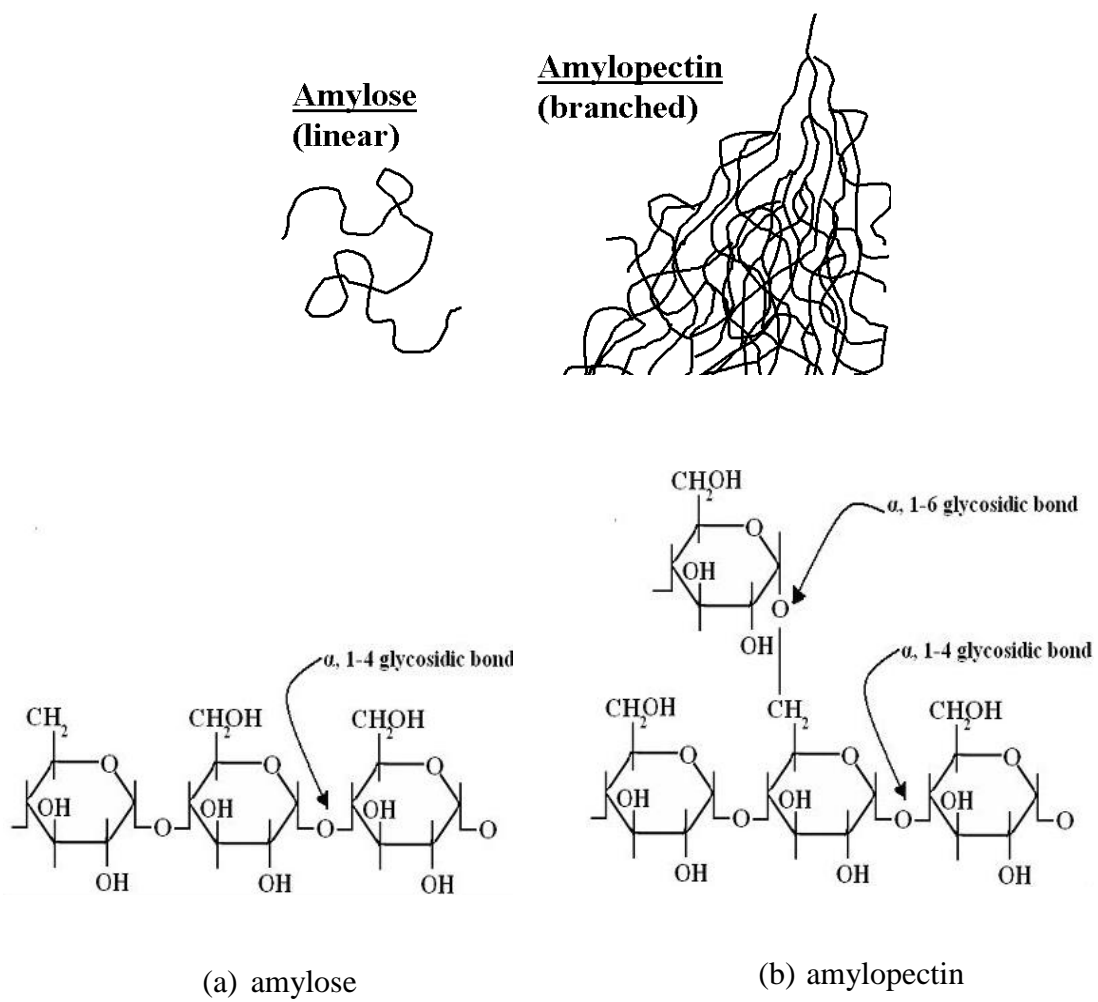
**Figure 1.5** The modern utilization of the SF in various forms

Silk fibroin has been widely used in many areas such as medical, pharmaceutical, cosmetic and agricultural applications in the forms of wound dressing, film matrices, hydrogels, *etc.* Recently, many researchers have reported the study of silk fibroin for various applications [28-32].

## 1.4 Starch

Starch is a polysaccharide produced by higher plants as a means of storing energy. It is stored intracellular in the form of spherical granules that are 2–100  $\mu\text{m}$  in diameter [33]. Most commercially available starches are isolated from grains such as corn, rice, wheat, and from tubers such as potato and tapioca. It is a consisting of anhydroglucose units linked together primarily through an  $\alpha$ -(1-4) glucosidic bonds. Although the detailed microstructures of starch are still being elucidated, it has been generally established that starch is a heterogeneous material containing at the extremes two microstructures according to their chain structure: amylose and amylopectin. Amylose has an essentially linear structure of  $\alpha$ -1,4-linked glucose units and amylopectin has a highly branched structure of short  $\alpha$ -1,4 chains linked by  $\alpha$ -1,6 bonds. The structure of amylose and amylopectin are shown in **Figure 1.6**.

The linear structure of amylose makes its behavior closer to that of conventional synthetic polymers. The molecular weight of amylose is about  $10^6$  (200–2000 anhydroglucose units) depending on the source and processing conditions. Amylopectin, on the other hand, is a branched polymer. The molecular weight of amylopectin is much larger than that of amylose. Light-scattering measurements indicate a molecular weight in millions. The large size and branched structure of amylopectin reduce the mobility of the polymer chains and interfere with any tendency for them to become oriented closely enough to permit significant levels of hydrogen bonding.



**Figure 1.6** The structure of (a) amylose and (b) amylopectin

### **Rice starch**

Rice starch (RS), another renewable and biodegradable biopolymer, has been increasingly utilized in many applications. Primarily because it is a low cost polysaccharide, vastly available and is one of the cheapest biodegradable polymers. The hydrophilicity of starch can be used to improve the degradation rate of some degradable

hydrophobic polymers [34]. Principally, there are three ways how starch can be used for biodegradable polymer production. The first one is the preparation of starch composition with other plastics with a low amount of starch to enhance the biodegradability of traditional oil-based polymer materials. The second way of starch application is the preparation of starch composite with starch content being more than half by mass and third way of starch biodegradable polymers preparation uses the extrusion processing of mixtures of granular starch [35].

In recent years, interest in using the PVA hydrogel has significantly increased related to its high biocompatibility, hydrophilicity, harmlessness, sterility, transparency and the good gel forming capability [36-41]. Several methods have been used for preparing PVA hydrogels [42]. One of them is the successive freeze-thaw cycles which result in a physically cross-linked gel called as cryogel [43]. However, the PVA gel degradation rate and oxygen gas permeability are not satisfactory for further applications. Thus, more improvement of these properties is required. One of the methods used to improving PVA properties is blending PVA with other materials. Natural polymers are usually preferred in biomaterials due to their excellent biological properties, including cell adhesion, mechanical properties similar to the natural tissue, biodegradability and biocompatibility. Thus many researchers prepared PVA blends with various natural polymers. Rice starch (RS) and silk fibroin (SF) are two interesting natural polymers with several advantages that can be utilized for blending with PVA. SF has been widely used in many areas such

as medical, pharmaceutical, cosmetic and agricultural applications in the forms of wound dressing, film matrices, hydrogels, etc. Recently, many researchers have reported the study of silk fibroin for various applications [44-49]. Regenerated SF can either be blended or chemically cross-linked with other natural or synthetic polymers to form hydrogels with improved properties. SF composite gels have been prepared with PVA, gelatin, collagen, poloxamer-407, modified polyethylene glycol (PEG), N-isopropylacrylamide (NIPAAm), polyacrylamide, etc. [50-60]. Rice starch (RS), another renewable and biodegradable biopolymer, has been increasingly utilized in many applications, primarily because of its low cost and ease of availability [61]. Only a few studies on starch polymer blend hydrogels have been reported [62]. PVA/starch blend hydrogels were prepared by chemical cross-linking [63] and also irradiation technique [64]. Our previous work demonstrated that such prepared PVA/RS/SF film exhibited some improved properties such as lower water solubility, higher oxygen permeability and degradability compared to PVA itself [65, 66].

In this work demonstrates further surface properties improvement of the previously prepared PVA/RS/SF film using glycerol treatment for generating a hydrophobic film. Additionally, the PVA/RS/SF blends were used to prepare PVA/RS/SF hydrogels using the freezing/thawing method to increase porosity in the hydrogels. The optimization of the SF content for preparing both the hydrogel and the glycerol-modified film was done through by firstly blending a PVA:RS mixture (2:1 by weight) with various amounts of SF ranging from 2.67-13.3 php (part per hundred of polymer), and then the characterization of some hydrogel properties. Finally,

depending on the proven self-healing property of PVA gels [67], a PVA/RS/SF hybrid material was prepared through the coupling of the prepared PVA/RS/SF hydrogel with the glycerol-modified PVA/RS/SF film by simply layering the hydrogel onto the glycerol-modified film, and leaving them at room temperature until completely adhered to each other. The surface properties of both layers of the hydrogel and the glycerol-modified film of the PVA/RS/SF hybrid material were determined through the water contact angle measurement. The prepared hybrid material has opposite water affinity on each surface. We presume that the opposite water affinity on each surface can give a bandage the advantage of isolating a wound from the external environment while protecting the wound bed from dehydration to facilitate the healing process.

## **1.5 Objective of this thesis**

The main objectives of this research project are:

- 1) To improve the surface properties of the PVA/RS/SF film using glycerol and water treatment for generating a hydrophobic PVA/RS/SF film.
- 2) To prepare PVA/RS/SF hydrogels using the freezing/thawing method to increase porosity in the hydrogels.
- 3) To prepare a PVA/RS/SF bio-hybrid material with special water affinity through the coupling of the prepared PVA/RS/SF hydrogel with the glycerol-modified PVA/RS/SF film for use as wound dressing materials and characterize their physical and mechanical properties.

## CHAPTER 2

### EXPERIMENTAL

#### 2.1 Chemicals, materials and equipment

##### 2.1.1 Chemicals and materials

Lists of various chemicals and materials used in this research are shown in **Table 2.1** and **Table 2.2**, respectively.

**Table 2.1** Chemicals

Chemicals	Grade	Manufacturer
Calcium chloride dihydrate (CaCl <sub>2</sub> · 2H <sub>2</sub> O)	AR	Ajax Finechem, Sydney, Australia
Ethanol (C <sub>2</sub> H <sub>5</sub> OH)	AR	Merck, Darmstadt, Germany
Glycerol (C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> )	AR	Sigma Chemicals, St. Louis, U.S.A
Hexane (C <sub>6</sub> H <sub>14</sub> )	AR	Kanto Chemical Co. Inc., Tokyo, Japan.
Poly(vinyl alcohol) (PVA) 99% hydrolyzed, MW; 85,000-124,000	AR	Aldrich Chemical Co. Inc., St. Louis, U.S.A.
Sodium carbonate (Na <sub>2</sub> CO <sub>3</sub> )	AR	Fisher Scientific, Leicester, UK



**Table 2.2** Materials

<b>Materials</b>	<b>Grade</b>	<b>Manufacturer</b>
Rice starch (Era-Tab)  Lot # T510405,  Anal. # T0405	AR	Erawan Pharmaceutical Research and Laboratory Co., Ltd., Nakhon Pathom, Thailand
Silk waste	Commercial	Jun Thai Silk Group Co., Phetchabun, Thailand

### 2.1.2 Equipment

The main equipment used were as listed in **Table 2.3**.

**Table 2.3** Equipment

<b>Equipment</b>	<b>Model</b>	<b>Manufacturer</b>
Contact angle analyzer	DM 300N	KYOWA, Saitama, Japan
Furier Transform Infrared Spectrometer	FTIR-8400S	SHIMADZU, Kyoto, Japan
Scanning electron microscope	JCM-6000	JEOL, Tokyo, Japan
SnakeSkin <sup>TM</sup> pleated dialysis tubing 10000 MWCO	-	Thermo Fisher Scientific, Rockford, U.S.A
Force measurement machine	MX2-500N	IMADA Co. Ltd., Toyohashi, Japan

## 2.2 Preparation of materials and solutions

### 2.2.1 Preparation of silk fibroin powder

Silk waste was purchased from Jun Thai Silk Group Co., Thailand. Silk fibroin (SF) powder was prepared from the silk waste by the method of Moonsri *et al* [68], without further characterization. A 15 g silk waste was boiled in 500 mL of 5 g/L  $\text{Na}_2\text{CO}_3$  solution at 98-100 °C for 30 min, the boiling process was triplicately repeated. Then rinsed by distilled water and dried at 60 °C for 6 h. This is done to remove a sericin (a gum like protein) coating on the silk fiber. After that a 10 g degummed SF was dissolved in 100 mL of a ternary solvent,  $\text{CaCl}_2$ :  $\text{C}_2\text{H}_5\text{OH}$ :  $\text{H}_2\text{O}$  (1:8:2 mole ratio, 60 % v/v  $\text{C}_2\text{H}_5\text{OH}$ ), at  $110 \pm 5$  °C for 2 h. Then the SF solution was filtered and dialyzed continuously in a dialysis tube (molecular cutoff 10,000) against distilled water for 3 days at room temperature to remove salt. Then, the SF solution was frozen and freeze-dried to obtain SF powder. The SF powder should be stored in a desiccator at room temperature for further experimental use.

### 2.2.2 Preparation of solutions

The chemicals were weighed at appropriate weights conformed to the particular solution concentrations. Then each weighed sample was dissolved in distilled water at various temperatures, and the volume was adjusted with distilled water to the required volumes in volumetric flasks. The weights of chemicals and volumes of the solvent used for solution preparation were listed in **Table 2.4**.

**Table 2.4** Solution preparation

<b>Solution</b>	<b>Chemical</b>	<b>Solvent</b>	<b>Container</b>
5 g/L Na <sub>2</sub> CO <sub>3</sub>	10 g anhydrous Na <sub>2</sub> CO <sub>3</sub>	distilled water at room temperature	2000 mL volumetric flask
CaCl <sub>2</sub> :C <sub>2</sub> H <sub>5</sub> OH:H <sub>2</sub> O (mole ratio of 1:2:8)	100 g CaCl <sub>2</sub> , 60 mL C <sub>2</sub> H <sub>5</sub> OH and 100 mL H <sub>2</sub> O	-	2000 mL beaker
5 and 10 % w/v RS solutions	5.00 g and 10.00 g RS powder	distilled water at 80 °C, stirring for 30 min *	100 mL volumetric flask
10 % w/v PVA solution	10.00 g PVA powder	distilled water at 95 °C, stirring for 45 min *	100 mL volumetric flask

\* In the case of RS and PVA solutions, the solutions were cooled down to room temperature before adjusting the volume in volumetric flasks

## 2.3 Procedures

### Part I: Preliminary study: Preparation of PVA, RS and PVA/RS hydrogels

#### I.1 Preparation of PVA, RS and PVA/RS hydrogels

The PVA, RS and PVA/RS hydrogels with various % hydrolysis and molecular weight of PVA were prepared by freezing-thawing method. Aqueous solutions of

10 % w/v PVA and RS were prepared at 95 °C and 80 °C, respectively. The 1:1 weight ratio of PVA/RS solution for preparing the PVA/RS hydrogel were prepared by the mixing of the 10 mL of 10 % w/v PVA (with various % hydrolysis and molecular weight as shown in **Table 2.5**) and the 10 mL of 10 % w/v RS solution. The solutions were poured in the moulds (5 cm diameter) and the solutions were exposed to successive cycles of freezing at -17 °C for 30 mins and thawing at 25 °C for 30 mins. After that the hydrogels were peeled off and its physical appearance was observed. This is done in order to test for the appropriate process of the PVA/RS/SF hydrogel preparation.

**Table 2.5** PVA samples with various % hydrolysis and molecular weight used for preparing PVA and PVA/RS hydrogels

<b>PVA Samples</b>	<b>% Hydrolysis (%mol)</b>	<b>MW (g/mol)</b>
PVA 1	78-82 %	2000
PVA 2	86.5-89 %	500
PVA 3	86-90 %	900-1000
PVA 4	Completely hydrolysis (96 %)	900-1000
PVA 5	Completely hydrolysis ( $\geq 99$ %)	85000-124000

## **Part II: Preparation and characterization of PVA/RS/SF hydrogels**

### **II.1 Preparation of PVA/RS/SF hydrogels**

A mixture of 10% w/v PVA and 5% w/v RS solutions (30 mL each) was stirred for 60 min at 80 °C before adding various amounts of SF (0.0200-0.1000 g) into each of the mixture (10 mL) at room temperature. After that the mixed solutions were stirred for 60 min more, then poured into the moulds (20×20×5 mm<sup>3</sup>) and processed for 7 cycles of freezing at –20 °C for 45 min and further thawing at 25 °C for 45 min. All obtained hydrogels of PVA:RS (2:1 by weight) with the SF contents ranging from 2.67-13.3 php were optimized for obtaining an appropriate SF content for further preparation of glycerol modified PVA/RS/SF film and PVA/RS/SF hybrid material.

### **II.2 Characterization of PVA/RS/SF hydrogels**

#### **II.2.1 Percentages of gel fraction and water absorption**

The gel fraction (G) of the PVA/RS/SF gels with various contents of SF was estimated [69] using equation (1). The dried hydrogel and the water-removed hydrogel were weighed. Water uptake of the hydrogel samples was measured at 25 °C. All dried samples were immersed in distilled water at different time intervals, then the swollen samples were removed and immediately weighed after blotting out excess water off the surface. The equilibrium water saturation ( $W_{eq}$ ) in the swollen samples was calculated [43] using equation (2).

$$G(\%) = \frac{W_2}{W_1} \times 100 \quad (1)$$

$$W_{eq} = \frac{W_3 - W_1}{W_3} \times 100 \quad (2)$$

where  $W_1$ ,  $W_2$  and  $W_3$  are the weight of dried hydrogel, the weight of the water-removed hydrogel, and the weight of the swollen sample, respectively.

### II.2.2 Morphology

The morphology of the PVA/RS/SF gels was examined using a Scanning Electron Microscope (SEM; JCM-6000, JEOL). The dried gel samples were cut to expose the cross-sectional part. These samples were then gold coated and their morphological appearance was observed.

### II.2.3 Hydrogel porosity

Percent porosity of the PVA/RS/SF hydrogels was measured by the liquid displacement method [70]. The porosity,  $\varepsilon$ , is defined as the total volume of the pores divided by the total volume of the porous sample. The sample was immersed in a known volume ( $V_1$ ) of hexane in a graduated cylinder for 10 min. Then the total volume of hexane was recorded again as  $V_2$ . Later on, the hexane imbibed sample was carefully removed from the cylinder and the remaining hexane volume was recorded as  $V_3$ . Porosity of the samples was calculated from equation (3).

$$\varepsilon = \frac{V_1 - V_3}{V_2 - V_3} \times 100 \quad (3)$$

## **II.2.4 Mechanical properties**

The stress at break and % elongation at break of five selected pieces of the PVA/RS/SF hydrogels were measured using a IMADA force measurement (MX2-500N), with a cross-head speed of 10 mm/min. The results were reported as the average values.

## **Part III: Preparation and characterization of glycerol modified PVA/RS/SF films**

### **III.1 Preparation of glycerol modified PVA/RS/SF films**

A blended film of PVA/RS/SF modified with glycerol in aqueous medium was prepared as the procedure previously mentioned by Kuchaiyaphum *et al.* [66] with the optimal composition as the above mentioned in the PVA/RS/SF hydrogel preparation. A 1.67 g of glycerol was added into 100 mL of PVA:RS mixture (2:1 by weight) containing 8.00 php of SF to obtain the concentration of 20 % w/w and stirred for 40 min more. Then the mixture was casted in the moulds (10 cm diameter) and the solvent was evaporated at room temperature in a laminar flow hood for a day. After solvent evaporation, the glycerol-modified film was peeled off and soaked in water for various time intervals (15, 30, 60 and 90 min). The soaking time resulted in generating the glycerol-modified film to be more hydrophobic was selected so that this modified film could be used further for a hybrid material preparation.

## **III.2 Characterization of glycerol modified PVA/RS/SF films**

### **III.2.1 Film hydrophobicity**

The hydrophobicity of the film was evaluated from water contact angle measurement and it was measured by dropping 1  $\mu\text{L}$  of deionized water onto the glycerol-modified film surface using a KYOWA DM 300N Contact Angle Analyzer at 25 °C. The contact angles at 5 second were monitored and the average of at least three measurements was calculated. The remaining glycerol on the PVA/RS/SF films surface after soaking in water at various time intervals were also confirmed through functional group analysis using ATR spectroscopy. The ATR spectra of film samples were recorded with a FTIR-8400S spectrometer (SHIMADZU, Japan) in the range between 4000 to 650  $\text{cm}^{-1}$ .

### **III.2.2 Mechanical properties**

Each dried glycerol-modified film sample was cut into small pieces ( $1 \times 5 \text{ cm}^2$ ) with a film thickness of  $0.060 \pm 0.010 \text{ mm}$ . The stress at break and % elongation at break of five selected pieces of the samples were measured in the same fashion as done with the hydrogels and the average values were reported.



## **Part IV: Preparation and characterization of PVA/RS/SF hybrid material**

### **IV.1 Preparation of PVA/RS/SF hybrid material**

The PVA/RS/SF hybrid materials were prepared by 2 methods;

(1) UV-light exposure: the PVA/RS/SF hybrid material was fabricated by placing the PVA/RS/SF gel ( $2 \times 2 \times 0.5 \text{ cm}^3$ ) on the glycerol modified PVA/RS/SF film ( $50 \times 50 \times 0.060 \text{ mm}^3$ ). Then the hybrid material was exposed to the UV-light at 365 nm at various exposure times (6, 12, 18 and 24 h).

(2) Simple placing (Self-healing): placing the PVA/RS/SF hydrogel on the glycerol modified PVA/RS/SF film without UV-light exposure. The interface between the surfaces of the hydrogel and the glycerol-modified film was investigated after allowing them to adhere for various time periods.

### **IV.2 Characterization of PVA/RS/SF hybrid material**

The cross-sectional morphology of the interface of the PVA/RS/SF hybrid material was observed using a Scanning Electron Microscope (SEM; JCM-6000, JEOL). For measuring the stress at break and % elongation at break, five selected pieces of the hybrid material were clamped across the bound interface and pulled parallel to the interface using IMADA force measurement (MX2-500N), with a cross-head speed of 10 mm/min. The results were reported as the average values. In addition, gel fraction and % porosity of the hybrid material were also determined using equations (1) and (3), respectively. Finally the surface properties on each surface of the hybrid material were investigated by measuring the water contact angle.



## CHAPTER 3

### RESULTS AND DISCUSSION


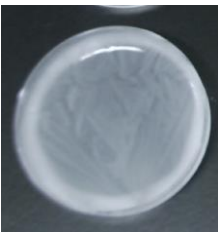








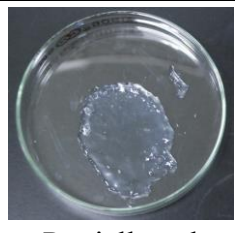

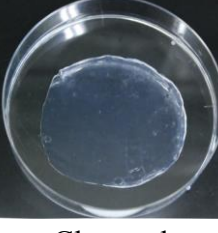
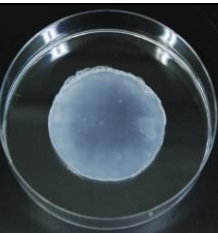
As described in the previous chapter, this study was carried out in four parts: (1) preliminary study: the PVA, RS and PVA/RS hydrogels were prepared and observed their physical properties to check for the appropriate preparation process, (2) the PVA/RS/SF hydrogels were prepared with various contents of SF addition by freezing-thawing method. The properties of the PVA/RS/SF hydrogels were characterized in order to determine an appropriate SF content for further preparation of glycerol modified PVA/RS/SF film and PVA/RS/SF hybrid material. (3) In order to improve the surface properties of the PVA/RS/SF hydrogels, the hydrophobic glycerol modified PVA/RS/SF blended films were prepared by water treatment and characterized their properties. The effect of water soaking time on hydrophobicity and mechanical properties of the hydrophobic films were investigated. (4) The PVA/RS/SF hybrid material was prepared by placing the PVA/RS/SF hydrogel on the glycerol modified PVA/RS/SF film. The interface between the surfaces of the hydrogel and the glycerol-modified film was investigated after allowing them to adhere for various time periods. The obtained results are reported and discussed as following.

#### **Part I: Preliminary study: Preparation of PVA, RS and PVA/RS hydrogels**

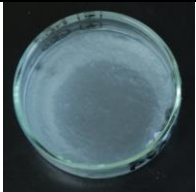
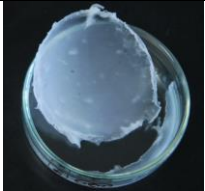
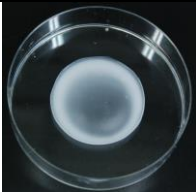
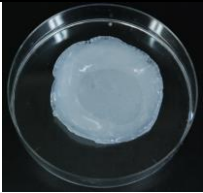
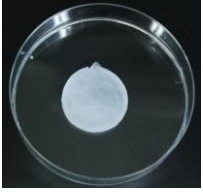
The PVA and PVA/RS hydrogels with various % hydrolysis and molecular weights were prepared by the freezing-thawing method. Some physical appearances of these PVA and PVA/RS hydrogels are shown in **Table 3.1** and **Table 3.2**, respectively.

From the observed results in **Table 3.1**, the PVA gel could be achieved at the content of PVA in water solution of about 10 % w/v by using the freezing-thawing method. The gel forming depends on the concentration, type of polymer (% hydrolysis and molecular weight) and number of freezing-thawing cycles. The partially hydrolysis polymers were poorly formed gel (PVA 3) and also did not form gel (PVA 1 and PVA 2) but the PVA 4 and PVA 5 which were the completely hydrolysis polymers can be successfully prepared as the gel. In the case of the partially hydrolysis polymers at the same % hydrolysis of about 86-90 % mol (PVA 2 and PVA 3), the PVA 3 had the better gel form than PVA 2 because of its high molecular weight. However, the polymers with the same molecular weight of 900-1000 g/mol (PVA 3 and PVA 4) but different % hydrolysis, they had different gel forming properties. The PVA 4 gel is a good gel form while the PVA 3 gel is a poor gel form due to the % hydrolysis. In addition, the increase of freezing-thawing cycles affects the gel properties that can be noticed for the PVA 5 (a completely hydrolysis and high molecular weight). The PVA 5 gel, with increasing freezing-thawing cycle, becomes a turbid gel at 8 cycles. From **Table 3.2**, the addition of RS may be able to reduce the freezing-thawing cycle for the gel preparation because the RS structure is large in its molecular chain having the –OH groups capable of interacting with the –OH groups of PVA. The obtained PVA/RS gels were a quite turbid gel.

**Table 3.1** The physical appearances of PVA hydrogels with various % hydrolysis and molecular weight after freezing-thawing at various cycles

PVA gels	Number of freezing-thawing cycles			
	5	8	15	25
PVA 1 gel 78-82 % 2000 g/mol	 Clear solution	 Clear solution	 Clear solution	 Clear solution
PVA 2 gel 86.5-89 % 500 g/mol	 Clear solution	 Clear solution	 Clear solution	 Clear solution
PVA 3 gel 86-90 % 900-1000 g/mol	 Clear solution	 Clear solution	 Partially gel	-
PVA 4 gel 96 % 900-1000 g/mol	 Clear solution	 Clear gel	-	-
PVA 5 gel ≥ 99 % 85000- 124000 g/mol	Clear gel	 Turbid gel	-	-

**Table 3.2** The physical appearances of PVA/RS hydrogels with various % hydrolysis and molecular weights after freezing-thawing at various cycles

PVA/RS gels	Number of freezing-thawing cycles		
	3	6	15
PVA 1/RS gel 78-82 % 2000 g/mol	Turbid solution	Turbid solution	 Poorly gel
PVA 2 /RS gel 86.5-89 % 500 g/mol	Turbid solution	Turbid solution	High viscosity turbid solution
PVA 3/RS gel 86-90 % 900-1000 g/mol	Turbid solution	High viscosity turbid solution	 Partially gel
PVA 4 /RS gel 96 % (Completely) 900-100 g/mol	Turbid solution	 Turbid gel	-
PVA 5/RS gel ≥ 99 % (Completely) 85000-124000 g/mol	Turbid solution	 Turbid gel	-
RS gel	 Turbid gel	-	-

From the results in Part I, the gel forming depends on the concentration, type of polymer (% hydrolysis and molecular weight) and number of freezing-thawing cycles. The addition of RS can help reduce the number of freezing-thawing cycles. Therefore, in the next step, 10 % w/v of PVA 5 ( $\geq 99$  % hydrolysis (completely), 85000-124000 g/mol) that have high % hydrolysis and molecular weight was used for preparing the PVA/RS/SF hydrogels with various SF contents.

## **Part II: Preparation and characterization of PVA/RS/SF hydrogels**

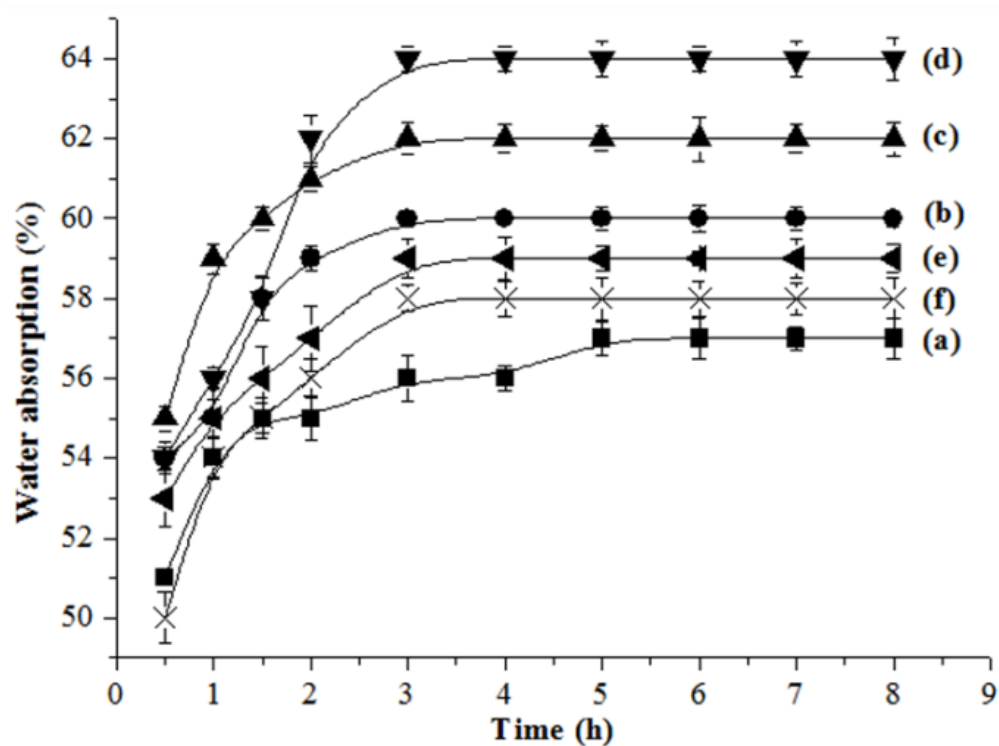
Properties of all obtained hydrogels of PVA:RS (2:1 by weight) with the SF contents ranging from 2.67-13.3 php were characterized in order to obtain an appropriate SF content for preparing glycerol modified PVA/RS/SF film and PVA/RS/SF hybrid material further.

### **II.1 Percentages of gel fraction and water absorption**

The optimum conditions for preparing the PVA/RS/SF hydrogel can be evaluated from the gel fraction values and water absorption data shown in **Table 3.3** and **Figure 3.1**, respectively.

**Table 3.3** Gel fraction (G) of PVA/RS/SF hydrogel with various SF contents

	SF content (php) in the PVA/RS/SF hydrogel					
	0.00	2.67	5.33	8.00	10.7	13.3
% gel fraction	85.2 $\pm$ 0.8	86.4 $\pm$ 0.5	87.3 $\pm$ 0.3	89.4 $\pm$ 0.5	84.7 $\pm$ 0.6	81.9 $\pm$ 0.7



**Figure 3.1** Water absorption of (a) the PVA/RS hydrogel, and the PVA/RS/SF hydrogels with the SF contents of (b) 2.67 php (c) 5.33 php (d) 8.00 php (e) 11.7 php and (f) 13.3 php after freezing/thawing for 7 cycles

Results in **Table 3.3** demonstrate that the percentages of gel fraction of the PVA/RS/SF mixtures at various SF contents are in the range of 82-89 %. Within this range, it appears that the gel fraction values increase with the increase of SF content until reaching the maximum value at the SF content of 8.00 php. Afterward, the more SF is added, the lower the values of gel fraction of the hydrogel are observed. As the SF content increases, more SF molecules enter the network of PVA/RS blend to form gel. However after the maximum amount of SF is reached, the rest of the increased SF could not be part of the gel formation and is washed out later, thus lowering the gel

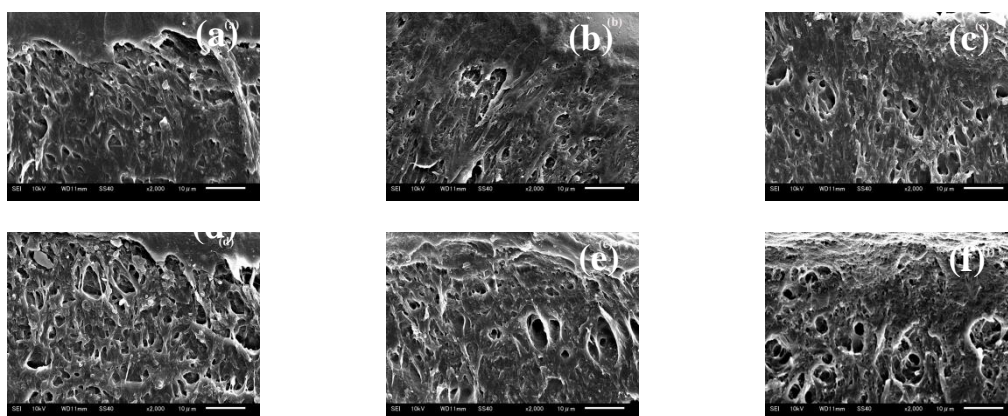


fraction values.

Since water can be absorbed by the PVA/RS/SF hydrogels quite easily due to the hydrophilic nature of the hydrogel, so the extent of water absorption of the PVA/RS/SF hydrogels in **Figure 3.1** agrees with the result of the gel fraction that the largest water absorption occurs at the highest gel fraction.

## II.2 Morphology

The SEM images in **Figure 3.2** reveal the porous structures of the PVA/RS/SF gels with various SF contents. As expected from the water absorption study, porosity within the PVA/RS/SF gel seems to increase with the increase of the SF content up to 8.00 php. However, when the SF content is more than 8.00 php, **Figure 3.2** (e-f), the pore sizes of the gels decrease with the increase of the SF content. This result shows that SF could help forming more porosity of the PVA/RS/SF gel.



**Figure 3.2** SEM images of (a) the PVA/RS hydrogel, and the PV/RS/SF hydrogels with the SF contents of (b) 2.67 php (c) 5.33 php (d) 8.00 php (e) 11.7 php and (f) 13.3 php after freezing/thawing for 7 cycles

### II.3 Hydrogel porosity and mechanical properties

To compare the porosity and mechanical properties of the PVA/RS/SF hydrogels with various SF contents, the porosity, stress at break and elongation at break of the PVA/RS/SF hydrogels with various SF contents were tested and the results are shown in **Table 3.4**.

**Table 3.4** Porosity and mechanical properties of PVA/RS/SF hydrogels with various SF contents

SF contents (php) in the PVA/RS/SF hydrogel	Mechanical properties		
	Porosity (%)	Stress at break (MPa)	% Elongation at break
0.00	50.0±0.8	0.64±0.03	132.89±7.50
2.67	52.3±0.7	0.29±0.07	76.98±2.49
5.33	60.0±1.2	0.35±0.04	85.74±5.28
8.00	71.4±0.7	0.45±0.12	105.45±2.72
10.7	66.7±1.0	0.43±0.10	78.31±3.70
13.3	66.4±0.5	0.42±0.19	76.41±2.12

From **Table 3.4**, the PVA/RS/SF hydrogels show similar trend of their porosity and mechanical properties on being SF dependent. The hydrogel with 8.00 php SF also exhibits the highest porosity which is agreeable with the gel fraction and water absorption values. Moreover, the maximum values of stress at break and % elongation at break in the presence of SF are also found when 8.00 php of SF is added. However, the stress at break and % elongation at break of the PVA/RS/SF hydrogels are lower

than those of the PVA/RS hydrogel without SF. This can be justified by the generated porosity due to the addition of SF and because most of the silk materials developed from silk fibroin solution are weak and brittle [48].

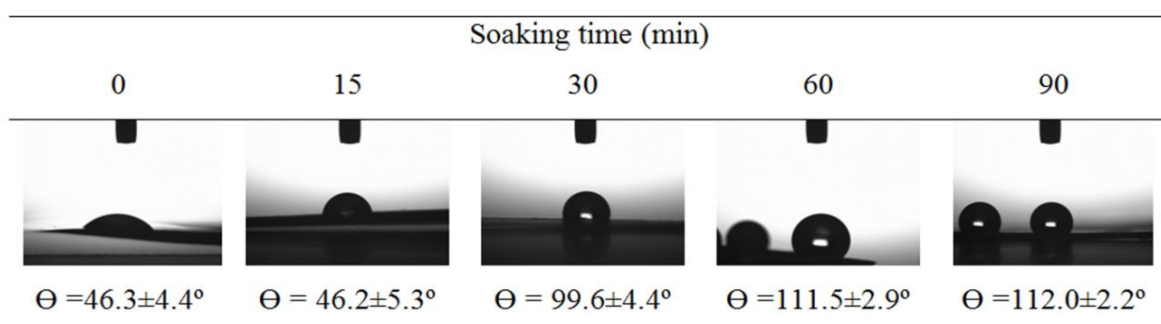
From the above mentioned results, the optimal content of SF in the PVA/RS/SF hydrogel, with 2:1 weight ratio of PVA:RS, was therefore selected at 8.00 php. This optimal condition was used for preparing the glycerol-modified PVA/RS/SF film and also the PVA/RS/SF hybrid material with an opposite water affinity on each surface.

### Part III: Preparation and characterization of glycerol modified PVA/RS/SF films

#### III.1 Film hydrophobicity

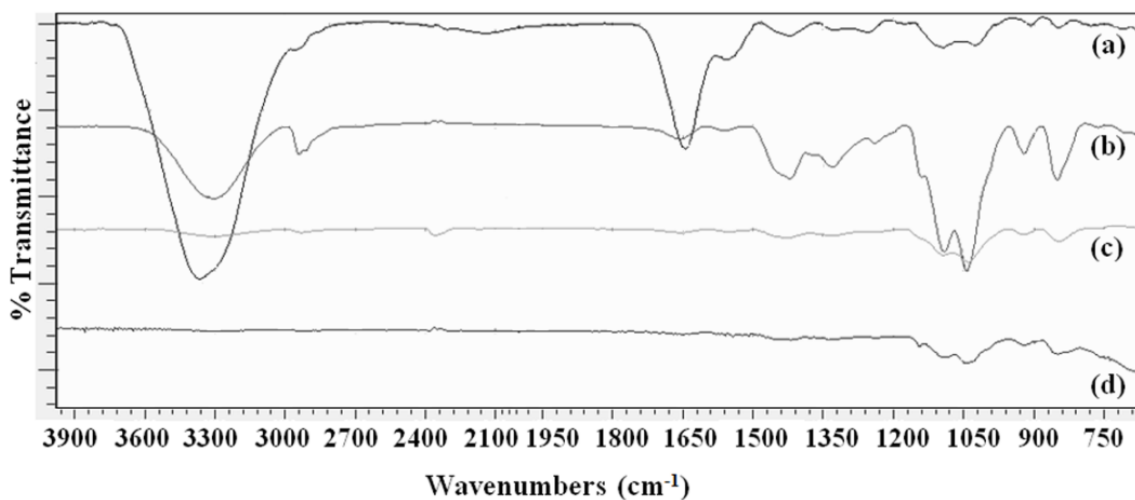
From our previous study [66], hydrophobicity of the glycerol-modified films was successfully achieved by ethanol treatment. However, in order to avoid using an organic solvent, water was used to replace ethanol in enhancing hydrophobicity of the film. Then its hydrophobicity was investigated through the measurement of water contact angle after soaking the glycerol-modified film in water at various soaking times.

The water contact angle on the glycerol-modified PVA/RS/SF films after soaking in water at various soaking times are shown in **Figure 3.3**.



**Figure 3.3** Water contact angles on the glycerol modified PVA/RS/SF films after soaking in water at different soaking times

Results in **Figure 3.3** reveal that the glycerol-modified films become hydrophobic after soaking in water for 30 min and their hydrophobicity slowly increases at longer soaking time, evidenced by the increase of water contact angles. This is because the glycerol molecules interact with PVA, RS and SF molecules in the blend through hydrogen bonding between the hydroxyl groups of glycerol, PVA, RS and the amide groups of SF [71, 72]. However, some of the glycerol molecules remain unbound, causing more interaction of the hydroxyl groups of unbound glycerol molecules in the glycerol-modified film with water molecules in the water drop. Thus, the water drop would be attracted and adhered more to the surface of the film resulting in less contact angle which indicates less hydrophobic character. Once the films are soaked in water, water molecules are allowed to penetrate the blend and remove more of the unbound glycerol molecules. It was found that 60 min is long enough to remove such glycerol.

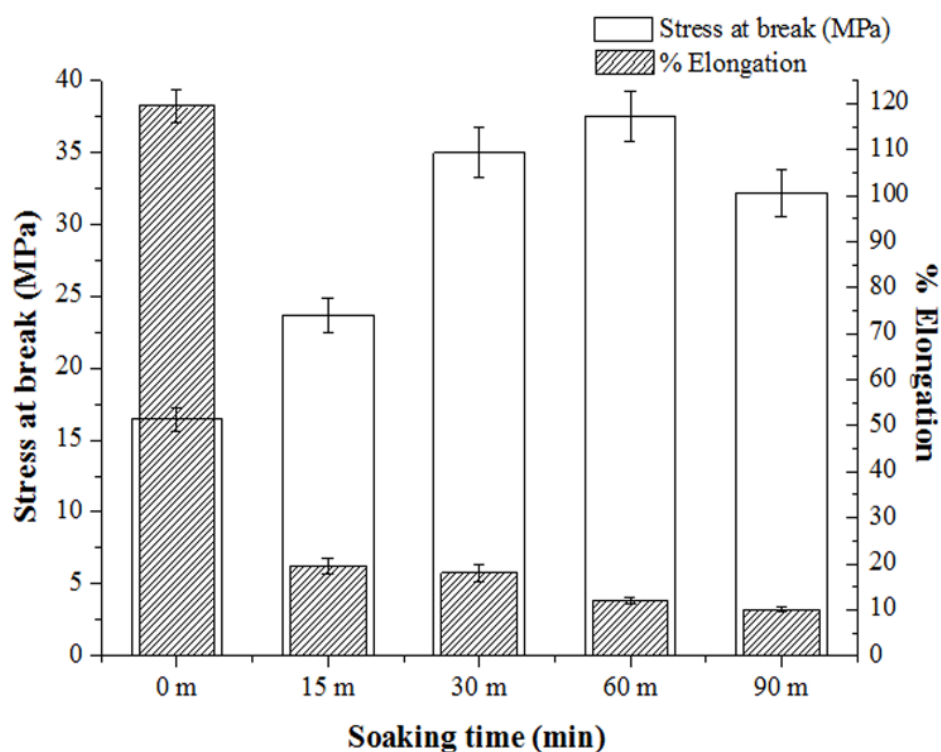


**Figure 3.4** ATR spectra of (a) the glycerol-modified PVA/RS/SF, and the glycerol-modified films after soaking in water for various time intervals of (b) 15 min, (c) 30 min and (d) 60 min

As the water contact angle of the glycerol-modified PVA/RS/SF film increases with the increase of soaking time, therefore in order to confirm the removal of glycerol, its content in the PVA/RS/SF films after soaking in water at various time intervals was followed from the ATR spectra shown in **Figure 3.4**. Considering the  $\text{-OH}$  group stretching vibration in the region of  $3600\text{-}3000\text{ cm}^{-1}$ , after soaking the modified film in water for longer time, the spectra show more disappearance of  $\text{-OH}$  band indicating that more unbound glycerol molecules are removed from the films.

### III.2 Mechanical properties

Mechanical properties in terms of the stress at break and % elongation of the glycerol-modified films after soaking in water at various soaking times are shown in **Figure 3.5**.



**Figure 3.5** Stress at break and % elongation at break of the glycerol modified PVA/RS/SF films after soaking in water

From **Figure 3.5**, the increase in soaking time causes the increase of the film strength but decreases its flexibility. Since glycerol is a plasticizer, its function is to reduce the phase separation between PVA, RS and SF by forming hydrogen bonding between the blended materials so that elongation at break of the film is improved. After water-treatment, the amount of glycerol is reduced which causes the higher strength and

less flexibility in the film. However, at soaking times longer than 60 min, the film strength is decreased because the increasing soaking time allows more glycerol within the blend to be removed, in addition to the unbound glycerol molecules.

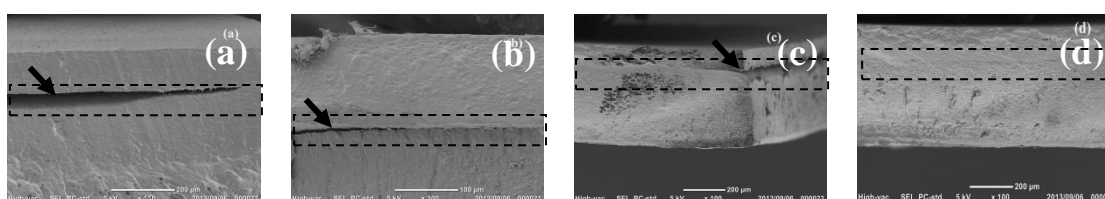
From the above results, 60 min was considered to be appropriate for soaking the glycerol modified film in water so that it can be used further for preparing the hybrid material.

## Part IV: Preparation and characterization of PVA/RS/SF hybrid material

### IV.1 UV-light exposing

#### IV.1.1 Morphology

The cross-sectional SEM images of the PVA/RS/SF hybrid material after exposing to UV-light at various times was observed and the images are shown in **Figure 3.6**.



**Figure 3.6** Cross-sectional SEM images of the PVA/RS/SF hybrid material after exposing to UV-light for (a) 6 h (b) 12 h (c) 18 h and (d) 24 h

The cross-sectional SEM images in **Figure 3.6** reveal the interface between the PVA/RS/SF gel and the glycerol modified PVA/RS/SF film of the PVA/RS/SF hybrid materials after exposing to the UV-light at various exposure periods. The hybrid material happened to be homogeneously blended after 24 h exposure.

#### IV.1.2 Porosity, hydrophilicity, hydrophobicity and mechanical properties

**Table 3.5** Porosity, water contact angle and mechanical properties of PVA/RS/SF hybrid materials with various UV-light exposure periods

Hybrid materials with various UV-light exposure periods (h)				
	6	12	18	24
Porosity (%)	71.8	71.7	71.5	71.4
Water contact angle ( $\Theta$ )				
<i>Gel side</i>	ND	ND	ND	ND
<i>Film side</i>	103.3 $\pm$ 2.3 $^\circ$	103.8 $\pm$ 3.7 $^\circ$	104.4 $\pm$ 1.9 $^\circ$	106.5 $\pm$ 1.4 $^\circ$
Mechanical properties				
<i>Stress at break (MPa)</i>	-	-	-	45.37 $\pm$ 2.4
<i>% Elongation</i>	-	-	-	17.75 $\pm$ 2.0

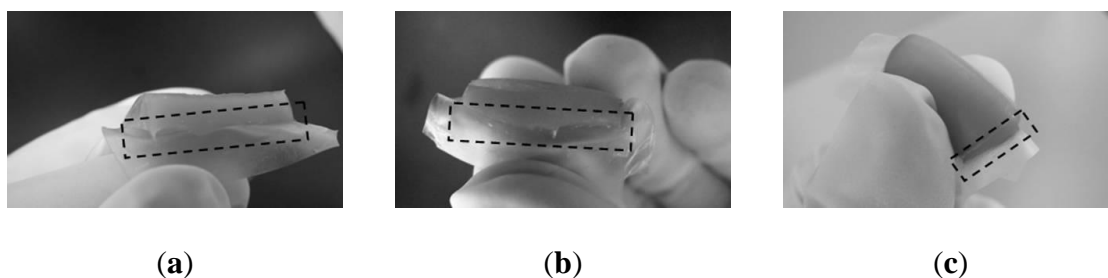
Result in **Table 3.5** demonstrates that the porosity of the hybrid material is not affected by UV-light exposure, whereas the water contact angles of the surface of the film side slightly increase up to 106.5 $\pm$ 1.4 $^\circ$  after 24 h UV-light exposure. The mechanical properties of the hybrid material after 24 h UV-light exposure (homogeneously blended) are quite low due to its dried state material.



## IV.2 Simple placing (Self healing)

### IV.2.1 Physical appearance and interfacial morphology

The physical appearance of the PVA/RS/SF hybrid material after layering at various times was observed and the images are shown in **Figure 3.7**.

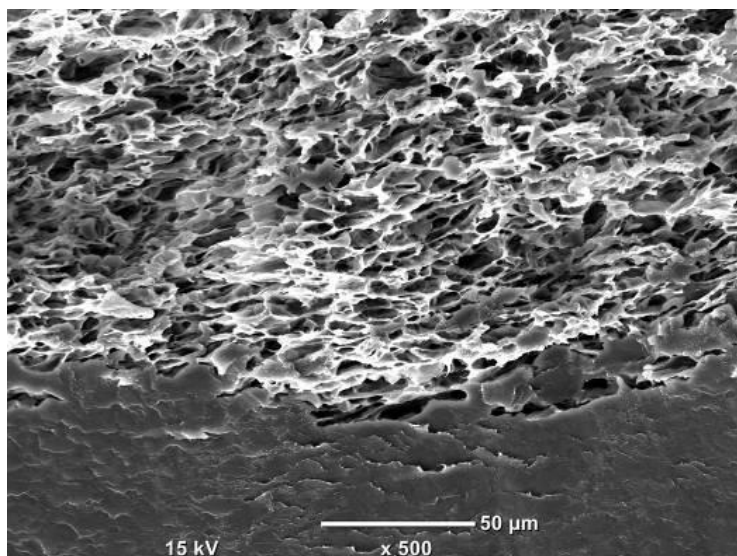


**Figure 3.7** Photos of the PVA/RS/SF hybrid material after layering at room temperature for (a) 18 h, (b) 24 h and (c) 30 h

The photos in **Figure 3.7** show the interface between the PVA/RS/SF hydrogel and the glycerol modified PVA/RS/SF film within the PVA/RS/SF hybrid material after layering at various times. The two layers of the hybrid material are completely bound together after layering the hydrogel on top of the glycerol-modified film for 30 h in which there is no cleavage between the layers observed. This can be justified by the fact that the bonding process occurs through the formation of hydrogen bonding between the hydroxyl groups of the PVA chains. It is easy to picture that the hydrogen bonds responsible for the bonding should essentially be those formed between PVA chains on both sides of the interface and/or those between PVA chains on one side and PVA chains diffusing across the interface from the other side when the two surfaces are

brought into contact [67].

The morphology of the PVA/RS/SF hybrid material after layering for 30 h was observed by SEM technique and the images are shown in **Figure 3.8**.



**Figure 3.8** Cross-sectional SEM images showing the interface within the PVA/RS/SF hybrid material after layering for 30 h

The cross-sectional SEM image in **Figure 3.8** also confirms the existing bound interface within the hybrid material after 30 h of layering. This image also reveals that the structures of the hydrogel layer appear to be porous whereas the blended film layer is more dense.

#### IV.2.2 Gel fraction, porosity and mechanical properties

To compare some properties of the PVA/RS/SF hybrid material with the PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film, the gel fraction, porosity and mechanical properties of the PVA/RS/SF hybrid material, the PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film were tested and the results are shown in **Table 3.6**.

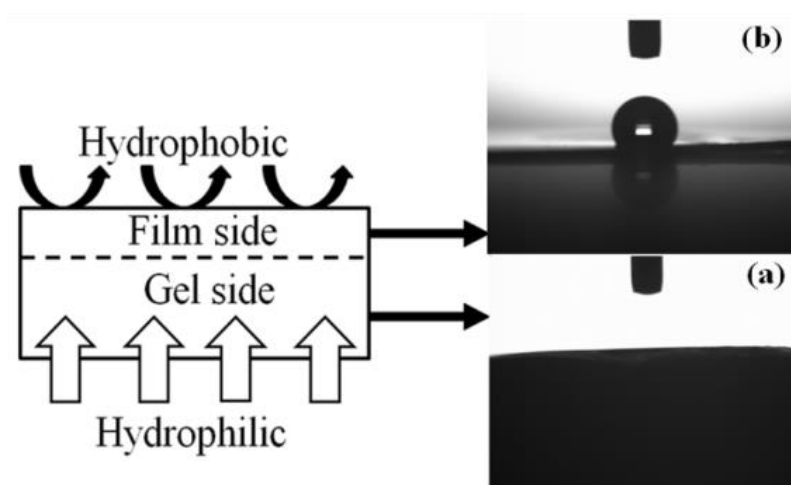
**Table 3.6** Comparison of some properties of the PVA/RS/SF hydrogel, the glycerol-modified PVA/RS/SF film, and the PVA/RS/SF hybrid material at the same optimal composition of 2:1 weight ratio of PVA:RS and 8.00 php SF

Properties	PVA/RS/SF		
	Hydrogel	Glycerol-modified film	Hybrid material
Gel fraction (%)	89.4±0.5	-	90.1±0.3
Porosity (%)	71.4±0.7	-	71.0±1.1
Mechanical properties			
- <i>Stress at break (MPa)</i>	0.45±0.12	37.53±1.75	1.7±0.1
- <i>Elongation at break (%)</i>	105.45±2.72	12.01±0.60	118.4±1.6

Results in **Table 3.6** reveals that after coupling the PVA/RS/SF hydrogel with the glycerol-modified PVA/RS/SF film, the gel fraction and percent porosity of the PVA/RS/SF hybrid material do not change that much. However, the stress at break and % elongation at break of the hybrid material are a bit higher than those of the

hydrogel itself, whereas, the elongation at break of the hybrid material is higher than that the glycerol-modified film. Hence, these results show the improvement of the mechanical properties of the PVA/RS/SF hybrid material indicating that the structure of the PVA/RS/SF hybrid material is stronger than that of the PVA/RS/SF hydrogel and more flexible than both PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film.

#### IV.2.3 Surface property and water absorption



**Figure 3.9** Demonstration of water affinity behavior of the PVA/RS/SF hybrid material and water absorption on each surface of the (a) PVA/RS/SF hydrogel and (b) glycerol modified PVA/RS/SF film, after complete adhering

**Figure 3.9** depicts the water affinity behavior of the PVA/RS/SF hybrid material. As evidenced by a large value ( $110.4 \pm 3.4^\circ$ ) of water contact angle and water drop formation observed on the surface of the glycerol-modified film, this indicates the

hydrophobic nature of the surface. On contrary, when water was dropped on the surface of the hydrogel layer, it was quickly absorbed, thus its water contact angle of the PVA/RS/SF hydrogel surface could not be detected. So the hydrogel layer of the hybrid material is very strongly hydrophilic. These results of the water affinity behavior of the prepared PVA/RS/SF hybrid material suggest an approach to produce a bio-hybrid material with opposite water affinity on its surfaces.

## CHAPTER 4

### CONCLUSION

The PVA, RS and PVA/RS hydrogels were prepared by the freezing-thawing method. The gel forming depended on the polymer concentration, types of polymer (% hydrolysis and molecular weight) and number of freezing-thawing cycles. The addition of RS could help reduce the number of freezing-thawing cycles. Therefore, the 10 % w/v of PVA with 99 % hydrolysis (completely) and molecular weight of 85000-124000 g/mol was used for preparing the PVA/RS/SF hydrogels with various SF contents and the PVA/RS/SF hybrid material.

In order to improve the oxygen permeability of PVA/RS hydrogel, the SF was selected for adding into the PVA/RS gel with various contents because of its good oxygen permeability. Some properties of the PVA/RS/SF hydrogels after SF addition were investigated. At 8.00 php of SF, the PVA/RS/SF hydrogels showed the highest value of % gel fraction, water absorption, porosity and mechanical properties. The optimal content of SF in the PVA/RS/SF hydrogel, with 2:1 weight ratio of PVA:RS, was therefore selected at 8.00 php. This optimal condition was used for preparing the glycerol-modified PVA/RS/SF film and also the PVA/RS/SF hybrid material with an opposite water affinity on each surface.

To improve the surface properties of the PVA/RS/SF hydrogel to be hydrophobic, the hydrophobic glycerol-modified PVA/RS/SF films were prepared by the addition of

glycerol then the glycerol was removed by treating with water to improve the hydrophobicity of the blended films. In addition, the effect of glycerol addition and also soaking time on hydrophobicity and mechanical properties of the hydrophobic films were investigated. The hydrophobic PVA/RS/SF films were successfully prepared by the addition of glycerol and followed by water treatment. The water contact angle of the glycerol-modified film clearly increased from  $46.3\pm 4.4^\circ$  to  $111.5\pm 2.9^\circ$  after soaking in water for 60 min. Moreover, the stress at break of the water-treated glycerol-modified film became highest at 60 min of water soaking time.

The PVA/RS/SF hybrid material with opposite water affinity on each surface was successfully prepared. The PVA/RS/SF hydrogel was coupled to the glycerol modified PVA/RS/SF film and they were left for bonding for 30 h at room temperature. The content of SF for preparing the layers of hybrid material was initially optimized from the specific properties of the PVA/RS/SF hydrogel. The addition of SF produced more porosity in the hydrogel. In addition, the water absorption, gel fraction and mechanical properties of the hydrogel were also dependent on the porosity of the hydrogel. The optimal SF content was selected at 8.00 php in which the gel fraction of the PVA/RS/SF hydrogel was about 89% after 7 cycles of freezing/thawing. The PVA/RS/SF film modified with glycerol became hydrophobic by simply soaking it in water. The hydrophobicity of the glycerol-modified film was slowly increased by increasing the soaking time up to 60 min as the excess glycerol was leached out. The obtained PVA/RS/SF hybrid material had better mechanical properties than the PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film itself. Results from the measurement of water contact angle on both surfaces of the hybrid material indicated

that the surface of the PVA/RS/SF hydrogel layer was hydrophilic while the layer of glycerol-modified PVA/RS/SF film was hydrophobic with a water contact angle of about 110°.

We presume that the opposite water affinity on each surface can give a bandage the advantage of isolating a wound from the external environment while protecting the wound bed from dehydration to facilitate the healing process. Therefore, this study provided an approach for preparing a novel bio-hybrid material with special water affinity for wound dressing application that could be obtained from renewable sources.



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- 1) Kuchaiyaphum P., Punyodom W., Watanesk S. and Watanesk R., “Composition optimization of polyvinyl alcohol/rice starch/silk fibroin-blended films for improving its eco-friendly packaging properties”, *J. Appl. Polym. Sci.*, 129, 2013, 2614-2620.
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- 3) Kuchaiyaphum P., Watanesk R., Watanesk S. and Yamauchi T., “A bio-hybrid material with special water affinity made from polyvinyl alcohol, rice starch and silk fibroin”, *IJASR*, 2, 2014, 1-12.

## CURRICULUM VITAE

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#### Publications

- 1) Kuchaiyaphum P., Punyodom W., Watanesk S. and Watanesk R., "Composition optimization of polyvinyl alcohol/rice starch/silk fibroin-blended films for improving its eco-friendly packaging properties", *J. Appl. Polym. Sci.*, 129, 2013, 2614-2620.
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- 3) Kuchaiyaphum P., Watanesk R., Watanesk S. and Yamauchi T., "A bio-hybrid material with special water affinity made from polyvinyl alcohol, rice starch and silk fibroin", *IJASR*, 2, 2014, 1-12.

#### Experience

- 1) Research on "Development of eco-friendly film made from silk fibroin" in the Graduate School of Science and Technology from October 2012 to March 2013 at Niigata University, Niigata, Japan.

2) Further study in the Double degree program between Niigata University and Chiang Mai University at the Graduate School of Science and Technology from May 2013 to June 2014 at Niigata University, Niigata, Japan.

## Conferences

### International Conference Participation:

Chiang Mai International Conference on Biomaterials & Applications 2011 (CMICBA 2011) between August 9-10, 2011 at The Empress Hotel, Chiang Mai, Thailand.

The 14th Asian Chemical Congress 2011 (14ACC) between September 5-8, 2011 at Queen Sirikit National Convention Center, Bangkok, Thailand.

Pure and Applied Chemistry International Conference (PACCON 2012): Chemistry Beyond Boundaries between January 11-13, 2012 at The Empress Hotel Convention Center, Chiang Mai, Thailand.

IUPAC World Polymer Congress (MACRO 2014) between January 6-11, 2014 at Chiangmai International Convention and Exhibition Centre, Chiang Mai, Thailand.

### International Conference Presentation:

Oral presentation on "Optimization of PVA/RS/SF composition for improving its eco-friendly packaging properties" and participation in The 1st ASEAN Plus Three Graduate Research Congress (AGRC 2012) between March 1-2, 2012 at The Empress Hotel Convention Center, Chiang Mai, Thailand.

Oral presentation on “Hydrophobicity enhancement of eco-friendly films made from poly(vinyl alcohol), rice starch and silk fibroin” and participated in 2013 Pre-Fusion Tech Conference on February 20, 2013 at Hanyang University, Seoul, Korea.

Oral presentation on “Hydrophobicity enhancement of the polyvinyl alcohol/rice starch/silk fibroin films by glycerol” and participation in The 3rd International Conference on Nanomaterials and Electronics Engineering (ICNEE 2013) between October 5–6, 2013 at Bayview Hotel Melaka, Malaysia.

Oral presentation on “A new approach for preparing bio-hybrid material of PVA/RS/SF with special water affinity” and participation in The 3rd International Conference on Engineering and Applied Science (2013 ICEAS) between November 7-9, 2013, RIHGA Royal Hotel, Osaka, Japan.

Oral presentation on “Preparation of PVA/RS/SF bio-hybrid material with special water affinity” and participation in The 4th International Symposium on Fusion Tech 2013-2014 between January 15-17, 2014, Hanyang University, Seoul, Korea.

Poster presentation on “An approach on blending silk fibroin with polymers for eco-friendly food packaging films” and participated in International Congress for Innovative Chemistry (PERCH-CIC VII): Chemistry, Environment and Society, between May 4-7, 2011 at Jomtien Palm Beach Hotel & Resort, Pattaya, Thailand.

Poster presentation on “Water solubility improvement of the eco-friendly films made from poly(vinyl alcohol), rice starch and silk fibroin” and participation in the International Chemistry PERCH-CIC Congress VIII between May 5-8, 2013 at Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, Thailand.

Poster presentation on “The effect of silk fibroin content on the properties of polyvinyl alcohol/rice starch gel” and participation in the International Symposium on Advanced Soft Materials between October 15-17, 2013 at Hokkaido University, Hokkaido, Japan.