STUDY OF MECHANICAL AND THERMAL PROPERTIES OF HYDROGEL FILM FROM SILK WASTE AND POLY (VINYL ALCOHOL)

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หัวข้อวิทยานิพนธ์	การศึกษาสมบัติเชิงกลและสมบัติทางความร้อนของฟิล์มไฮโดรเจลจากเศษไหมเหลือทิ้งและพอลิไวนิลแอลกอฮอล์
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บทคัดย่อ

การใช้ฟิล์มไฮโดรเจลเป็นวัสดุที่ใช้อย่างกว้างขวางทางด้านการแพทย์ นั่นคือใช้เป็นผ้าปิดบาดแผล เนื่องจากฟิล์มไฮโดรเจลมีโครงสร้างเป็นตาข่ายสามมิติ จึงช่วยในการระบายอากาศและกำจัดน้ำส่วนเกินในบาดแผลได้ดี ในการทดลองนี้ทำการผลิตฟิล์มไฮโดรเจลระหว่างเศษไหมเหลือทิ้งและพอลิไวนิลแอลกอฮอล์ อัตราส่วนของเศษไหมเหลือทิ้งและพอลิไวนิลแอลกอฮอล์คือ 1:3 โดยปริมาตร งานวิจัยนี้เป็นการศึกษาการเตรียมฟิล์มไฮโดรเจลหลายวิธี วิธีแรกเตรียมฟิล์ม PVA วิธีที่สองเพิ่มความเป็นผลึกของฟิล์มผสมโดยการให้ความร้อนที่อุณหภูมิ 100 °C เป็นเวลา 4 นาที วิธีที่สามฉายรังสีแกมมาในปริมาณ 20 กิโลเกรย์ (kGy) ภายใต้ไนโตรเจน วิธีที่สี่เตรียมฟิล์มผสมระหว่าง PVA และไหม วิธีที่ห้าใช้ผงไหมบดเป็นวัตถุดิบในการผสมกับพอลิไวนิลแอลกอฮอล์ วิธีสุดท้ายเตรียมฟิล์มจากไฟโบรอิน พบว่าเปอร์เซ็นต์การดูดซึมน้ำของฟิล์มที่ได้จากวิธีให้ความร้อนแผ่นฟิล์มมีค่าสูงที่สุด ส่วนฟิล์มที่ผ่านการฉายรังสีและการใช้ผงไหมมีสมบัติการดูดซึมน้ำได้น้อย โดยรวมแล้วฟิล์มทุกชนิดมีการดูดซึมน้ำได้เร็วมากในช่วง 24 – 48 ชั่วโมงแรก การศึกษาค่าความแข็งแรงดึงและเปอร์เซ็นต์การยืดออกพบว่าฟิล์มที่ผ่านกระบวนการทางความร้อนมีสมบัติความแข็งแรงดึงและเปอร์เซ็นต์การยืดสูงกว่าฟิล์มชนิดอื่น ซึ่งตรงกับผลของ XRD แสดงให้เห็นว่าฟิล์มที่ผ่านความร้อนมีปริมาณผลึกมากขึ้น ผลทาง TGA และ DSC แสดงให้เห็นว่าค่าความเสถียรทางความร้อนของ PVA ลดลงเมื่อมีไหมผสมอยู่ในฟิล์ม การวิเคราะห์ด้วยแสงอินฟราเรดพบว่าการเกิดพันธะไฮโดรเจนในสองเฟสยังไม่ชัดเจน ภาพถ่ายจากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกวาด (SEM) พบว่าพื้นผิวของฟิล์มผสมไม่เกิดการแยกเฟสอย่างชัดเจน แต่เมื่อผ่านความร้อนทำให้ผิวหน้าของฟิล์มแตก ส่วนฟิล์มที่ทำจากผงไหมมีผิวเรียบกว่าชนิดอื่นๆ

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ABSTRACT

Hydrogel film widely used for medical devices such as wound dressing. The three dimensional network structure of this film enhance wound breathing and releasing of exudate from wet wound. In this experiment, the ratio of silk waste and poly (vinyl alcohol) (PVA) is 1:3 by volume. Several methods were used to develop good silk/PVA film hydrogel. First method is preparation of PVA film. Second method, increase the close packing and crystallzation of blend film, thermal treament at 100 °C for 4 minutes. Third method, PVA/SF solution was gamma irradiated at 20 kGy under nitrogen. Forth method, prepared PVA/SF blend film. Fifth method, milled silk powder blended with poly (vinyl alcohol). The final method is preparation of silk fibroin film. The results indicated that percentage of water uptake of the first method film was highest. While irradiated and silk powder film showed low percentage of water uptake. Generally, all blends were rapid water uptake at first 24 – 48 hours. The study of tensile strength and elongation at break of the blend was found that the heat treated film was the highest. The results of XRD showed that crystallinity of heat treated film was increased. Results from TGA and DSC showed that thermal stability of blend film was decreased when used silk on blending film. The thermal decomposition of blend films trended to slightly increase. Infrared analysis was found that hydrogen bonding of two phase was not fully investigated. SEM images of the blend showed not clear on phase separation. After heat treatment, the surface of blend film occurred many cracks while the silk powder film was smoother surface than other films.

Keywords : hydrogel, wound dressing, silk waste, poly (vinyl alcohol), blend

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Nopaklao Tongbanbor

CONTENTS

CONTENTS (CONTINUED)

CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

LIST OF ABBREVIATIONS

A0	Poly (vinyl alcohol) film
A1	Heat treated film
A2	Irradiated film
A3	Untreated film
A4	Silk powder film
A5	Silk fibroin film
Ala	Alanine
DNA	Deoxyribonucleic Acid
DSC	Differential Scanning Calorimetry
FT-IR	Fourier Transfer Infrared
Gly	Glycine
PAA-K	Poly (potassium acrylate)
PEEK	Poly (aryl ether ether ketone)
PEG	Poly (ethylene glycol)
PVA	Poly (vinyl alcohol)
PVAc	Poly (vinyl acetate)
SEM	Scanning Electron Microscope
Ser	Serine
SF	Silk Fibroin
TGA	Thermo Gravimetric Analysis
XRD	X-ray Diffraction

CHAPTER 1
INTRODUCTION

1.1 Motivation

During the past century, silk has been used for human garment. The ancient silk route was discovered by the great traveler whose name is Marco Polo (1254 – 1324) [1]. He had journeyed along this route from 1271 – 1275. In nineteenth century, commerce of silk products was decreased when the world war began. Silk road well-known as travelling route from the Mediterranean to Asia. The legacy of the past is induced many wanderers from around the world to visit this road. At present, silk is still a luxury garment for everyone who wore it. From this reason, many designers of the famous boutiques in Paris have chosen them in their cloth collection. Anyway, silk is considered not only in a luxury textile industry but also for non-textile application.

Silk fiber is excluded by silkworm which species of *Bombyx mori* and others that is widely used in many application. Silkworm is a common name for the silk-producing larvae of any of several species of moths. The larvae is not really a worm at all but a caterpillar. Silkworms possess a pair of specially modified salivery glands called silk glands or sericteries which are used for the production of silk cocoons. These glands secrete a clear, viscous, proteinous fluid that is forced through openings called spinnerets on the mouthpart of the larvae. As the fluid comes in contact with the air it hardens. The diameter of the spinneret determines the thickness of the silk thread. The silk is a continuous filament fiber consisting of fibroin protein secreted from two salivery glands in the head of each larvae and a gum called sericin which cements the two filaments together. Silk must be reeled off the cocoon quickly before the pupae begin to rot and taint the thread with unpleasant smells. Cocoons are then softened in hot water to remove the sericin which frees the silk filament ends for reeling or filature. Single filaments are drawn from cocoons in water bowls and combined to form yarn. This yarn is drawn under tension through several guides and eventually wound on to reels. The yarn is dried, packed according to quality and is now raw silk ready for marketing. One cocoon can be made up of thread as much as 3,000 meters long which is very fine and

strong. As it is well-known, the composition of this filament is 70-80 % fibroin and 20-30 % sericin, silk fibroin (SF) is available not only in form of fiber but can also prepared in form of gel, powder and compact. Surgical sutures and biosensors are developed by the enzyme immobilization technique [2]. Wound-repairing and bone-binding functions are some of the applications developed from silk.

Modern wound dressing used hydrogel film to keep the wound moist, therefore the sores are allowed to breathe. By healing via this film, they do not require frequent changing like gauze dressings which absorbed exudation without drying out the wound. Hydrogel can hydrate wounds and keep them clean. This wound care products is embranced worldwide by patients, physicians, nurses, therapists, researchers, medical instructors and health care administrators.

Hydrogels are cross-linked three-dimensional hydroplilic polymer networks that swell, but do not dissolve when brought into contact with water. With good physical and chemical properties as a membrane material, poly (vinyl alcohol), (PVA) has been widely used for the preparation of several kinds of membranes.

In this study, the characterizations of film prepared from blending silk solution and PVA solution were investigated. Several methods were used to develop good silk/PVA film hydrogels.

For the first experiment, to increase the close packing and crystallization of blend film, the film hydrogels were thermally treated at 100 $^{\circ}$C for 4 minutes [3].

Second, wet hydrogels were gamma irradiated at 20 kGy under nitrogen.

Third, the film hydrogels were made of PVA and silk powder.

1.2 Objectives

1) to prepare SF/PVA film hydrogel by casting method.
2) to study the effect of thermal treatment on film strength, water uptake and thermal stability.
3) to study the influence of radiation dose on film strength, water uptake and thermal stability.
4) to study the influence of silk powder that used to be in placed of dialysted silk in film preparation.

1.3 Scopes of study

1) preparation of 3 % silk fibroin solution from silk waste.
2) preparation of 3 % PVA solution.
3) preparation of film hydrogels by mixing 1:3 ratio of 3 % silk and 3% PVA solution.
4) study on the properties of SF/PVA film hydrogel after modifying by thermal treatment, gamma irradiation and silk powder using.

1.4 Expected Results

1) To improve value added of natural renewable polymer such as silk fibroin.
2) To get knowledge and know the way to manage silk waste.
3) To prepared a new type of hydrogel from natural polymer for medical usage.

CHAPTER 2
LITERATURE REVIEWS AND THEORY

In this introductory chapter, the detail about utilities of silk membrane, hydrogel forming and the effects of radiation on polymeric materials will be presented.

Hydrogel is a three dimensions swollen cross-linked polymer network. In the present of water, the gel will swell due to their hydrophilic components. Chemical crosslinks are occurred the insoluble parts of hydrogel film. Porous structure of hydrogel allowed for uses in the biomedical field. Some hydrogels are sensitive to the surrounding environmental conditions such as pH or temperature. Sensitivity to local environment helps in controlled release of packaged proteins, drugs or DNA. The solute packaged in the hydrogel diffuses via a concentration gradient. Hydrogels are presently used in contraceptive medicine and cancer treatment.

In the past few years, blend of silk fibroin (SF) with other natural polymers (for example algae) or synthetic polymers such as poly (ethylene glycol) (PEG) [2], poly acrylamide [4], and poly (acrylonitrile – *co* – methyl acrylate) [5] were abled to improve their properties and applications. Although, hydrogel films of homopolymer such as poly (α-amino acid) [6] was used in the areas of biomedical and biochemical.

Tsukada Masuhiro (1989) cooled the silk solution to form a hydrogel. With this method, hydrogel can be prepared without using special apparatus. He found that the strength of hydrogel can be controlled by the temperature for freezing, the fibroin concentration, the temperature of thawing and the number of thaw cycles [7].

Agarwal N., Hoagland D.A. and Farris R.J. (1996) had studied to quatify the effect of moisture on the thermal properties of regenerated silk. They examined the moisture sensitivity of the glass transition temperature (T_g). They found that the differential scanning calorimetry (DSC) thermograms depended strongly on relative humidity (RH). At 75% RH provided a T_g of 39°C. While a sample of dry film exhibited a T_g of 178°C [8].

Zhengzhong Shao, Yuyu Sun, Ping Zhou and Tongyin Yu (1998) studied on interaction of SF and poly (potassium acrylate) (PAA-K) blend. They found that there were two points which indicated blend of SF/PAA-K with FT-IR spectroscopy and DSC

results. DSC curves of homopolymer and SF/PAA-K blend from 160°C to 250°C showed the introduction of SF in PAA-K. In addition, FT-IR spectroscopy was used to study the specific interaction of SF/PAA-K blend [9].

Doo Hyun Lee, Jin Woong Kim and Kyung Do Sun (1999) a group of researchers from division of chemical engineering, Hanyang University focus on drug-releasing and swelling behaviors of amphiphilic urethane acrylate hydrogels. They compared the results from using water and organic solvent. In the drug-releasing study it was found that were controlled by the swelling behaviors. Comparison of pH 4 and pH 9 showed that at higher pH the swelling ratio were increased [10].

Sung Jin Park, Kuen Yong Lee, Wan Shik Ha and Soo Young Park (1999) studied on structural changes of SF by the addition of chitosan. The results of investigation with IR spectroscopy showed that the introduction of chitosan on SF were induced the random coil structure of SF to β pleated sheet structure. From this results, crystallinity and density of the blend films is increase [11].

Bahia Hardev S. and Burrow Thomas R. (1999) used carboxymethyl cellulose filament on the wound-contacting surface. This wound dressing can be absorbed aqueous saline solution at least 15 times by weight. The range of a cord of textile fibers were used from 3 to 15 millimeters [12].

Fumio Y., Tamikazu K., Keizo M. and Fusamitsu S. (2000) had studied on the characteristics of rubberlike elasticity and long-lasting water retention by blending a water-soluble polymer with silk protein and irradiated to cause crosslinking. They have chosen poly vinyl alcohol, poly vinyl pyrrolidone and poly ethyleneoxide as water-soluble polymer. The source of silk protein was from a silkworm powder or silk fibers. A containing water-soluble parts were mixed together. The concentration of water-soluble polymer was 5 to 15%. While percentage of silk protein was 10-20 %. Gamma ray was used for irradiated mixed solution [13].

Zhengqui G. and Jiumei X. (2000) worked for the preparation of hemicrystalline poly vinyl alcohol hydrogel to body's joint cartilage with crystallinity over 50%. Poly vinyl alcohol was irradiated by gamma ray. For replacing joint cartilage of human body, the hydrogel would have microporous structure, enough strength, toughness and low elastic modulus [14].

Samih T.Y. and Kadem A.L., (2001) had prepared a gel for as wound dressing. The gel includes a monovalent salt of a poly galacturonic, acid derivative (such as sodium pectate) a carboxy poly saccharide and multivalent ions as ionic cross-links between the monovalent salt and the carboxy poly saccharide [15].

2.1 Silk fibroin (SF)

Fine, horny, translucent and yellowish fiber produced by the silkworm in making its cocoon and covered with gum silk. In silk manufacture, the first operation is reeling. The cocoons, having been sorted for color and texture are steamed or placed in warm water to soften the natural gum. They are then unwound; each cocoon may give from 4 to 18 strands of which are reeled or twisted together to make an even thread strong enough to handle. The next step, called throwing is preparing the raw silk for the loom by twisting and doubling it to the required strength and thickness. This process also is now mostly done in large mills with specialized machinery. After throwing, they have three forms. First form is singles which are untwisted used for the warp of very delicate fabrics. Second form is tram (two or more singles) twisted and doubled used for the weft of various fabrics. Final form is organzine which made of singles twisted one way. They doubled and twisted in the opposite direction. Organzine used for the warp of heavy fabrics. For sewing and embroidery thread, more doubled and smoother twists are made. In modern factories spinning frames complete the preparation for the loom. Silk is boiled off in soapsuds to remove gum and prepare it for dyeing. For white and pale tints it must be bleached. Scouring or boiling causing loss of weight, sometimes made up by loading with metallic salts as tin which has an affinity for silk and can be adsorbed to excess, causing weakening of the fiber. Dyeing may be done in the yarn or in the piece. Finishing processes are varying and important as in making moires.

Main silk production in the world is still from China. Silk is a natural evolution of the silk moth which is forms its cocoon on the leaves of the Mulberry tree. This is a seasonal activity, like all things in nature and occurs in the early fall in east central China. Man have been breeding the chineses silkworn *Bombyx mori* for over five thousands year. Larva of the *Bombyx mori* or mulberry silkworm is bleed for commerce. While the wild

species of the silkworm *Bombyx mandarina* [16] still exists in nature. Today they have over 500 different mutations of intentional and unintentional selection.

Physical properties of silk fiber such as water absorptivity [17] is considered in percentage of water regain and percentage of swelling in water. At 65 % of relative humidity and 21°C, water regain of silk is 11.0% while percentage of swelling in water is 30 – 41.

Table 2.1 Physical properties of silk fiber [17]

Physical Property	Silk
Abrasive resistance	Fair
Moisture regain, % at 65% rh	11
Drapability	Excellent
Elongation at break, % at 65% rh, 25 °C	20
Environmental resistance	
Mildew resistance	Good
Renovation	Wash, Dry clean
Safe iron limit, °C	148
Sunlight resistance	Poor
Hand	Excellent
Pilling resistance	Good
Resiliency	Fair
Specific gravity, g/cm^3	1.30
Static resistance	Fair
Strength loss when wet, %	15

Several species of silkworm produce silk, a substance that consists of the fibrous protein fibroin embedded in an amorphous matrix. Each silk fiber is composed of double fiber of single filament which are parallel and a silk glue surrounds them. The composition of filament is 70-80 % silk material which called *fibroin* and 20-30 % silk glue which called *sericin*. By boiling the silk in hot water, the gum (sericin) is removed from the yarn or fabric. By doing this, the luster of the silk is enhanced. It is very lightweight. One fibroin contained of many thousands of bundle of fibril. Many bundles of microfibril are consisted on one fibril.

Figure 2.1 The structure of Microfibril [18].

In fibroin, which is considered to be a β-keratin, the polypeptide chains are arranged in anti-parallel β - pleated sheet conformations (Figure 2.2). Beta pleated sheets form because of fibroin's large content of amino acids with relatively small R groups such as glycine (Gly) and alanine (Ala) or serine (Ser). Bulky R groups would distort the almost crystalline regularity of silk protein. Silk is a strong fabric because the chains are fully extended. Because the pleated sheets are loosely bonded to each

other (primary with weak van der Waals forces), they slide over each other easily. This arrangement gives silk fibers their flexibility.

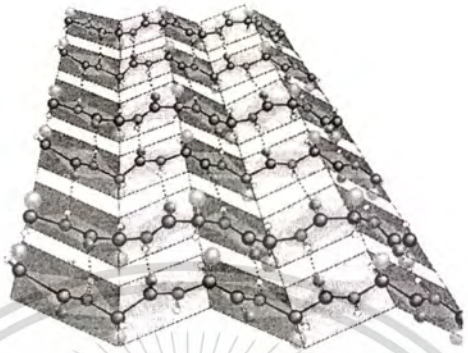


Figure 2.2 The structure of Beta pleated sheet [18].

Beta pleated sheet [18] is form when two or more polypeptide chain segments line up side by side. Each individual segment is refer to as a β-strand. Each beta-strand is fully extended by hydrogen bonds between the polypeptide backbone N-H and carbonyl groups of adjacent chains. There are two kinds of beta-pleated sheets: parallel and anti-parallel. In parallel beta-pleated sheet structures, the polypeptide chains are arranged in the same direction. When polypeptide chains is arranged in opposite direction then called anti-parallel beta-pleated sheet structures.

SF is a structural protein produced by the *Bombyx mori* silkworm and consists mainly of very simple amino acids such as glycine, alanine and some of serine. Alanine and glycine forming is only crystalline regions of SF. While the other is a chian containing polar and bulky amino acids forming only amorphous region [19]. A part of the primary structure of SF has been determined by sequencing a number of DNA of *Bombyx mori* silkworm.

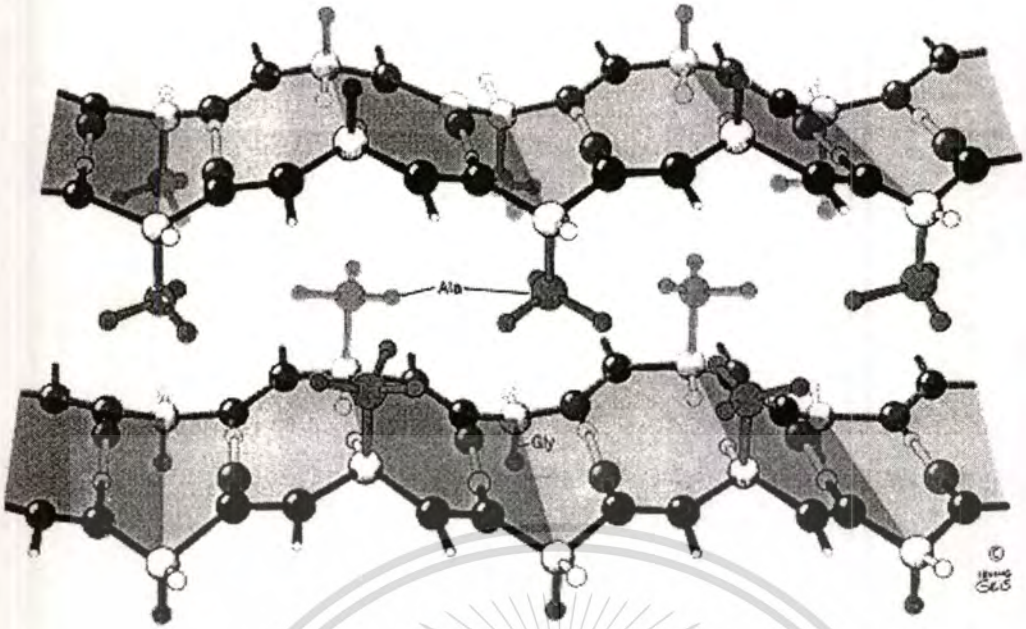


Figure 2.3 The structure of Silk Fibroin

Absorption bands of the IR spectra of SF exhibited at 1631 (amide I), 1528 (amide II) [20] and 696 cm^{-1} (amide V) [2] which were characteristic of a β-sheet structure.

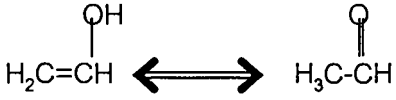
Differential scanning calorimetry (DSC) curves of amorphous silk fibroin occurs the endothermic peak at 175 °C and the exothermic peak at 212 °C. While the endothermic peak at 280 °C is shown to be the degradation [21].

In this experiment, raw silk fibers were degummed by Ajisawa's method [22]. By this method, the fiber was dissolved in aqueous $CaCl_2$-ethanol (Ajisawa's reagent) to prepare a fibroin solution.

2.2 Poly (vinyl alcohol) (PVA) [23]

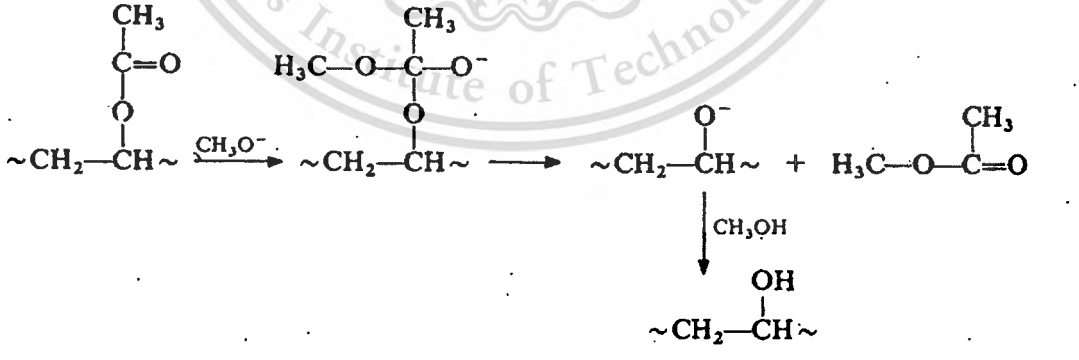
Poly (vinyl alcohol) (PVA) is polymerized from vinyl acetate monomer. Major uses of PVA are textile sizing, adhesive, paper coating, joint cements, water-soluble films, nonwoven fabric binders and paper treatment. The trade names of PVA are different from each company such as Vinol (Air products), Elvanol (Du Pont), Gelvatol (Monsanto), etc.

Vinyl alcohol has not been isolated in the free state; the keto tautomer, acetaldehyde, is much the more stable form and is always obtained.



(2.3.1)

Thus poly (vinyl alcohol) cannot be prepared from its monomer by the usual techniques, although the polymerization of acetaldehyde with sodium amalgam at –80 to –20°C has been found to give poly (vinyl alcohol) is obtained exclusively from poly(vinyl acetate) [24]. PVA is manufactured by controlled hydrolysis of poly (vinyl acetate) using base (sodium hydroxide) catalyzed methanolysis. Poly (vinyl alcetate) (PVAc) is readily hydrolysed by treating an alcohol solution with aqueous acid or alkali. Acid hydrolysis results in traces of acid in the PVA which are difficult to remove and which lead to instability of the polymer. Alkaline hydrolysis results in contamination of the product by a large amount of sodium acetate which is also difficult to remove and which has little intrinsic value. These difficulties are avoided if PVA is prepared from PVAc by alcoholysis using a small amount of base as catalyst. The reaction is commonly carried out by treating PVAc with methanol in the presence of sodium methoxide.



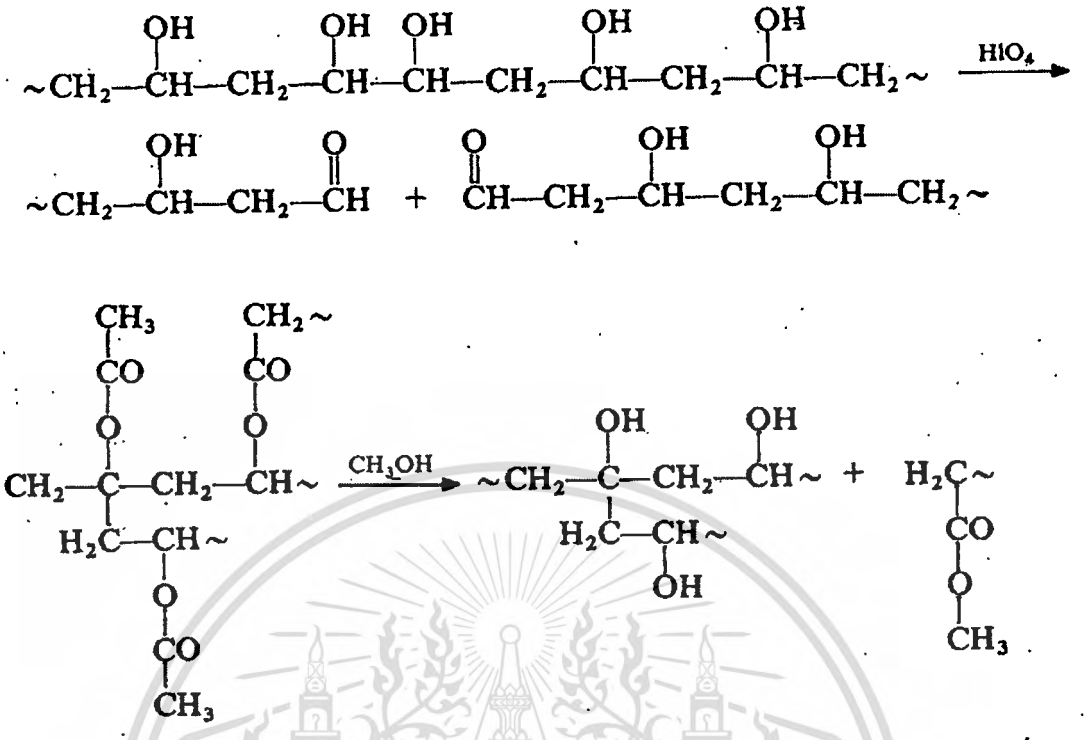
The preferred methods of preparing PVAc for conversion to PVA are solution and suspension polymerization. The former technique has the advantage that if

polymerization is conducted in methanol the resulting solution can be used directly without the need for isolating the polymer. This method is the most suitable for continuous processes. Bulk polymerized PVAc tends to give low molecular weight PVA of poor colour.

In one continuous process, a solution of PVAc in methanol (about 20%) is mixed with the catalyst solution in a high speed in-line mixer. The mixture then passes through a 'gelling zone' on a conveyor belt. Typically, the material is kept at 40°C for 10 minutes in this zone during which time the alcoholysis reaction occurs. The gel is chopped up and neutralized with acetic acid to stop reaction. The liquid content (which is mainly methanol and methyl acetate) is then expressed and recovered. The residual solid is washed with methanol, dried and pulverized.

It is possible to control the extent to which acetate groups are replaced by hydroxyl groups by changing the reaction conditions. In particular, the catalyst concentration and the time of reaction have a major effect on the degree of alcoholysis. The most common commercial types of PVA are the so-called partially hydrolyzed grades in which 87-89% of the acetate groups have been replaced and the completely hydrolysed grades in which 99-100 % of the acetate groups have been replaced. The degree of alcoholysis has an effect on the properties of the polymer.

It follows that PVA has a substantially head-to-tail structure since neither periodic acid nor lead tetraacetate, reagents which cleave1,2-diols, are consumed to any measurable extent by the polymer. However, after treatment with these reagents the polymer does have a reduced solution viscosity, indicating the presence of a few head-to head linkages (1-2 % of the total linkages).



Two types of branching may occur in PVAc. The alcoholysis of PVAc causes removal of branches resulting from chain transfer to the acetate group but not those arising from transfer to chain hydrogen.

Thus it is found that PVA has a degree of polymerization considerably lower than that of the PVAc from which it was derived. There appears to be little branching in PVA, from which it may be deduced that most of the branching in PVAc arises from transfer to the acetate group. Generally speaking, PVA is commercially available in four molecular weight ranges which are commonly referred to as super-high-, high-, medium- and low-viscosity poly (vinyl alcohol)s. The corresponding average molecular weight (M_v) are 250,000 – 300,000; 170,000 – 220,000; 120,000 – 150,000 and 250,000 – 350,000 respectively. Since PVAc as normally prepared, is atactic PVA is also atactic. However, although PVAc is amorphous PVA exhibits crystallinity. The hydroxyl group is small enough to fit into a crystal lattice which is essentially the same as that of polyethylene.

The physical properties of PVA are somewhat dependent on the degree of alcoholysis. Thus completely hydrolysed PVA has a higher tensile strength and tear

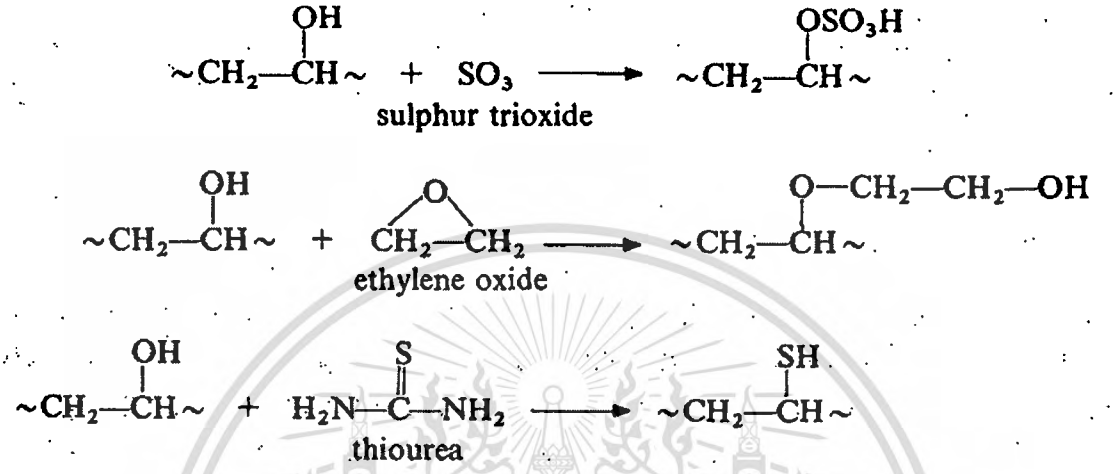
resistance than the partially hydrolysed material, in which crystallnity and hydrogen bonding are less extensive. Physical properties are also affected by environmental humidity. Water acts as a plasticizer and PVA conditioned at, for example, 50% relative humidity shows a decrease in tensile strength but an increase in elongation compared to material conditioned in a moisture-free atmosphere. Molecular weight also has an effect on physical properties and the low viscosity grade polymers have appreciably lower tensile strength and tear resistance than the higher viscosity grades. The specific gravity of PVA is 1.27 to 1.31 g/cm^3, and the glass transition temperature is 75 to 85 °C. PVA is water soluble polymer and it adheres well to cellulose surfaces. An important characteristic of PVA is its solubility in water. As the acetate groups of PVAc are replaced by hydroxyl groups the water sensitivity of the polymer increases. Maximum sensitivity occurs at a degree of alcoholysis in the range 87-89 % are readily soluble in cold water. At higher degrees of alcoholysis, hydrogen bonding becomes more appreciable and results in a reduction in the ease of solubility. The completely hydrolysed grades of PVA are dissolved in water only by heating to above 85°C.

Poly (vinyl alcohol) is resistant to a very wide range of organic solvents. In general, resistance to organic solvents increases with degree of alcoholysis. All grades are substantially unaffected by most aliphatic and aromatic hydrocarbons, chlorinated hydrocarbons, higher monohydric alcohols, esters, ethers and ketones. Lower monohydric alcohols have some effect on the partially hydrolysed grades of polymer but negligible effect on completely hydrolysed grades. The only effective solvents are those capable of hydrogen bonding with the hydroxyl groups. Glycerol is commonly used as a plasticizer for poly (vinyl alcohol).

Recently, using of organic solvent such as hexamethylene diisocyanate as crosslinked agent for PVA can be increased formation of a chemical network and introduction of flexible moiety [25].

The secondary hydroxyl groups in PVA are reactive and many derivatives have been prepared. The most important commercial derivatives are the acetals. While the other derivatives which have found application are the acid sulphates (as ion exchange

resins), the hydroxyethyl ethers which have better low temperature flexibility than the parent alcohols and the thiols. These derivatives are prepared as follows.



It will be appreciated that PVA has an unusual combination of properties. In particular, it has much greater tensile strength than is normally associated with water-soluble materials. By adding it has outstanding chemical resistance. This combination results in a wide variety of applications. Then PVA film is used for water-soluble packages for materials such as bath salts, disinfectants and insecticides. This film is also employed as a release agent in the production of reinforced plastics. PVA may be spun into fibres which have been developed particularly in Japan.

2.3 Hydrogel

Advantages of hydrogel film are faster healing than gauze dressing, easier to change the dressing, no dressing material remains on the wound, transparency, no adhesive to adhere the wound and won't tear outer layer of wound.

The normal commercial hydrogel films are transparent occlusive wound dressing consisting of synthetic polymer film into which has been grafted hydrophilic polymers. It is notes for its ability to allow transport of excessive exudate out of the wound, and to permit penetration of small molecule antiseptic and antibiotic agents into the wound through the dressing.

The impetus for commercial hydrogel film came about considering the risk to health care workers in ambulances, emergency rooms, or surgeries, where a wound can be quickly covered with hydrogel film to protect the worker from infected blood. While such workers are generally wearing gloves and masks, the additional protection at the wound area maybe of considerable value.

Hydrogels or water-containing gels are polymers characterized by hydrophilicity and insolubility in water. In water they swell to an equilibrium volume, but preserve their shape. The hydrophilicity is due to the presence of water-solubilizing groups, such as –OH, -COOH, $-CONH_2$, -CONH-, $-SO_3H$, etc.

This film consists of polymer network. This network may absorb water from the wound exudate. The space in the polymer network is very small. They allow the passage of small molecules, including the drug [26] while excluding large molecules like proteins and microorganism.

One method of manufacturing hydrogel dressings from synthetic and natural polymers by polymerization and cross-linking involves pouring aqueous solutions of synthetic polymers, such as poly (acrylamide) and poly (vinyl pyrolidone), their monomers or their mixtures, natural polymers, such as agar or silk fibroin.

Hydrogels of poly (acrylamide) and poly (ehtylene oxide) are convenient and biocompatible media for use as medical device. Biosensor [27] or biological sensor [28] is an interesting film of hydrogel which is used to define glucose, sulfite and phenytoin by immobilized enzyme on surface of electroactive probe. Changing of ions on reference and auxiliary electrode verify volumetric result of anything above. Anyway, immobilized methods are used in difference.

2.4 Irradiation

Nuclear reactions differ from normal chemical reactions. When normal chemical reactions occurs, electron configurations are altered with changing of atomic energy. The nuclear reactivity of an atom is independent of its chemical state.

There are four types of nuclear reactions. Artificial transmutation is the first type of nuclear reactions. The second type is nuclear fission. Nuclear fission is used in areas of military and peaceful purpose. The third type of nuclear reactions is nuclear fusion. Many developed countries use nuclear fusion as a long-term of energy source. The final type is radioactive decay. All atoms which contain of the same number of protons and neutrons are called nuclides. Thus the term nuclide is similar to the term isotope except that isotopes are atoms of the same element whereas nuclides refer to the different elements. When mass is destroyed in the process the destroyed mass is converted into energy, as Albert Einstein predicted in 1905 AC. He said that energy and mass are related by the equation where C is the speed of light

$$E = mC^2 \qquad (2.5.1)$$

In nuclear fusion, two small nuclides come together to form one larger and more stable nuclide. On the other hand, unstable nuclides in nuclear fission are split into smaller ones. Fusion reactions have a much higher energy than fission reactions because a greater percentage of the mass of the reactants is converted into energy. Although fusion reaction is very exothermic, they also have very high activation energies because two positively charged particle must come together. The electrostatic repulsive energy must be overcome before the particles can get close enough to be fused together. This high activation energy causes serious problems. It has been estimated that a temperature of at least several million degrees Celsius is necessary to supply thermally the energy needed to start a fusion reaction.

The classification of radiation of radioactive substances as alpha (α), beta (β) and gamma (γ) rays was based on their intensity could be reduced by absorbers [29]. When a nuclear reaction such as radioactive decay occurs, energy is released because the

products are more stable than reactants. The energy given off is manifested in several ways. Some is given off as heat energy and some is transferred to the decay products as kinetic energy to propel them out of the nucleus. Some of the energy is given off as photons of electromagnetic radiation. The number of protons in the nucleus of nuclide equals the atomic number, Z, the number of neutrons equals the difference between the mass number, A, and the atomic number.

Therefor Z = number of protons

$N = A - Z$ = number of neutrons

Alpha particle is a positively charged particle. It made up of two neutrons and two protons. This particle is the least penetrating of the three common forms of radiation. Alpha particle can be stopped by a sheet of paper or the outer layer of person's skin. It is not dangerous to living things unless the alpha emitting substance is inhaled or ingested or comes into contact with the lens of the eye.

Beta particle is an electron of either positive charge or negative charge which has been emitted by an atomic nucleus or neutron in the process of a transformation. These charged particles emitted from a radioactive atom. In the process of beta decay a nucleus emits an electron. But there are no electrons in the nucleus. Nucleus in the process of beta decay is converted into a proton and an electron. While the electron is then ejected from the nucleus. It has a mass number of zero and a charge number of –1. Its symbol is ${}^{0}\beta_{-1}$. Beta particles are more penetrating than alpha particles but less than gamma rays or x-rays. They can be stopped with a sheet of aluminium foil or glass.

Gamma ray is a highly penetrating type of nuclear radiation, similar to x-ray. On the other hand, it comes from within the nucleus of an atom. In general, gamma ray has a shorter wavelength. This type of radiation is very penetrating and requires shielding with such materials as concrete, lead, steel or water to absorb its energy.

Source of gamma source in this experiment is from radioactive substance that is cobalt-60. The cobalt-60 source is general used to sterilize surgical instrument and to improve the safety and reliability of industrial fuel. On the agriculture field used this source for preserve poultry fruits and spices. This reduces the chance of the products

becoming re-contaminated. Irradiation is ideal for sterilizing heat-sensitive products, such as plastics, since irradiation is a cold process that generates very little heat so it does not significantly raise the temperature of the foods being processed.

In food industry, irradiation for destroys bacteria and yeast that cause spoilage in fresh foods such as fruit and vegetables. It destroys dangerous microorganisms such as Salmonella in poultry, E. coli in meat and Listeria in prepared meats. Spices and grains are irradiated to destroy insects and their eggs or larvae.

Irradiation induces modification of polymer properties as the basis of heat shrinkable films, cross-linked polymers and graft copolymers. Further technological advances require study of the interactions and processes taking place within a given polymer with the goal of designing appropriate chemistry for a specific application.

Several high technology industries require specially polymers that exhibit a specific response upon exposure to radiation. For example, some applications require materials for which efficient radiation-induced scission or cross-linking effects a change in solubility. Even small amounts of radiation can induce significant changes in the physical or mechanical structure of a particular polymer. In some cases, even a few crosslinks or scission sites per molecule can dramatically affect the strength or solubility of a polymer. Highly radiation stable materials are required for other applications. The design and development of appropriate chemistry for a given purpose requires full understanding of the effect of radiation induced chemical processes occurring in polymers. With this background the technological advances required by today's industries can be realized. Irradiation of polymers is widespread in many industries. The role of radiation sterilization of medical and pharmaceutical items, many of which are manufactured from polymeric materials, is increasing. This trend arises from both the convenience of the process and concern about the toxicity of chemical sterilants. Information about the radiolysis products of natural and synthetic polymers used in the biomedical industry is required for the evaluation of the safety of the process.

The radiation sterilization of biomedical polymeric materials, particularly implantable surgical devices, raises significant concerts. Polymers typically undergo some radiation-induced degradation leading to discoloration and associated deterioration in

properties. Knowledge of the relationship between the chemical composition of polymer materials and their radiation sensitivity is necessary to enable selection of appropriate materials for radiation sterilization.

Understanding the radiation chemical processes taking place in biomedical materials and evaluating the dependence of these reactions on dose and storage time is also important. As noted in the previous section, post-irradiation reactions resulting from trapped radicals within the polymer matrix are commonly observed upon high-energy radiation of polymers. Knowledge and understanding of such reactions in candidate polymers for biomedical applications is critical. Information on the radiolysis products from both natural and synthetic polymers is further required by the food industry as radiation sterilization of products related to this industry becomes more commonplace.

Irradiation of polymers causes modification of properties which is currently the basis of major industries in heat shrinkable film and tubing, crosslinked polymers and grafted copolymers. This area is now entering an era of new technology resulting from greater knowledge of the chemical processes. There are many applications of modified polymers including their use in the medical and health-care fields. For instance, these materials may be used as implantable materials, controlled release drug preparations, and hydrophilic wound dressings. Alternative applications include manufacture of light weight high strength ropes and high performance fabrics, rock bolts for mining and reinforcement of concrete.

High-energy radiation may be classified into photon and particulate radiation. Gamma radiation is utilized for fundamental studies and for low-dose rate irradiation with deep penetration. The radioactive isotopes, cobalt-60 and cesium-137 are the main sources of gamma radiation. Synthetic polymer hydrogel films are irradiated by cobalt–60 source at room temperature. Many researchers focus on effects of radiation grafting conditions, such as absorption dose, dose rate releasing of methylene blue and acid concentration.

Table 2.2 Radiation Effect of Biodegradable Polymers (Green Polymers)

Radiation Effect of Biodegradable Polymers (Green Polymer)	
Synthetic Aliphatic Polyesters	
Poly (ε-caprolactone) (PCL)	Crosslinking
Poly (butylene succinate)	Crosslinking
Poly (ethylene succinate)	Degradation
Polymers Produced by Bacteria	
Poly (3-hydroxybutylate) [P(3HB)]	Degradation
Poly (γ-glutamic acid) (PGA)	Crosslinking
Poly (aspartic acid)	Crosslinking
Natural Polymers	
Cellulose	Degradation
Starch	Degradation
Chitin / Chitosan	Degradation
Sodium Alginate	Degradation
Carrageenan	Degradation
Cellulose Derivertives (Carboxy methyl cellulose)	Crosslinking
Starch Derivertives (Carboxy methyl starch)	Crosslinking

Absorption of high-energy radiation by polymers produces excitation and it is these excited species that are initial chemical reactants. Although the absorption of radiation energy is dependent only on the electron density of the substrate and therefore occurs spatially at random on a molecular scale, the subsequent chemical changes are not random. Some chemical bonds and groups are particularly sensitive to radiation-induced reactions. They include COOH, C-X where X=halogens, $-SO_2-$, NH_2, and C=C. Spatial specificity of chemical reactions may result from intramolecular or intermolecular migration of energy or reactive species such as free radical. Some polymers such as isotactic polypropylene can be degraded when irradiate at higher doses. Degradation gave rise to decrease in melting temperature and enthalpy, as observed in the DSC

results [30]. For poly (aryl ether-ether-ketone) (PEEK) occurred not only chain scission but also structure changing in the main chain under irradiation at high-energy electron beam [31].

The molecular changes occuring in polymers as a result of radiation-induced chemical reactions may be classified as two effects. The first effect is chain crosslinking effecting an increase in molecular weight and formation of a macroscopic network (polymer solubility decreases with increased radiation dose) and the second effect is chain scission effecting a decrease in molecular weight and substantially changing a polymer materials properties. In addition to these changes, irradiation of polymers will frequently give rise to small molecular products, resulting from bond scission followed by abstraction or combination reactions.

Table 2.3 Ratio of Absorbed Dose in Common Polymers to Absorbed Dose in Water irradiated by Co-60 Radiation in Air and at Various Depth in Water-Equivalent Material

Substance	Depth (cm)			
	0	5	10	15
Polyethylene	1.026	1.021	1.015	1.008
Polypropylene	1.026	1.021	1.015	1.008
Polystyrene	.967	.963	.967	.951
Polychlorostyrene	.956	.980	1.008	1.043
Polyester	.937	.934	.931	.928
Polycarbonate	.948	.945	.939	.937
Polyvinyl acetate	.962	.959	.956	.953
Nylon	.980	.977	.973	.968
Polyvinyl chloride	.946	1.011	1.087	1.173
Polyvinylidene chloride	.923	1.005	1.107	1.221

Values for the ratio of absorption dose for some common polymers are given in table 2.3. The general pattern shows that the absorption characteristics of low atomic

number materials composed of H, C, N and O do not change appreciably with radiation quality.

In Thailand, irradiation inhibits unwanted sprouting in foods such as onions and garlic. Another products that have been irradiated with gamma source are mango, rice, wheat seasoning, spices, herb, frozen shrimp, frozen chicken, surgical instruments and cosmetics. It dose not make food radioactive or harmful in any way. Verify of quality and quantity of product irradiation are in controlled of Office of Atomic Energy for Peach at Thai Irradiation Center.

Although hydrogels play an important role in the new trends of biotechnology, gel chemistry is not only circumscribed to organic molecules. In fact there are many inorganic hydrogel, obviously derived from inorganic polymers, particularly silicones and polysilanes. There is another important branch of hydrogel. This is not the end of advantages of hydrogels. The future work for new applications of hydrogels is still induced many researchers from around the world to discover them.

CHAPTER 3

EXPERIMENTAL DETAILS

3.1 Materials

1. Silk waste (SF) : STB II : SHINANO THAI COMPANY
2. Poly (vinyl alcohol) (PVA) MW ≈ 80,000 – 100,000 : Sigma Chemical Company
3. Calcium chloride 2 – hydrate ($CaCl_2 . 2H_2O$) : BDH Laboratory Supplies
4. Sodium carbonate (Na_2CO_3) : BDH Laboratory Supplies
5. Ethanol (C_2H_5OH) : Merck
6. Methanol (CH_3OH) : Merck
7. Deionized water

3.2 Apparatus

1. Seamless cellulose tubing small size 24 (molecular weight cut off 12,000 – 14,000 ca.)
2. Fisher Scientific Beaker unstirred digital water bath
3. Centrifugal test tube
4. Volumetric flask
5. Indicator paper
6. Vacuum oven
7. Stirring rod
8. Petri-dish
9. Dessicator
10. Parafilm
11. Forcept
12. Oven
13. Melamine tray
14. Gamma cell 220

15. LLOYD tensiometer
16. LEO LEO 1455VP scanning electron microscope
17. Perkin-Elmer differential scanning calorimeter
18. Perkin-Elmer thermogravimetric analyzer
19. D8 Advance Bruker X-ray diffractometer
20. Spectrum GX Fourier transform infrared spectrophotometer.

3.3 Preparation of degummed silk fibroin

1. Boiled 5 grams of silk waste (STB II) for 2 hours in 500 ml of distilled water about 80 – 90 °C.
2. The insoluble silk was then filtered, after that soaked in 200 ml of methanol about 1 hour.
3. Filtered the methanol out and soaked the silk in 200 ml. Dilute detergent solution about 1 hour.
4. Wrung out, soaked in 200 ml of 0.5 % sodium chloride solution, stirred about 30 minutes, filtered and rinsed in distilled water until the distilled became neutral.
5. Dried cleaned silk in the vacuum oven at 70 °C about 4 hours.
6. Keep the dried silk in the desicator.

3.4 Preparation of calcium chloride solution

Table 3.1 Preparation of calcium chloride solution [32]

Calcium chloride	Ethanol	Deionized water
73 g.	47 ml.	54 ml.

3.5 Preparation of silk solution [32]

1. 30 grams of degummed silk fibroin was dissolved in 100 ml of calcium chloride solution at 100 °C.
2. The silk fibroin solution was dialyzed using seamless cellulose tube with deionized

water at least for three days.

Calculation:	if 30 g. of silk waste gave silk solution	300 ml.
	total 300 ml of silk solution gave fibroin	9 g.

3.6 Preparation of silk powder solution

1. Grounded silk powder with motor speed at for 24 hours.
2. Dissolved 1 gram of grounded silk powder with deionized water.
3. Autoclaved at 100 °C for 10 minutes in the sealed container.

3.7 Preparation of 3 % PVA solution

1. Dissolved 3 grams of PVA with deionized water.
2. Autoclaved at 121 °C for 25 minutes in the sealed container.

3.8 Preparation of PVA/silk fibroin hydrogel

3.8.1 Preparation of 3 % PVA film (A0)

1. Casted 3 % PVA solution on petri-dish.
2. Dried 3 % PVA solution in oven at 50°C about 4 hours.

3.8.2 Preparation of heat treated film (A1)

1. Mixed silk fibroin solution with 3 % PVA solution at the ratio 1:3 by volume in beaker.
2. Casted mixed solution on petri-dish.
3. Dried mixed solution in oven at 50°C about 4 hours.
4. Heated film in oven at 100 °C for 4 minutes.

3.8.3 Preparation of irradiated film (A2)

1. Gamma irradiated silk fibroin solution at 20 kGy for 4 hours under nitrogen.
2. Mixed viscous silk fibroin solution with 3 % PVA solution at the ratio 1:3 by volume in the beaker.
3. Casted mixed solution on petri-dish.
4. Dried mixed solution in oven at 50°C about 4 hours.

3.8.4 Preparation of untreated film (A3)

1. Mixed silk fibroin solution with 3 % PVA solution at the ratio 1:3 by volume in beaker.
2. Casted mixed solution on petri-dish.
3. Dried mixed solution in oven at 50°C about 4 hours.

3.8.5 Preparation of silk powder film (A4)

1. Mixed silk powder solution with 3 % PVA solution at the ratio 1:3 by volume in beaker.
2. Casted mixed solution on petri-dish.
3. Dried mixed solution in oven at 50°C about 4 hours.

3.8.6 Preparation of silk fibroin film (A5)

1. Casted silk fibroin solution on petri-dish.
2. Dried silk fibroin solution in oven at 50°C about 4 hours.

3.9 Measurement PVA/silk fibroin hydrogel

3.9.1 Percentage of gel fraction

1. Feed the resultant hydrogel in the net of aluminium bag.
2. The samples were immersed in water at room temperature for extraction of sol part, the water being occasionally changed for 8 hours.
3. After that samples were kept at 50°C in vacuum oven for 24 hours.

4. Measure the weight of the samples after 24 hours.

$$\text{Percentage of gel fraction} = \frac{\text{Weight of dry sample after extraction}}{\text{Weight of sample feed in the net}} \times 100$$

3.9.2 Percentage of water uptake and water content

1. The samples were immersed in water at room temperature.
2. Measure the weight of the samples after 1, 2, 3, 4, 5, 6, 7 and 8 hours and after 24, 48 and 72 hours.
3. Water uptake is the amount of water in the polymer will pick up during swelling, expressed as percent added, calculated as the following.

$$\text{Percentage of water uptake} = \frac{Ws - Wo}{Wo} \times 100$$

Where Ws = weight of swollen gel (g.)

Wo = weight of sample feed in the net (g.)

4. Water content is the amount of water in the swollen polymer, based on the total weight.

$$\text{Percentage of water content} = \frac{Ws - Wo}{Ws} \times 100$$

3.9.3 Percentage of water retention

1. The sample was immersed in the water at room temperature for 8 hours.
2. Measured the weight of the sample at 8 hours soaking time as weight of swollen gel.
3. Dried the sample at 50 °C for 1 hour and recorded the dried weight as weight of dried gel.
4. Measured the weight of the sample every 1 hour until 8 hours.
5. Water retention is the amount of water retained in the film, based on total weight.

$$\text{Percentage of water retention} = \frac{\text{Weight of dried gel}}{\text{Weight of swollen gel}} \times 100$$

3.10 IR spectra

The measurement of IR spectra were taken by using a Perkin-Elmer Spectrum GX Fourior Transform Infrared Spectrometer in the spectral region 4000 – 400 cm^{-1}.

3.11 X-ray Diffraction analysis

The measurements of XRD were interesting in crystal structure of PVA on silk fibroin films. X-ray diffraction patterns were obtained using an x-ray source with $CuK\alpha$ radiation. The conditions for the x-ray measurements was 30 kV. The specimen was scanned from $2\theta = 30^{\circ}$ to $2\theta = 10^{\circ}$ at $5^{\circ}/ min^{-1}$.

3.12 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was the method that used to study the thermal behavior of silk fibroin blended with poly (vinyl alcohol). The thermal degradation was investigated using a Perkin-Elmer DSC Pyris Diamond differential scanning calorimeter under nitrogen atmosphere. The film was placed in an aluminium DSC pan with the sample total weight of 5 - 10 mg.

3.13 TGA analysis

The measurement of TGA were studied on thermal stability of hydrogel films. The thermogravimetric analysis were run under nitrogen atmosphere on a Perkin-Elmer thermogravimetric system. Range of temperature from 50 °C to 400 °C at heating rate of $10^{\circ}C\ min^{-1}$.

3.14 Tensile properties

The tensile strength and elongation at break of the PVA/SF blends and pure PVA were measured with a LLYOD instrument Co. Ltd. with speed rate of 10 mm/min at 25 °C and relative humidity 65%. Gauge length of these samples were 7 cm at load cell 1 kN.

3.15 Scanning electron microscope (SEM)

The surface of the blends were examined, after gold coating, with a LEO LEO 1455VP scanning electron microscope at 30 kV acceleration voltage with magnification of 1000X for all samples.

CHAPTER IV
RESULTS AND DISCUSSION

The hydrogel film were prepared from solution of silk fibroin and poly (vinyl alcohol) in the ratio of 1 : 3 by casting on melamine tray under oven temperature 50°C. The concentration of PVA was 3 percent while SF used 3 percent.

In this experiment, there were six types of sample. First type was PVA film, (A0). The second was heat treated PVA/SF film, (A1). The third was irradiated PVA/SF film, (A2). The forth was untreated PVA/SF film, (A3). The fifth was film that made from solution of silk powder which received from SHINANO company limited (THAILAND) and PVA solution, (A4) and the final film was prepared from silk fibroin dialysis, (A5). During drying process, silk fibroin dialysis film, (A5) was cracked into a sliver. In addition, A0 dissolved after soaking in water, so A0 and A5 cannot determined water uptake behaviors.

4.1 Characteristics of Film Hydrogel

4.1.1 Percentage of gel fraction

Gel fraction is an undissolved part of the film after immersion film in water at each determining time. High percentage of gel fraction means high stability of hydrogel film against water. In this experiment, there were four types preparing of hydrogel film for studied. The abbreviation of A1, A2, A3 and A4 in the graph below were represented for different hydrogel films as above mentioned. Data in Figure 4.1 showed a rather high gel content of irradiated blend film. While another kinds of sample were lower gel fraction values at the same time. After dried for 24 hours in vacuum oven, the percentage of gel fraction of A1, A2, A3 and A4 were about 78%, 79%, 77% and 75% respectively. The order of gel fraction were A2 > A1 > A3 > A4. The high gel fraction film would have high structure of complex three dimensions network.

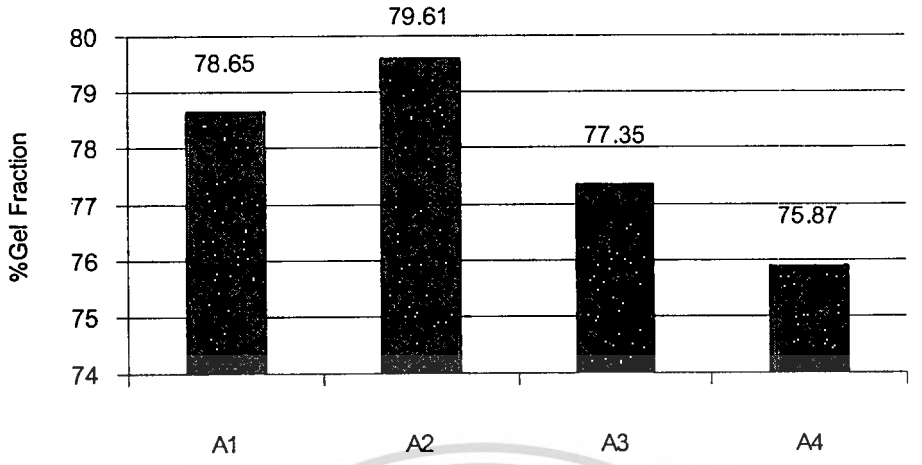


Figure 4.1 Percentage gel fraction of A1, A2, A3 and A4 for 8 hours of soaking time

4.1.2 Percentage of water uptake

Water uptake simply refers to how water is present. For each film, the relation between absorped water and soaking time is a basis for that film's ability to absorp water from wet wound. PVA film (A0) could not measured water uptake and water content properties because it dissolved in water during soaking process. The water uptake of blend films A1, A2, A3 and A4 at room temperature during soaking process were shown in Figure 4.2 - 4.4. The water uptake in initial stage was very rapidly. At first eight hours, Figure 4.2, the water uptake increased from 0% to 68 to 143% depended on each sample. Then films reached to about 73 to 163 % after 24 hours, Figure 4.3. This means that water could be absorbed quickly in the first eight hours.

The effect of films preparation on the water uptake is shown in the same figure. The results showed in Figure 4.2 can classified into two groups of high water uptake and group of low water uptake. The higher group included graph of heat treated PVA/SF film, (A1) and untreated PVA/SF film, (A3) while the lower group included graph of irradiated PVA/SF film, (A2) and silk powder blend/PVA film, (A4). It appears that the higher group showed significantly higher water uptake than the lower group. In higher group, heat treated film absorb water more than untreated PVA/SF film while in lower

group irradiated film showed slightly lower water uptake than PVA/silk powder blend film. This indicated that films preparation process is a very important factor affecting the water uptake of hydrogel film.

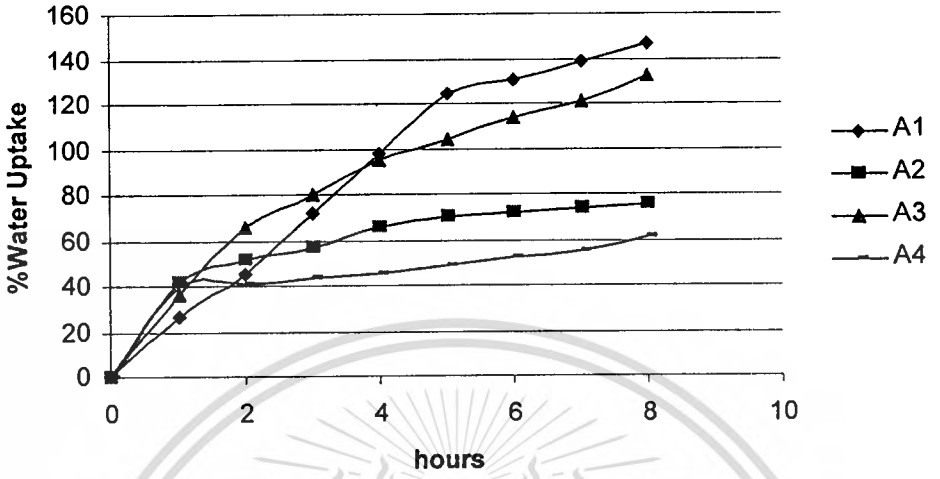


Figure 4.2 Percentage water uptake of A1, A2, A3 and A4 during soaking time from 0 to 8 hours

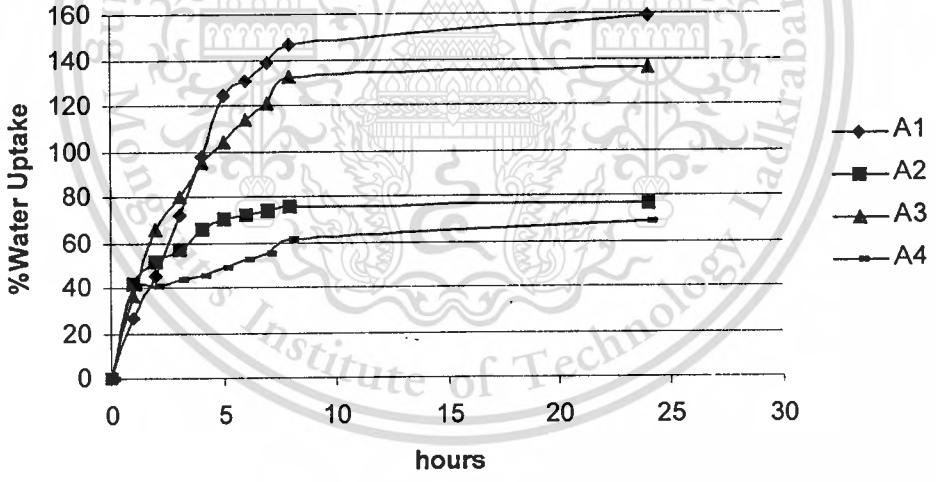


Figure 4.3 Percentage water uptake of A1, A2, A3 and A4 during soaking time from 0 to 24 hours

Figure 4.2 showed the water uptake of each films during eight hours. At first eight hours, percentage of water uptake of A1, A2, A3 and A4 were 143%, 75%, 132% and 61%, respectively. The order of water uptake at 24 hours shown as following A1 > A3

> A2 > A4. Percentage of water uptake of A1 was about 160%. A3 was 136%, A2 and A4 were 76% and 68%, respectively.

. Graph of water uptake at 72 hours showed in figure 4.4. The order of water uptake at 72 hours shown as following, A1 > A3 > A2 > A4. Percentage of water uptake of A1 was about 170%. A3 was 140%, A2 and A4 were 80% and 75%, respectively. The hihg rate of absorp water is promoted rapid healing, minimizes nursing time and provides comfort to patients. While the physical properties of the artificial wound dressing are affected by water uptake. Hydrogel film is hihgly elastic when wet. Therefore it is easy to attach it to various body contours.

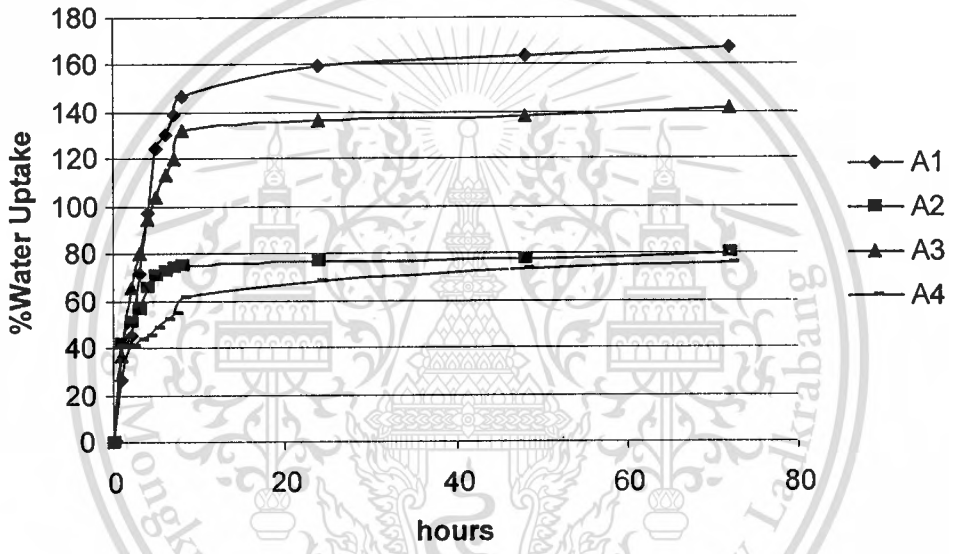


Figure 4.4 Percentage water uptake of A1, A2, A3 and A4 during soaking time from 0 to 72 hours

4.1.3 Percentage of water content

Percentage of water content was based on total weight of the swollen film. It is discribes how much water is in sample. This content can be expressed on the basis of sample and water mass. Thus, the mass water content is a ratio of water mass to wet sample mass. Normally it is visualized as grams of water per gram of wet sample. Water content is expressed as a fraction or as a percentage. Trend of curves in figure 4.5 – 4.7 were the same. The rapid increase in the first eight hours of water content in

swollen film exhibited in every types of film. Graphs of water content of blend films were shown in Figure 4.5. During first 8 hours, the order of water content as the following, A1 > A3 > A2 > A4. At first eight hours, percentage of water content of A1, A2, A3 and A4 were 59%, 42%, 51% and 29%, respectively. After 24 hours, percentage of water content seemed to be constant as showed in figure 4.6. The order of water uptake at 24 hours shown as following A1 > A3 > A2 > A4. Percentage of water uptake of A1 was about 61%. A2 was 43%, A3 and A4 were 52% and 32%, respectively.

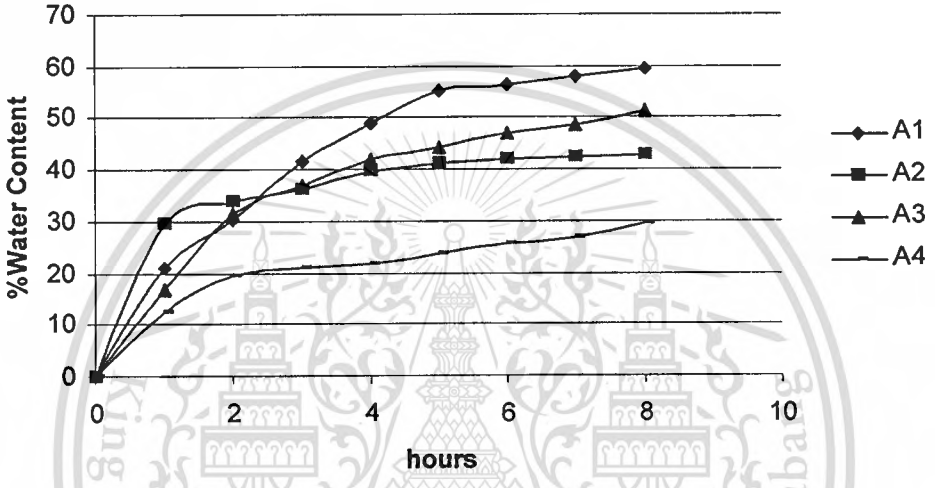


Figure 4.5 Percentage water content of A1, A2, A3 and A4 during soaking time from 0 to 8 hours

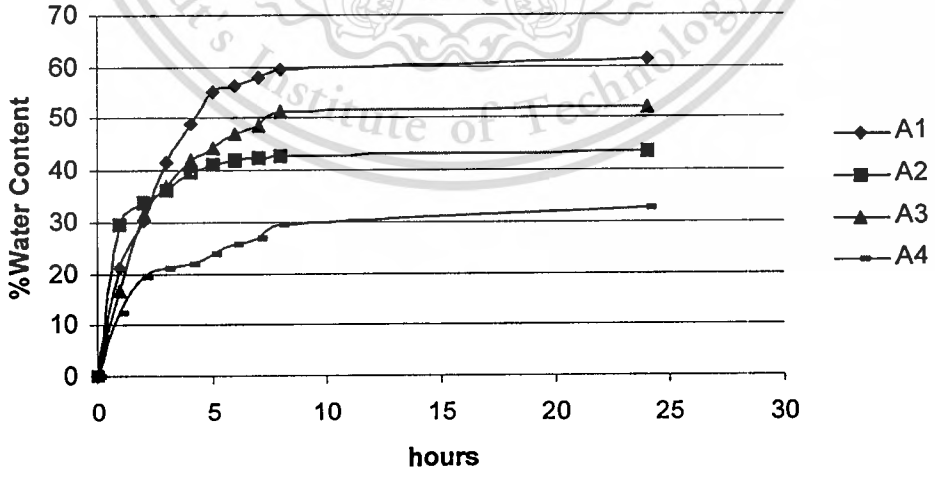


Figure 4.6 Percentage water content of A1, A2, A3 and A4 during soaking time from 0 to 24 hours

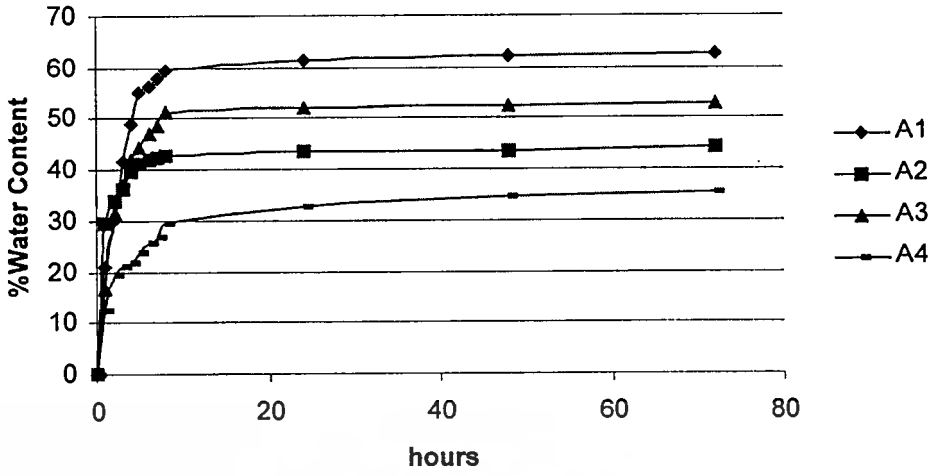


Figure 4.7 Percentage water content of A1, A2, A3 and A4 during soaking time from 0 to 72 hours

After 72 hours, trend of water content was resembled to the graph of 24 hours as shown in figure 4.7. The maximum of water content at 72 hours was A1 and the minimum of water content was A4. The order of water content at 72 hours shown as following A1 > A3 > A2 > A4. Percentage of water uptake of A1 was about 62%. A2 was 44%, A3 and A4 were 57% and 35%, respectively. This trend was similar to the percentage of water uptake. All blends were rapid increased the extent of water content at first 24 hours and still constant.

4.1.4 Water retention characteristics

The percentage of water retention were plotted against time. Figure 4.8 showed the results for the heat treated sample (A1), irradiated sample (A2), untreated blend sample (A3) and silk powder sample (A4). Water retention was determined in triplicate for each of the four film types using the same treatments.

From Figure 4.9 it was apparent that untreated PVA/SF blend film (A3) plots have a higher water content at any given time as compared to the other treatments. As can be seen before, the A1, A2 and A4 do not show any obvious differences. There are clear trends between the other film preparations. The results showed effect of differences of film preparation.

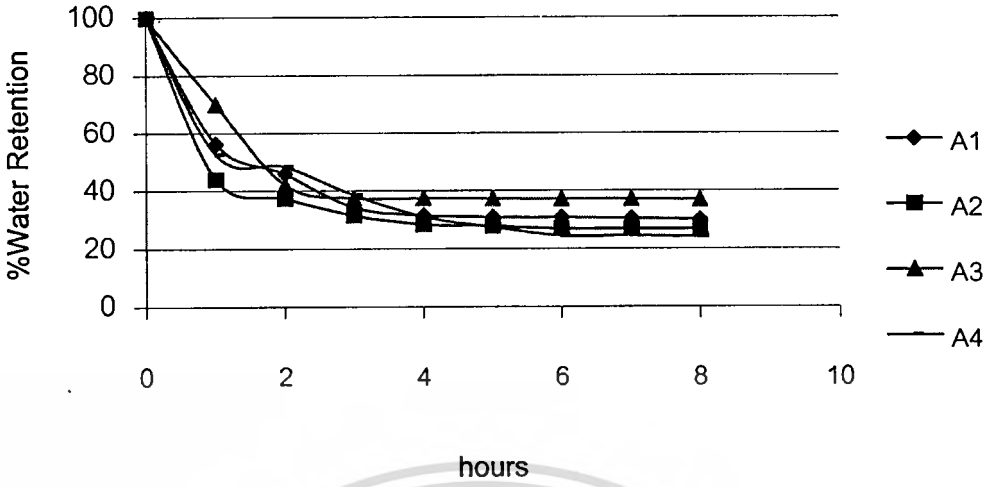


Figure 4.8 water retention curves of A1. A2, A3 and A4 during 8 hours

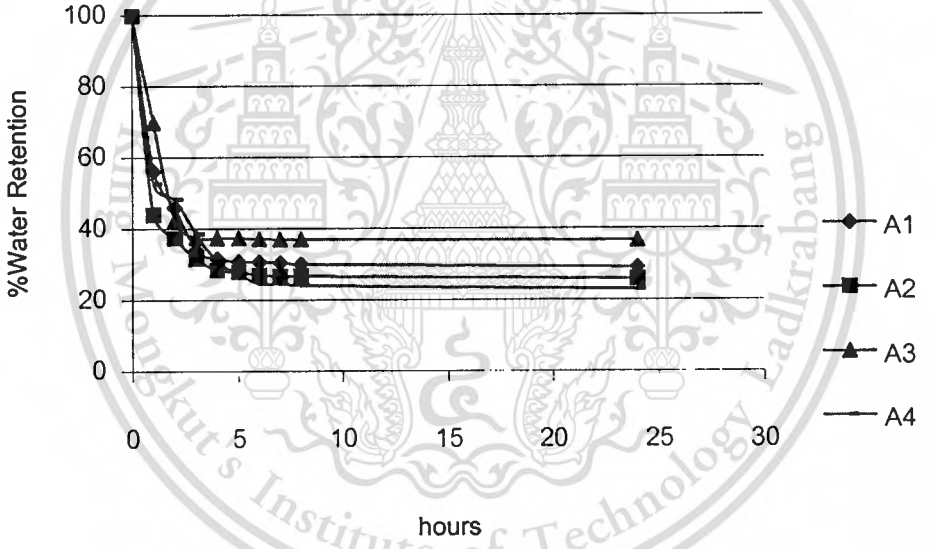


Figure 4.9 water retention curves of A1. A2, A3 and A4 during 24 hours

Figure 4.9 showed the percentage of water retention of A1, A2, A3 and A4. After 24 hours, the maximum water retention of A1 was about 29% and A2 was about 25%. The maximum water retention of A3 was about 36% and A4 was about 22%. The order of the water retention after 24 hours as the folllowing, A3 > A1 > A2 > A4. The untreated film exhibited the highest water retention because the structure still retent more free hydroxyl groups of PVA and some of carbonyl groups of silk. The water could be formed hydrogen bonding between water molecules and free functional groups of film.

While the affect of hydrogen bonding of the other blend films was observed by the quantity of retent water of each film types.

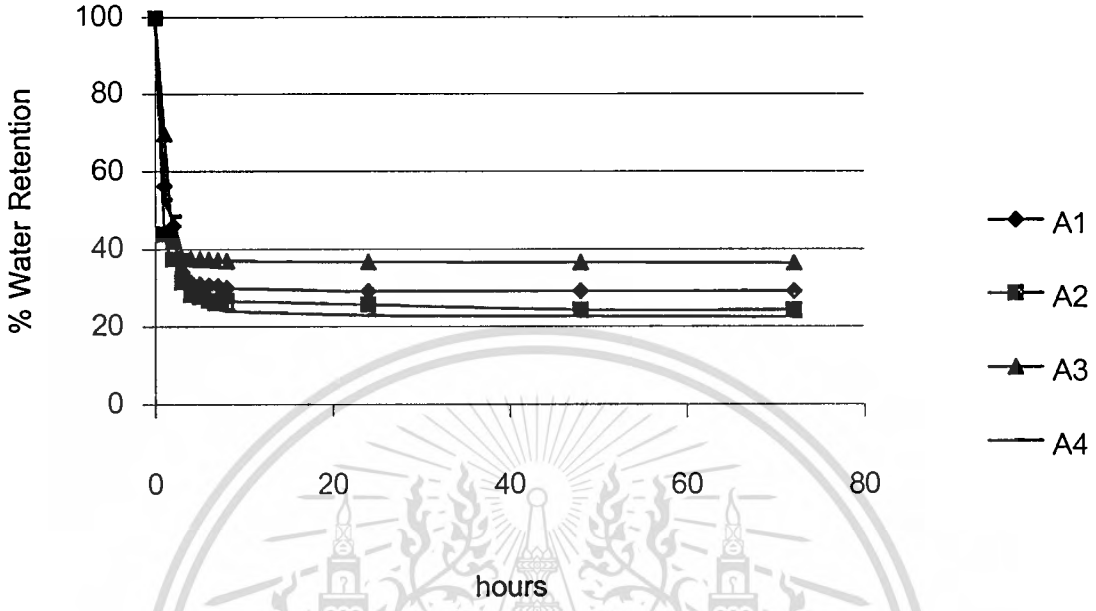


Figure 4.10 water retention curves of A1. A2, A3 and A4 during 72 hours

Figure 4.10 showed the water retention of A1, A2, A3 and A4 in the range of 72 hours. After 24 hours, trend of water retention of all blend was the same until 72 hours. The slightly change of water retention of each film was observed by the figure 4.10. The order of the water retention after 72 hours as the folllowing, A3 > A1 > A2 > A4. A3 was shown the maximum water retention (36%) and this behavior was occurred since after 24 hours. A4 was the minimum water retention (22%) of this group. It was indicated that the silk powder might be random dispersed into the polymer matrix. Hydrogel allows low molecular weights of fluids and gases to penetrate the film but not the larger molecular weights of microbes. The wound stays moist promoting migration of epidermal cells necessary for healing

4.2 Structure and Thermal properties

Fourier transform infrared spectroscopy and thermal gravimetric analysis were used for indicated the thermal properties of films. For FT-IR spectroscopy and TGA, six samples were used in the experiments.

4.2.1 Infrared spectroscopy

Infrared spectra were measured with an Spectrum GX IR spectrophotometer from Mattson Instruments in the spectral region 4000 – 400 cm^{-1}. Figure 4.11 shown the IR spectra for the pure PVA, SF and blend films. The form of β sheet structure exhibited absorption bands at around 1630 (amide I), 1530 (amide II) and around 699 cm^{-1} (amide IV) [33]. Absorption bands of SF film, (A5) showed at 2328 cm^{-1}, attributed to the –OH group, the peak at 1701 attributed at C=O, the peak at 1630 cm^{-1} attributed to N-H stretching (amide I), the peak at 1518 cm^{-1} attributed to amide II and 698 cm^{-1} attributed to amide IV. The same figure showed absorption bands of pure PVA at 3600 - 3300 cm^{-1}, 2925 cm^{-1}, 1440 – 1420 cm^{-1}, 1321 cm^{-1}, 1235 cm^{-1}, 1089 cm^{-1} and 914 cm^{-1} were attributed to OH stretching, CH streching, CH_2, CH, C-O and CH, respectively.

The spectra of the PVA/SF untreated blend film was characterized by the presence of absorption bands typical of the pure components, whose intensity was roughly between that of pure PVA and pure SF films. However, the change could be observed in the –OH stretching region of PVA. The width of the –OH absorption broad peak decreased as shown in Figure 4.11. After blending peak at 3283 cm^{-1} of SF shifted to 3417 cm^{-1}. This means that there is some interaction between SF and PVA.

Figure 4.11 showed infrared spectra of heat treated film, (A1) irradiated film, (A2) untreated blend films, (A3) and PVA/SF film, (A4). General spectra of the blends were shown a medium band at about 3500 – 3200 cm^{-1} of NH, the absorption bands of –CH stretching at 3000 cm^{-1}, the width intense band at about 1710 – 1620 cm^{-1} of carbonyl group, the amide I band at around 1640 cm^{-1} and small peak at about 1019 cm^{-1} of C-O. The amide II absorption bands, 1518 cm^{-1} and 1544 cm^{-1}, could be observed clearly on the samples of heat treatment and silk powder film, respectively. In addition, bands at

698 cm^{-1} of amide IV were exhibited on heat treated sample. The change of width in PVA/SF films at –OH stretching region, 3600 – 3300 cm^{-1}, increased with heat treatment process. . The decrease of the –OH absorption broad peak is indicated that there is some interaction between SF and PVA. The results indicated that the intermolecular interactions between hydroxyl groups of PVA and amide groups of SF was not fully occurred.

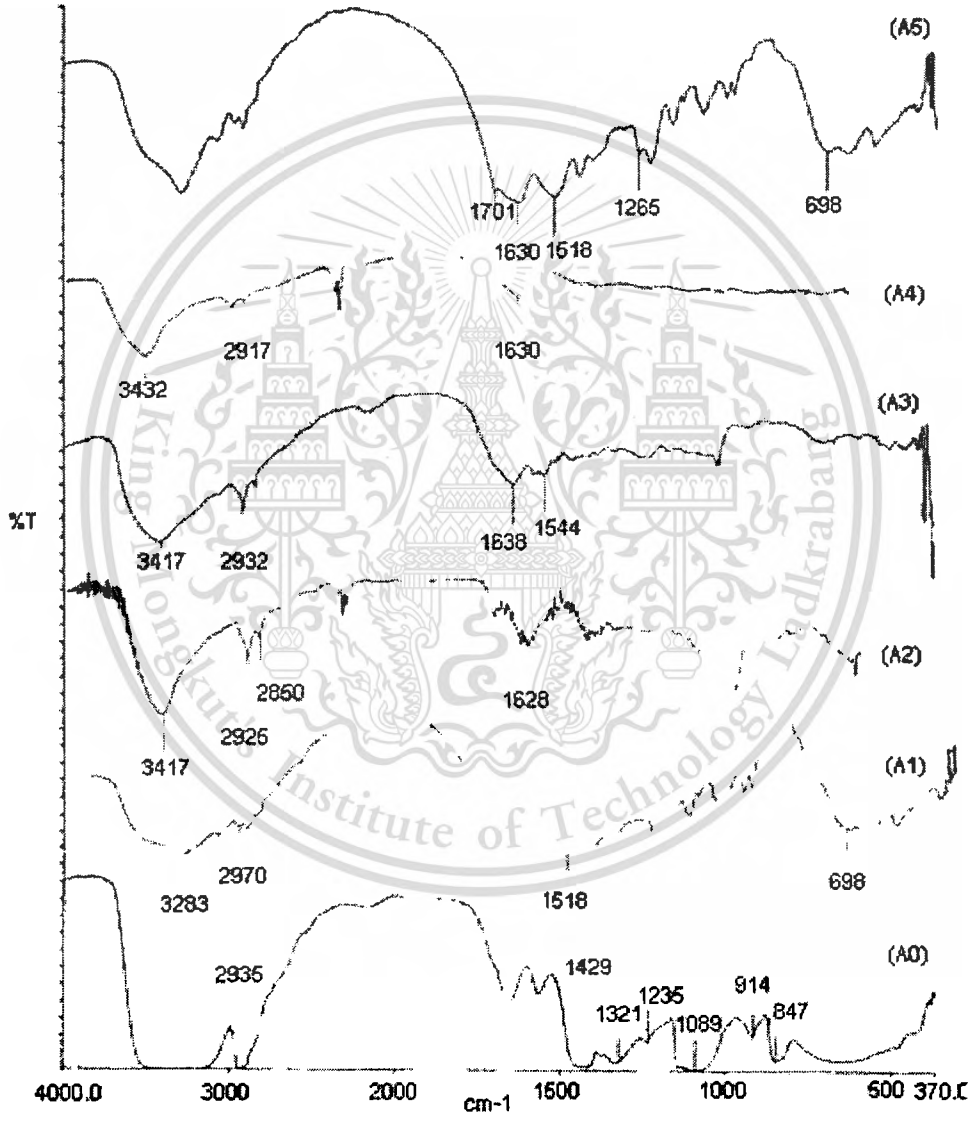


Figure 4.11 FTIR scans of PVA/SF blend films; SF film, (A5) silk powder film, (A4) untreated PVA/SF blend film, (A3) irradiated film, (A2) heat treated film (A1) and PVA film, (A0)

The IR measurements indicated that PVA and the amide group of fibroin were interacted with each other. After heating, it was found that some part free hydroxyl and amide group was recovered. This means that heat treatment process decreased hydrogen bonding between these two groups.

4.2.2 X-ray diffraction method (XRD)

X-ray diffraction patterns were recorded on a D8 Advance Bruker X-ray apparatus. Cu Kα radiation was used at 30 kV and 30 mV. The specimen was scanned from 2θ = 30° to 2θ = 10° at 5°/min. X-ray patterns indicated that crystalline structure of films which depended on each technique.

. The microstructures of hydrogel films under different film preparations were studied with X-ray diffractometer. The x-ray diffraction curves of pure PVA, (A0) silk fibroin solution, (A5) and blend films with different film preparation techniques were examined. In ordered to ascertain if any changes in the fine structure were induced by preparation techniques. The inter planar spacings, d, were determined from the diffraction distribution by using Bragg's equation.

The XRD pattern indicated crystalline structure of films depended on each technique. Pure PVA film, (A0) showed a less intense peak at 2θ = 11.9°, in addition to a major diffraction peak at 2θ = 20.5°. The x-ray diffraction curve of SF film, (A5) exhibited a broad 2θ peak at 20.6° that was assigned to the silk crystalline structure [34]. The diffraction curve of untreated blend film (A3) showed a minor peak at 11.2° and major peak 20.2°, corresponding to the peaks in the diffraction curves of PVA and SF.

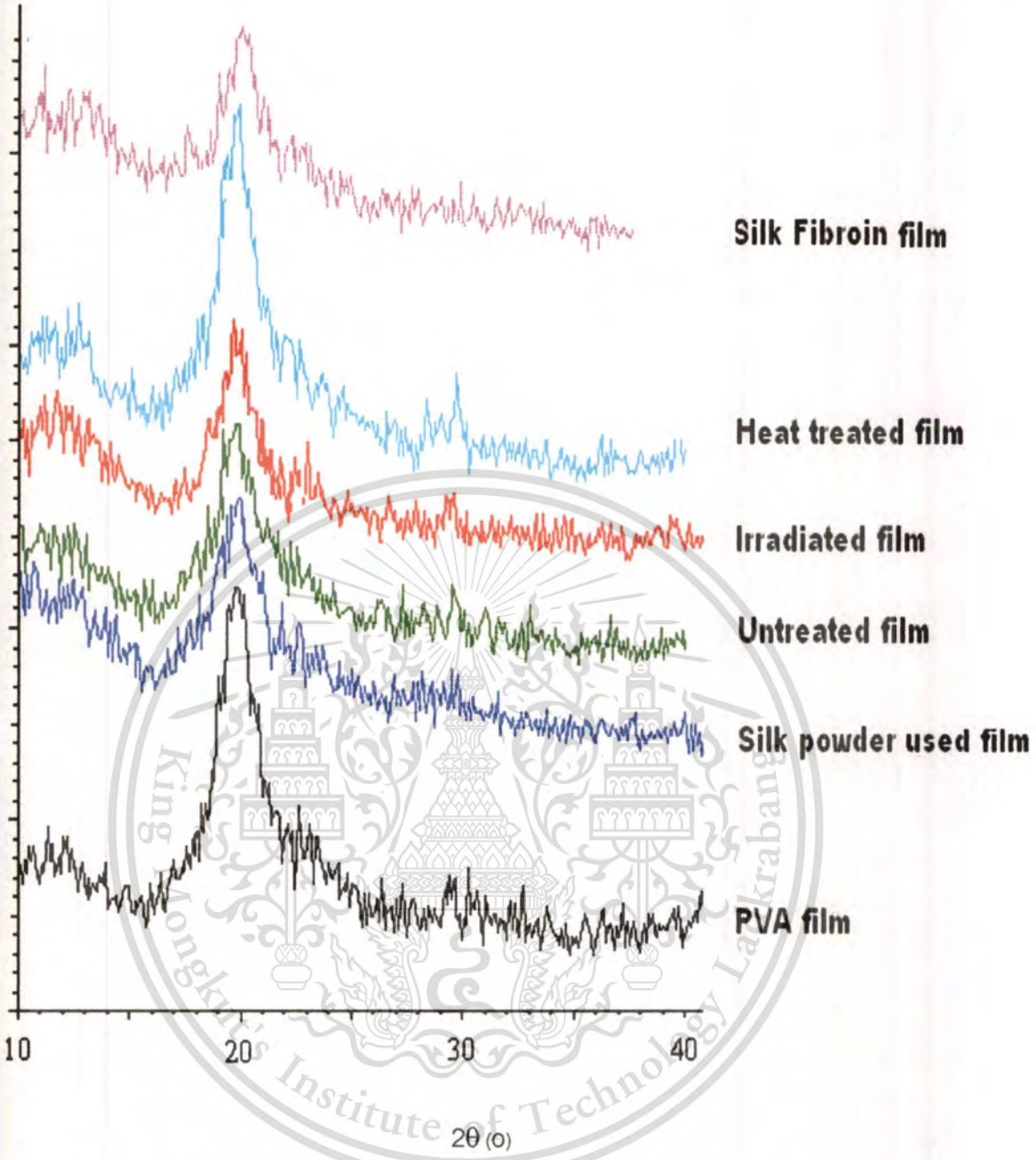


Figure 4.12 X-ray diffraction pattern of silk fibroin film, PVA film and blend films.

X-ray patterns showed that the blending served to decrease the peak intensity at around $2\theta = 20^{\circ}$ of PVA. The addition of heat treatment on blend film changed the pattern of peak at around $2\theta = 20^{\circ}$ whereas the irradiated film (A2), untreated film (A3) and PVA/SF films (A4) showed less intense peak at around $2\theta = 20^{\circ}$ than heat treated film. Accordingly, the strength of irradiated and silk powder used films were less strength than heat treated

4.2.3 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was the method that used to study the thermal behavior of silk fibroin blended with poly (vinyl alcohol). The thermal degradation was investigated using a Perkin-Elmer DSC Pyris Diamond differential scanning calorimeter under nitrogen atmosphere. The film was placed in an aluminium DSC pan with the sample total weight of 5 - 10 mg. The melting temperature (T_m) is meaningful for thermal behavior. Figure 4.13 showed the DSC thermogram of PVA, SF and blend films with various techniques. The pure PVA film, (A0) displayed a major endothermic peak at about 225 °C which is attributed to the melting of PVA. The single endothermic peak of A5 at about 298 °C attributed to the melting temperature of silk fibroin. All of the blends exhibited peak at around 290 °C of silk and peaks around 228 °C for the melt of PVA. Peaks of silk in blend film were decreased from about 298 to 300 °C. Melting temperature of PVA for A1, A2, A3 and A4 were about 225 °C. Melting temperature of silk for A1, A2, A3 and A4 were about 290 °C, 280 °C, 295 °C and 280 °C, respectively. This results indicated both PVA and silk fibroin interfered the crystallinity of each other.

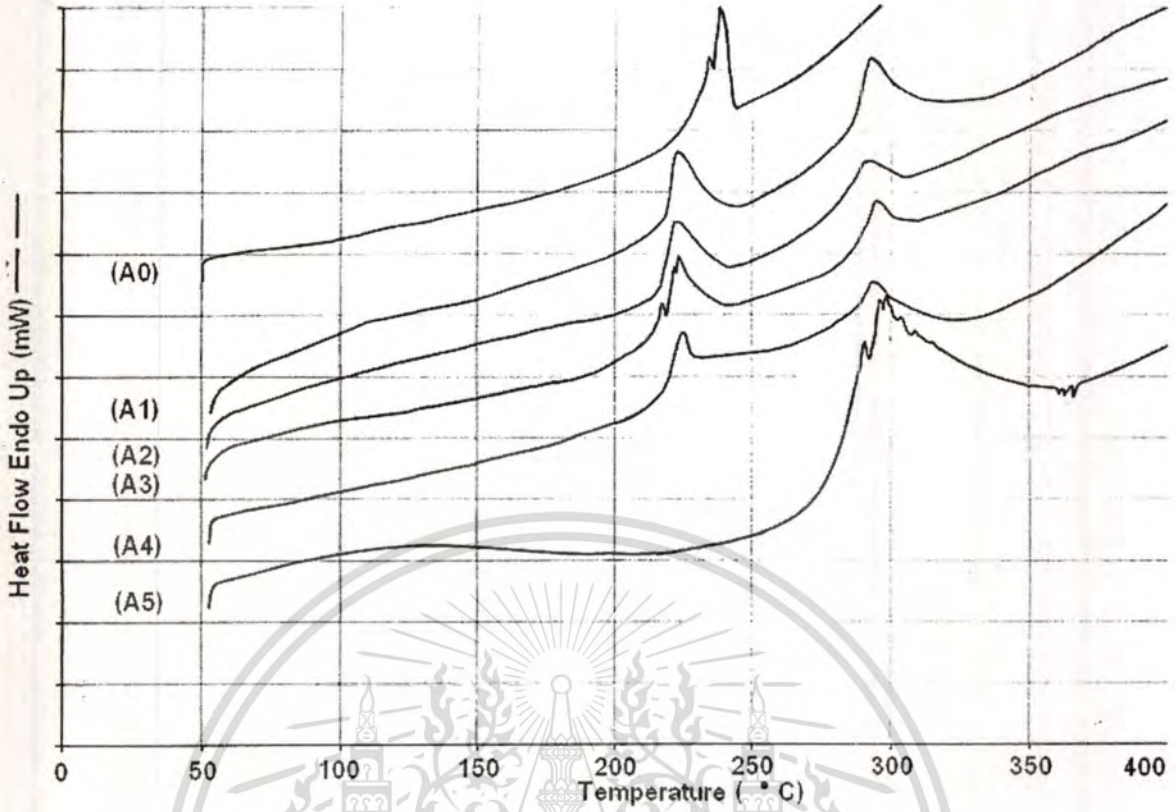


Figure 4.13 DSC thermograms of PVA/SF blend films; PVA film, (A0) Heat treated film, (A1) irradiated film, (A2) untreated film, (A3) PVA/silk powder film, (A4) and SF film, (A5)

4.2.4 Thermal Gravimetic Analysis

All thermogravimetric analysis (TGA) test were carried out by a Perkin-Elmer Pyris thermal analyzer at heating rate of 10 $^{\circ}$C min^{-1} under nitrogen atmosphere. Temperature range of sample was from 50 $^{\circ}$C to 400 $^{\circ}$C. Figure 4.14 showed thermogravimetric analysis curves of TG of PVA/SF blends and their components such as silk fibroin and PVA. The maximum rate of weight loss was used to illustrate the qualitative characterization of the degradation process. Initial degradation temperature of all samples were shown in Table 4.1. The two curves at the first step of heat treatment and irradiation process were nearly overlapping.

The weight loss curves of A2, A3 and A4 displayed multi-stages, Figure 4.14. The weight loss at around 100 $^{\circ}$C corresponding to the water evaporation in the materials.

The second and third step should be the degradation of PVA and of silk. However, the further decomposition of the residues appeared at above 350 °C. Untreated PVA/SF blend film, (A3) showed lower initial and maximum degradation rate temperatures compared with silk fibroin film. Thus, the thermal stability of silk was decreased with increasing PVA. The heat treatment film and PVA/silk powder film steadily increased the decomposition temperature.

Table 4.1 Initial degradation temperature of samples, (T_{onset})

Sample	Initial degradation temperature (°C)
A0 (PVA film)	256
A1 (heat treated film)	262
A2 (irradiated film)	231
A3 (untreated film)	248
A4 (silk powder film)	251
A5 (silk fibroin film)	197

Figure 4.15, A0 showed the decomposition point of PVA at 240 °C. A5 showed the decomposition temperature of SF at 290 °C. The blend A2, A3 and A4 were showed multiple peak indicated that it should have many materials with various melting temperature in various quantity.

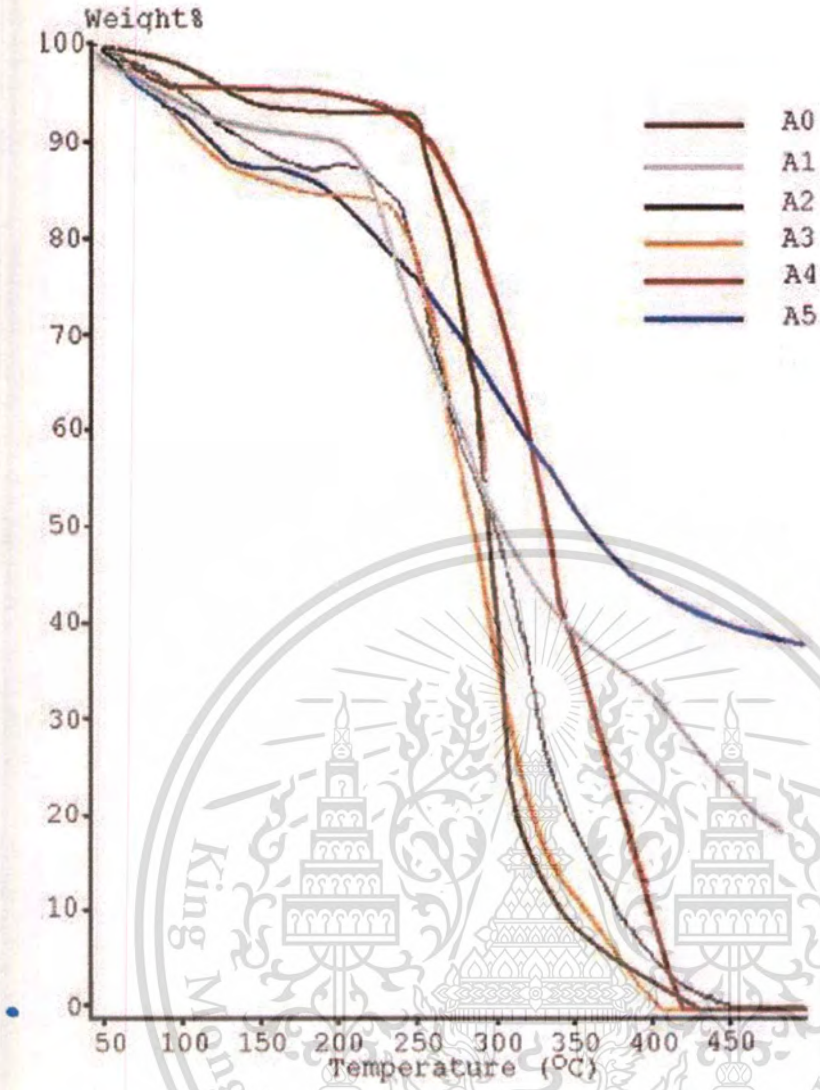


Figure 4.14 TGA curves of PVA/SF blend films; PVA film, (A0) Heat treated film, (A1) Irradiated film, (A2) Untreated film, (A3) PVA/Silk powder film, (A4) and SF film, (A5)

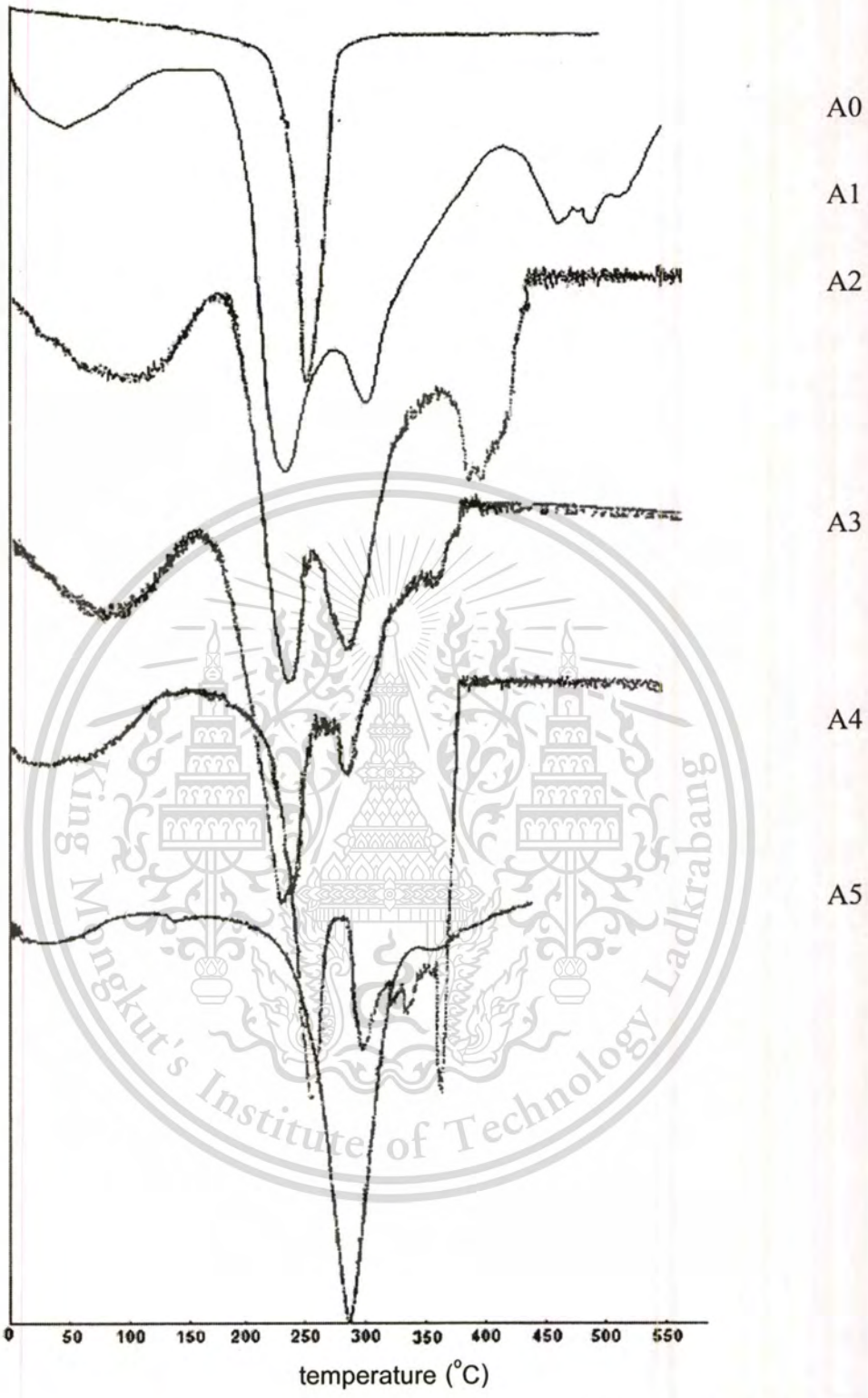


Figure 4.15 DTG curves of PVA/SF blend films; PVA film, (A0) Heat treated film, (A1) irradiated film, (A2) untreated film, (A3) PVA/silk powder film, (A4) and SF film, (A5)

4.3 Mechanical properties

Tensile tests were performed using a LLYOD INSTRUMENT LTD. tensile tester at a crosshead speed of 10 mm./min at 25 °C and under the relative humidity of 65%. Films used for study of mechanical properties were PVA film (A0), heat treated PVA/SF film (A1), irradiated PVA/SF film (A2), untreated PVA/SF film (A3) and PVA/silk powder films (A4). Changes of tensile properties were showed in figure 4.16. The effect of heat treament, silk powder and radiation techniques were compared with film of untreated blend. The order of tensile strength were A1 > A2 > A0 > A3 > A4, respectively. Because heat tretment incresed crystallinity on polymer matrix and then corresponding to increase tensile strength. However, the other blend films decreased their tensile strength when compare with PVA film (A0).

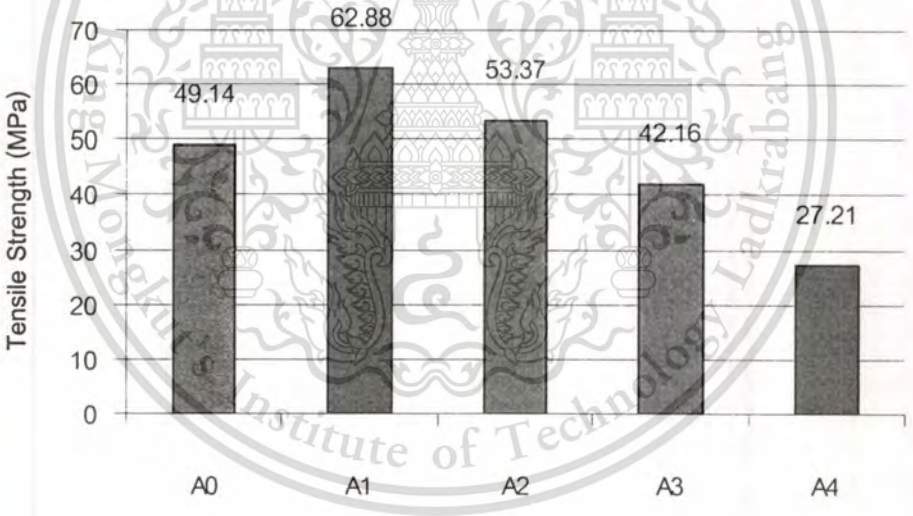


Figure 4.16 Tensile strength of A0, A1, A2, A3 and A4

Data of the tensile measurements imply that the tensile strength of blend film decreased mainly attributed to the adding of silk fibroin, especially silk powder. On the other hand, the increase of tensile strength should be attributed to the heat treament and radiation techniques. Structure of close packing on polymer after heat treatment process (A1) induced to increase tensile strength. It has been reported that good interfacial adhesion increase tensile properties. Because poor interfacial adhesion

leading to poor mechanical properties [35]. The results of tensile strength indicated that heat treated process is the suitable method for improvement film.

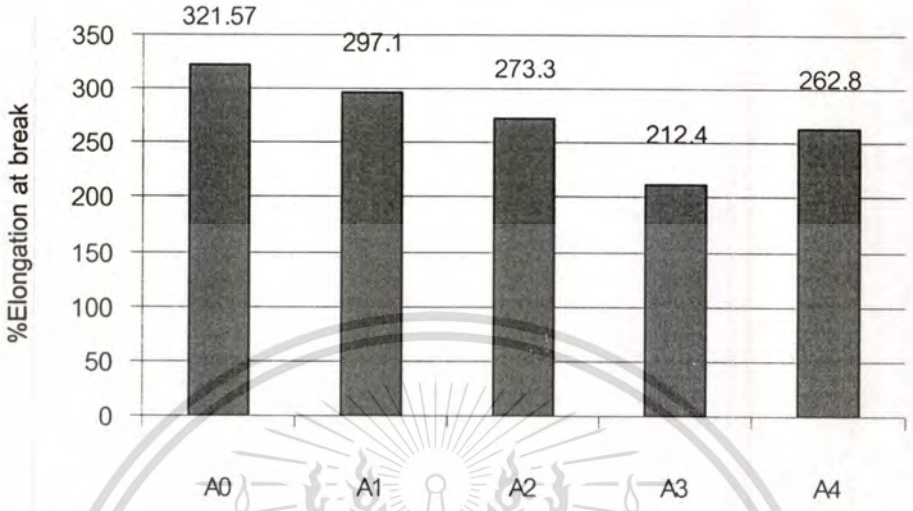


Figure 4.17 Percentage elongation at break of A0, A1, A2, A3 and A4

Figure 4.17 showed elongation at break of blend films and PVA film. Generally, PVA given a flexible film. After blending with silk, the elongation at break of the blend films were lower than the pure PVA film. The elongation of pure PVA film showed the highest value 321%. In the groups of blend films, heat treated sample (A1) reached a maximum value of elongation while the lowest was untreated film (A3). The order of elongation at break were A0 > A1 > A2 > A4 > A3, respectively.

Modulus of A1 and A2 were higher than PVA film. It indicated that these films were more brittle than PVA film. On the other hand, films which prepared from silk powder (A4) were lower modulus than PVA film. It indicated that this film is softer than PVA.

Table 4.2 Mechanical properties of samples

Samples	Tensile Strength (MPa)	Elongation at break (%)	Modulus (MPa)
A0	49.14	321.5	30.89
A1	62.88	291.1	35.07
A2	53.37	273.3	33.26
A3	42.16	212.4	27.72
A4	27.21	262.8	24.43

4.4 Scanning electron microscopy (SEM)

A LEO LEO-1455VP low-voltage, high resolution SEM with a field emission gun was used to examine the morphology of hydrogel films. PVA/silk fibroin blend films were attached to sample stubs coated with approximately 15 nm of gold prior to being imaged at 30 kV. The magnification of all sample is 10,000 times.

In polymer blends, it was essential to study the morphology of the final product since most of its properties, especially its mechanical properties depend on it. In most cases, the major component of the blend formed the continuous phase, whereas the minor component formed the dispersed phase. The blend morphology was controlled by parameters such as the nature of the polymers, the composition of the blend as well as the processing conditions.

The four types of sample that were heat treated film (A1), irradiated film (A2), untreated PVA/SF film (A3) and PVA/silk powder (A4) were used for this study. SEM micrographs of PVA/SF film with various film preparation were shown in Figure 4.18. The blends of A4 and A2 exhibited a similar surface morphology but the former had slightly smoother surface than the later.

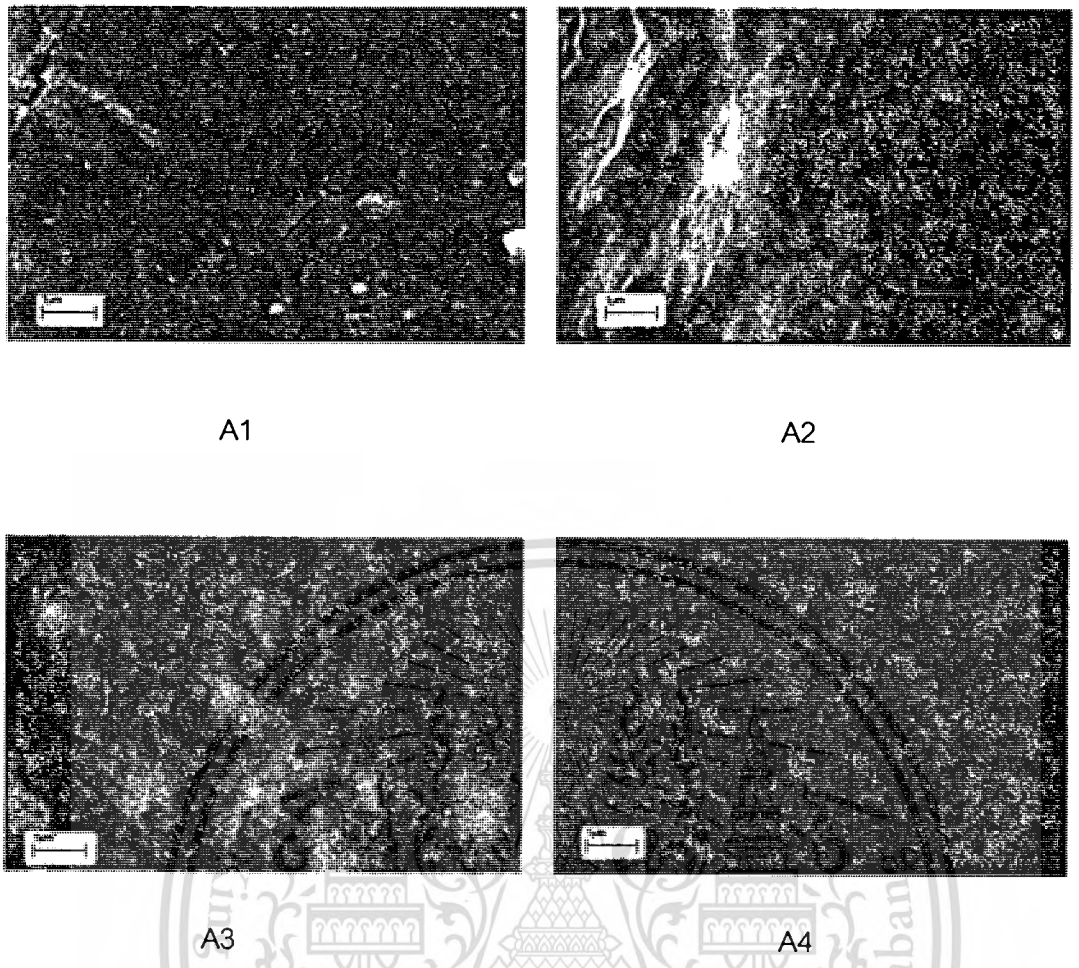


Figure 4.18 SEM images of surface of blend films (X10k). (A1) heat treated film, (A2) irradiated film, (A3) untreated blend PVA/SF film and (A4) silk powder used film

The different phase morphology of A1, A2 and A4 should be seen clearly when compared with A3. The results showed that smooth surface film, A1, A2 and A4 seemed to provided good mechanical properties. As mentioned earlier, the type of morphology did not depend only on the nature of the polymers and their volume but on the processing conditions as well. All images were not significantly exhibited the separated phase on film surface.

CHAPTER 5
CONCLUSIONS AND SUGGESTION

5.1 Conclusions

This research is focused on the effects of silk fibroin, special techniques such as heat treatment and irradiation and the differences of silk solution sources such as silk dialysis and silk powder on blend films.

5.1.1 Effects of silk fibroin on blend films

In this study, the effects of silk fibroin as used to blend with poly (vinyl alcohol) were investigated.

1. The results of water uptake and water retention measurements of all blends were increased compared with that of another blends. With this properties is important for dressing material. The rapid water absorbed, the water loaded and shapable under water condition are expected to be dressing material.

2. The melting temperature of PVA was decreased when blended silk with PVA for all blend films. Silk is distributed the structure of blend film as decreased melting temperature of PVA while less distributed to the melting point of silk.

3. The tensile strength and modulus of blend with heat treatment and irradiation techniques were increased while blend with untreated process was decreased. Silk powder film was developed the properties of elongation but tensile strength was decreased.

5.1.2 Comparison of films with heat treatment process and irradiation technique

This step was studied and comparison the properties of heat treated film and irradiated film.

1. Percentage of water uptake and water content of heat treated film was higher than irradiated film because the heat treated film was not fully occurred crosslinking into polymer matrix while irradiated film should have crosslinking.

2. The melting temperature of PVA and SF on polymer blends were decreased when used both heat treatment process and irradiation technique.

3. Adding heat treatment process in blend gave the significantly increased in mechanical properties. The tensile strength, elongation at break and modulus of heat treated film were higher than irradiated film.

5.1.3 Effects of silk solution sources versus silk dialysis and silk powder

In this study, the effects of silk solution sources between untreated film (A3) as used silk dialysis and silk powder film (A4) for carrying out mechanical properties of blend film.

1. Percentage of water uptake and water retention of untreated film are higher than silk powder film. Then untreated film gave the better properties of water uptake and water retention.

2. Melting temperature of untreated film and silk powder film were decreased. Adding silk into polymer blend was decreased melting temperature of PVA but less decreased melting temperature of silk.

3. The film which made from silk powder had dominant property in elongation. Then the silk powder film gave higher elongation at break than film that used silk dialysis. On the other hand, tensile strength and modulus of untreated film were higher than silk powder film.

In table 5.1 showed all of the sample results from A0 to A5. It is included of percentage of gel fraction, percentage of water uptake, percentage of water content, percentage of water retention, melting temperature, initial degradation temperature, tensile strength, elongation at break and modulus.

5.2 Suggestion for further work

1. The water absorption properties was concentrated only at the ambient air. In fact, the dressing is used on human skin which had 37 °C. Then the influence of body temperature as above room temperature should be investigated.

2. Due to the difficult to peel film off the tray, the operator had to carefully peel film off the tray without film damage. Accordingly, it needed compatibilizer for improving properties. .

3. In this research, all films were examined under dry condition. The strength of them were not good. After improving their strength, it was very interesting to experiment under wet condition. Some additives would have to used for this purpose.

4. The expected advantages from this study are to improve process of casting hydrogel film for higher water absorption and higher strenght with natural resource, i.e. using silk waste or algae. In the meantime, because of some unique properties, such as nontoxicity and biodegradability, natural polymers have been paid a great deal of attention by many researchers. Since silk fibroin contribute substantially to the biodegradability of hydrogel film this means also a waste reduction.

Table 5.1 Synopsis of sample results from A0, A1, A2, A3, A4 and A5

Sample	%Gel Fraction	%Water Uptake	%Water Content	%Water Retention	T_m (°C)		Initial degradation Temperature (°C)	Tensile Strength (MPa)	Elongation at break (%)	Modulus (MPa)
					PVA	SF				
A0	N.D.	N.D.	N.D.	N.D.	225	-	256	49.14	321.5	30.89
A1	78.65	146.33	59.34	29.94	225	290	262	62.88	297.1	35.07
A2	79.61	75.27	42.94	26.57	225	280	231	53.77	273.3	33.26
A3	77.35	132.52	56.98	36.93	225	295	248	42.16	212.4	27.72
A4	75.87	61.07	37.95	24.02	225	280	251	27.21	262.8	24.43
A5	N.D.	N.D.	N.D.	N.D.	-	290	197	N.D.	N.D.	N.D.

Notice : N.D. = Non detectable

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APPENDICES

APPENDIX A

EXPERIMENTAL DATA FOR CALCULATION OF % WATER UPTAKE

Table A.1 Wet weight and %water uptake of blend film during soaking process between 0 to 72 hours.

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
1	2.0546	2.3747	2.1641	1.8967	27.8500	39.6882	44.3733	26.4466
	2.0110	2.4202	2.0025	1.9021	25.6875	42.3647	33.5000	26.8066
	2.0025	2.4430	1.9706	2.0404	25.1562	43.7058	31.3733	36.0266
Total average					26.2312	41.9195	36.3822	39.7599
2	2.3276	2.5266	2.5211	2.0839	45.4750	48.6235	68.0733	38.9266
	2.2177	2.5944	2.5089	2.1254	38.6062	62.6117	67.2600	41.6933
	2.4441	2.5946	2.4169	2.1267	52.7562	52.6235	61.1266	41.7800
Total average					45.6124	51.2862	65.4866	40.7999
3	2.9150	2.6238	2.7225	2.1460	82.1875	54.3411	81.5000	43.0666
	2.7156	2.6779	2.7336	2.1565	69.7250	57.5823	82.2400	43.7666
	2.6132	2.6893	2.6427	2.1585	63.6232	58.0176	76.1800	43.9000
Total average					71.7458	56.6470	79.9733	43.5777
4	3.4101	2.7853	2.9742	2.1602	113.1312	63.8411	98.2800	44.0133
	3.2555	2.8073	2.9025	2.1729	103.4687	65.1352	93.5000	44.8600
	2.8128	2.9147	2.9003	2.1915	75.8000	67.1823	93.3533	46.2000
Total average					97.4666	70.6372	95.0444	45.0244

Notices: 1) initial weight of A1 = 1.600 ± 0.005 g.

2) initial weight of A2 = 1.700 ± 0.005 g

3) initial weights of A3 and A4 = 1.500 ± 0.005 g

Table A.1 (continued)

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
5	3.8597	2.8630	3.1197	2.2136	141.2312	68.4117	107.9800	47.5733
	3.5226	2.9147	3.0141	2.2397	120.1625	71.4529	100.9400	49.3133
	3.4007	2.9248	3.0385	2.2424	112.5437	72.0470	102.5666	49.4933
Total average					124.6458	70.6372	103.8288	48.7933
6	3.9006	2.8838	3.2115	2.2591	143.7875	69.6352	114.1000	50.6066
	3.6783	2.9389	3.1617	2.2752	129.8937	72.8764	110.7800	51.6800
	3.4748	2.9547	3.2485	2.3105	117.1750	73.8054	116.5666	54.0333
Total average					130.2854	72.1056	113.8155	52.1066
7	4.0566	2.9285	3.3439	2.3114	153.5375	72.2647	122.9266	54.0933
	3.7236	2.9677	3.2894	2.3141	132.7250	74.5705	119.2933	54.2733
	3.6767	2.9734	3.2948	2.3411	129.7937	74.9058	119.6533	56.0733
Total average					138.6854	73.9136	120.6244	54.8133
8	4.1375	2.9553	3.5427	2.3726	158.5937	73.8411	136.1800	58.1733
	3.9078	2.9814	3.4882	2.4131	144.2375	75.3764	132.5466	60.8733
	3.7787	3.0025	3.4326	2.4687	136.1687	76.6176	128.8400	64.5800
Total average					146.3333	75.2783	132.5222	61.0755
24	4.2625	2.9906	3.6488	2.4805	166.4062	75.9176	143.2533	65.3666
	4.1131	2.9944	3.5302	2.5473	157.0687	76.1411	135.3466	69.8200
	4.0710	3.0225	3.4549	2.5553	154.4375	77.7941	130.3266	70.3533
Total average					159.3041	76.6176	136.3088	68.5133
48	4.3263	2.9949	3.6743	2.5634	170.3937	76.1705	144.9533	70.8933
	4.2194	3.0294	3.5676	2.6127	163.7125	78.2000	137.8400	74.2800
	4.1041	3.0407	3.4940	2.6168	156.5062	78.8647	132.9333	74.4533
Total average					163.5374	77.7450	138.5753	73.2088

Table A.1 (continued)

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
72	4.4843	3.0389	3.7385	2.6219	180.2687	78.7588	149.2333	74.7933
	4.2447	3.0653	3.6118	2.6266	165.2937	80.3117	140.7866	75.1066
	4.1109	3.0740	3.5140	2.6518	156.9312	80.8235	134.2666	76.7866
Total average					167.4978	79.9646	141.4288	75.5621

APPENDIX B

EXPERIMENTAL DATA FOR CALCULATION OF %WATER CONTENT

Table B.1 Wet weight and %water content of blend film during soaking process between 0 to 72 hours.

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
1	2.0546	2.3747	2.1641	1.8967	21.6879	28.4119	30.7564	20.9152
	2.0110	2.4202	2.0025	1.9021	20.4375	29.7579	25.0936	21.1397
	2.0025	2.4430	1.9706	2.0404	20.0998	30.4134	23.8810	26.4849
Total average					20.7417	29.5277	26.5770	22.8466
2	2.3276	2.5266	2.5211	2.0839	31.2597	32.7159	40.5021	28.0195
	2.2177	2.5944	2.5089	2.1254	27.8532	41.0268	40.2128	29.4250
	2.4441	2.5946	2.4169	2.1267	34.5362	34.4793	37.9370	29.4682
Total average					31.2163	36.0740	39.5506	28.9709
3	2.9150	2.6238	2.7225	2.1460	45.1115	35.2084	44.9036	30.1025
	2.7156	2.6779	2.7336	2.1565	41.0812	36.5547	45.1273	30.4428
	2.6132	2.6893	2.6427	2.1585	39.9550	36.6749	43.2399	30.5073
Total average					42.0492	36.1460	44.4236	30.3508
4	3.4101	2.7853	2.9742	2.1602	53.0805	38.9652	49.5662	30.5619
	3.2555	2.8073	2.9025	2.1729	50.8524	39.4435	48.3204	30.9678
	2.8128	2.9147	2.9003	2.1915	43.1172	39.1841	48.2812	31.6221
Total average					49.0167	39.1976	48.7226	31.0506

Notices: 1) initial weight of A1 = 1.600 $\pm$ 0.005 g.

2) initial weight of A2 = 1.700 $\pm$ 0.005 g

3) initial weight of A3 and A4 = 1.500 $\pm$ 0.005 g

Table B.1 (continued)

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
	3.8597	2.8630	3.1197	2.2136	58.5459	40.6216	51.9184	32.2370
5	3.5226	2.9147	3.0141	2.2397	54.5790	41.6749	50.2339	33.0267
	3.4007	2.9248	3.0385	2.2424	52.9508	41.8763	50.6335	33.1073
Total average					55.3585	41.3909	50.9286	32.7903
	3.9006	2.8838	3.2115	2.2591	58.9806	41.0499	53.2928	33.6018
6	3.6783	2.9389	3.1617	2.2752	56.5016	42.1551	52.5571	34.0717
	3.4748	2.9547	3.2485	2.3105	53.9541	42.4642	53.8248	35.0789
Total average					56.4788	41.8898	53.2249	34.2508
	4.0566	2.9285	3.3439	2.3114	60.5581	41.9498	55.1421	35.1042
7	3.7236	2.9677	3.2894	2.3141	57.0308	42.7165	54.3989	35.1799
	3.6767	2.9734	3.2948	2.3411	56.4826	42.8263	54.4737	35.9275
Total average					58.0238	42.4975	54.6716	35.3705
	4.1375	2.9553	3.5427	2.3726	61.3293	42.4792	57.6594	36.7782
8	3.9078	2.9814	3.4882	2.4131	59.0563	42.9798	56.9979	37.8393
	3.7787	3.0025	3.4326	2.4687	57.6574	43.3805	56.3013	39.2393
Total average					59.3476	42.9454	56.9858	37.9522
	4.2625	2.9906	3.6488	2.4805	62.4633	43.1552	58.8906	39.5283
24	4.1131	2.9944	3.5302	2.5473	61.0999	43.2273	57.5095	41.1141
	4.0710	3.0225	3.4549	2.5553	60.6976	43.7552	56.5834	41.2985
Total average					61.4202	43.3791	57.6611	40.6469
	4.3263	2.9949	3.6743	2.5634	63.0169	43.2368	59.1759	41.4839
48	4.2194	3.0294	3.5676	2.6127	62.0799	43.8833	57.9549	42.6455
	4.1041	3.0407	3.4940	2.6168	61.0146	44.0918	57.0693	42.6871
Total average					62.0271	43.7372	58.0666	42.2692

Table B.1 (continued)

Time	Wet weight (g)				Water uptake (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
	4.4843	3.0389	3.7385	2.6219	64.3199	44.0587	59.8769	42.7896
72	4.2447	3.0653	3.6118	2.6266	62.3059	44.5405	58.4694	42.8919
	4.1109	3.0740	3.5140	2.6518	61.0791	44.6975	57.3136	43.4346
Total average					62.5683	44.4322	58.5533	43.0386

APPENDIX C

EXPERIMENTAL DATA FOR CALCULATION OF % WATER RETENTION

Table C.1 Dried weight and %water retention of blend film during soaking process between 0 to 72 hours.

Time	Dried weight (g)				Water retention (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
1	0.7457	0.8238	0.7879	0.8074	56.4924	48.8068	70.0978	52.8057
	0.7426	0.7428	0.7871	0.8043	56.2575	42.2045	70.0266	52.6030
	0.7401	0.7150	0.7779	0.7966	56.0681	40.6250	69.2081	52.0994
Total average					56.2723	43.8787	69.7775	52.5027
2	0.6074	0.6592	0.4936	0.7405	46.0151	37.4545	43.9145	48.4303
	0.6058	0.6570	0.4704	0.7398	45.8939	37.3295	41.8505	48.3845
	0.6042	0.6563	0.4567	0.7359	45.7727	37.2897	40.6316	48.1295
Total average					45.8939	37.3579	42.1322	48.3147
3	0.5227	0.5611	0.4228	0.5938	34.1812	31.8806	37.6156	38.8358
	0.5219	0.5562	0.4219	0.5889	34.1289	31.6022	37.5355	38.5153
	0.5213	0.5460	0.4218	0.5863	34.0897	31.0227	37.5266	38.3453
Total average					34.1332	31.5018	37.5592	38.5654
4	0.5154	0.4996	0.4215	0.4936	31.5809	28.3863	37.5000	32.2825
	0.5136	0.4973	0.4213	0.4704	31.4706	28.2556	37.4822	30.7652
	0.5129	0.4965	0.4211	0.4567	31.4277	28.2102	37.4644	29.8692
Total average					31.4930	28.2840	37.4822	30.9723

Notices: 1) Initial weight of all samples = 0.600 $\pm$ 0.05 g.

2) Swollen weight of A1, A2, A3 and A4 = 1.6320, 1.7600, 1.1240 and 1.5290 $\pm$ 0.005 g. respectively

Table C.1 (continued)

Time	Dried weight (g)				Water retention (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
5	0.5040	0.4877	0.4205	0.4257	30.8824	27.7102	37.4110	27.8417
	0.5031	0.4875	0.4199	0.4202	30.8273	27.6988	37.3576	27.4820
	0.5023	0.4869	0.4198	0.4066	30.7782	27.6647	37.3487	26.5925
Total average					30.8293	27.6912	37.3724	27.3054
6	0.5019	0.4724	0.4187	0.3752	30.7537	26.8409	37.2508	24.5389
	0.5009	0.4721	0.4182	0.3743	30.6925	26.8238	37.2064	24.4768
	0.4974	0.4719	0.4178	0.3738	30.4780	26.8125	37.1708	24.4441
Total average					30.6414	26.8257	37.2093	24.4866
7	0.4973	0.4689	0.4168	0.3736	30.4719	26.6420	37.0818	24.4310
	0.4965	0.4685	0.4167	0.3734	30.4228	26.6193	37.0729	24.4180
	0.4953	0.4682	0.4162	0.3717	30.3493	26.6022	37.0284	24.3068
Total average					30.4146	26.6135	37.0610	24.3852
8	0.4943	0.4681	0.4156	0.3683	30.2880	26.5965	36.9750	24.0844
	0.4880	0.4677	0.4149	0.3674	29.9020	26.5738	36.9306	24.0256
	0.4840	0.4672	0.4147	0.3664	29.6569	26.5454	36.9039	23.9602
Total average					29.9489	26.5719	36.9365	24.0234
24	0.4815	0.4552	0.4132	0.3514	29.5037	25.8636	36.7615	22.9793
	0.4721	0.4529	0.4127	0.3501	28.9277	25.7329	36.7170	22.8943
	0.4213	0.4480	0.4119	0.3492	28.8787	25.4545	36.6459	22.8354
Total average					29.1033	25.6836	36.7081	22.9030
48	0.4803	0.4281	0.4109	0.3459	29.4302	24.3238	36.5569	22.6196.
	0.4715	0.4277	0.4107	0.3454	28.8910	24.3011	36.5391	22.5869
	0.4702	0.4272	0.4106	0.3452	28.8113	24.2727	36.5302	22.5738
Total average					29.0441	24.2992	36.5420	22.5934

Table C.1 (continued)

Time	Dried weight (g)				Water retention (%)			
(hrs)	A1	A2	A3	A4	A1	A2	A3	A4
72	0.4803	0.4271	0.4098	0.3446	29.4302	24.2670	36.4590	22.5346
	0.4715	0.4267	0.4093	0.3443	28.8910	24.2443	36.4145	22.5150
	0.4702	0.4266	0.4084	0.3439	28.8113	24.2386	36.3345	22.4888
Total average					29.0441	24.2499	36.4026	22.5128

APPENDIX D

CHARACTERISTIC INFRARED AMIDE BANDS OF PROTEINS

Table D.1 Characteristic infrared amide bands of protein [32].

Designation	Approximate Frequency (cm^{-1})	Nature of vibration
I	1630	C=O Streching
II	1530	In-plane N-H Bending coupled with C-N Streching
III	1299	Coupled N-H Bending and C-N Streching As in amide II but in opposite plane
IV	699	In-plane C=O Bending
V	725	Out-of-plane N-H Bending
VI	600	Out-of-plane C=O Bending
VII	200	Out-of-plane C-N

APPENDIX E

FTIR SCAN OF SILK FIBROIN FILM AND SILK POWDER

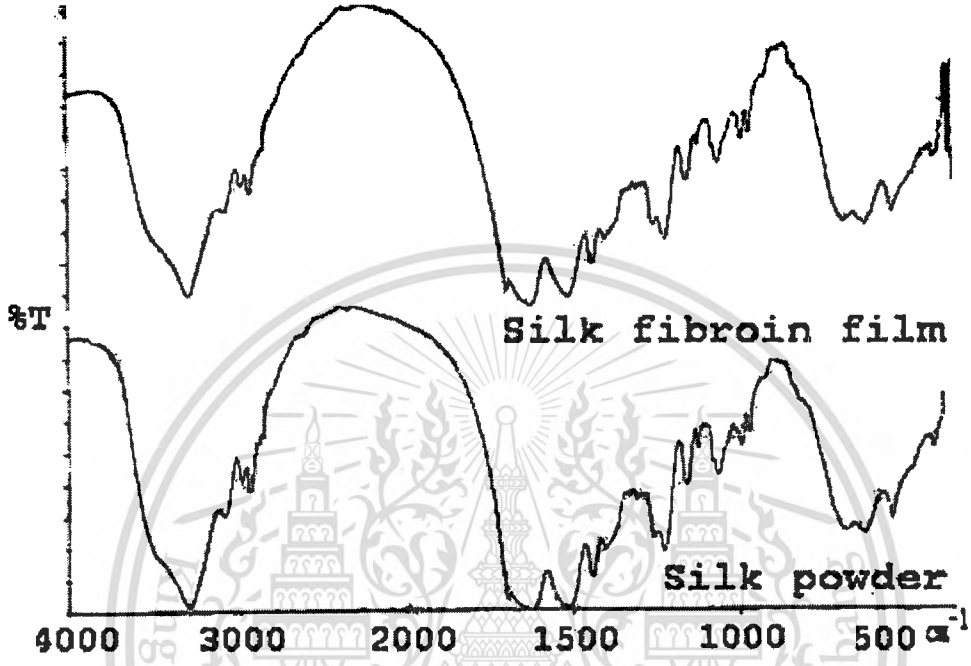

Figure D.1 FTIR scan of silk fibroin film and silk powder

APPENDIX F

SEM MICROGRAPH OF SILK POWDER

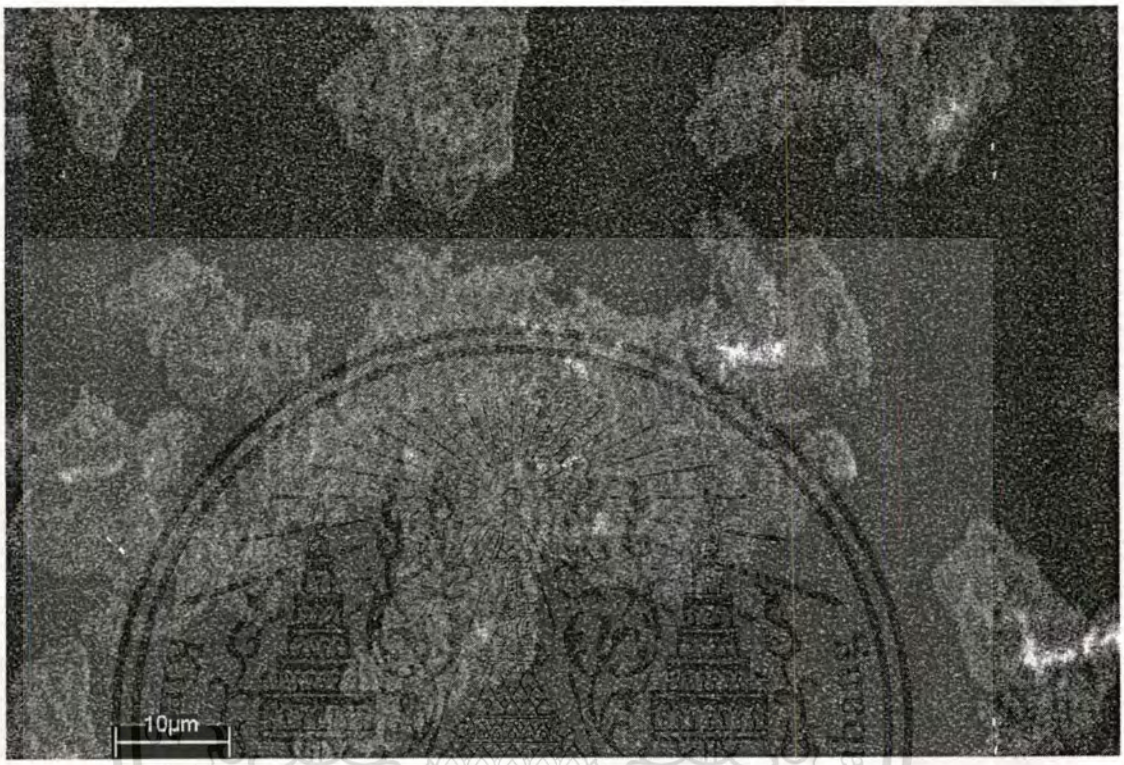

Figure E.1 SEM micrograph of silk powder X1000

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