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THESIS

HYDROGEL PREPARATION FROM REGENERATED CELLULOSE AND SILK FIBROIN

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A Thesis Submitted in Partial Fulfillment of
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This study was on the preparation of the blended hydrogel from the regenerated cellulose obtained from rayon fiber residue, the industrial waste fiber, and *Bombyx mori* silk fibroin. The regenerated cellulose possessed cellulose II crystalline form while the β-sheet structure of silk fibroin was observed. The infrared spectra of the blends showed changes in shape and intensity of IR absorption frequency characteristics in the region of 3500-3300 cm^{-1} as compared with those of the starting materials, cellulose and pure silk fibroin. This indicates the occurrence of the intermolecular interactions between the –OH groups of cellulose and silk fibroin by forming hydrogen bond. The tensile properties of the blends with cellulose content lower than 50 % could not be measured due to the weakness of the prepared hydrogel. The stronger and more flexible hydrogel was obtained with increasing the regenerated cellulose content and the pure cellulose hydrogels had the swelling ratios higher than the blends. The 75 and 100% cellulose hydrogels with a crosslinking agent, NHDT, could absorb higher water molecules to their structures and exhibit a higher initial degradation temperature as compared with those without NHDT. The study on the effect of NaCl and $CaCl_2$ salt addition aiming to establish a porous structure to the blends showed that adding 2.5 and 5%$CaCl_2$ did not affect the structure of hydrogels while the 10%$CaCl_2$ did result in a rough and porous structure. In addition, hydrogels with added NaCl showed macroporous structure and the highest swelling ratios at about 150% while the pure cellulose hydrogels displayed the lowest swelling ratio around 40%.

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Laksanawadee Saikhao
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LIST OF ABBREVIATIONS

Ala = Alanine

A^o = Angstrom

ASTM = American society for testing and materials

ATR-FTIR = Attenuated total reflection fourier transform infrared

cm = Centimetre

cS = Centisiemens

FTIR = Fourier transformed infrared spectroscopy

Gly = Glycine

GPa = Gigapascal

kDa = Kilodalton

M = Molarity

MPa = Megapascal

M_v = Molecular weight

nm = Nanometre

SEM = Scanning electron microscope

Ser = Serine

SF = Silk fibroin

SR = Swelling ratios

TGA = Thermal gravimetric analysis

WAXD = Wide-angle x-ray diffraction

XRD = X-ray diffraction

β = *Beta-*

η = The intrinsic viscosity

HYDROGEL PREPARATION FROM REGENERATED CELLULOSE AND SILK FIBROIN

INTRODUCTION

Hydrogel is a chemically or physically cross-linked structure composed of hydrophilic polymers in a three dimensional network and has the ability to absorb a large amount of water and swell while maintaining their three-dimensional structure (Yang *et al*., 2010). The hydrogels derived from natural polysaccharides have been found in several applications such as food industry, biomedicals, and cosmetics because of their unique properties, viz. high swelling capacity, biocompatibility and biodegradability (Zhou *et al*., 2007).

Cellulose is the most abundant naturally occurring polymer being found as the main constituent of plants and natural fibers such as cotton and linen (Sannino *et al*., 2009). Cellulose, a poly-β-1,4-d-glucopyranose, is biodegradable, nontoxic, biocompatible, hydrophilic, safe and has high moisture retentivity. Thus, making use of cellulose to produce various products does not only help protecting the environment from pollution but also can save limited oil resources because of its biodegradability and potential to substitute for some petrochemicals. Moreover, cellulose has many uses as emulsifier, stabilizer, dispersing agent, thickener, and gelling agent for the preparation of textiles. However, cellulose has not reached its potential application in many areas because of its infusibility and insolubility (Abou-Zeid *et al*., 2011).

Regenerated cellulose fibers are made of cellulose from wood pulp through a process of fiber forming. The examples of regenerated cellulose fibers are viscose rayon, lyocell and cellulose acetate. Each type of cellulose fibers has a different manufacturing process. Typically, dissolving cellulose in an appropriate solvent yields a viscous solution that can be formed into fiber. Chemical modification of cellulose can also be done in order to gain the fiber with different properties, for example cellulose acetate. In the industrial process of regenerated cellulose fiber spinning, the cellulose solution is pressed through a fine spinneret and the cellulose filaments are continuously produced. These filaments are then cut into staple fiber at a proper length. During this process, the fibers at the beginning and the end are eliminated and left behind as a waste. Consequently, there are quite a large quantity of cellulose fiber waste generated from production of the regenerated cellulose fiber.

Silk from *Bombyx mori* silkworm is a natural fibrous protein consisting of sericin and fibroin proteins. Silk fibroin (SF) is fibrous protein fibers in a cocoon of silk worm being held together by a glue-like protein, sericin. SF fibers are composed of a repetitive sequence of amino acids: glycine (Gly), alanine (Ala) and serine (Ser). As all fibrous proteins, SF is not soluble in water because of its high concentration of hydrophobic amino acids (Moraes *et al*., 2010). SF consists of heavy (350 kDa) and light (25 kDa) polypeptide fractions connected by disulfide linkages. The amino acids comprising of the high-molar-mass chains of SF are capable of organizing into crystalline β-sheet structures (Gil *et al*., 2007).

This research focuses on the preparation of hydrogels made from regenerated cellulose fiber, the waste from textile industries, in order to transform fiber waste into the potentially value-added products. The preparation of a blended hydrogel from regenerated cellulose and silk fibroin was investigated. Moreover, porous blended hydrogels from the addition of salt and the crosslinked blended hydrogels were also prepared. The properties of the obtained hydrogels, i.e. tensile properties, swelling ratios, surface characteristics, thermal stability were examined.

OBJECTIVES

1. To study the preparation of the hydrogels from regenerated cellulose fiber waste and silk fibroin.

2. To investigate the properties of the hydrogels prepared from regenerated cellulose fiber waste and silk fibroin.

3. To study the effect of salt addition and chemical crosslinking on the properties of the hydrogels.

LITERATURE REVIEW

1. Hydrogels

Hydrogels are hydrophilic, crosslinked, network polymers which can absorb a large amount of water (Chang *et al*., 2010b). Hydrogels can be synthesized from natural and synthetic polymers, which are crosslinked by physical and/or chemical methods. Molecular self-assembly through ionic interactions and hydrogen bondings could provide physical crosslinked hydrogels, while the chemical crosslinked hydrogels are formed through covalent bonding (Chang and Zhang, 2011). Hydrogels have been found using in drug delivery, contact lenses (Darwis, 2009), biosensors, absorbents for removal of heavy metals, agriculture and biomedical materials (Chang *et al.*, 2010a).

The degree of crosslinking and porosity influence on the integrity and swelling properties of hydrogels. Greater extent of crosslinking brings about a less flexible and lower swellable hydrogel (Rimmer, 2011). In addition, a high degree of crosslinking causes the fragileness of hydrogels (Peppas *et al.*, 2000). The fabrication of polymer hydrogel can be done by three different methods, viz crosslinking of high molecular-weight polymers, copolymerization of polymer with multi-functional monomers and coupling of polymer end groups with telechelic oligomer (Rimmer, 2011).

2. Cellulose

Cellulose is a natural polymer, which is found in natural fibers and plants (Rimmer, 2011). Cellulose is the most abundant polysaccharide and considered as an environmentally friendly, biocompatible (Chang *et al.*, 2010a) and hydrophilic material (Ruan *et al.*, 2008). The latter one is because of the hydroxyl groups on the cellulose chain. The structure of cellulose consists of β-(1-4)-glycosidic linkages as demonstrated in Figure 1 (Phonwong *et al.*, 2000).

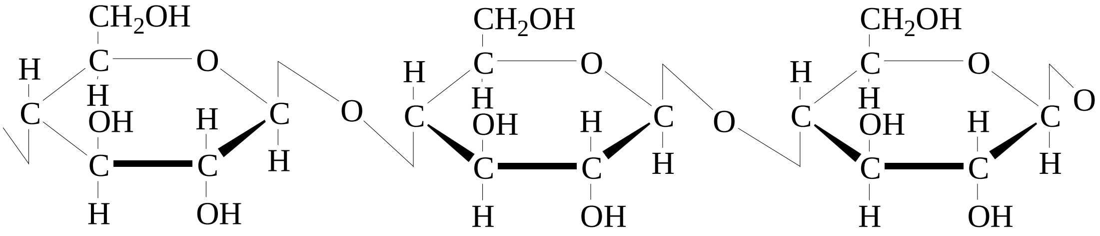


Figure 1 The structure of cellulose.

At present, cellulose is widely used for production of textiles fiber (Marsano *et al.,* 2007). However, as cellulose has hydroxyl groups in its structure, it can simply be used for hydrogel preparation. Cellulose hydrogels can be prepared from a cellulose solution through physical crosslinking. Therefore, cellulose fibers are needed to be dissolved in solvent for hydrogel fabrication. One of the best possible solvent systems to dissolve cellulose was LiCl/DMAc, discovered in the preparation of unsubstituted cellulose hydrogel (Oliveira and Glasser, 1996). In addition, this cellulose solvent system was a faster and easier direct dissolution method of cellulose (Dupont, 2003). There are many researchers interested in studying the cellulose dissolution using LiCl/DMAc as follows.

Dupont (2003) studied the dissolution methods of cellulose from paper substrate by using 8%LiCl/DMAc at 4°C. He found that the optimum dissolution conditions concerned the activation by a solvent exchange with a water/methanol/DMAc series. This experiments led to the optimun method for the dissolution of paper containing different types of additives. The suitability and efficiency obtained in the different trials were evaluated. The experiments carried out to test the stability of the solutions over the period of time proved that the dissolution method and the storage at low temperature were sufficient to bring no significant depolymerisation of cellulose over a period of many months.

Marsano *et al.* (2007) used 7%LiCl/DMAc to dissolve cellulose and silk fibroin for the preparation of cellulose/silk fibroin blended fibers from wet spinning. Various coagulation baths (water and ethanol) and spinning conditions were used. By using water as the coagulant, a partial dissolution of silk fibroin occurred and negligible variation of the mechanical properties of cellulose and silk fibroin fibers with respect to the cellulose fibers was found. Fibers coagulated in ethanol were dimensionally homogeneous and showed better properties. A modulus of about 13 GPa and elongation at break of 16% for the blend containing 30%(w/w) silk fibroin were obtained with a 20-mm air gap. The X-ray results showed that cellulose/silk fibroin fibers were amorphous with a homogeneous dispersion of small silk fibroin domains (1.3nm) in the cellulose matrix. These results ensured the good compatibility between the two natural polymers.

2.1 Hydrogels prepared from cellulose

Cellulose has hydroxyl group in its structure, so that it could easily be used to prepare hydrogels. Cellulose hydrogels could simply be prepared from a cellulose solution through physical crosslinks between hydroxyl groups in the cellulose. Many researchers have been interested in preparing cellulose-based hydrogels (Chang and Zhang, 2011).

Oliveira and Glasser (1996) reported the preparation of hydrogel beads from cellulose by dropwise addition method. The properties of hydrogel beads were examined in relation to cellulose solution concentration, viscosity, and molecular weight. Bead properties were defined in terms of solids content, flow characteristics, mechanical strength, bead size, and pore dimensions. They found that hydrogel beads obtained had the solids content between 2 to 12 wt%, the shape was uniformly spherical, and the viscosity ranged between 100 and 300 cS. In addition, the bead size was different, being in between 70 to 350 cm/min and the pore size of the beads was between 8 to 200 A°.

Saito *et al.* (2003) have successfully prepared a highly transparent hydrogel from cellulose by dissolution in nonaqueous organic solvents, namely, LiCl/DMAc, paraformaldehyde/dimethyl sulfoxide and triethylammonium chloride/dimethyl sulfoxide. A highly transparent hydrogel (97%transparency) was obtained from nonaqueous organic solution. While the hydrogel from viscose provided a very high tensile strength of 13.7 MPa.

Zhou *et al.* (2007) studied the cellulose hydrogel preparation by treating the unsubstituted cellulose in NaOH/urea aqueous solution and using epichlorohydrin as a crosslinking agent. Transparent hydrogels with internal macroporous structure were obtained. The rapid gel formation of the cellulose solution could be sped up by increasing the temperature and the cellulose content. The hydrogels had the equilibrium swelling ratios at 25^{o}C in the range between 30 to 60%(w/w) which slowly decreased with an increase in temperature. The reswelling water uptake was more than 70% relative to their initial stage due to the excellent hydrophilicity of the rigid cellulose backbone and the macroporous structure.

Lu *et al.* (2009) prepared the regenerated cellulose hydrogels by ionic liquid solution method. The resulting hydrogels were transparent and possessed good chemical stability, thermal resistance and suitable mechanical properties. These hydrogels were claimed to be able to use as a biomaterial in the future.

Chang *et al.* (2010a) focused on preparing superabsorbent cellulose-base hydrogels for smart swelling and controllable delivery. The hydrogels were prepared from cellulose and epichlorohydrin was used as a crosslinking agent. The results showed that the stiff cellulose molecules acted as a strong backbone in hydrogel structure, rendering higher swelling ratios as 1000 compared relative to their hydrogel structures. When they were immersed in NaCl and $CaCl_2$ aqueous solutions, smart swelling and shrinking were exhibited. This could be applied in biomaterial fields.

Abe and Yano (2011) reported the preparation of hydrogels from an aqueous suspension of cellulose nanofibers by alkali treatment. They found that cellulose hydrogels were formed by aggregating the nanofibers having the initial structure of cellulose I in 6-9 wt% NaOH. However, at the concentration of 15 wt% NaOH, it showed cellulose II structure, which had higher degree of crystallinity and the axial shrinkage of the cellulose nanofibers in NaOH aqueous solutions occurred as a result of the gel formation of cellulose hydrogels.

2.2 Hydrogels prepared from cellulose blends

Preparation of polymer blend hydrogels aiming to produce new materials with improved physicochemical and mechanical properties, has gained considerable attention from researchers in the past several decades. Hydrogels prepared from different sources of cellulose fibers have been investigated for the optimum preparation conditions and their properties. Moreover, the preparation of blended films and hydrogels from different natural polymers such as cellulose/silk fibroin, cellulose/chitosan, cellulose/chitin, have also been reported, as follows.

Freddi *et al*. (1995) reported the preparation, structure and physical properties of silk fibroin and cellulose blended films. The dissolution of the fibers with a metal complex solution was studied. The average molecular weight of silk fibroin slightly decreased, while cellulose was almost unaffected. The transparent films were obtained by blending silk fibroin and cellulose in various proportions. From the x-ray diffraction (XRD), it was found that the structure of regenerated silk fibroin was in β-sheet form and the structure of cellulose was cellulose II. Density values increased with the cellulose content, even they were less than expected from a pure cellulose behavior. Moisture regain increased due to the addition of a small amount of cellulose to silk fibroin. The better properties of the silk fibroin films in both strength and elongation at break were developed by blending with cellulose. The film showed better mechanical properties when cellulose was added. The IR spectra showed that the intermolecular interactions between silk fibroin and cellulose was hydrogen bonding.

Phonwong *et al.* (2000) reported the preparation and characterization of chitin/cellulose blended films. They found that chitin could be dissolved in 5%LiCl/ DMAc at room temperature and cellulose was dissolved in 9%LiCl/DMAc by repeated heating at 150°C and cooling down to room temperature. The tensile strength and elongation at break of the chitin/cellulose blended films increased with increasing the content of cellulose to 80%. The cellulose content of 60% had the maximum degree of swelling in the solution containing salts, namely NaCl, LiCl, $CaCl_2$, $AlCl_3$ and $FeCl_3$. Among these salts, the blended film immersed in LiCl solution had the highest degree of swelling. The effect of pH on the degree of swelling of the films was studied at pH 3, 5, 7 and 10. The degree of swelling of the blended films decreased as the pH of the solutions increased. They also found that the blended films had lower oxygen permeability than those of pure chitin and cellulose films.

Li and Bai (2005) studied the hydrogel beads prepared from chitosan and cellulose for the application as an adsorbent for Cu in aqueous solutions. They found that the blended hydrogel beads could be crosslinked by ethylene glycol diglycidyl ether. The crosslinking agent improved the chemical stability of the hydrogel beads in acid solutions with pH down to 1. The adsorption experiments showed that the blends had high adsorption capacities for Cu, with the optimum pH in the range around neutral, although the crosslinked chitosan–cellulose beads always exhibited slightly lower adsorption capacities than the non-crosslinked beads.

Chang *et al.* (2008) studied the hydrogels simply prepared from cellulose and poly(vinyl alcohol) in NaOH/urea aqueous solution by both physical and chemical crosslinking. For chemical hydrogels, epichlorohydrin was used as a chemical crosslinking agent. The results showed the homogenous porous structures in the hydrogel. In addition, the use of the chemical crosslinker provided higher swelling ratios and water uptake than physical crosslinker. For the interaction between cellulose and poly(vinyl alcohol), it exhibited a weak hydrogen bonding and the compact structure in chemical hydrogels. The physical crosslinking hydrogel had a more regular structure and a stronger interaction between cellulose and poly(vinyl alcohol).

Marsano *et al.* (2008) reported the preparation of fiber blends from regenerated cellulose and silk fibroin. The fibers were dissolved in *N*-methylmorpholine *N*-oxide hydrates ($NMMO/H_2O$) and later coagulated in ethanol.

They found that preparation of cellulose and fibroin solutions showed a phase separation in all blends compositions. The tensile characterization, however, illustrated that the properties of the blends fibers were higher than the pure polymers. The results confirmed a good compatibility of the blends fibers. For 75% of cellulose content, it showed better mechanical properties (23 GPa of modulus and 307 MPa of the strength to break) than the pure cellulose.

Shih *et al.* (2009) prepared and characterized cellulose and chitosan blended films. The results showed the formation of transparent orange viscose films. After rinsing with deionized water and drying, transparent yellowish blend films were obtained. Scanning electron microscope (SEM) indicated that when the chitosan content in the blend increased up to 3%(w/w), the surface structure became smoother. However, the film containing 5%(w/w) chitosan became coarse probably because of phase separation. Tensile strength was consistent with the change in the film structure. Antibacterial assessment proved that addition of chitosan to the films resulted in slightly better antibacterial properties. The halo zone test confirmed that the blend films had non-diffusible antibacterial properties.

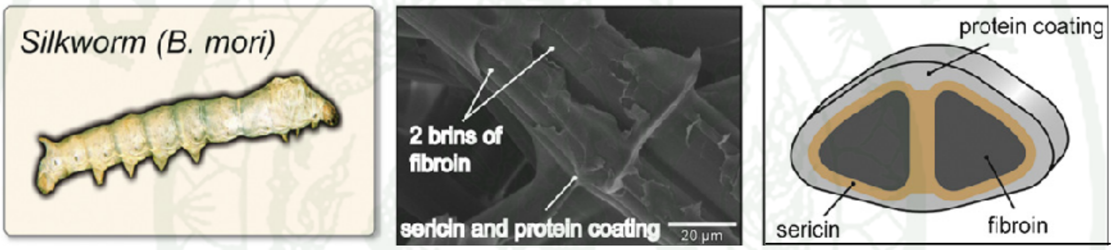
Moraes *et al.* (2010) prepared silk fibroin and chitosan blend films and then characterized them. Crystallographic analysis (XRD) indicated that the addition of chitosan enhanced a higher structural organization. Fourier transformed infrared spectroscopy (FTIR) showed that silk fibroin exhibited a more stable form in the presence of a chitosan content of only 25%(w/w). Thermal analysis specified that silk fibroin was thermally stable and when its proportion in the blend increased, the temperature at which degradation was initiated also increased.

Wu *et al.* (2010) reported structures and properties of cellulose/chitin blended hydrogel membranes fabricated via a solution pre-gelation technique. They found that the hydrogel had a denser structure with lower crystallinity compared with those from solution casting method.

Shang *et al*. (2011) studied physical properties of silk fibroin/cellulose blended films regenerated from the hydrophilic ionic liquid. They found that interactions between silk fibroin and cellulose in all hydrogel samples induced the conformation transition of random coil structure (silk I) to β-sheet structure (silk II). The ratio of 75% cellulose content had the stronger interactions, higher tensile strength, higher thermal stability and higher water stability. However, the ratio of 50% cellulose content had a more homogeneous surface, better miscibility and higher elongation at break.

3. Silk

Silk is a natural protein produced in fiber form by *Bombyx mori* silkworms (Figure 2a). Silk fiber is composed of two types of protein: sericin 26.5-33.5% and fibroin 66.5-73.5% as shown in Figures 2b and 2c (Sashina *et a.*, 2006). The structural protein of silk fibers is fibroin and the water-soluble glue that binds the fibroin fibers is sericin (Kim *et al.*, 2004). The unique properties of silk are nontoxicity, degradability, and biological compatibility (Kweon *et al.*, 2001). The general structure of silk is β-sheet because of the dominant hydrophobic domains with short side chain amino acids in the primary sequence (Vepari and Kaplan, 2007).



a) b) c)

Figure 2 Photograph of *Bombyx mori* silkworms and silk fiber.

Source: Hardy *et al.* (2010)

3.1 Silk fibroin

Silk fibroin consists of glycine, alanine and serine (Gly-Ala-Gly-Ala-Ser) as shown in Figure 3. The chemical composition of silk fibroin is shown in Table 1. The silk fibroin contains both light and heavy chain polypeptides of ~26 kDa and ~390 kDa, respectively. They are connected by a disulfide linkage at the C-terminus of the two subunits and are associated by hydrophobic interaction of the heavy-light complex primary structures (Zhang *et al.*, 2009). From the adaptability of the fibroin proteins, they have been widely used as tissue engineering scaffolds (Chao *et al.*, 2010), contact lenses (Freddi *et al.,* 1995) and biomaterials such as films, membranes, gels, sponges and powders (Cao and Wang, 2009).

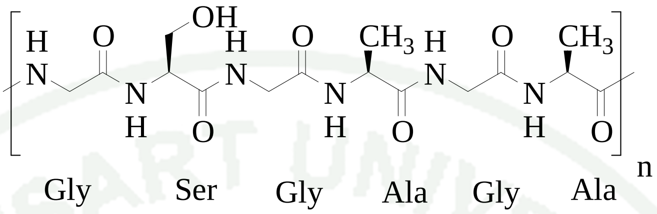


Figure 3 The structure of silk fibroin.

Table 1 Amino acid composition of silk fibroin from *Bombyx mori*.

Amino acid	**Composition, mol %**		
	total	**heavy areas**	**light areas**
Glycine	42.9	49.4	10
Alanine	30.0	29.8	16.9
Serine	12.2	11.3	7.9
Tyrosine	4.8	4.6	3.4
Valine	2.5	2	7.4
Aspartic acid	1.9	0.65	15.4
Glutamic acid	1.4	0.7	8.4
Threonine	0.92	0.45	2.8
Phenylalanine	0.67	0.39	2.7
Methionine	0.37	-	0.37
Isoleucine	0.64	0.14	7.3
Leucine	0.55	0.09	7.2
Proline	0.45	0.31	3
Arginine	0.51	0.18	3.8
Histidine	0.19	0.09	1.6
Lysine	0.38	0.06	1.5

Source: Sashina *et al.* (2006)

Two major types of secondary structures of silk fibroin are well-known today, namely silk I and silk II. The metastable form of silk fibroin is silk I which is a non-crystalline, water soluble part. Thus, α-helix and random coil conformations are usually called silk I. Silk I (α-helix structure) is formed by intramolecular hydrogen bonding and the hydrophobic fragments displaced to the periphery. The silk I can be changed to silk II when it is treated in methanol (Cao *et al.,* 2009). In contrast, silk II is crystalline, highly stable and insoluble in water. The β-sheet (antiparallel forms) conformation is called silk II which has hydrogen bonding interactions between polypeptide chains (Moraes *et al.*, 2010). The α-helix and the β-sheet structure of silk fibroin are shown in Figures 4 and 5, respectively.

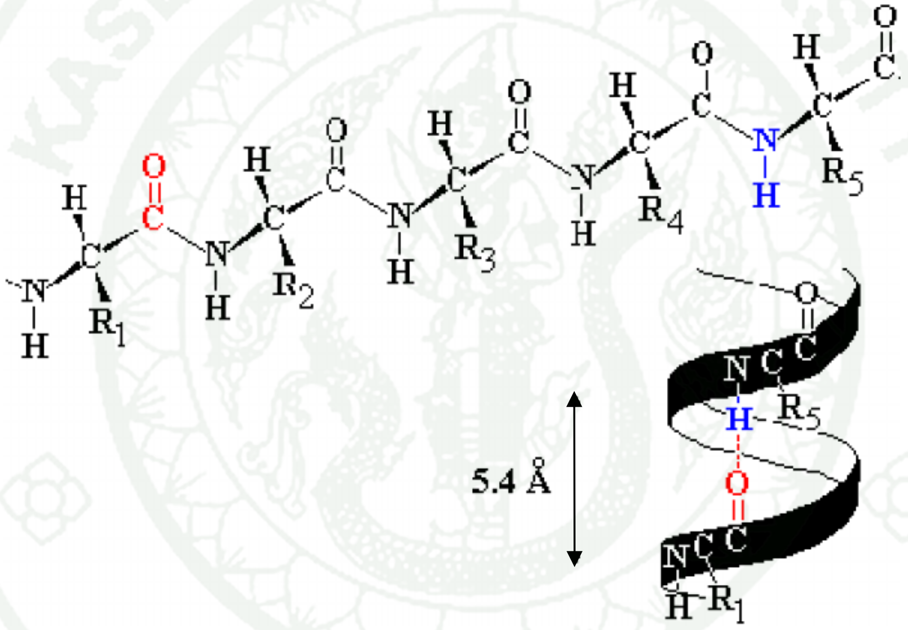


Figure 4 The α-helix conformation structure of silk fibroin.

Source: Geourjon and Deleage (1994)

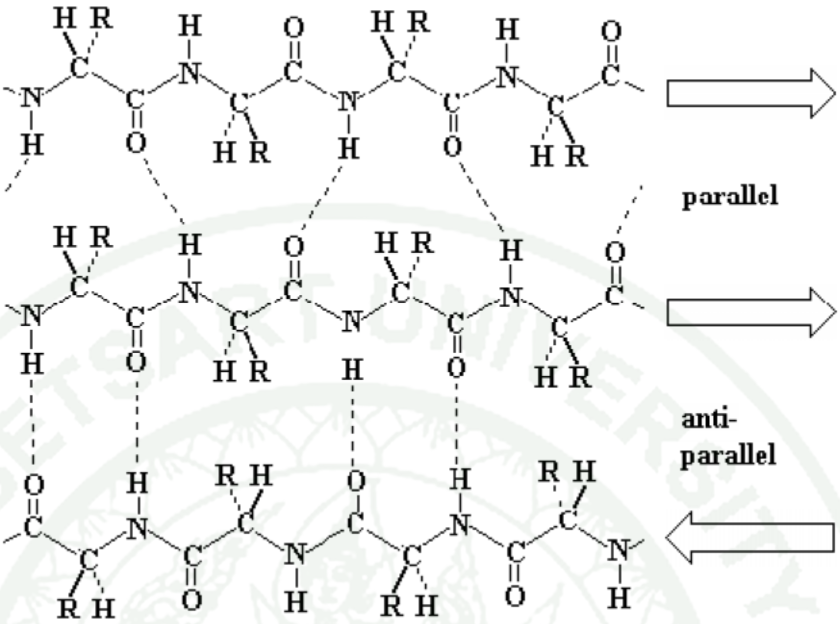


Figure 5 The β-sheet conformation structure of silk fibroin.

Source: Geourjon and Deleage (1994)

MATERIALS AND METHODS

Materials

1. Fibers

1.1 Rayon fibers from Thai Rayon Co. Ltd., Thailand

Figure 6 Rayon fiber.

1.2 Silk cocoons (*Bombyx mori*) from the local silk sericulture group in Thailand

Figure 7 Silk cocoons *(Bombyx mori)*.

2. Reagents

2.1 Acetone (AR grade, Carlo)
2.2 Calcium chloride dehydrate (AR grade, Ajax)
2.3 Copper(II)-Ethylenediamine complex solution (AR grade, Acros)
2.4 Cyanuric chloride, 99% (AR grade, Acros)
2.5 Ferric chloride (AR grade, Carlo)
2.6 Hydrochloric acid (AR grade, Carlo)
2.7 Hydroxylamine hydrochloride (AR grade, Carlo)
2.8 Isopropyl alcohol (AR grade, Carlo)
2.9 Kimwipes paper (Kimberly-Clark EX-L, USA)
2.10 Lithium bromide, 99% (AR grade, Acros)
2.11 Lithium chloride (AR grade, Merck)
2.12 Methanol (AR grade, Acros)
2.13 *N*,*N*-Dimethylacetamide, 99% (AR grade, Acros)
2.14 Potassium hydroxide (AR grade, Carlo)
2.15 Sera Wash M-SF (nonionic surfactant, DyStar Thai Ltd., Thailand)
2.16 Silver nitrate (AR grade, Carlo)
2.17 Sodium carbonate anhydrous (AR grade, Carlo)
2.18 Sodium chloride (AR grade, Merck)

3. Equipments

3.1 Attenuated total reflection fourier transform infrared (ATR-FTIR) Spectroscopy (EQUINOX 55, Bruker)
3.2 Hotplate/stirrer (SciLution Co., Ltd., Thailand)
3.3 Oven (Memmert)
3.4 pH meter (SciLution Co., Ltd., Thailand)
3.5 Scanning electron microscope (SEM) (JEOL JSM-5800 LV)
3.6 Thermal gravimetric analysis (model PERKIN-ELMER7 Series)
3.7 Universal testing machine (Lloyd) (model LRX tester)
3.8 Viscometer

3.9 X-ray diffractometer (Philips X'PERT PW3050 diffractometer)

Methods

1. Preparation of the raw materials and determination of their molecular weights

The obtained regenerated cellulose, rayon fiber, was scoured in a bath containing 3 g/L Sera Wash M-SF (nonionic wetting agent) and 2 g/L sodium carbonate (Na_2CO_3) at 80°C for 1 h. After scouring, the fiber was rinsed with water, oven-dried at 60°C for 24 h. Silk cocoons were degummed by boiling twice in 2 g/L Na_2CO_3 for 30 min each time and rinsed thoroughly with distilled water to remove the glue-like sericin proteins. The obtained silk fibroin fiber was dried at 60°C for 48 h. Figure 8 shows the degummed silk fibroin fiber.

Silk fibroin solution was prepared by dissolving the fiber in 9.3M lithium bromide (LiBr) at 60°C for 3 h while the cellulose was dissolved in 0.5M cupriethylenediamine. The observed viscosities [η] of the solutions were used for viscosity-average molecular weight (M_v) calculation according to Mark-Houwink equation (Eqn.1) where $a = 0.95$ and $K = 2.95 \times 10^{-3}$ for silk fibroin and $a = 0.905$ and $K = 1.33 \times 10^{-4}$ for regenerated cellulose.

$$[\eta] = KM^a \qquad \text{Eqn. 1}$$

Figure 8 The degummed silk fibroin fiber.

2. Preparation of regenerated cellulose/silk fibroin blended hydrogels

The scoured regenerated cellulose was heated and stirred in *N,N*-dimethylacetamide (DMAc) at 80-100^{o}C for 30 min. The swollen fibers were filtered to subsequently use in the preparation of the regenerated cellulose solution. The picture of the swollen rayon fibers is shown in Figure 9. The regenerated cellulose and silk fibroin solutions were prepared separately by dissolving each fiber in the mixture of 7% (w/v) lithium chloride in dimethylacetamide (LiCl/DMAc) at 140-160^{o}C for 45 min (Marsano *et al.,* 2007). The two solutions were then mixed together at the cellulose to silk ratios of 0/100, 25/75, 50/50, 75/25 and 0/100 coded by R0/100, R25/75, R50/50, R75/25, and R100/0, respectively (Table 2). The blended solutions were poured into petri dishes allowing the gel formation for 24 h (Figure 10). After that, the gel was immersed in isopropyl alcohol for 3 days to remove LiCl/DMAc. A complete removal of the solvent was checked according to Thipamon (1999: 122). The regenerated cellulose/silk fibroin blended hydrogels were washed with methanol and then air-dried for 24 h. The hydrogels were washed again with distilled water and also air-dried for 24 h (Oliveira and Glasser, 1996).

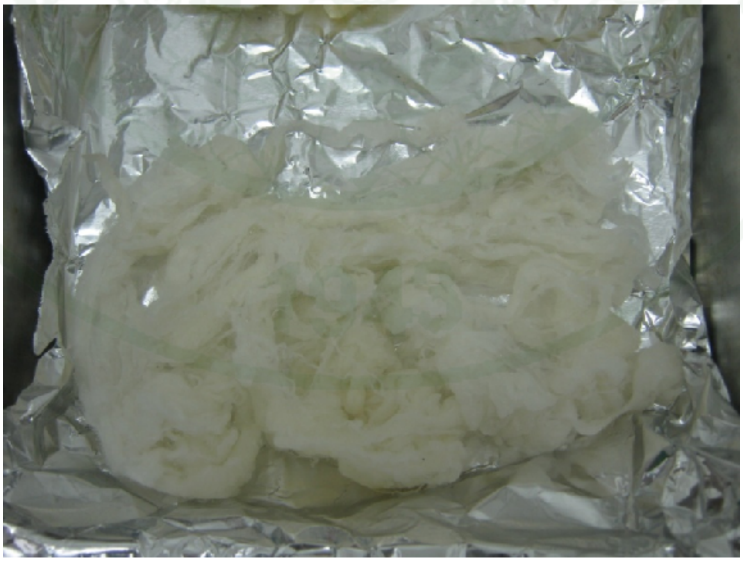


Figure 9 The swollen rayon fibers.

Table 2 The codes of prepared hydrogels at different weight ratios of cellulose to silk fibroin.

Codes	**Cellulose (%v/v)**	**Silk fibroin (%v/v)**
R0/100	0	100
R25/75	25	75
R50/50	50	50
R75/25	75	25
R100/0	100	0

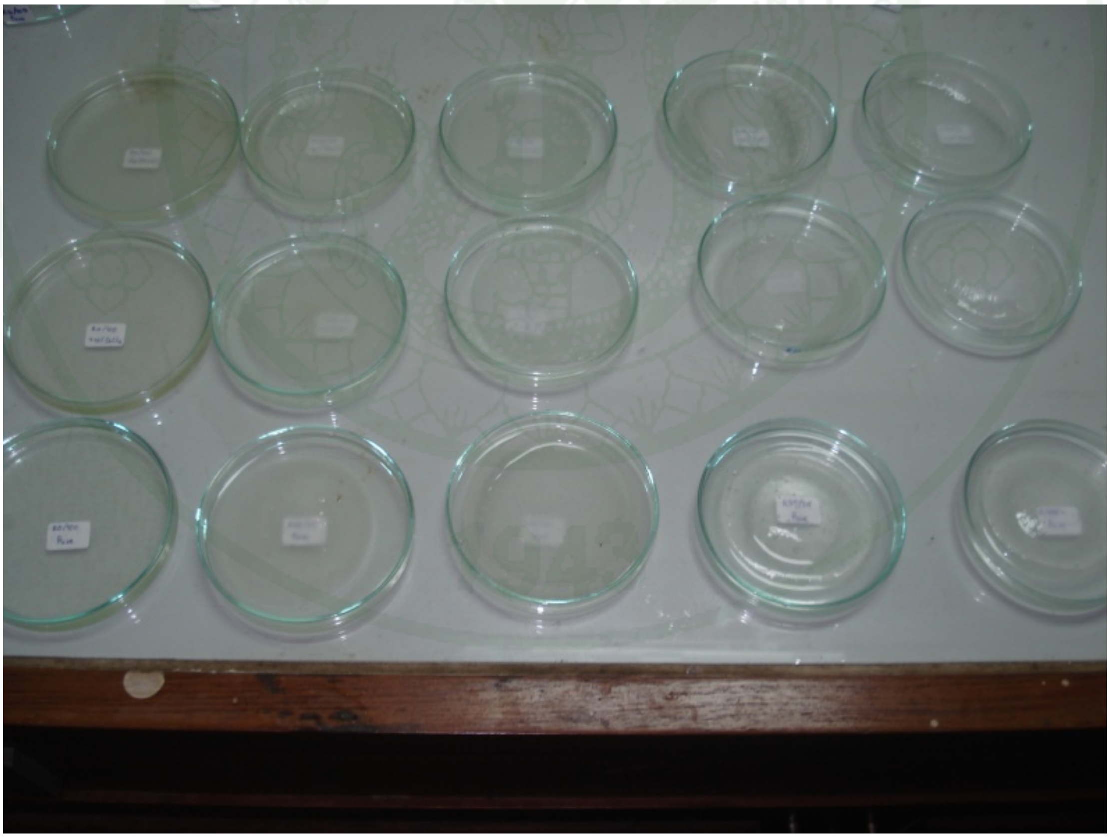


Figure 10 The gel formation.

3. Effect of salt addition on the regenerated cellulose/silk fibroin hydrogels

R100/0 and R75/25 hydrogels were chosen for this study. A study on preparing the porous hydrogel of regenerated cellulose and silk fibroin was performed using calcium chloride ($CaCl_2$) and sodium chloride (NaCl) as a porogen. Each salt was added into the blends at 2.5, 5 and 10% (w/v) before the blends were left for gelling. After that, the gel was immersed in isopropyl alcohol for 24 h, washed with methanol and air-dried. The salts were removed from all samples by immersing the hydrogel in distilled water for 3 days and a complete removal of salt was tested by using 0.1M silver nitrate ($AgNO_3$) and then air-dried for 24 h.

4. Effect of a crosslinking agent on the regenerated cellulose/silk fibroin hydrogels

4.1 Synthesis of a crosslinking agent

In this experiment, monohydroxydichlorotriazine (NHDT) was used as a crosslinking agent. The synthesis of NHDT is shown in Figure 11. Cyanuric chloride was dissolved in a mixture of acetone and water. The weight ratio of cyanuric chloride:water:acetone used was 1:4:4. The mixture was stirred and the pH was adjusted to 8 by the addition of 1M Na_2CO_3 within 1 h.

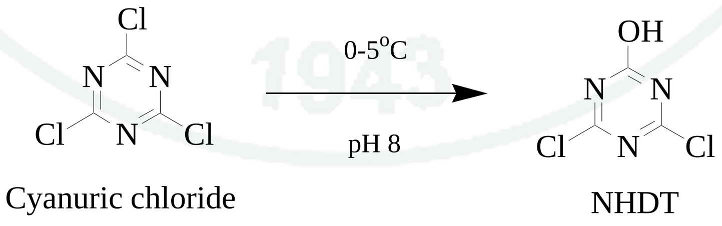


Figure 11 The synthesis of NHDT.

4.2 Preparation of the crosslinked hydrogels

Vacuum-dried R100/0 and R75/25 hydrogels were used in this experiment. The hydrogels were immersed in 10, 20, 30 and 50 g/L crosslinking agent for 24 h and then air-dried for 48 h. The preparation of the crosslinked hydrogel is shown in Figure 12.

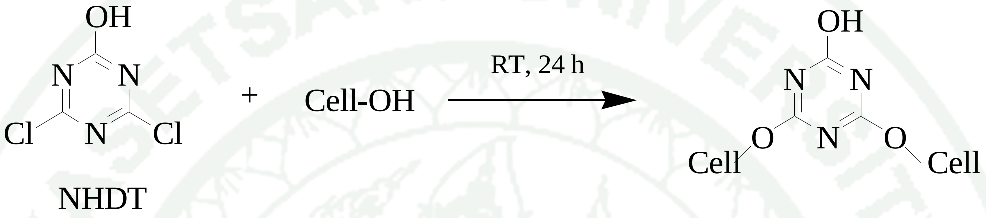


Figure 12 Preparation of the crosslinked hydrogels.

5. Characterization of the regenerated cellulose/silk fibroin hydrogels

The properties of the obtained regenerated cellulose and silk fibroin hydrogels were characterized as follows.

5.1 Tensile properties

Tensile strength and %elongation were examined using Lloyd (Universal testing machine) model LRX tester (Figure 13) by following the ASTM D638 standard at the gauge length of 30 mm and strain rate of 10 mm/min.

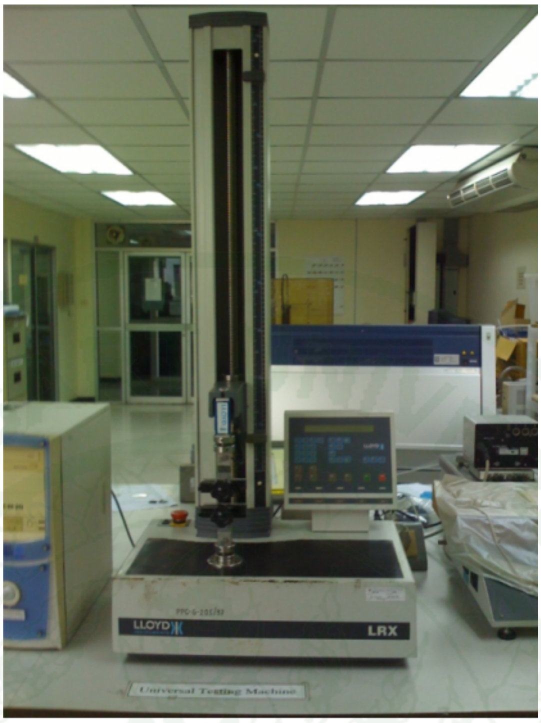


Figure 13 Universal testing machine.

5.2 Scanning electron microscope

The surface characteristics of the hydrogels were investigated by JEOL JSM-5800 LV scanning electron microscope (Figure 14) after gold coating. All hydrogel samples were fractured in liquid nitrogen. The fractured cross-section of the hydrogels was also examined.

Figure 14 JEOL JSM-5800 LV scanning electron microscope (SEM).

5.3 FTIR analysis

The functional groups in the hydrogel samples were investigated by ATR-FTIR. All spectra were collected with a resolution of 4 cm^{-1} in the spectral range of 4000-650 cm^{-1}.

5.4 X-ray diffraction analysis

Wide-angle x-ray diffraction (WAXD) profiles of hydrogel samples were examined by Philips X’PERT PW3050 diffractometer and the measurement range was between 5-40°. Figure 15 is the picture of Philips X’PERT PW3050 diffractometer.

Figure 15 Philips X'PERT PW3050 diffractometer.

5.5 Thermal gravimetric analysis

The thermogravimetric analyzer (TGA) was used to examine the thermal stability of hydrogels and was run under nitrogen atmosphere. Each sample was heated at a heating rate of 10°C/min from 50 – 450°C.

6. Swelling properties

The swelling ratios (SR) of the cellulose/silk fibroin blended hydrogels were measured by immersing the hydrogel in 50 ml distilled water at room temperature. Then, all samples were removed after 30, 60, 120, 180 and 240 min. After excess water at the surface of the hydrogels was blotted out with Kimwipes paper, the weights of the swollen hydrogels were measured. The swelling ratio of the hydrogel was calculated using Eqn. 2 (Chang *et al*., 2010).

$$SR\ (\%) = \frac{W_s - W_d}{W_d} \times 100 \qquad \text{Eqn. 2}$$

Where

W_d : The weight of dried hydrogels
W_s : The weight of swollen hydrogels

Summary of research methodology is shown in Figure 16.

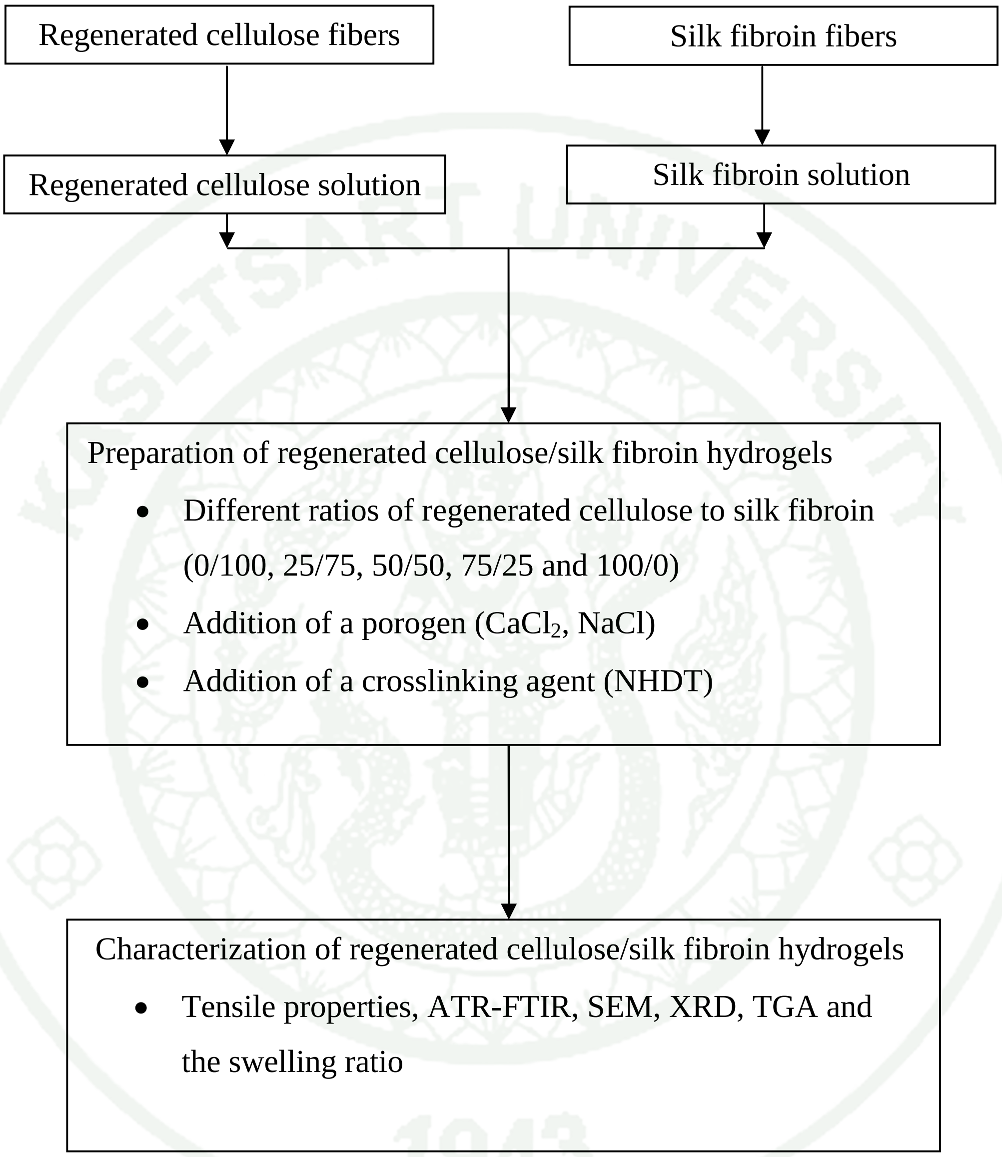


Figure 16 Research plan.

RESULTS AND DISCUSSION

1. Preparation and physical property characterization of the hydrogels from regenerated cellulose fiber waste and silk fibroin

In this experiment, the regenerated cellulose and silk fibroin used had the molecular weight of 500,000 and 19,300, respectively. The regenerated cellulose and silk fibroin were blended together at various blend ratios as mentioned in the experimental. The blends coded as R0/100, R25/75, R50/50, R75/25 and R100/0 were corresponded to those having the cellulose to silk ratio of 0:100, 25:75, 50:50, 75:25 and 100:0, respectively. In Table 3, the blends having less than 50% cellulose took a longer time for gel formation and the gel obtained was unstable and very fragile. Silk fibroin alone did not form gel (R0/100). Incorporating a high amount of silk fibroin in the blend would impair gelation capacity of the blend.

Table 3 Physical properties of regenerated cellulose/silk fibroin hydrogels.

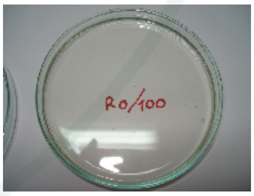
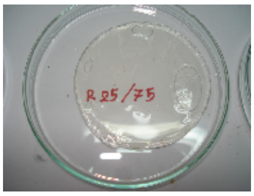
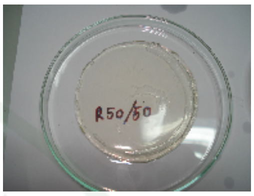
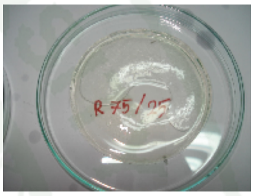
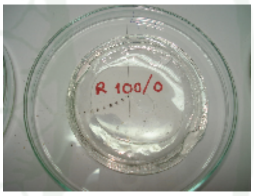
Sample	**The ratios of hydrogels (%)**		**Gel formation**	**Pictures**
	Regenerated cellulose	**Silk fibroin**		
R0/100	0	100	Transparent, pale yellow liquid	
R25/75	25	75	Slow gel formation, very soft, transparent pale yellow gel with unstable shape	

Table 3 (Continued)

Sample	The ratios of hydrogels (%)		Gel formation	Pictures
	Regenerated cellulose	Silk fibroin		
R50/50	50	50	Fast gel formation, transparent pale yellow gel with stable shape	
R75/25	75	25	Fast gel formation, transparent pale yellow gel with very stable shape	
R100/0	100	0	Fast gel formation, transparent colorless gel with the most stable shape	

1.1 Characterization of hydrogels

1.1.1 Wide-angle X-ray diffraction characterization

The x-ray diffraction patterns of the blends (R25/75, R50/50 and R75/25) are illustrated in Figure 17 in comparison with the pure ones (R0/100 and R100/0). The x-ray diffraction patterns of pure silk fibroin hydrogel exhibited the presence of β-sheet structure at $2\theta = 20^{o}$. Meanwhile, the characteristic diffraction peaks at $2\theta s = 12$ and 20^{o} of the pure regenerated cellulose hydrogel represented $(1\bar{1}0)$ and (110) crystal reflections. This is a typical diffraction pattern of the cellulose II crystalline form (Freddi *et al.*, 1995). By blending regenerated cellulose into silk fibroin hydrogel, the diffraction patterns showed the combination of both regenerated cellulose and silk fibroin characteristic peaks in different intensities depending on their blended ratios.

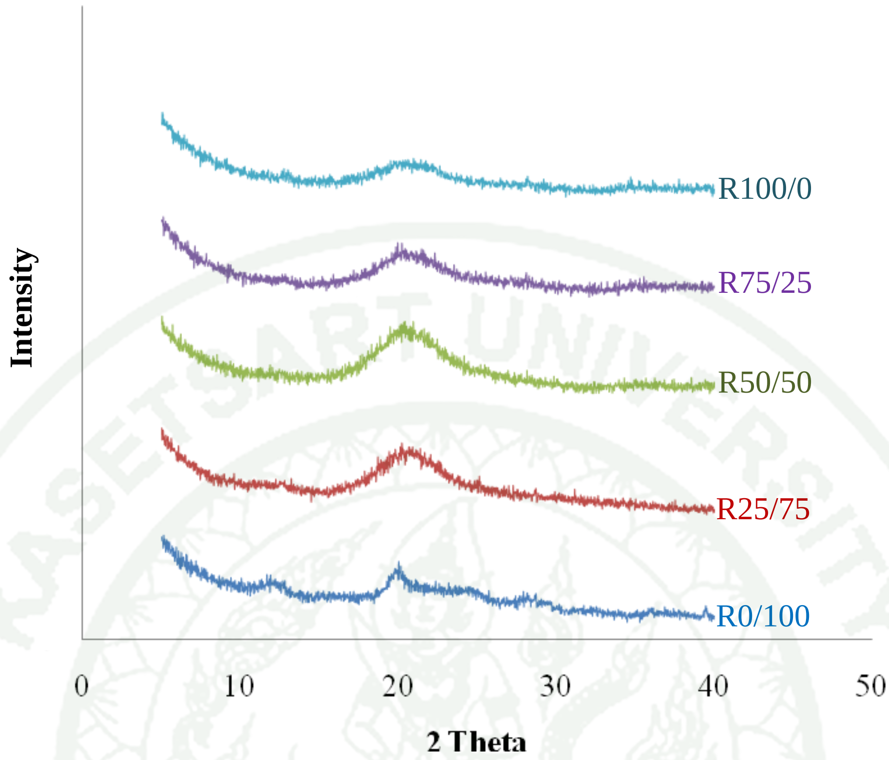


Figure 17 WAXD profiles of R0/100, R25/75, R50/50, R75/25 and R100/0 regenerated cellulose/silk fibroin hydrogels.

1.1.2 FT-IR spectra

The ATR-FTIR spectra of the blended hydrogels are depicted in Figure 18. Pure silk fibroin hydrogel presented the absorption bands at 1630 cm^{-1}(amide I), 1530 cm^{-1}(amide II), and 1265 cm^{-1}(amide III), corresponding to the silk II structural conformation (β-sheet) (Yang *et al.*, 2000). In case of R100/0, the spectrum displayed absorption bands at 1423 cm^{-1}, 1367 cm^{-1}, 1262 cm^{-1}, 1154 cm^{-1}, 1116 cm^{-1} and 1018 cm^{-1}, corresponding to the structure of cellulose II (Marsano *et al.*, 2008). For the blends, the spectra in the region of 3500-3300 cm^{-1} exhibited changes in shape and intensity of IR absorption frequency characteristics from pure silk fibroin and regenerated cellulose. This resulted from the occurrence of intermolecular interactions between the two polymers within the amorphous regions. The –OH groups of regenerated cellulose and –CONH- groups of silk fibroin were able to form hydrogen bonding between the polymer chains (Freddi *et al.*, 1995).

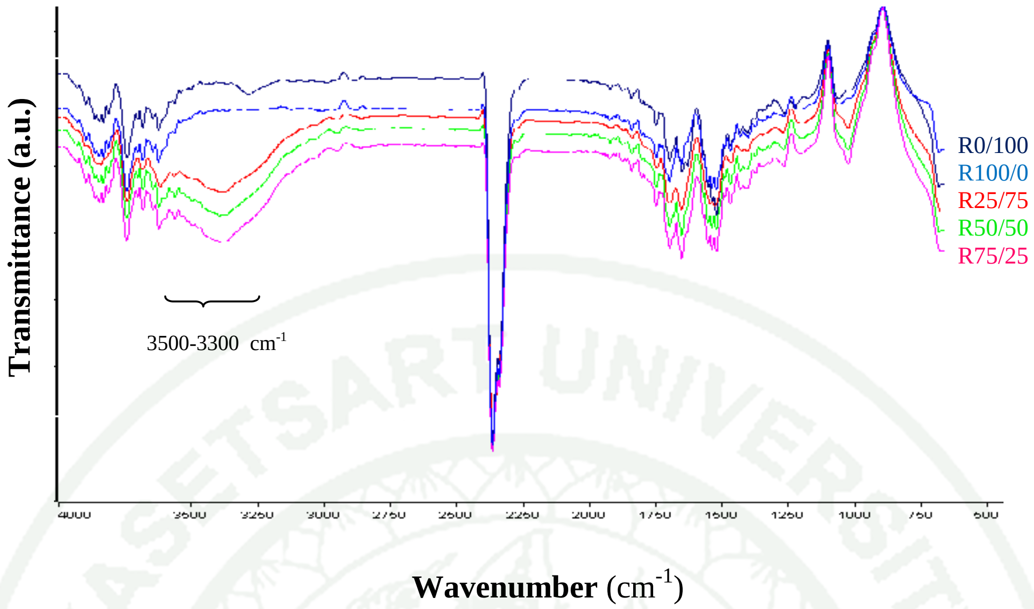
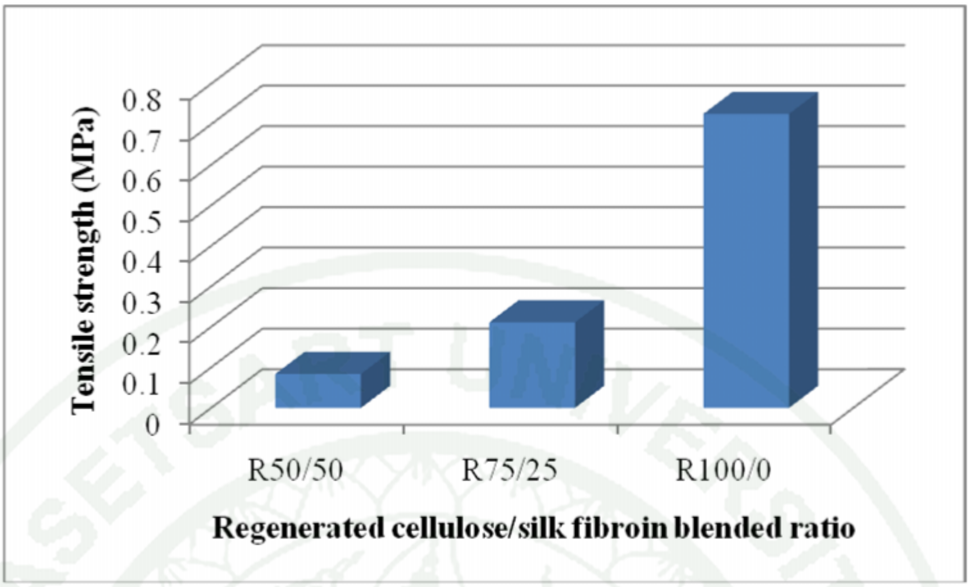


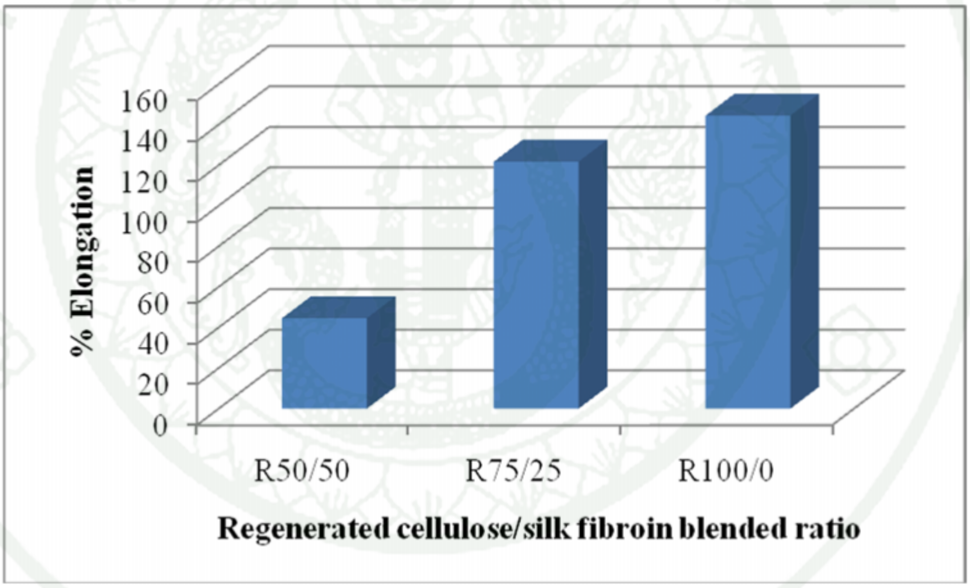
Figure 18 ATR-FTIR spectra of regenerated cellulose/silk fibroin hydrogels at various regenerated cellulose to silk fibroin ratios.

1.1.3 Tensile properties

The tensile properties of the blends were measured and the results are demonstrated in Figures 19 a) and b). Due to a lack of sufficient strength of the pure silk fibroin, the blends with high silk fibroin content were too fragile to measure their tensile properties. With increasing regenerated cellulose content to at least 50% in the blends, the hydrogels were stronger and their tensile strength could be measured as seen in Figure 19. The blends with higher regenerated cellulose content exhibited a higher tensile strength and % elongation. The stronger and more flexible hydrogel was obtained with increasing regenerated cellulose content. This can be said that the regenerated cellulose enhances the strength and flexibility to the blends. However, the tensile properties of the blended hydrogel were still lower than the pure regenerated cellulose hydrogel.



a)

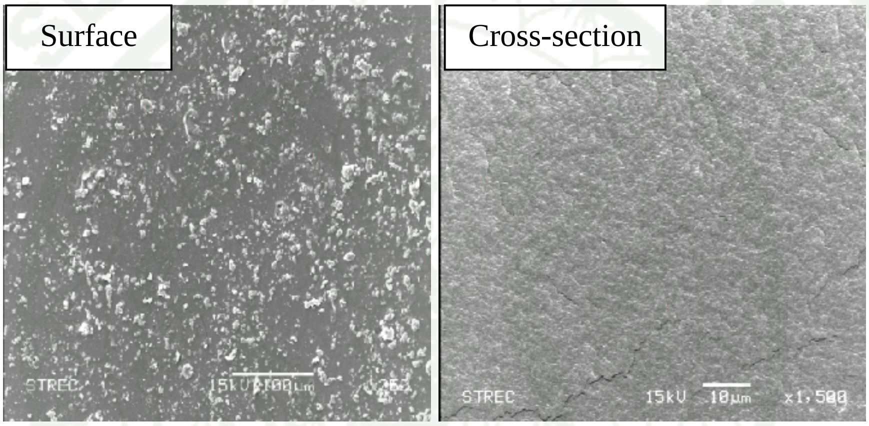


b)

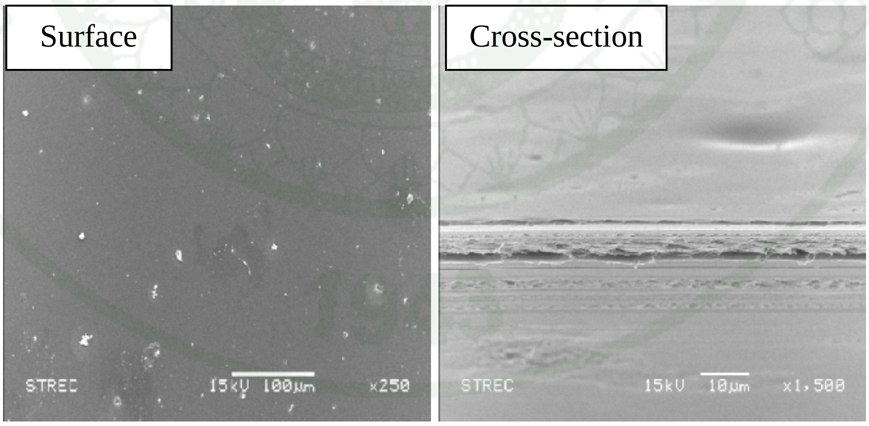
Figure 19 Tensile strength, a) and %elongation, b) of regenerated cellulose/silk blended hydrogels.

1.1.4 Surface and cross-sectional morphology of the hydrogels

The SEM images showed a granular appearance of R75/25 surface (Figure 20 a)), while the R100/0 surface was smooth (Figure 20 c)). Moreover, the cross-section morphology of R75/25 and R100/0 was also investigated as shown in Figure 20 b) and d), respectively. The cross-sectional morphology of the R75/25 was rough. The regenerated cellulose hydrogels displayed smooth and lamellar phase in Figure 20 d) (Freddi *et al.*, 1995).



a) R75/25 b)



c) R100/0 d)

Figure 20 SEM images of the surface and cross-section of regenerated cellulose/silk fibroin hydrogels of R75/25, a) and b) and R100/0 c) and d).

1.1.5 Swelling ratios

The swelling ratios of the R75/25 and R100/0 hydrogels from 0 to 240 min are shown in Figure 21. Both hydrogels showed about 40-45% swelling ratios with a slightly higher swelling for R100/0. The hydrogels absorbed water readily in the first 30 min and marginally increased afterward. The relatively low water swelling of the two hydrogels may be due to the β-sheet structure of the silk fibroin which hindered the water binding ability of the hydrogels (Shang *et al.*, 2011). The pure regenerated cellulose hydrogel exhibited about 5% higher swelling ratio than the blended hydrogel. This may be caused by a higher interaction between water molecules and the –OH groups on regenerated cellulose, interrupting the intermolecular bonding between cellulose chains and facilitating water absorption.

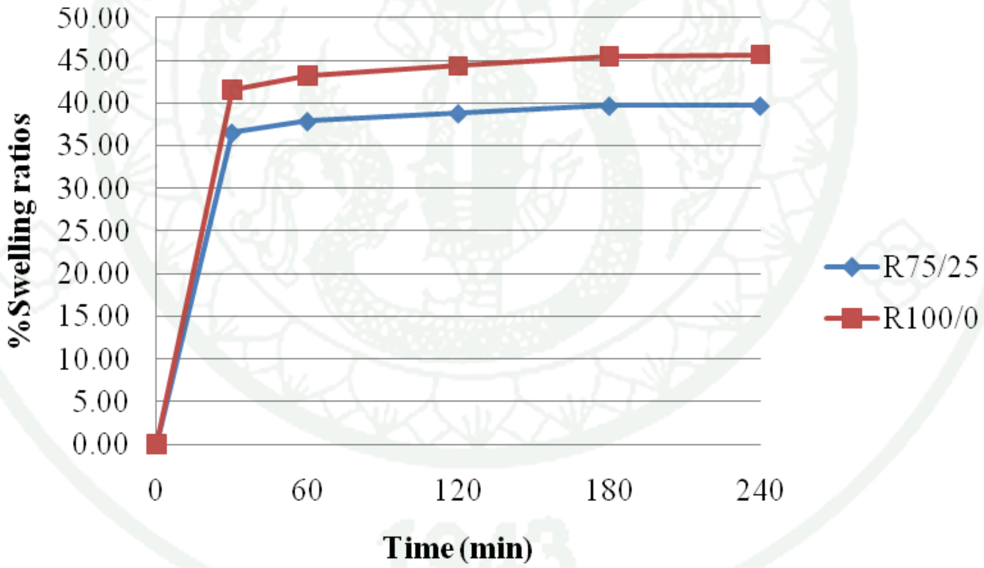
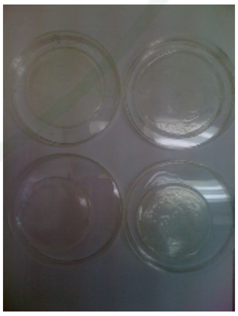


Figure 21 Swelling ratios of R75/25 and R100/0 regenerated cellulose/silk fibroin hydrogels from 0 to 240 min.

2. Effect of a crosslinking agent on the regenerated cellulose/silk fibroin hydrogels

In Table 4, pictures and properties of the pure and the blended hydrogels after being immersed in various concentrations of the crosslinking agent (NHDT) for 24 h are shown. The hydrogels did not show any change in the physical properties as compared with their original states.

Table 4 Physical properties of R100/0 and R75/25 regenerated cellulose/silk fibroin hydrogels immersed in NHDT.

Sample	**The ratios of hydrogels (%)**		**After immersed in NHDT**	**Pictures**
	Regenerated cellulose	**Silk fibroin**		
R0/100	0	100	Transparent colorless gel, stable shape, very rigid	
R75/25	75	25	Transparent pale yellow gel, stable shape, rigid	

2.1 Characterization of crosslinked hydrogels

2.1.1 Swelling ratios

R75/25 and R100/0 hydrogels were immersed in five different concentrations of NHDT solution. From Figure 22, both crosslinked hydrogels had higher %swelling ratios than the non-crosslinked counterparts. Without a crosslinking agent, the hydrogels had %swelling ratio around 40-45%. After crosslinking, the NHDT crosslinked hydrogels were in the range of 60-70% swelling ratios. The hydrophilic enhancement obtained from the crosslinking agent, brought a higher water absorption in the hydrogel structure (Chang *et al.*, 2010a). However, the effect of crosslinks on swelling ratio of the hydrogel was more pronounced in R75/25. The blended hydrogel after NHDT treatment can absorb water better than the pure regenerated cellulose hydrogel after treatment.

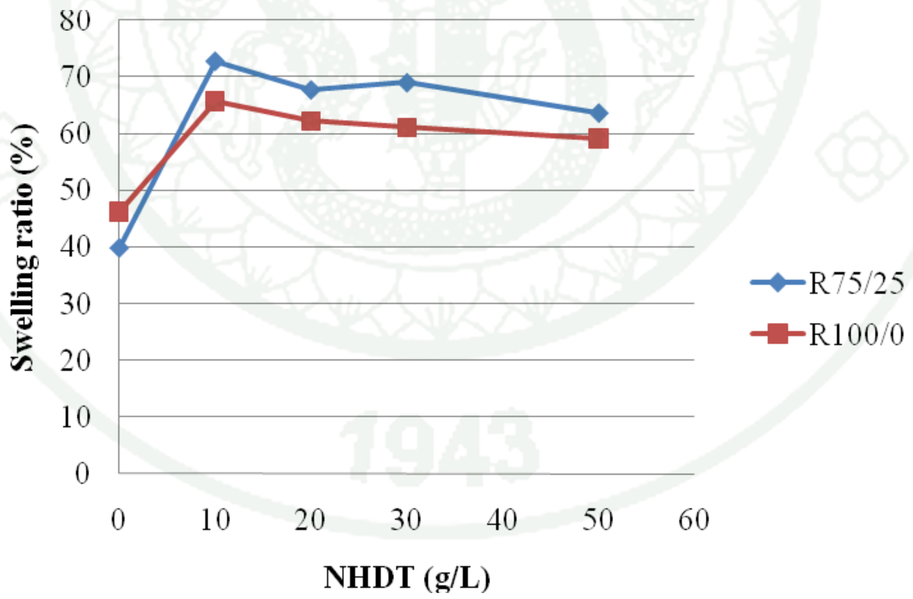


Figure 22 Swelling ratios of R75/25 and R100/0 regenerated cellulose/silk fibroin hydrogels immersed in various concentrations of NHDT for 24 h.

2.1.2 Thermal gravimetric analysis

The thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of the pure regenerated cellulose hydrogels, and the regenerated cellulose hydrogel added with 10% and 50% crosslinking agent are shown in Figures 23 and 24, respectively. The maximum rate of weight loss was used to illustrate the quantitative characterization of the degradation process. The pure regenerated cellulose hydrogels with no crosslinking agent, with 10 and 50%NHDT crosslinking agent showed the initial degradation temperature at 223^{o}C, 289^{o}C and 303^{o}C, respectively. As the crosslinking agent content increased, the degradation temperature of the regenerated cellulose hydrogels increased. It may be said that the addition of the crosslinking agent improved the thermal properties of the hydrogels. This could be explained by the occurrence of the intermolecular crosslinking of the cellulose chains. NHDT which was used as a crosslinking agent, covalently bonded to the cellulose chains and held the cellulose chains together. The crosslinked regenerated cellulose hydrogel had a higher initial degradation temperature as compared with the no-crosslink one.

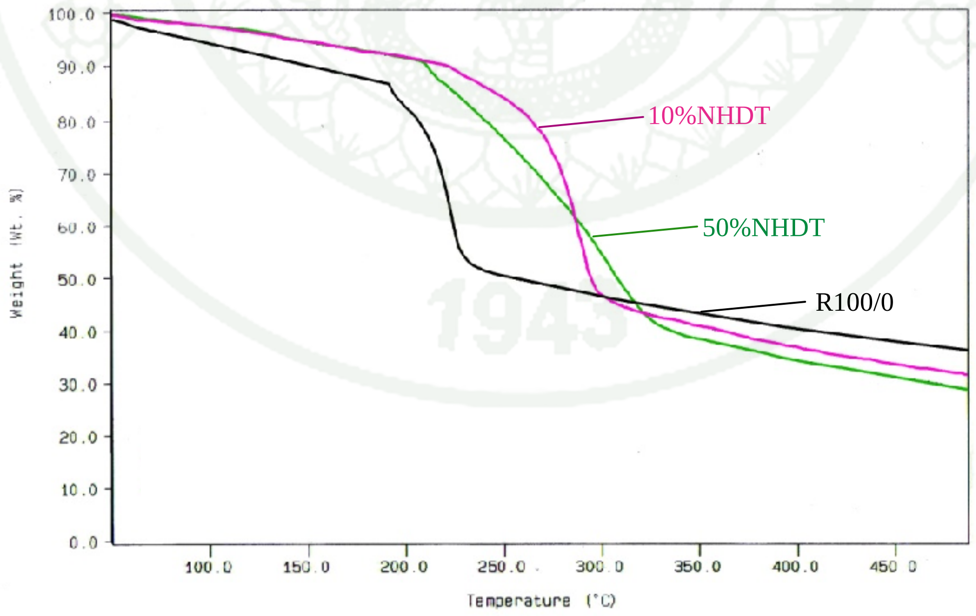


Figure 23 TG curves of R100/0 regenerated cellulose/silk fibroin hydrogels and R100/0 hydrogels with 10 and 50%NHDT.

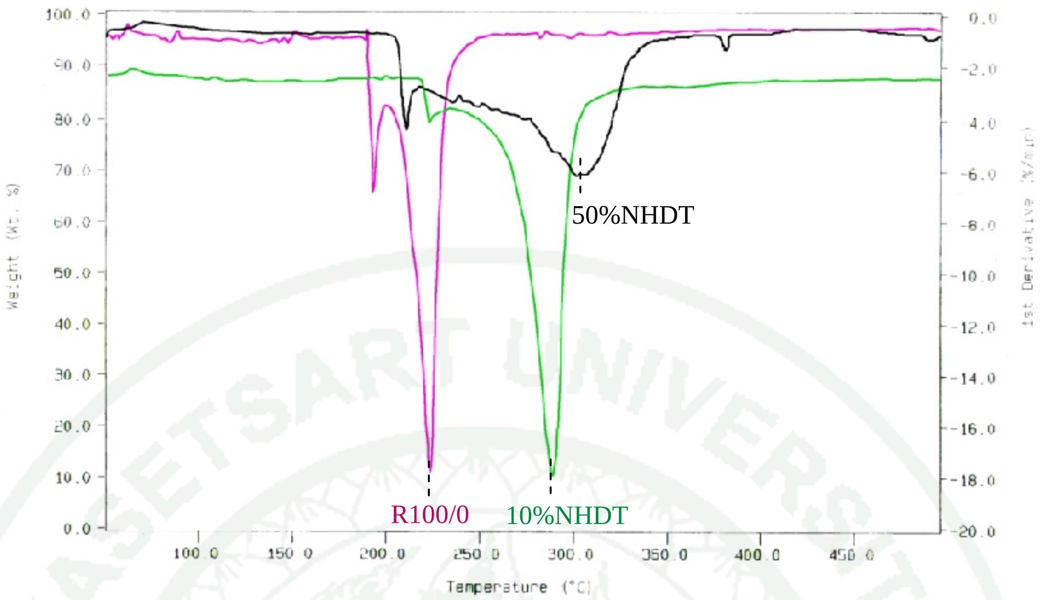


Figure 24 DTG curves of R100/0 regenerated cellulose/silk fibroin hydrogels and R100/0 hydrogels with 10 and 50%NHDT.

The curves of the blended hydrogels are shown in Figures 25 and 26. The blended hydrogel exhibited a degradation temperature at 235°C whereas the hydrogels with 10 and 50%NHDT degraded at 295°C and 300°C, respectively. This result indicated that a higher thermal stability could be enhanced by crosslinking of cellulose chains by NHDT. As compared with R100/0 regenerated cellulose hydrogel (Figures 23 and 24), the R75/25 blended hydrogel had higher thermal stability (higher degradation temperature). This may be due to a stronger interaction between regenerated cellulose and silk fibroin and molecular arrangement in the blend (Shang *et al.*, 2011).

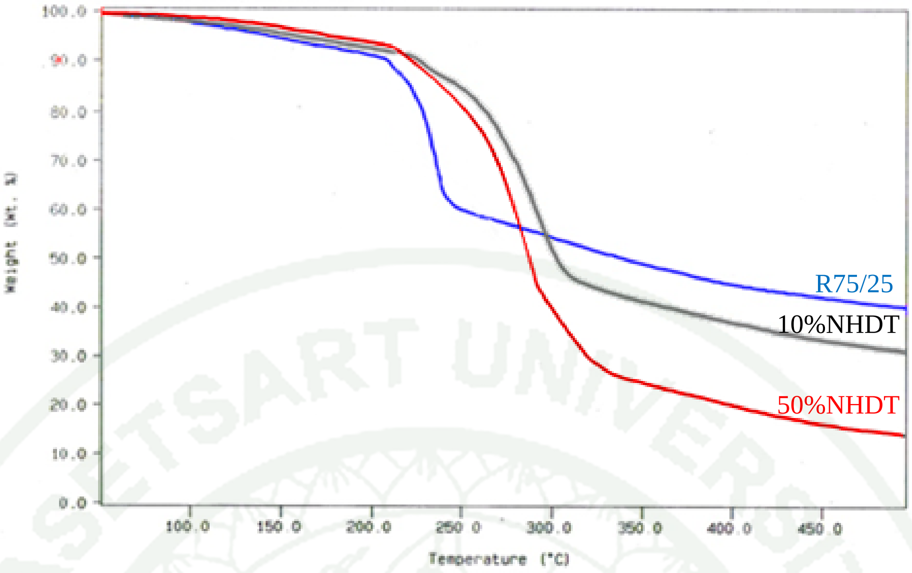


Figure 25 TG curves of R75/25 regenerated cellulose/silk fibroin hydrogels and R75/25 hydrogels with 10 and 50%NHDT.

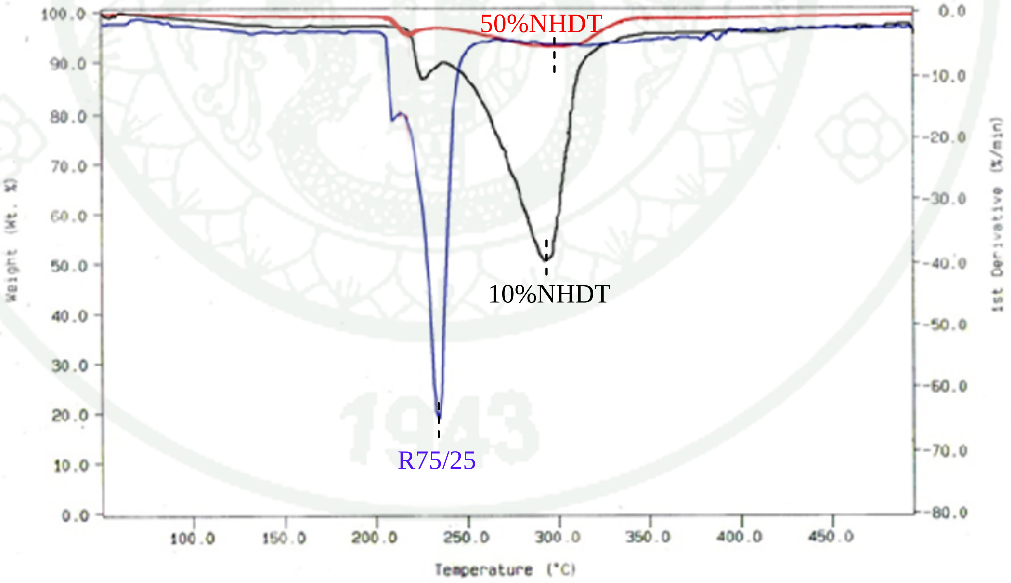


Figure 26 DTG curves of R75/25 regenerated cellulose/silk fibroin hydrogels and R75/25 hydrogels with 10 and 50%NHDT.

3. Effect of salts addition on the regenerated cellulose/silk fibroin hydrogels

3.1 Characterization of hydrogels

3.1.1 Wide-angle X-ray diffraction characterization

The XRD patterns of R75/25 regenerated cellulose/silk fibroin hydrogels at various $CaCl_2$ and NaCl additions are shown in Figures 27 and 28. The blended hydrogels without salts presented the diffraction peak at $2\theta = 20.5^o$ which corresponded to the β-sheet form of silk fibroin and displayed the diffraction peak at $2\theta = 20^o$ and 12^o, which corresponded to cellulose II form (Freddi *et al.*, 1995). In addition, the blended hydrogels with salt addition showed similar diffraction peaks at $2\theta = 20^o$ and 12^o, corresponding to cellulose II form. The diffraction peak at $2\theta = 20.5^o$ of the β-sheet form was overlapped with $2\theta = 20^o$ of cellulose II form. However, the results from IR spectra would support the existence of the β-sheet form (Figure 31).

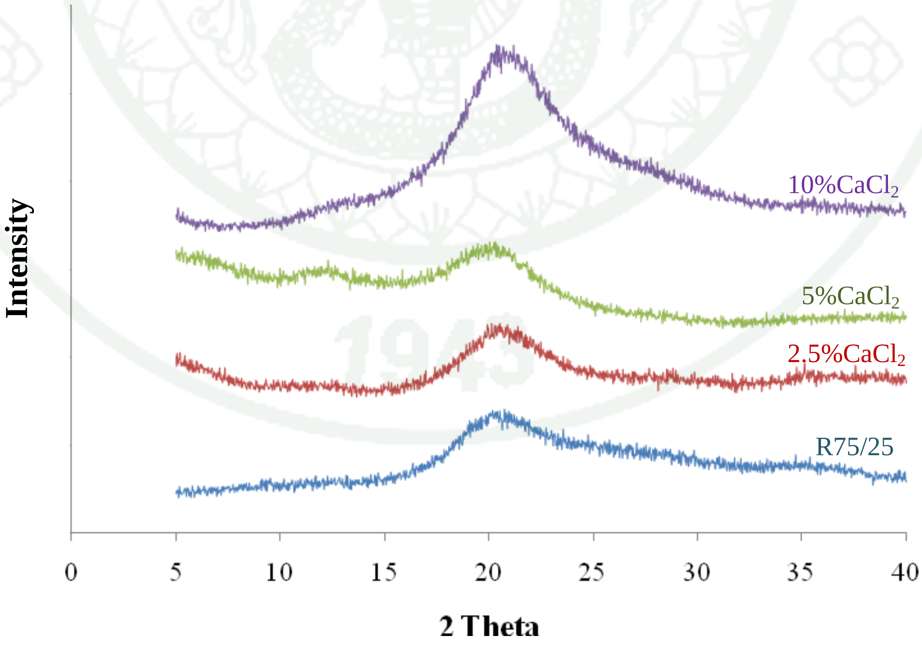


Figure 27 WAXD profiles of R75/25 regenerated cellulose/silk fibroin hydrogels at various $CaCl_2$ addition.

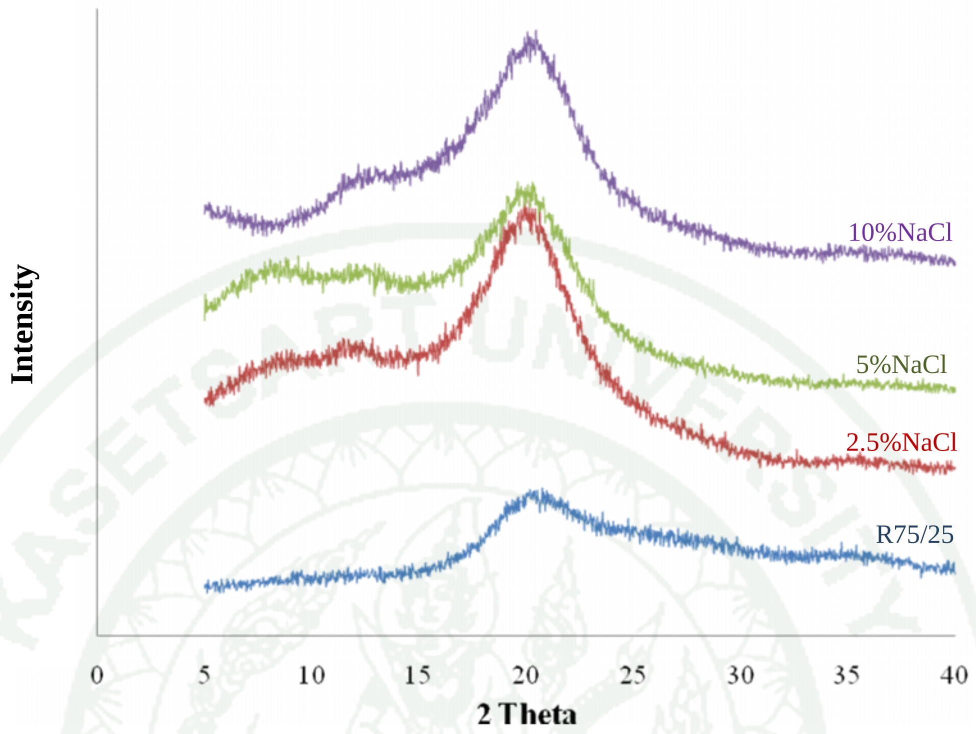


Figure 28 WAXD profiles of R75/25 regenerated cellulose/silk fibroin hydrogels at various NaCl addition.

The XRD patterns of R100/0 regenerated cellulose/silk fibroin hydrogels at various $CaCl_2$ and NaCl additions are shown in Figures 29 and 30. The pure regenerated cellulose hydrogels with and without salt addition showed similar diffraction peaks at $2\theta = 20^{o}$ and 12^{o}, which corresponded to cellulose II form.

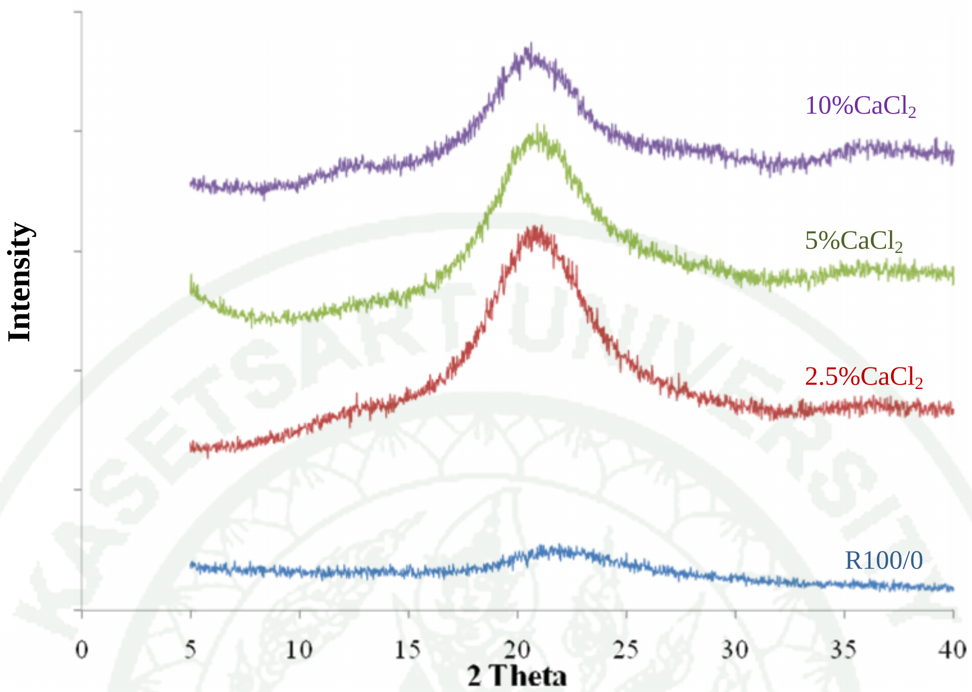


Figure 29 WAXD profiles of R100/0 regenerated cellulose/silk fibroin hydrogels at various CaCl$_2$ addition.

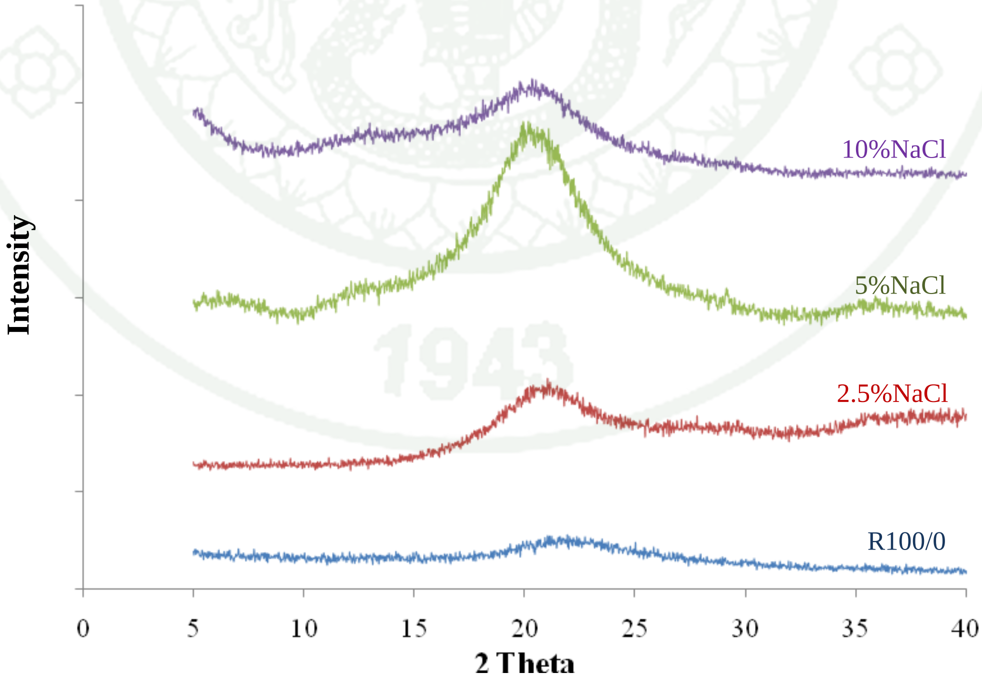


Figure 30 WAXD profiles of R100/0 regenerated cellulose/silk fibroin hydrogels at various NaCl addition.

3.1.2 FT-IR spectra

Figures 31 and 32 show the FTIR spectra of the blended hydrogels and those added $CaCl_2$ and NaCl salts. The absorption bands of the blended hydrogels with and without salts displayed at 1634 cm^{-1}(amide I), 1529 cm^{-1}(amide II) and 1259 cm^{-1}(amide III) which belong to the β-structure of silk II while the absorption bands at 1374 cm^{-1}, 1156 cm^{-1}, 1018 cm^{-1} and 900 cm^{-1} were the characteristic peaks of cellulose II structure (Shang *et al.*, 2011). In the absence of salt, the blend exhibited a strong –OH absorption in the region of 3500-3000 cm^{-1}.

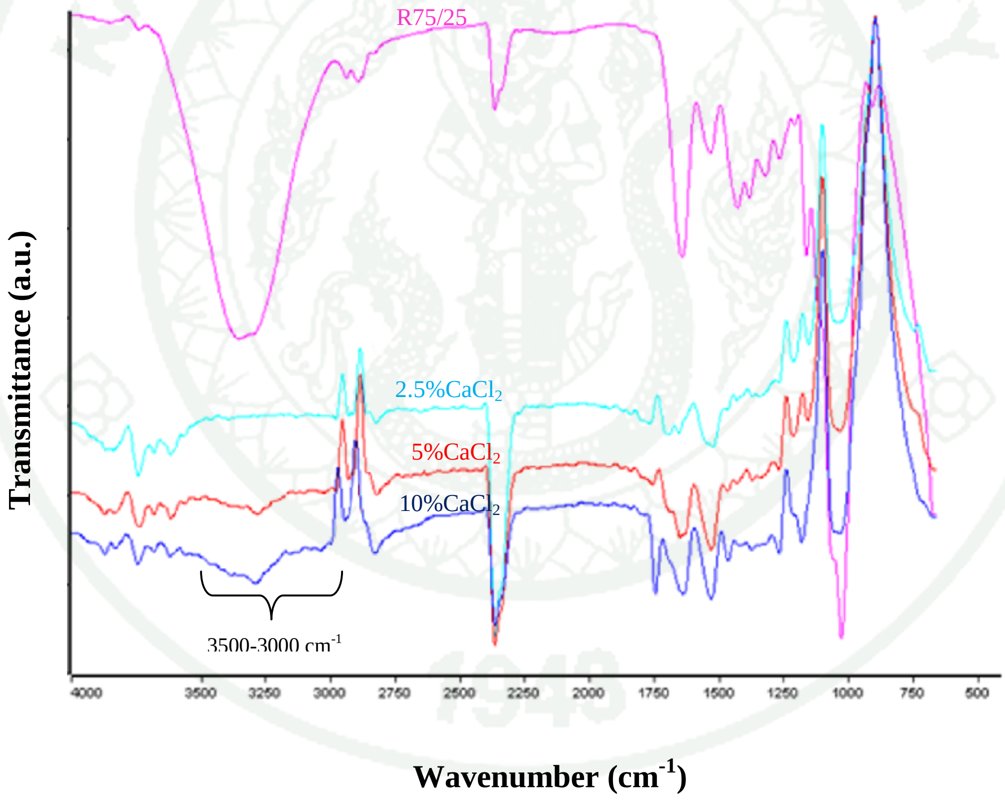


Figure 31 ATR-FTIR spectra of R75/25 regenerated cellulose/silk fibroin blended hydrogels at various $CaCl_2$ additions.

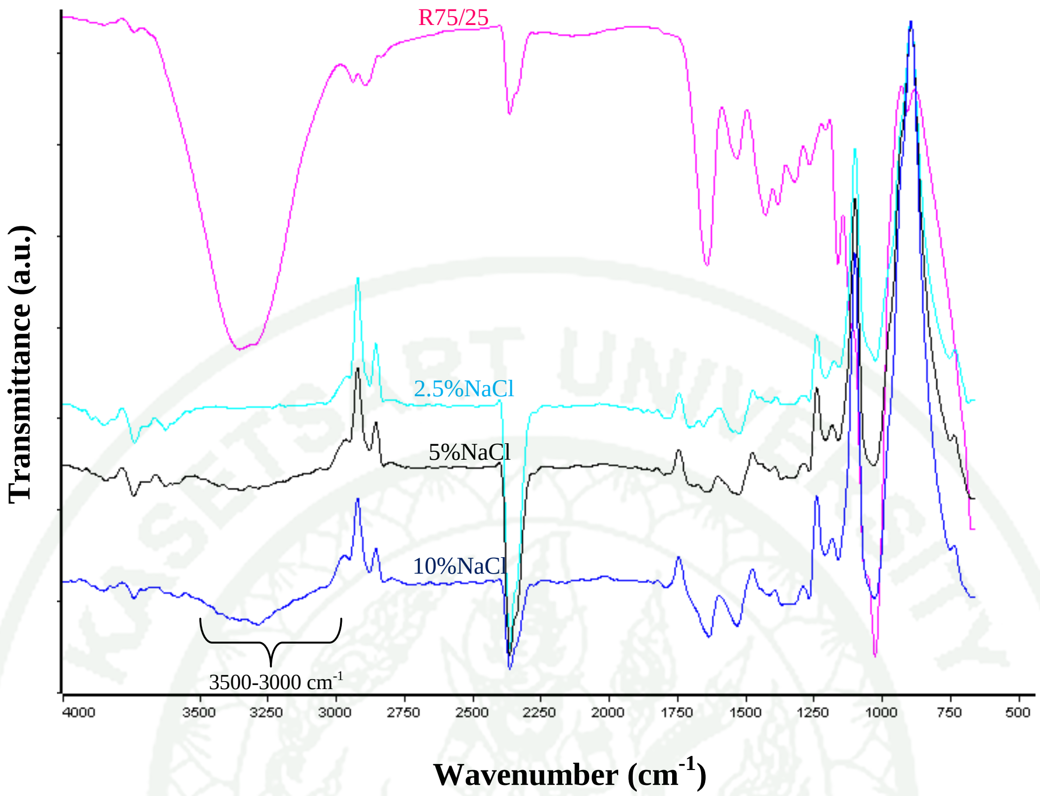


Figure 32 ATR-FTIR spectra of R75/25 regenerated cellulose/silk fibroin blended hydrogels at various NaCl additions.

Figures 33 and 34 show the FTIR spectra of the pure regenerated cellulose hydrogels and those added $CaCl_2$ and NaCl. The results showed the same absorption bands as seen before for the 75/25 blended hydrogels (Figures 31 and 32). It displayed the absorption bands at 1374 cm^{-1}, 1151 cm^{-1}, 1026 cm^{-1} and 748 cm^{-1} (Shang *et al.*, 2011) which were form of the cellulose II structure exhibited absorption bands. In the absence of salt, the blend exhibited a strong –OH absorption in the region of 3500-3000 cm^{-1}.

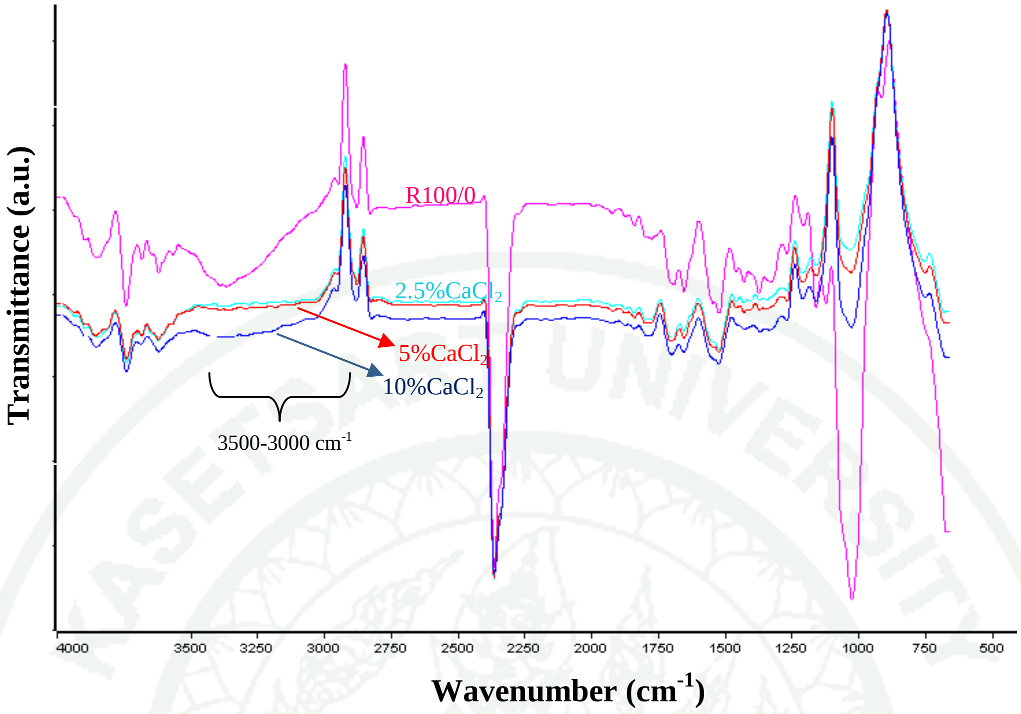


Figure 33 ATR-FTIR spectra profiles of R100/0 regenerated cellulose/silk fibroin blended hydrogels at various $CaCl_2$ additions.

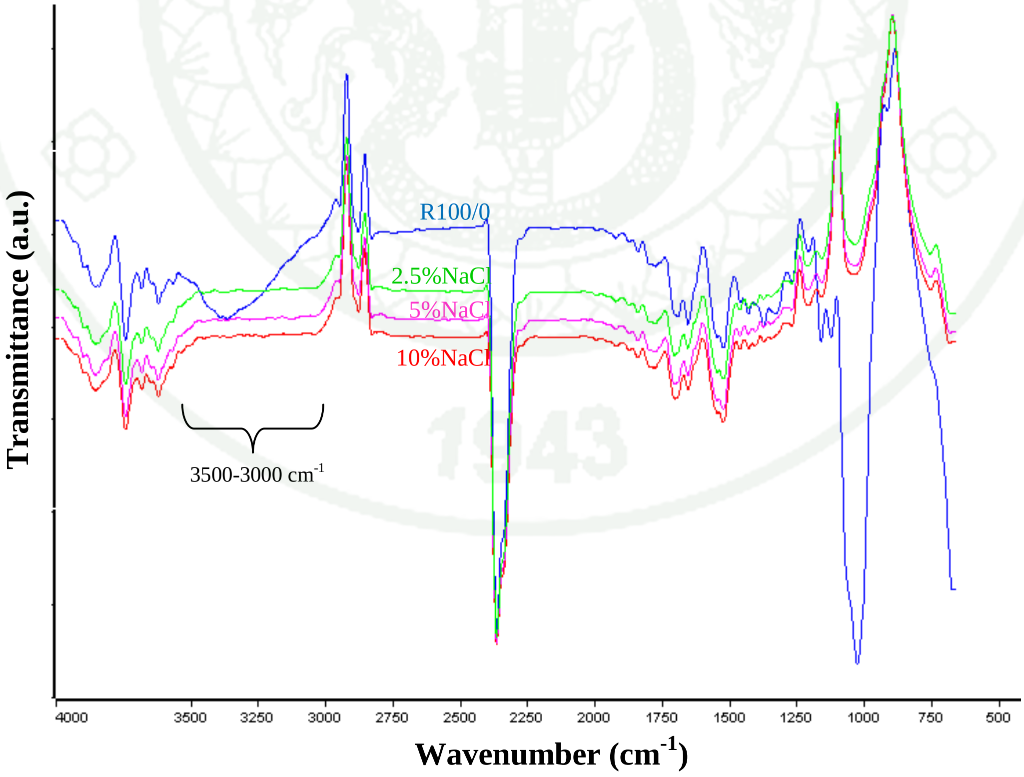
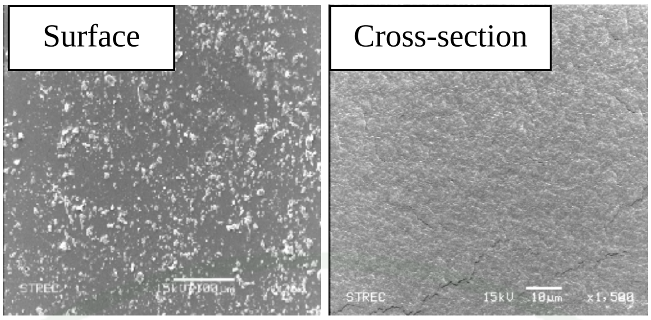


Figure 34 ATR-FTIR spectra of R100/0 regenerated cellulose/silk fibroin blended hydrogels at various NaCl additions.

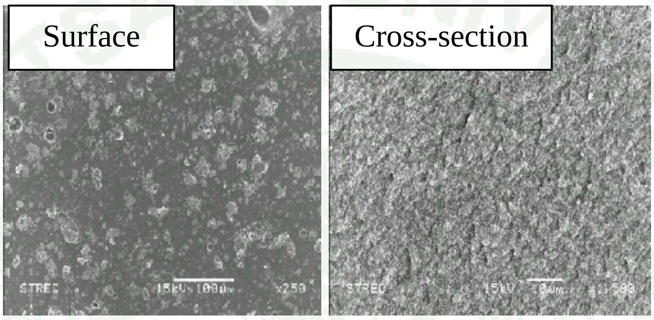
3.1.3 Surface and cross-sectional morphology of the hydrogels

The surface and cross-sectional morphology of R75/25 regenerated cellulose/silk fibroin hydrogels with $CaCl_2$ addition are shown in Figure 35. With adding $CaCl_2$ salt, the surface of the hydrogel exhibited a more porosity and roughness as compared with that without salt. The cross-section of the hydrogel showed a rough surface with some crack marks. This cross-sectional characteristics observed may caused by phase separation between regenerated cellulose and silk fibroin generating a grainy rough surface morphology (Marsano *et al.,* 2008). However, no porosity was obtained in the internal structure of the blend. From this result, it indicates that $CaCl_2$ did affect the morphology of the blended hydrogels. A change in surface and cross-sectional characteristics was observed but no porosity was obtained in the hydrogel structure.

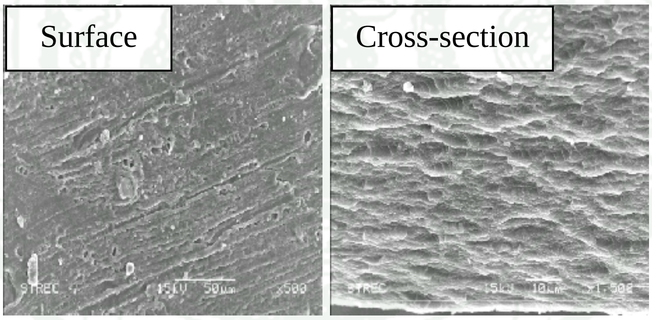
The surface and cross-sectional morphology of R75/25 hydrogels with NaCl addition are shown in Figure 36. The results showed an apparently different surface and cross-sectional morphology with NaCl addition as compared with those with $CaCl_2$ salt. In the presence of NaCl salt, the hydrogels exhibited a rougher and porous structures both at the surface and across their internal part. A macroporous structure observed in all blends with NaCl content enhanced a more porous and rougher morphology to the blend.



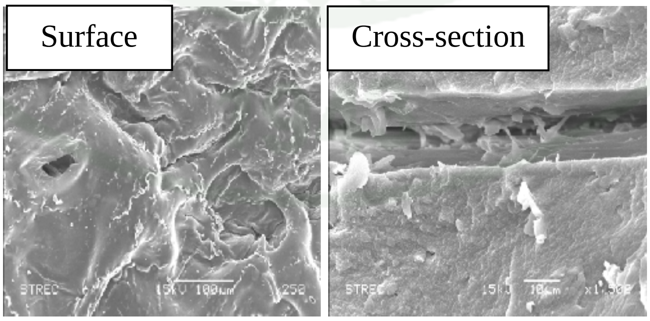
a) No salt



b) 2.5% $CaCl_2$

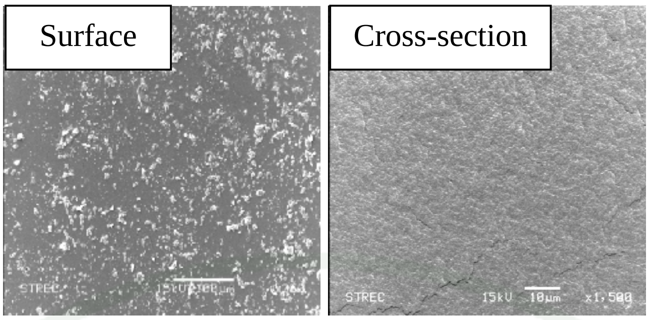


c) 5% $CaCl_2$

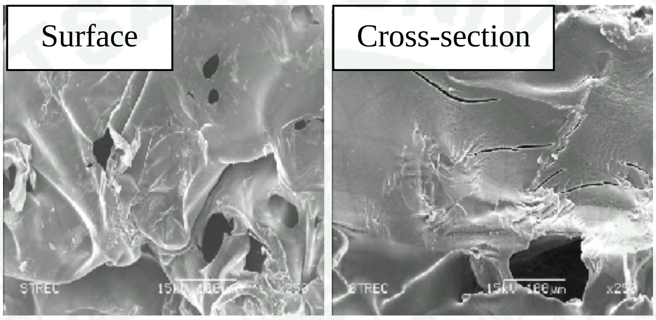


d) 10% $CaCl_2$

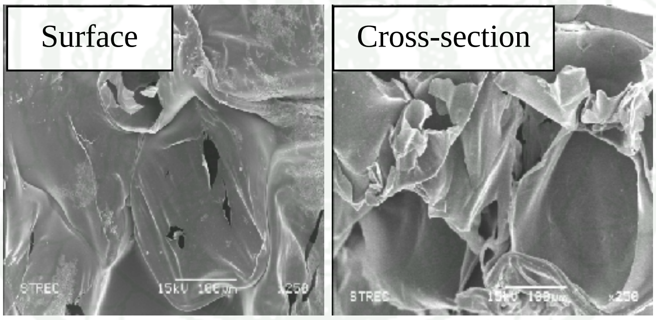
Figure 35 SEM images of the surface and cross-section of R75/25 regenerated cellulose/silk fibroin hydrogels $CaCl_{2;}$ a) No salt; b) 2.5% $CaCl_2$; c) 5% $CaCl_2$ and d) 10% $CaCl_2$.



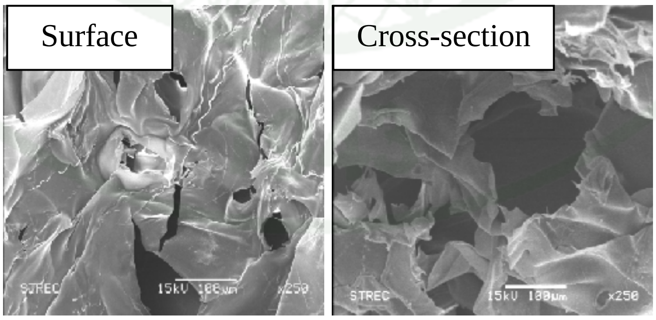
a) No salts



b) 2.5% NaCl



c) 5% NaCl

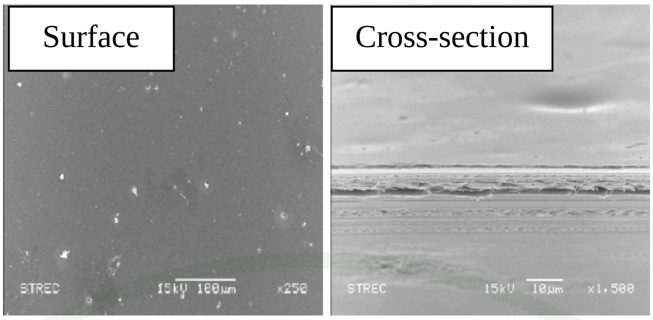


d) 10% NaCl

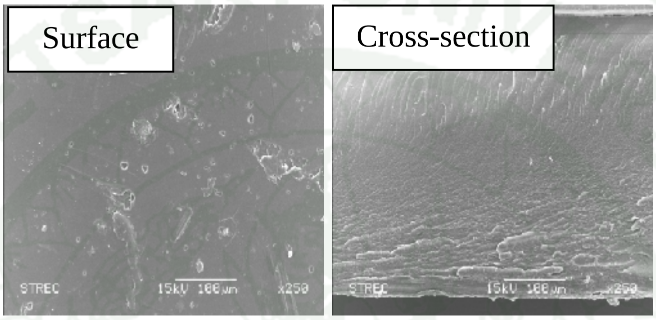
Figure 36 SEM images of the surface and cross-section of R75/25 regenerated cellulose/silk fibroin hydrogels : a) No salt, b) 2.5% NaCl; c) 5% NaCl and d) 10% NaCl.

The surface and cross-sectional morphology of R100/0 hydrogels with $CaCl_2$ addition are shown in Figure 37. It could be seen that addition of $CaCl_2$ at 2.5 and 5% did not have a signified effect on the surface and cross-sectional morphology of the regenerated cellulose hydrogel. A higher amount of $CaCl_2$ was needed to cause a change in hydrogel structure. At 10%$CaCl_2$, a change in surface roughness and internal morphology of the hydrogel was found. From this result, it can be said that a small amount of $CaCl_2$ salt (2.5 and 5%) did not affect the structure and cross-sectional morphology of the pure hydrogel whereas the 10%$CaCl_2$ did result in a rough and porous structure which is expected to enhance water absorptivity to the pure hydrogel (Chang *et al.*, 2008).

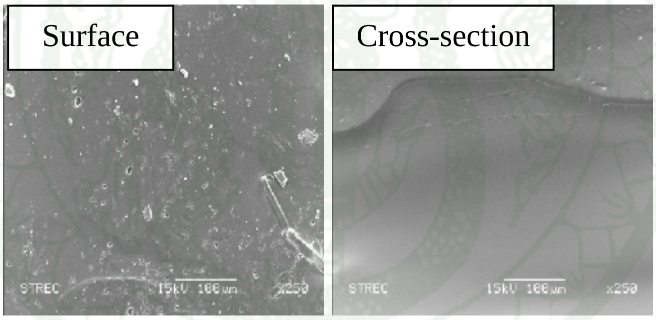
The surface and cross-sectional morphology of the pure regenerated cellulose hydrogels with NaCl addition are shown in Figure 38. The surface morphology displayed a higher porosity with increasing NaCl content. Also, the cross-sectional morphology of hydrogels showed a macroporous structure due to the replacement of NaCl particles in the hydrogel structure. As compared with $CaCl_2$ salt, NaCl provided a stronger effect of the change in the surface and cross-sectional morphology of the hydrogel with more porous and rougher structure obtained. The pure cellulose hydrogel with NaCl addition are expected to exhibit a higher water absorption ability due to a less compact and more porous structure, facilitating an accessibility of water molecules into the hydrogel structure (Chang *et al.*, 2008).



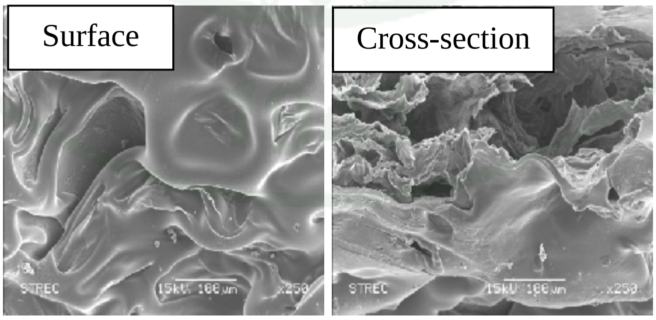
a) No salt



b) 2.5% $CaCl_2$

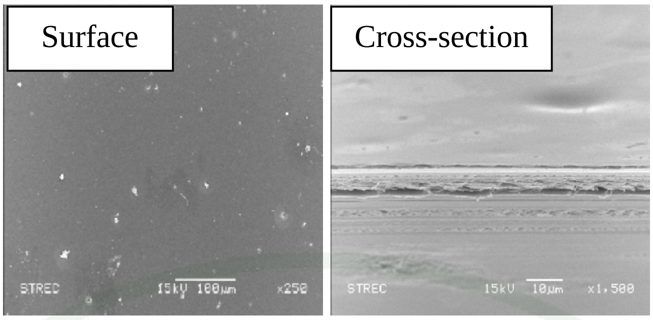


c) 5% $CaCl_2$

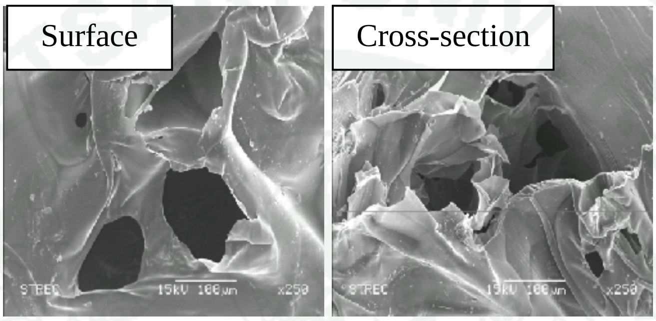


d) 10% $CaCl_2$

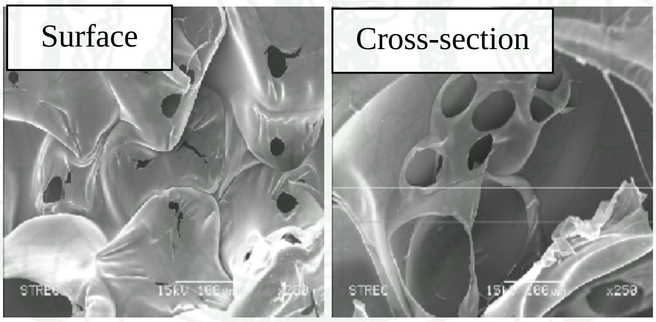
Figure 37 SEM images of the surface and cross-section of R100/0 regenerated cellulose/silk fibroin hydrogels : a) No salt, b) 2.5% $CaCl_2$; c) 5%,$CaCl_2$ and d) 10% $CaCl_2$.



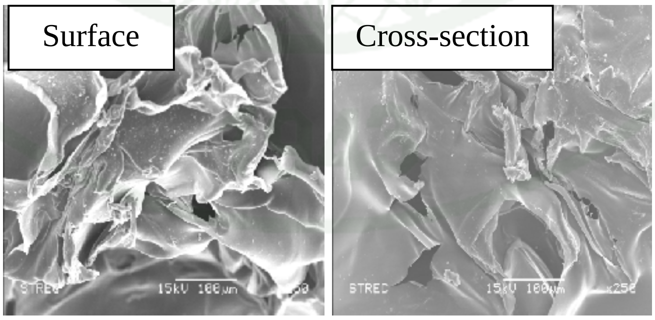
a) No salt



b) 2.5% NaCl



c) 5% NaCl



d) 10% NaCl

Figure 38 SEM images of the surface and cross-section of R100/0 regenerated cellulose/silk fibroin hydrogels : a) No salt, b) 2.5% NaCl; c) 5% NaCl and d) 10% NaCl.

3.1.3 Swelling ratios

The swelling ratios of the 75/25 regenerated cellulose/silk fibroin blended hydrogels are shown in Figure 39. The swelling ratios of the blended hydrogels added with 10%$CaCl_2$ had the highest swelling ratios at about 90% whereas the pure ones showed the lowest (40%). The swelling ratio increased with increasing $CaCl_2$ content in the hydrogels. This indicates an influence of $CaCl_2$ salt on the water absorption ability of the hydrogel. The salt may interrupt the interaction between the polymer chains, resulting in a less ordered molecular arrangement of the polymer. The swelling results corresponded with the SEM images (Figure 35).

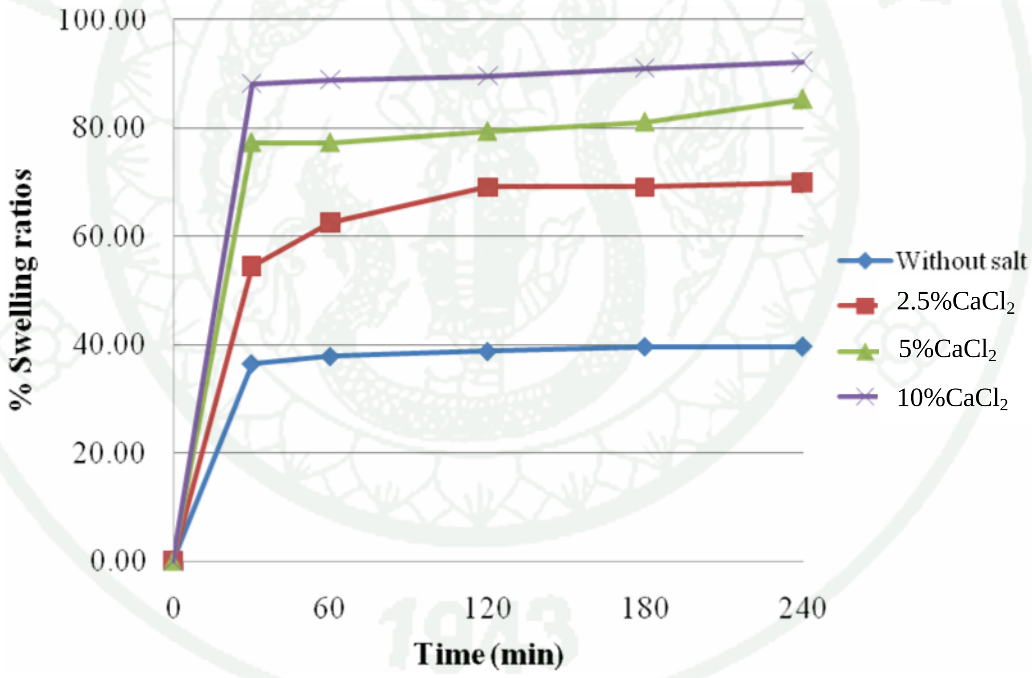


Figure 39 Effect of $CaCl_2$ salt content on the swelling ratios of R75/25 regenerated cellulose/silk fibroin hydrogels at different periods of immersion time.

The influence of NaCl salt on the swelling ratio of the 75/25 regenerated cellulose/silk fibroin blended hydrogel is shown in Figure 40. The hydrogels with salts added had higher swelling ratios than that without salt due to a formation of porous structure in the hydrogel, resulting in an increase in better water absorption capacity of the hydrogel. The effect of the NaCl concentration on the swelling ratios was not significantly different. An addition of just 2.5 %NaCl could provide a similar level of the swelling ratios to those added with 5 and 10%NaCl. Thus, addition of 2.5%NaCl is enough for preparing the hydrogel with improved swelling properties. The swelling results corresponded with the SEM images (Figure 36).

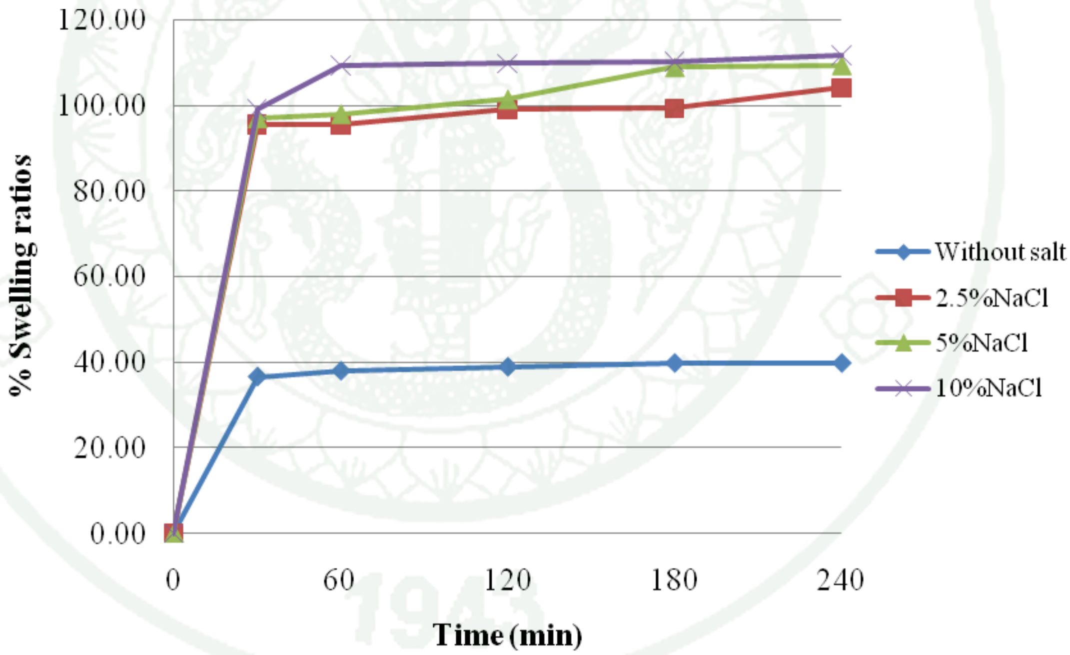


Figure 40 Effect of NaCl salt content on the swelling ratios of R75/25 regenerated cellulose/silk fibroin hydrogels at different periods of immersion time.

The swelling ratios of the 100/0 pure regenerated cellulose hydrogels are shown in Figure 41. The swelling ratios of the blended hydrogels added with 10%$CaCl_2$ had the highest swelling ratios at about 110% whereas the pure regenerated cellulose hydrogels showed the lowest 40%. The swelling ratio increased with increasing $CaCl_2$ content in the hydrogels. This indicates an influence of $CaCl_2$ salt on the water absorption ability of the hydrogel. As mentioned before, the $CaCl_2$ salt may interrupt the interaction between the polymer chains, resulting in a less ordered molecular arrangement and higher water accessibility was induced. There was a correspondence between these swelling properties and the morphology of pure cellulose hydrogel with and without $CaCl_2$ as shown in SEM images in Figure 37.

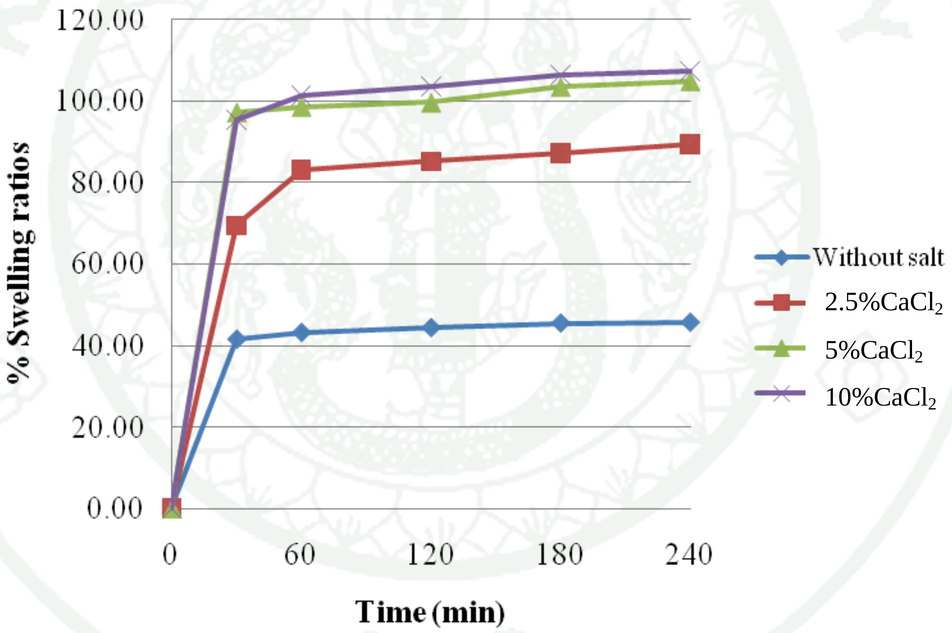


Figure 41 Effect of $CaCl_2$ salt content on the swelling ratios of R100/0 regenerated cellulose/silk fibroin hydrogels at different period of immersion time.

The swelling ratios of the 100/0 pure regenerated cellulose hydrogels are shown in Figure 42. The swelling ratios of the pure regenerated cellulose hydrogels added with 10%NaCl had the highest swelling ratios at about 150% whereas the pure hydrogels showed the lowest swelling ratio 40%. The swelling ratio increased with increasing NaCl content in the hydrogels. This indicates an influence of NaCl salt on the water absorption ability of the hydrogel being the same as those seen in the case of 75/25 blend hydrogel. The interaction between the polymer chains may be interrupted, resulting in less ordered molecular arrangement, or more water accessibility. Adding 10%NaCl content in pure regenerated cellulose hydrogels could increased the swelling ratio of the hydrogels to 50% higher than that with 10%$CaCl_2$ (Figure 41). Therefore, NaCl is more suited for use as a porogen to enhance a change in morphology of the hydrogel which facilitates a high swelling properties in water to the hydrogel. There was a correspondence between these swelling properties and the morphology of pure cellulose hydrogel with and without NaCl as shown in SEM images in Figure 38.

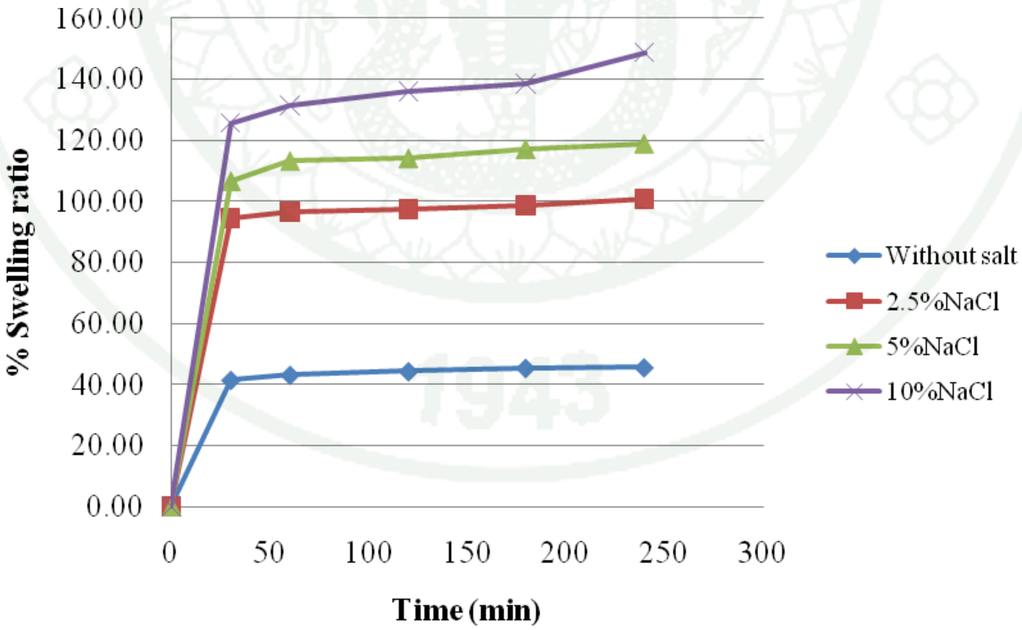


Figure 42 Effect of NaCl salt content on the swelling ratios of R100/0 regenerated cellulose/silk fibroin hydrogels at different period of immersion time.

CONCLUSION

This work was focused on the preparation of blended hydrogels from regenerated cellulose (rayon fiber residues) and silk fibroin. The hydrogels were prepared at different ratios of regenerated cellulose to silk fibroin which are 0/100, 25/75, 50/50, 75/25, and 100/0. The samples R0/100 and R100/0 from pure silk fibroin and pure regenerated cellulose, respectively, were used to compare their properties with the blends, R25/75, R50/50, and R75/25. Regenerated cellulose and silk fibroin were first prepared as solutions separately in 7%LiCl/DMAc at 140-160ºC for 30 min before mixing them together. After the gel was formed at room temperature for 24 h, the gel was immersed in isopropanol for 24 h and then washed with methanol. Silk fibroin alone could not form gel and the higher amount of silk fibroin in the blends caused a slower gel formation with unstable resulting hydrogels. The WAXD pattern of the blends showed the combination of both regenerated cellulose and silk fibroin characteristic peaks at 2θs = 12º and 20º and 2θ = 20º, respectively. These are the diffraction patterns of the β-sheet structure from silk fibroin and cellulose II form of regenerated cellulose. The ATR-FTIR spectra of the blended hydrogels were similar to the pure cellulose and silk hydrogels. Nevertheless, there were somewhat differences in the region of 3500-3300 cm^{-1} resulting from the hydrogen bonds between the hydroxy groups of regenerated cellulose and –CONH- groups of silk fibroin. The blends with higher regenerated cellulose content also exhibited a higher tensile strength and % elongation. The pure regenerated cellulose hydrogel (R100/0) was the strongest and the most flexible hydrogel among all samples. From SEM images, R100/0 surface and cross-section were smooth whereas R75/25 showed a granular surface and rough cross section. The study of swelling ratios comparing between R75/25 and R100/0 hydrogels indicated that the hydrogel could absorb water readily within 30 min and insignificantly increased afterward. R100/0 had about a 5% swelling ratio higher than the R75/25 hydrogel. The β-sheet structure of the silk fibroin may hinder the water accessibility into the hydrogel.

The hydrogel after the treatment with a crosslinking agent, NHDT, did not have any noteworthy change in their physical appearance as compared with the one before the treatment. However, the crosslinked hydrogel could absorb a higher amount of water. The swelling ratios of the hydrogels with and without NHDT were in the range of 40-45% and 60-70%, respectively. The effect of crosslinks on the swelling ratio of hydrogel was more pronounced in the blends (R75/25) than the pure regenerated cellulose hydrogel (R100/0). The crosslinked hydrogels also had a higher initial degradation temperature as compared with the non-crosslinked one.

The porous hydrogels were also prepared by using NaCl and $CaCl_2$ as a porogen. The prepared regenerated cellulose/silk fibroin blended hydrogel can absorb a higher amount of water as compared with the pure regenerated cellulose hydrogel. A small amount of $CaCl_2$ (2.5 and 5%) did not affect the structure and cross-sectional morphology of the blend whereas the 10%$CaCl_2$ did result in a rough and porous structure which is expected to enhance water absorptivity to the pure and the blended hydrogels. On the other hand, hydrogels with a NaCl addition exhibited higher water absorption ability even at 2.5%. This might be due to a less impact and more porous structure which facilitated an accessibility of water into the hydrogels. The addition of 10%NaCl gave the highest swelling ratio at about 150% whereas the pure regenerated cellulose hydrogel showed the lowest swelling ratio at around 40%.

Therefore, the regenerated cellulose residues which are fiber waste from the textile industry and the silk fibroin which is the abundant natural fiber in Thailand could be used to prepare the blended hydrogels. NHDT treatment could provide a higher initial degradation temperature and also % swelling ratio. Using NaCl as a porogen in this experiment gave the best result for % swelling ratio. NaCl would be a good choice to enhance absorptivity of the blended hydrogels. The prepared hydrogel blends have a potential to be used in adsorbent application.

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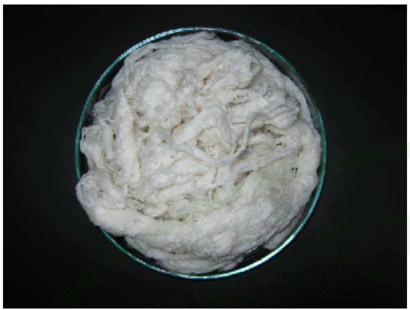
APPENDICES

Appendix A

The preparation of regenerated cellulose and silk fibroin fibers

1. The scouring steps regenerated cellulose fiber



1. Regenerated cellulose fiber, rayon.

2. Scoured at 80^{o}C, 1 h 3 g/L Sera Wash M-SF and 2 g/L Na_2CO_3.

3. Rinsed with distillated water and oven-dried at 60^{o}C for 24 h.

Appendix Figure A1 The scouring of regenerated cellulose fiber.

2. The degumming steps of silk cocoons

1. Silk cocoons.

2. Degummed at boil in 2 g/L Na_2CO_3 for 30 min twice.

3. Rinsed with distillated water and oven-dried at 60°C for 48 h.

Appendix Figure A2 The degumming of silk cocoons.

Appendix B
The data of the swelling ratios

Appendix Table B1 Dried weight and %swelling ratios of R75/25 and R100/0 cellulose/silk fibroin hydrogels at different period of immersion time.

Time (min)	R75/25		R100/0	
	Dried weight (g)	**Swelling ratios (%)**	**Dried weight (g)**	**Swelling ratios (%)**
0	0.2371	0.00	0.1612	0.00
30	0.3237	36.52	0.2281	41.50
60	0.3269	37.87	0.2308	43.18
120	0.3291	38.80	0.2327	44.35
180	0.3312	39.69	0.2345	45.47
240	0.3312	39.69	0.2348	45.66

Appendix Table B2 Dried weight and %swelling ratios of R75/25 and R100/0 cellulose/silk fibroin hydrogels immersed in various concentrations of NHDT for 24 h.

Concentrations of NHDT (g/L)	Dried weight (g)	Swollen weight (g)	Swelling ratios (%)
	R75/25		
0	0.2371	0.3317	46.22
10	0.0580	0.1002	72.76
20	0.0638	0.1070	67.71
30	0.0245	0.0414	68.98
50	0.0572	0.0936	63.64
	R100/0		
0	0.1612	0.2357	39.90
10	0.0531	0.0880	65.73
20	0.0873	0.1416	62.20
30	0.0624	0.1005	61.06
50	0.0411	0.0654	59.12

Appendix Table B3 Dried weight and %swelling ratios of R75/25 cellulose/silk fibroin hydrogels with added $CaCl_2$ at different period of immersion time.

$CaCl_2$ concentration (%)	**After treatment**	**Time (min)**					
		0	**30**	**60**	**120**	**180**	**240**
0	Dried weight (g)	0.2371	0.3237	0.3269	0.3291	0.3312	0.3312
	%Swelling ratios	0.0000	36.52	37.87	38.80	39.69	39.69
2.5	Dried weight (g)	0.0262	0.0405	0.0426	0.0443	0.0443	0.0445
	%Swelling ratios	0.000	54.58	62.60	69.08	69.08	69.85
5	Dried weight (g)	0.0291	0.0516	0.0516	0.0522	0.0527	0.0539
	%Swelling ratios	0.0000	77.32	77.32	79.38	81.10	85.22
10	Dried weight (g)	0.0280	0.0527	0.0529	0.0531	0.0535	0.0538
	%Swelling ratios	0.0000	88.21	88.93	89.64	91.07	92.14

Appendix Table B4 Dried weight and %swelling ratios of R75/25 cellulose/silk fibroin hydrogels with added NaCl at different period of immersion time.

NaCl concentration (%)	After treatment	Time (min)					
		0	30	60	120	180	240
0	Dried weight (g)	0.2371	0.3237	0.3269	0.3291	0.3312	0.3312
	%Swelling ratios	0.0000	36.52	37.87	38.80	39.69	39.69
2.5	Dried weight (g)	0.0201	0.0396	0.0398	0.0405	0.0421	0.0420
	%Swelling ratios	0.0000	95.56	95.56	98.98	99.32	104.10
5	Dried weight (g)	0.0293	0.0573	0.0573	0.0583	0.0584	0.0598
	%Swelling ratios	0.0000	97.01	98.01	101.49	108.96	109.45
10	Dried weight (g)	0.0214	0.0426	0.0448	0.0449	0.0450	0.0453
	%Swelling ratios	0.0000	99.07	109.35	109.81	110.28	111.68

Appendix Table B5 Dried weight and %swelling ratios of R100/0 cellulose/silk fibroin hydrogels with added $CaCl_2$ at different period of immersion time.

$CaCl_2$ concentration (%)	**After treatment**	**Time (min)**					
		0	**30**	**60**	**120**	**180**	**240**
0	Dried weight (g)	0.1612	0.2281	0.2308	0.2327	0.2345	0.2348
	%Swelling ratios	0.0000	41.50	43.18	44.35	45.47	45.66
2.5	Dried weight (g)	0.0236	0.0400	0.0432	0.0437	0.0442	0.0447
	%Swelling ratios	0.0000	69.49	83.05	85.17	87.29	89.41
5	Dried weight (g)	0.0284	0.0570	0.0574	0.0577	0.0588	0.0582
	%Swelling ratios	0.0000	97.23	98.62	99.65	103.46	104.93
10	Dried weight (g)	0.0289	0.0555	0.0572	0.0578	0.0586	0.0599
	%Swelling ratios	0.0000	95.42	101.41	103.52	106.34	107.27

Appendix Table B6 Dried weight and %swelling ratios of R100/0 cellulose/silk fibroin hydrogels with added NaCl at different period of immersion time.

NaCl concentration (%)	**After treatment**	**Time (min)**					
		0	**30**	**60**	**120**	**180**	**240**
0	Dried weight (g)	0.1612	0.2281	0.2308	0.2327	0.2345	0.2348
	%Swelling ratios	0.0000	41.50	43.18	44.35	45.47	45.66
2.5	Dried weight (g)	0.0232	0.0451	0.0456	0.0458	0.0461	0.0466
	%Swelling ratios	0.00	94.40	96.55	97.41	98.71	100.86
5	Dried weight (g)	0.0228	0.0471	0.0486	0.0488	0.0495	0.0499
	%Swelling ratios	0.00	106.58	113.16	114.04	117.11	118.86
10	Dried weight (g)	0.0156	0.0352	0.0361	0.0368	0.0372	0.0388
	%Swelling ratios	0.00	125.64	131.41	135.90	138.46	148.72

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