PREPARATION OF HYDROGEL FROM MODIFIED SILK WASTE AND POLY(VINYL ALCOHOL)

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หัวข้อวิทยานิพนธ์	การเตรียมไฮโดรเจลจากเศษไหมเหลือทิ้งที่ผ่านการปรับปรุงกับพอลิไวนิลแอลกอฮอล์
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บทคัดย่อ

ในประเทศไทยมีเศษไหมที่เหลือทิ้งจากอุตสาหกรรมสิ่งทอมากมาย งานวิจัยนี้จึงเป็นการนำเศษไหมที่เหลือทิ้งนั้นมาใช้ใหม่เพื่อช่วยเพิ่มคุณค่าของเศษไหม โดยนำเศษไหมมาผสมกับพอลิไวนิลแอลกอฮอล์ แล้วทำการฉายรังสีเพื่อก่อให้เกิดไฮโดรเจล จากนั้นศึกษาถึงผลของปริมาณรังสี อัตราส่วนของไหมและพอลิไวนิลแอลกอฮอล์ และน้ำหนักโมเลกุลของพอลิไวนิลแอลกอฮอล์ที่มีต่อสมบัติทางกายภาพและสมบัติเชิงกลของไฮโดรเจล

จากผลการทดลอง พบว่าลักษณะทางกายภาพที่แตกต่างกันของไฮโดรเจลเป็นผลเนื่องมาจากความเข้มข้นของไหม ความเข้มข้นของพอลิไวนิลแอลกอฮอล์ อัตราส่วนของไหมและพอลิไวนิลแอลกอฮอล์ น้ำหนักโมเลกุลของพอลิไวนิลแอลกอฮอล์ และความเข้มของรังสี นอกจากนี้ยังพบว่าปริมาณการเป็นเจล การดูดซับน้ำ และความแข็งแรงของเจล เป็นผลเนื่องจากพอลิไวนิลแอลกอฮอล์มากกว่าไหม การผสมพอลิไวนิลแอลกอฮอล์กับไหมช่วยเพิ่มคุณสมบัติดูดซับน้ำของไฮโดรเจล พอลิไวนิลแอลกอฮอล์ชนิดน้ำหนักโมเลกุลต่ำ(11,000-31,000) ไม่เหมาะต่อการเตรียมไฮโดรเจล การผสมไหม 1% และพอลิไวนิลแอลกอฮอล์ 3% ในอัตราส่วน 1:3 พร้อมทั้งทำการฉายรังสีด้วยความเข้ม 30 กิโลเกรย์ เป็นสูตรที่เหมาะสมในการเตรียมไฮโดรเจลเนื่องจากมีการดูดซับน้ำที่ดี มีความแข็งแรงพอสมควร และใช้ความเข้มรังสีไม่สูงจนเกินไป

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ABSTRACT

In Thailand, there is much of silk waste from textile industry. This research focused on preparation of hydrogel from modified silk waste(SF) and poly(vinyl alcohol)(PVA) for value added to the silk waste. The hydrogels were prepared by γ-irradiation technique. The research aimed to examine the influence of radiation dose, ratio of SF/PVA, and molecular weight of PVA on physical and mechanical properties of the hydrogels. It was found that physical appearance of the hydrogels depended on concentration of the silk and PVA, ratio of silk/PVA ,molecular weight of PVA ,and irradiation dose. Gel fraction ,water absorption ,and gel strength of the hydrogels depended on PVA more than silk. Addition of PVA into silk could increase %water absorption of hydrogel. Low molecular weight PVA (11,000 – 31,000) was not suitable for preparing the hydrogel. The appropriate formula was 1%silk / 3%PVA at ratio 1:3 and irradiated at 30 kGy because it gave high water absorption, appropriate gel strength, at an appropriate irradiation dose.

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Jirapan Naikhorn

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Silk fibroin(SF) fibers are water insoluble fibrous protein. Nevertheless fibroin fibers are dissolved easily in concentrated aqueous solution of salts, such as lithium bromide and calcium chloride, when the interchain hydrogen bonding is weakened. Thus, a regenerated silk fibroin solution is obtained. Native silk fibroin can be prepared by dispersing in water, the liquid silk proteins collected from the posterior silk gland of mature larvea. In recent years it was demonstrated that the silk fibroin has good compatibility and that the fabricated silk membrane has good oxygen permeability in the wet state. Studies on the structure and physical properties of silk fibroin are very important, therefore, in paving the way for potential future applications. Nowadays, silk fibroins derived from silkworms, *Bombyx mori* and philosamia cynthia ricini have been used as excellent enzyme immobilization materials as well as excellent fibers. The recognized advantages of the silk fibroins for use as biomaterials are as follows: [1]

1) Purification of the silk fibroin is relatively simple and easy compared with other biomaterials.
2) The specific activity of the enzyme entrapped in the silk fibroin and the substrate permeability of the silk fibroin membrane are controlled by the structural transition of the silk fibroin, such as occurrence of β-structure induced by methanol.
3) There is no appreciable interaction between the silk fibroin and the enzymes such as glucose oxidase.
4) The functional residues in the protein can be used for chemical modification.

In particular, the chemical modification of the protein provides a variety of biomaterials with unique character. The applications of silk fibrion(SF) films as fungible of the skin and enzyme immobilized films, etc. However, SF films are very brittle by themselves.

Poly(vinyl alcohol)(PVA) is a nontoxic water-soluble synthetic polymer, which is widely used in biochemical and biomedical applications. Water-soluble PVA is rendered insoluble by the introduction of crosslinks into the specimen (called hydrogel). PVA hydrogels are being studied in place of biological tissues and used as a matrix for immobilization of microorganisms, enzymes, and heparin.[8]

It is considered that the SF/PVA blends prepared using both of these two potentially important functional polymers could contribute to produce new functional polymers which can be used in various industrial domains. Blends of these two have been implicated as functionally important in a wide range of biological phenomena. There were many researches about SF/PVA blend. Their properties have been reported such as water absorption, degree of swelling, permeability by neutral salts, and their structure.

A hydrogel is defined as "a polymeric material which exhibits the ability to swell in water and retain a significant fraction of water within its structure, but which will not dissolve in water". Recently an interest has developed to use the hydrogel as biomedical materials because of its excellent biocompatibility, its high water permeability, its characteristics desirable for enzyme supports, its controlled release of drugs incorporated in the hydrogel, etc. A radiation technique seems to be promising for preparation of hydrogels, because a polymer in aqueous solution or water-swollen film readily undergoes crosslinking on irradiation technique to yield a gel-like material highly swollen with water. Since this hydrogel, the water content of which can be varied over a wide range with the irradiation dose, is not contaminated with foreign additives and the crosslinks must be composed of chemically stable C-C bonds. It is considered that the preparation of hydrogels by radiation crosslinking is a very interesting method.

In the present work the irradiation of solution blends of water-soluble polymers(PVA) / silk fibroin(SF) seperated from silk waste was carried out (PVA/SF hydrogel). Physical, Mechanical, Thermal properties and water absorption ability of SF/PVA hydrogel would be investigated.

1.2 Objectives

1) To prepare a new type of hydrogel from silk waste for biomedical usage.
2) To study the effect of radiation dose on silk fibroin.
3) To study on radiation dose that is suitable for preparing the hydrogel.
4) To study the ratio of SF/PVA that is the suitable ratio.
5) To study the appropriate molecular weight of PVA for prepare the hydrogel.

1.3 Scopes of Study

1) Preparation of silk fibroin solution, poly(vinyl alcohol) solution and the mixtures of SF/PVA solution.
2) Preparation of hydrogel from SF, PVA and SF/PVA blends by γ-irradiation.
3) Study on various doses which produce the crosslinked hydrogel.
4) Study on various ratios of SF/PVA.
5) Study on the effect of molecular weight of PVA in SF/PVA blends under γ-irradiation.
6) Study on mechanical and physical properties of the hydrogel.

1.4 Expected Results

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

CHAPTER 2

LITERATURE REVIEW AND THEORY

2.1 Literature Review

Jun Magoshi and Shigeo Nakamura (1975) studied on physical properties, structure of silk, glass transition and crystallization of silk fibroin. They found that amorphous silk fibroin with random coil conformation showed endothermic and exothermic peaks. From DSC (differential scanning calorimetry) curve, endothermic shift observed at 175 °C was due to the crystallization, which later was confirmed by x-ray diffraction pattern. The endothermic peak at 280 °C was shown to be the degradation. In the addition, the β-form of silk showed endothermic peak at about 283 °C [2].

Jun Magoshi, et al.(1977) studied on physical properties, structure of silk, and thermal behavior of silk fibroin in the random-coil conformation. They found that intramolecular and intermolecular hydrogen bonds of silk were broken between 150 and 180 °C. The glass transition was observed at 173 °C. The random coil changed to β-form transition accompanied by reformation of hydrogen bonds above 180 °C [3].

Yoshito Ikada, et al.(1977) prepared hydrogels by radiation technique. Poly (vinyl alcohol)(PVA), poly(ethylene oxide), poly acrylamide, poly (vinyl pyrrolidone), and methyl cellulose were cast on a glass plate and irradiated with electron beams to yield crosslinked hydrogels. The measurement of tensile properties of the hydrogels revealed that the hydrogel from PVA gave the highest tensile strength among the hydrogels [4].

Jun magoshi, et. al. (1979) studied about silk fibroin films. They found that when treated silk fibroin films with water below 60 °C, the random-coil conformation converted to the α-form. When the water was above 70 °C, there were mixture of α and β conformations. The β-form content increased as the immersion temperature was raised [5].

Makoto Demura, et al.(1988) investigated styrene-grafted *Bombyx mori* silk fibroin membrane. It was revealed from the water absorption and ^{1}H nuclear magnetic resonance(NMR) measurements that the amounts of water absorbed on the silk fibroin membranes decreased by the styrene grafting and the states of water adsorbed on the styrene-grafted silk fibroin membranes were not homogeneous. The fraction of fast component decreased with increasing styrene grafting. The membrane potentials increased with increasing of the grafting. The potassium chloride permeability of the membrane strongly depended on the degree of styrene grafting. Application of these membranes was the seperation of substrates or the immobilization of biocatalysts [6].

Toshihiro Hirai, et al. (1989) studied on the properties and permeation characteristics of poly(vinyl alcohol) hydrogel membranes. They found that the membranes had excellent elasticity and showed reversible compaction by operation pressure. The membrane performance was also changed reversibly by pressure, and was held stable at ambient temperature for more than 12 months. The hydrogel membrane was stable against temperature up to 40 $^{\circ}$C, and was melted down at 70 $^{\circ}$C. Rejection increased with operating pressure. In the case of 1%(w/w) aqueous solution of poly(ethylene glycol) whose degree of polymerization is 400, the rejection increased from 30 to 85% by changing oprerating pressure from 5 kg/cm^{2} to 60 kg/cm^{2}. These results suggested the possibility for the pressure controlled fractionation of molecular weight [7].

Kazuo Yamaura, et al. (1990) studied permeabilities of water, several alcohols, and water/alcohol mixtures through thin films of syndiotactic – rich poly (vinyl alcohol). They compared the permeabilities of liquids through syndiotactic - rich poly(vinyl alcohol)(s-PVA); very thin films(about 0.3 μm) scooped up from the interface of air/aqueous solution; with the casting s-PVA films (about 15 μm). They found that the permeability of water decreased with time and the volume of permeate leveled off after 4 or 5 days in both films. In the case of the very thin films, the permeabilities of alcohols decreased with the increase in the molecular weight of alcohol, whereas, in the case of casting films, the permeability of methanol was lower than that of ethanol. In the case of the very thin films, the permeabilities of any water/alcohol (50/50) mixtures were lower than those of pure water and alcohols. These results were correlated to rearrangement of s-PVA molecules, formation of bound water, hydrophobic interaction, and clusterization of small molecules [8].

Hiroaki Yoshimizu and Tetsuo Asakura(1990) prepared and characterized of silk fibroin powder and its application to enzyme immobilization. The powders of *Bombyx mori* silk fibroin were prepared from aqueous solution of silk fibroin by several kinds of the insolubilization methods, some of which used methanol. The insolubilization appeared to be affected by the conformational transition of silk fibroin from the random coil to antiparallel β-sheet form, which was monitored with IR spectroscopy. In order to characterize the structure of the powder on the swollen state in water, a spin-labelled silk fibroin powder was prepared and the ESR spectra were observed, as well as ^{13}C NMR. The heterogeneous structure was analyzed quantitatively in terms of the fraction of fast, slow, and very slow components on the ESR spectra. Finally an enzyme, invertase, was immobilized with the silk fibroin powder. The thermal stability of the enzyme was much improved by the immobilization [9].

Norihiko Minoura, et al. (1990) studied physico-chemical properties of silk fibroin membrane as a biometerial. A water-insoluble silk fibroin membranes was prepared by immersing a silk fibroin membrane as cast in 50 vol% aqueous methanol solution for different periods of time at 25 °C. To use the membranes as a biomaterial, oxygen and water vapor permeability, transparency, mechanical property and enzymatic degradation behavior in vitro of the membrane in the wet state were investigated. It was supposed that in the process of the enzymatic degradation, silk fibroin molecules in the amorphous region of the membrane surface were digested by the enzyme and that crystalline region or highly dense region remained. The silk fibroin membranes treated with methanol had high oxygen permeability, high water vapour permeability, high transparency, good mechanical property and bio-degradability. The crystalline region in the membrane plays an important role in the mechanical property, as dose the amorphous region in the permeability [10].

Kazuo Yamaura, et al. (1990) prepared mixtures of Silk Fibroin (SF) / Syndiotactic-Rich Poly(vinyl alcohol). The thermal analysis of dry-gels and the various properties of blend films were investigated. The rate of gelation of a mixed solution was lower than that of each SF or s-PVA solution at the same concentration. In the DSC thermogram of mixed dry-gel (SF/ s-PVA = 2/8 - 8/2) 2 endothermic peaks corresponding to SF (at 288-299 °C: melting) were found. Namely, microphase segregation took place. This was confirmed by optical and electron microscopes. The mechanical properties of blend films did not have additive properties. The degree of swelling in water of blend films was independent on water temperature or methanol treatment. The mechanochemical behaviors were berely observed by the pH exchange between pH·2 and 12 only under loads. The addition of SF into s-PVA films promoted that the permeation of neutral salts [11].

Chuan Xin Liang and Kiyoshi Hirabayashi (1992) improved the physical properties of silk fibroin membranes with sodium alginate. The experimental results showed the β-form crystal. The β-form molecular conformation increased due to the increase of intermolecular hydrogen bonds, such as C=O----HN and C=O----HO. The kinds of intermolecular interaction and β-form molecular conformation of polymer blends were clarified by x-ray analysis. The water absorbability, mechanical properties, and thermal stability of fibroin membranes were improved by blending with sodium alginate. The rupture elongation also increased greatly with the increase of sodium alginate content [12].

Jin Hong Kim, et al. (1992) prepared cross-linked poly(vinyl alcohol) / chitosan blend membrane from a solvent-casting technique. They characterized for their intermolecular interactions by infrared and x-ray diffraction methods. The blends were crosslinked by glutaraldehyde. A crosslinked membrane showed lower crystallinity and smaller swelling degree, but having improved thermostability and mechanical properties. The presented blend membranes show a pH-dependent swelling characteristic [13].

Masuhiro Tsukada, et al. (1994) prepared poly(vinyl alcohol) - silk fibroin (PVA/SF) blend films. The structure of them was analyzed by differential scanning calorimetry(DSC), thermomechanical(TMA), thermogravimetric analysis(TGA), x-ray diffractometry, scanning(SEM) and transmission(TEM) electron microscopes. DSC curves of PVA/SF blend films showed a major endothermic peak at 220 °C, along with a peak at 280 °C. These endothermic peaks were assigned to the thermal decomposition of the ordered PVA and to the thermal degradation of silk fibroin, respectively. The PVA/SF blends behaved in a manner intermediate to the pure components, as suggested by both contraction-expansion and sample weight retention properties recorded by TMA and TGA measurements. The IR absorption spectra of the blends were identified as purely a composite of the absorption bands chracteristic of both PVA and SF pure polymers. The x-ray diffraction patterns of PVA/SF blends showed overlapping spacings due to PVA and SF. A dispersed phase formed by spherical particles of 3-7 μm diameter was observed by SEM and TEM. All these findings suggested that PVA and SF are incompatible [14].

Zhicheng Zhang and Jiegen Chu (1995) prepared radiation induced graft of acrylonitrile on silk protein in $ZnCl_2$ solution. Gamma-ray induced grafting of acrylonitrile on silk protein on the solution of 60 wt% $ZnCl_2$ was carried out. The effects of absorbed dose(D), dose rate(I), the concentrations of monomer, silk protein and certain metal ions (Cu^{2+} or Fe^{3+}) were examined. They found that the initial graft efficiency was mainly determined by

$$\text{Initial graft efficiency} = [S] / ([S]+[M]) \tag{2.1}$$

where [S] : concentrations of silk protein

[M] : concentrations of monomer

The graft efficiency increased with the increase of absorbed dose. It was noticed that the existence of Cu^{2+} or Fe^{3+} had a strong inhibition effect on the molecular weight of grafted chain, and almost no effect on graft yield and efficiency. An attempt to spin the filament of the copolymer by solution spinning method had been made [15].

Masao Kunioka and Hyuk Joon Choi (1995) studied on properties of biodegradable hydrogels by γ-irradiation of microbial poly(ε-lysine)(PL) aqueous solutions. When the dosage of γ-irradiation was 70 kGy or more and the concentration of PL in water was 1-7 wt%, transparent hydrogels (opaque hydrogels for 1-3 wt% PL concentration) could be produced. Specific water contents of PL hydrogels decreased markedly with an increase in the dosage of γ-irradiation. The specific water contents were increased with an increased in PL concentration in the irradiated solution. This result indicated the presence of a radical scavenger in the PL solution. Under acid conditions, the PL hydrogel swelled due to the ionic repulsion of the protonated amino groups in the PL molecules. The degree of deswelling in electrolyte solution was smaller than that of other ionic hydrogels [poly (γ-glutamic acid), poly(acrylic acid) etc.]. In addition, the enzymatic degradation of PL hydrogels were studied at 40 °C and pH 7.0 on an aqueous solution of the neutral protease [Protease A (Amano)] produced from a spergillus oryzae. The rate of enzymatic degradation of the respective PL hydrogels was much faster than the rate of simple hydrolytic degradation. The rate of enzymatic degradation decreased with the increase in γ-irradiation dose during preparation of the PL hydrogel [16].

Yongcheng Liu, et al. (1996) studied the structure and properties of the composite membrane of regenerated silk fibroin and PVA. Application of the membranes was amperometric tetrathiafulvalene-mediating glucose sensor. The structure of a composite of regenerated silk fibroin(RSF) and poly(vinyl alcohol) (PVA) was characterized with FT-IR spectra. PVA had little influence upon the structure of RSF in the composite since it exhibited the same structure as those of pure RSF. The water absorption property and maximum strength of the composite were dependent upon its component. An amperometric glucose sensor using tetrathiafulvalene as an electron transfer mediator was constructed to test the feasibility and workability of the composite of RSF and PVA as an immobilization matrix of glucose oxidase. The effects of scan speed, pH, and temperature on the electrocatalytic oxidation of glucose at the sensor were discussed. The enhanced hydrophilicity of the membrane resulted in fast response of the sensor to glucose (less than 40 seconds). Moreover, the sensor was useable for more than 2 months [17].

Xin Chen, et al. (1997) studied pH sensitivity and ion sensitivity of hydrogels based on complex-forming chitosan / silk fibroin interpenetrating polymer network. A novel natural polymer blend, namely, a semi-interpenetrating polymer network(semi-IPN) composed of crosslinked chitosan with glutaraldehyde and silk fibroin was prepared. The FTIR spectra of the semi-IPN manifested that the chitosan and silk fibroin had a strong hydrogen-bond interaction and formed an interpolymer complex. The semi-IPN showed good pH sensitivity and ion sensitivity. According to the different swelling ratios of the semi-IPN in the buffer solution with different pH values or the $AlCl_3$ aqueous solution with different concentration, the semi-IPN could swell and shrink while being put alternately into different pH buffer solutions or $AlCl_3$ aqueous with different concentrations. The semi-IPN could also act as an "artificial muscle" because its swelling-shrinking behavior exhibited a fine reversibility [18].

Jonggeon Jegal and Kew-ho Lee(1999) prepared nanofiltration membranes based on poly(vinyl alcohol)(PVA) and ionic polymers, such as sodium alginate (SA) and chitosan. They made these membrans by casting the respective polymer solutions, The membranes prepared from PVA or PVA-ionic polymer blend were crosslinked in a isopropanol solution using glutaraldehyde as a crosslinking agent. The membranes crosslinked through the acetal linkage formation between the -OH groups of PVA and the ionomer and glutaraldehyde appeared to be semicrystalline. To study the permeation properties, the membranes were tested with various feed solutions [sodium sulfate, sodium chloride, poly (ethylene glycol) with 600 g/mol of molecular weight (PEG600), and isopropyl alcohol]. The permeance and solute rejection of the 1,000 ppm sodium sulfate at 600 psi of upstream pressure through the PVA membranes were 0.55 m^3/m^2 day and over 99%, respectively. The addition of small amount of ionic polymers (5 wt%) made the PVA membranes more effective for the organic solute rejection without decrease in their fluxes. The rejection ratios of the PEG600 and isopropanol were increased substantially [19].

Malinee Chaisupakitsin (2000) prepared hydrogel from silk fibroin by gamma irradiation. It was found that water absorption of blend hydrogel depended on the composition of SF/PVA and irradiation dose. Addition PVA in 3% of silk fibroin enhances water absorption of hydrogel during 10-50 kGy, then decreased with increasing dose. Radiation dose at 50 kGy provided the highest water absorption in all samples, however, the amount of water uptake depended on PVA concentration. Hydrogels composed of 3% silk fibroin and 3%PVA exhibited water absorption higher than the others. The maximum gel strength was found at 20-30 kGy, suggesting an interaction between silk fibroin and PVA [20].

Sumitra Kaseamchainun and Surakiet Kumta (2000) studied on the effect of gamma radiation induced hydrogel from poly(vinyl alcohol) / poly(acrylic acid) and poly(vinyl alcohol) / poly(acrylic acid) / silk protein(SF) hydrogel blend film. Mixture of polymer solutions were gamma irradiated under nitrogen atmosphere. It was found that 20 kGy was suitable dose for preparation film. The results showed that swelling property reached equillibrium within one hour. In the case of high temperature, water absorption rate was decreased. It was observed that blend film with poly(acrylic acid) solution exhibited high rate of water absorption. On the other hand, blend film with silk protein solution provided the highest water absorption of salt solution compared to other film. The ability of water absorption of salt solution depended on the size of cation. The smaller size of cation the higher water absorption. The results received from DSC suggested that there was a combination between each component leading to decreased degree of crystallinity [21].

2.2 Silk Fibroin

Silk [22] is the solidified viscous fluid excreted from special glands or orifices by many types of insects and spiders. It is a protein; the amino acids glycine and alanine are the main components. The only significant source for textile usage is the silk-moth caterpillar, commonly refered to as the silkworm. Several varieties are known; the most valuable is the caterpillar of the moth *Bombyx mori* , which was domesticated.

Silk fibroin [23], is a protein. Kai Linderstrom-Lang, the Danish protein chemist, suggested that one could consider the structure of a protein on several levels:

a) Primary structure : the sequence of amino acids.

b) Secondary structure : the regular, repeating folding pattern (such as the α helix and β structures), stabilized mostly by hydrogen bonds between peptide groups close together in the sequence.

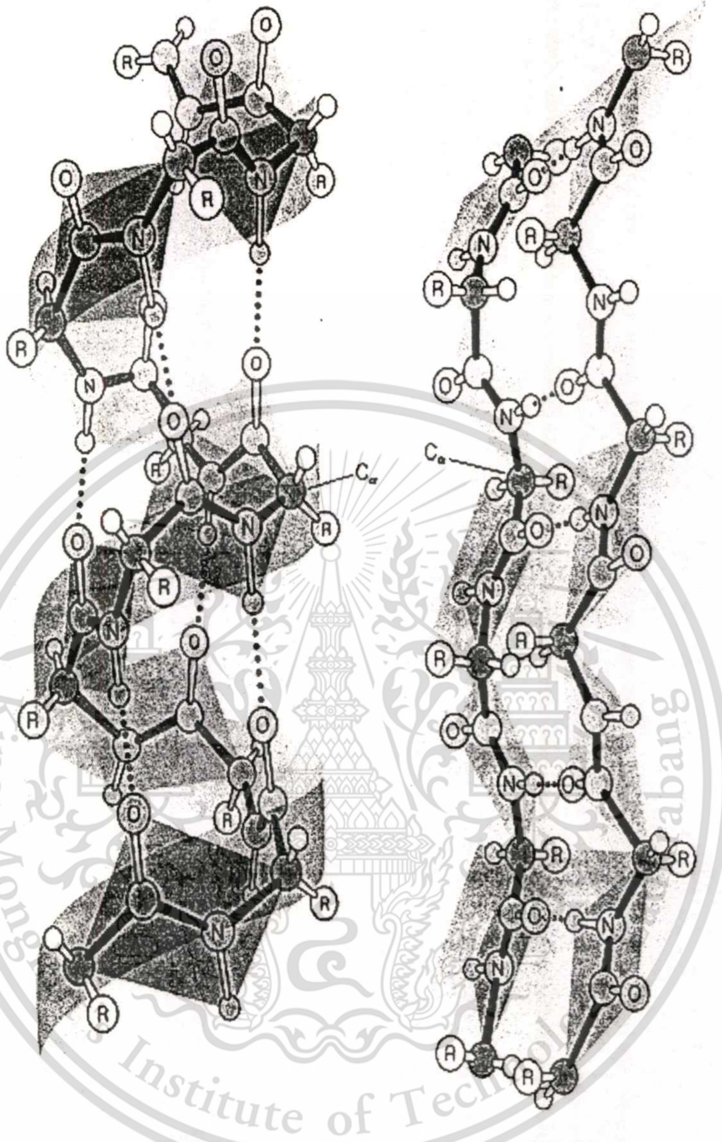
c) Tertiary structure : for a globular protein, the way that segments of secondary structure fold together in three dimensions, stabilized by interactions often far apart in the sequence. For those proteins with little or no detectable α helix or β structure, the tertiary structure can be considered to be the way the protein folds in these dimensions, stabilized by interactions between distant parts of the sequence.

d) Quaternary structure : the interaction between different polypeptide chains to produce an oligomeric structure, stabilized by noncovalent bonds only.

Figure 2.1 shows two most important regular secondary structures of polypeptides. Their existence was predicted by Linus pauling. [24]

a) In the α helix the hydrogen bonds are within a single polypeptide chain.

b) In the β sheet, the hydrogen bonds are between adjacent chains, of which only two are shown here.



(a) α helix (b) β sheet

Figure 2.1 The α helix and β sheet, the two most important regular secondary structures of polypeptides. [23]

Silk fibroins are subset of fibrous proteins [24]. The amino acid sequence of each of these proteins favors a particular kind of secondary structure, which in turn confers a particular set of appropriate mechanical properties on the substance. Table 2.1 lists the amino acid composition of four specific example of fibrous proteins.

Table 2.1 Amino acid compositions of some fibrous proteins.[24]

Amino Acid	α-Keratin (wool)	Fibroin (silk)	Collagen (Bovine Tenddon)	Elastin (Pig Aorta)
Gly	8.1	44.6	32.7	32.3
Ala	5.0	29.4	12.0	23.0
Ser	10.2	12.2	3.4	1.3
Glu+Gln	12.1	1.0	7.7	2.1
Cys	11.2	0	0	-
Pro	7.5	0.3	22.1	10.7
Arg	7.2	0.5	5.0	0.6
Leu	6.9	0.5	2.1	5.1
Thr	6.5	0.9	1.6	1.6
Asp+Asn	6.0	1.3	4.5	0.9
Val	5.1	2.2	1.8	12.1
Tyr	4.2	5.2	0.4	1.7
Ile	2.8	0.7	0.9	1.9
Phe	2.5	0.5	1.2	3.2
Lys	2.3	0.3	3.7	3.6
Trp	1.2	0.2	0	-
His	0.7	0.2	0.3	-
Met	0.5	0	0.7	-

Note : Values are given in mole percent.

2.2.1 Fibroin

The β sheet structure is most elegantly utilized in the fibers spun by silkworms and spiders. Silkworm fibroin(Figure 2.2) contains long regions of antiparallel β sheet, with the polypeptide chains running parallel to the fiber axis. The β sheet regions comprise almost exclusively multiple repetitions of the sequence

[Gly-Ala-Gly-Ala-Gly-Ser-Gly-Ala-Ala-Gly-(Ser-Gly-Ala-Gly-Ala-Gly)$_8$]

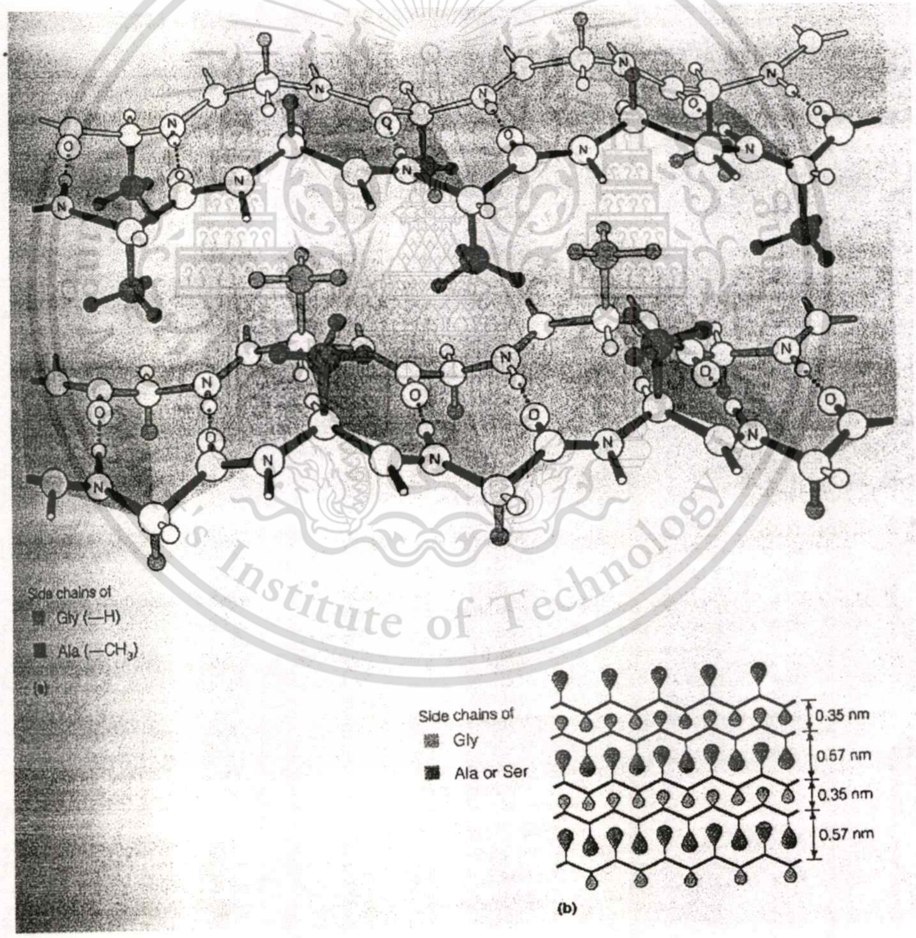


Figure 2.2 The structure of silk fibroin.[24]

From Figure 2.2, it shows:

a) A three-dimensional view of the stacked β sheets of fibroin.

b) Interdigitation of alanine or serine side chains and glycine side chains in fibroin. The plane of the section is perpendicular to the folded sheets.

Examining this sequence, it is noted that almost every other residue is Gly and that between them lie either Ala or Ser residues. This alternation allows the sheets to fit together and pack on top of one another in the manner shown in Figure 2.2 . The arrangement results in a fiber that is strong and relatievely inextensible, because the covalently bonded chains are stretched to nearly their maximum possible length. Yet the fibers are very flexible, because bonding between the sheets involves only the weak Vander Walls interactions between the side chains, which provide little resistance to bending.

Not all of the fibroin protein is in β sheets. As the amino acid composition in Table 2.1 shows, fibroin contains small amounts of other, bulky amino acids like valine and tyrosine, which would not fit into the structure shown. These are carried in compact folded regions that periodically interrupt the β sheet segments, and they probably account for the amount of stretchiness that silk fibers have. In fact, different species of silkworms produce fibroins which different extents of such non - β sheet structure and corresponding differences in elasticity. The overall fibroin structure is a beautiful example of a protein molecule that has evolved to perform a particular function-to provide a tough, yet flexible fiber for the silkworm's cocoon or the spider's web.

Naturally occuring silks [25] are characterized by β-sheet crystalline domains [e.g., (GlyAlaGlyAlaGlySer)$_n$ for B.mori silk, and (Ala)$_n$ for spider dragline silk] that are dispersed in a continuous amorphous domain composed of amino acids having bulky side groups. This characteristic feature, which imparts desirable properties such as high strength onto silks, is also responsible for their interactibility, very low solubility in common solvents, and tendency to aggregate.

Glysine is the most abundant amino acid composed of silk fibroin [26]. It is known that crystallinity of silk fibroin fiber very high, however, never 100%.

Crystalline phase of silk fibroin fiber mainly composed of glycine and alanine which are non polar amino acids. Sericin and tyrosine, water soluble amino acid, exist in amorphous phase.

It has been reported that amorphous silk fibroin with random - coil conformation showed two endothermic peaks at about 100 °C and 280 °C. The peak at 100 °C seems to be attributable to the evaporation of water in the specimens and the peak at 280 °C was prominent, suggesting the degradation of silk fibroin with random coil conformation [2]. The β-form of silk fibroin showed the endothermic peak at about 283 °C. The membrane from silk fibroin provides the suitable properties for permeability, molecular and catalyst transportation. However, dry film prepared from silk fibroin shows brittle property and difficult to handle. Many polymers such as chitosan, poly(sodium glutamate), and sodium alginate were used for improving this strength.

2.2.2 Structure of silk yarn's fibrils

The cross section of cocoon fiber[27] is shown in Figure 2.3. The left picture(1) shows that cocoon fiber consists of two fibroin fibers wrapped with sericin and so looks like a single line of fiber to our eyes. The right picture(2) show the microstructure of fibroin's cross section. If cocoon fiber is caused to swell and get wet with an alkali solution and is lightly rubbed after that, many fibrils become visible. Silk yarn is composed of several hundreds or more of fibrils and the structure of these fibrils is considered one reason for the gloss and adsorptive function of silk yarn. Silk yarn's fibrils are about 0.01 denier and equivalent to synthetic microfibers now available. So this yarn is expected to became a good adsorbent in the future.

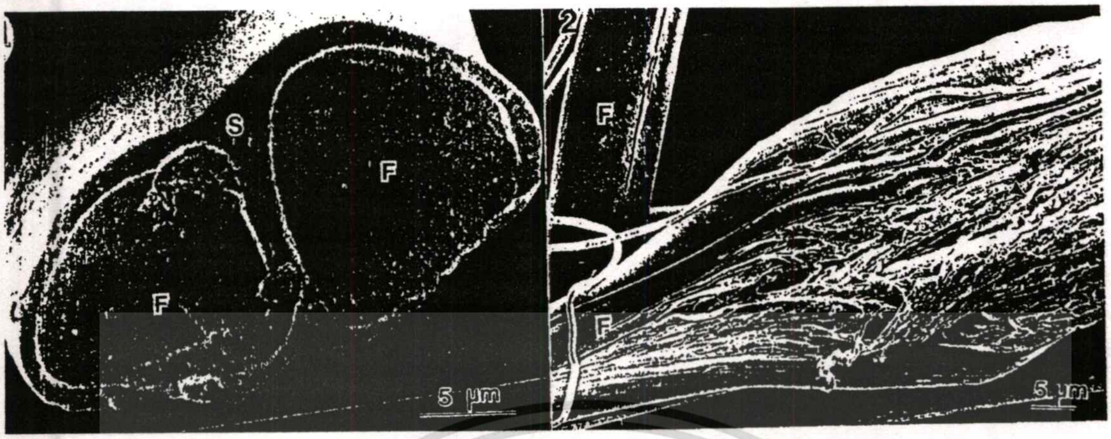


Figure 2.3 Cross section of a cocoon fiber (1) and fibrils of fibroin fiber (2) · F : fibroin, S : Sericin [27]

2.2.3 New applications of silk

For several thousand years[27], silk has enjoyed the highest rating as a "dream fiber" for its smooth texture and outer appearances, such as gloss. The world's silk production is increasing at present, too. Why is silk so popular even now when consumer needs have been very diverse? This is probably because it gives users a sense of content and physiological comfort in addition to a feeling of satisfaction comfort in addition to a feeling of satisfaction that they wear silk. From a different viewpoint, this will mean that silk has new applications as a bioplastic suited to living bodies. Recently published papers report that this fiber has many non-fiber effects, such as propagating cells, keeping lobsters and prawns fresh and promoting alcohol digestion. These are silk's internal functions, and since studies in this field have just been started, new silk functions will be discovered one after another in the future.

Figure 2.4 shows how a cocoon protects silk worm pupae. Some silkworms hibernate in this condition. During the long hibernation period, the cocoon layers cannot be washed unlike our clothes. It is thus considered that cocoons(and silk made of them) have so many functions because their layers have to protect the pupa in them from solar light, winds, rains, smoke, germs, etc. by other means than washing during this period. As noted, some of these functions are being discovered. Figure 2.4 shows the basic concepts of new materials development and these concepts can be regarded as properties suitable for living bodies. Silk yarn has long been and is still used as thread for surgical operations. This indicates itself that silk suits living bodies. So if we use this fiber not only for clothing but also for other purposes as an environment-friendly bioplastic by changing its highly ordered structure and molecular form, that will help us make our daily life more comfortable. The problem is how to change the form of silk and how to use its functions effectively. However, silk has a unique form of fiber and there id the possibility of developing this fiber into new materials. This deals with the characteristics of silk as a living body-suited material and its new applications. The powdered silk and use of silk for a coating material are the typical examples of these new uses.

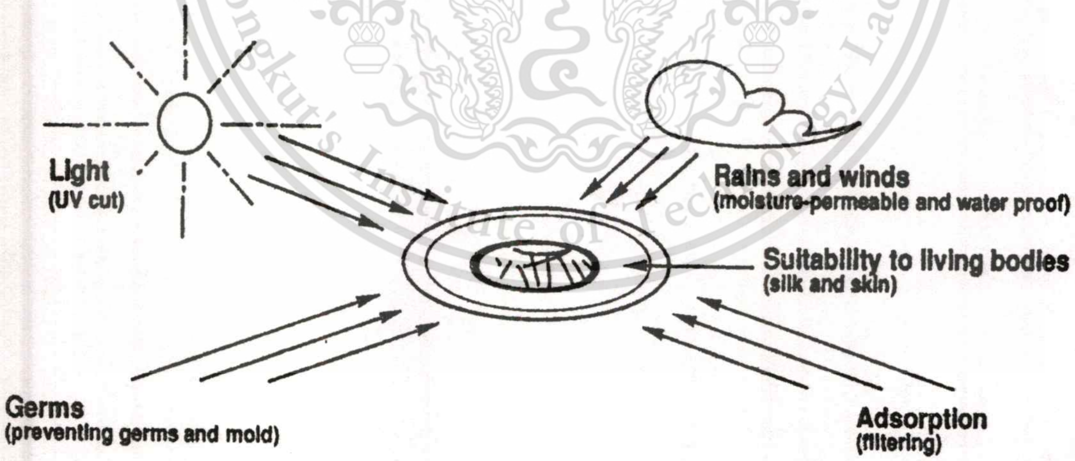


Figure 2.4 Cocoon layer's pupa protecting function. [27]

2.2.4 Processing and use of silk

To increase or decrease the diameter of silk or to strengthen, is the method of using silk as a bioplastic with fiber form. But more positive methods of new material development from silk include dissolution of silk to re-mold it into powder, films or fibers. Figure 2.5 shows the model chart of silk process.

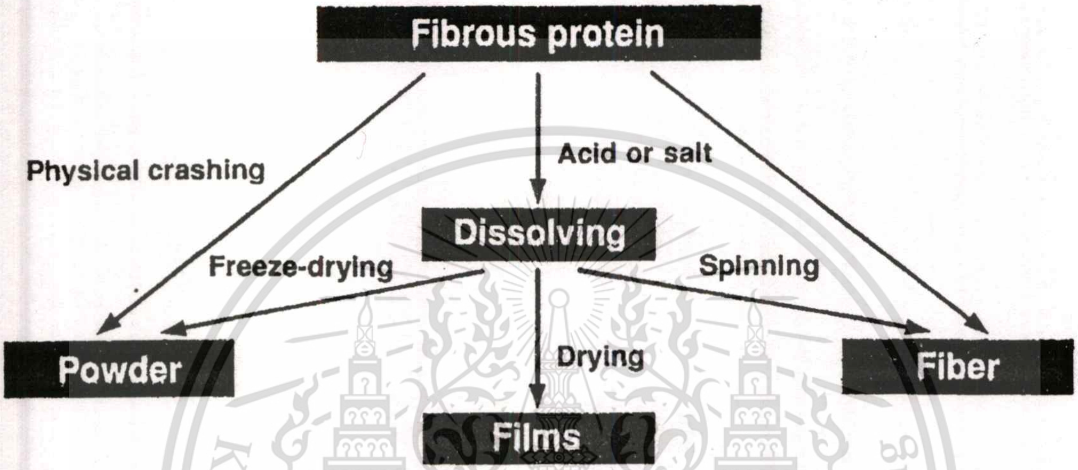


Figure 2.5 Model chart of the process of making silk powder, films and regenerated fiber of silk.[27]

1) Changing the shape of silk

Having an estimated molecular weight of about 350,000 , silk fibroin is water-insoluble without chemical treatment. This fibroin represents one of the extreme forms that fibroin has. By the chemical treatment, it has become possible to dissolve, gelate and solidify. We can reduce it to film and make it into regenerated fibers. Its crystalline degree can be controlled.

2) Chemical treatment

Recently, silk powder has come to be used for cosmetics and foods. Silk powder can easily be obtained by dissolving silk in a neutral salt or an acid, desalting it, making a fibroin solution and then freeze-dry or give other treatment to the solution. The silk powder has a non-crystalline molecular structure and is water-soluble. Another use of silk powder that has recently been introduced to the market is chemical fibers coated with silk powder. These fiber products coated with silk fibroin and sericine powder feel very smooth and give a very pleasant feeling to users.

3) Physical treatment

This section discusses the case where silk is physically converted into powder and put into practical use. Since silk is very strong, it is very hard to crash it in a physical means unless it is weakened by some methods. One of the techniques for this purpose is to process silk in an alkali and then crash weakened silk. The alkaline treatment of silk makes it possible to turn it into powder. It does not dissolve silk literally but produce tears to silk at right angles with its fiber direction, thus reducing its strength. To crash such a weakened silk physically will be to make its fibroin's molecular structure smaller without destroying it totally; so the physically produced silk powder has different properties from chemically made silk powder. The former is water-insoluble and has the same crystalline degree, moisture absorbing capacity and thermal properties as silk itself and its powder is white.

2.3 Poly(vinyl alcohol)(PVA)

Although the direct polymerization of vinyl alcohol(VA) was recently reported, this polymer [28] is obtained by the hydrolysis of Poly(vinyl alcohol) PVAc, since the monomer is the enolic non-stable tautomer of acetaldehyde and cannot be produced without reverting to the aldehyde form, Thus PVA is not synthesized by a polymerization process and its degree of polymerization depends primarily on the size of PVAc chains.

The transformation of PVAc to PVA is obtained by the base catalyzed alcoholysis or by the acid initiated hydrolysis of PVAc. In both cases, the acidic traces of sodium acetate formed in the base catalyzed alcoholysis affect the properties of PVA, primarily the stability. Such difficulties are avoided if PVA is produced using a small amount of base as catalyst. In methanol medium in the presence of sodium methoxide, the formation of PVA takes place as follows:

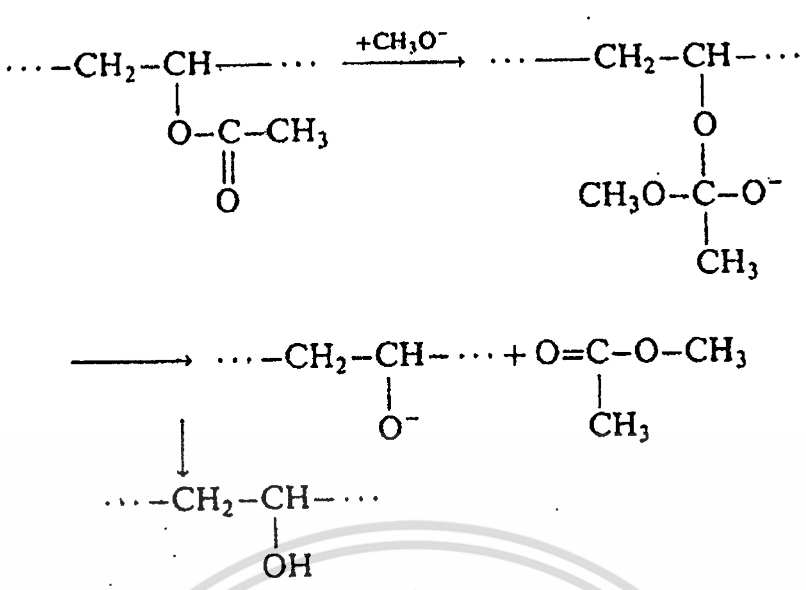


Figure 2.6 PVA from hydrolysis of Poly(vinyl acetate).[28]

With sodium methoxide, the alcoholysis is produced in 6-24 hours at a temperature in the range of 25-35 °C. If the process of alcoholysis is done without agitation, PVA is obtained in a gel form; alcoholysis with agitation leads to a polymer in powder form or pellets. Apart from sodium methoxide, alkaline hydroxides can be used, especially KOH. Saponification (hydrolysis degree) depends on the amount of hydroxide and the amount of water in the alcohol.

PVA produced through catalytic alcoholysis presents some advantages in comparison with that obtained through acidic hydrolysis; its stability is better and the polymer purification is easier. A pure PVA is obtained by precipitation with acetone (in excess) from as aqueous solution.

In the case of acidic saponification, the acid's nature influences the main characteristics of PVA; acids such as HCl, $HClO_4$, H_3PO_4 and $ClCH_2COOH$ can be used as sponifying agents. Hydrochloric acid is not recommended due to the fact that it leads to a colored PVA. Often sulfuric acid is used, because that agent cause a light colored powder which can be easily washed and dried; the formation of unstable esters constitutes the drawback of using sulfuric acid. Heating those esters causes a release of acid which degrades the polymer. The other three acids mentioned above do not have this drawback and thus they increase the stability of PVA. The pH and the temperature influence the rate of saponification. A high ratio polymer / liquid medium and an intense agitation lead to an increase of the fitness of the PVA powder.

a) Discontinuous hydrolysis of PVAc.

In the case of acidic hydrolysis, the saponification takes place at the boiling temperature of the solvent or at a lower temperature in 8-24 hours. The hydrolysis with alkalis occurs at 30-35 °C, and at the end of the process the system is brought to boiling point. The gel produced during the initial stage disintegrates after new solutions of alkaline catalyst are added. Saponification continues until the desired content of hydroxylic groups is obtained.

b) Continuous hydrolysis of PVAc.

The flow chart of this technology is shown in figure 2.4 . The process in the hydrolyzer needs 5-30 minutes at 50-60 °C and leads to a PVA with 77-95% degree of hydrolysis which can be increased during maturation. The supplementary hydrolysis is necessary when a PVA with high degree of hydrolysis (99.5% hydroxylic groups) is required.

c) Properties

The presence of secondary alcoholic groups allows the polymer to participate in reactions which are specific for secondary alcohols, such as oxidation, which leads to ketonic groups, formation of polyalcoholates with methallic sodium which can be used for the production of vinylic ethers. It also forms esters and the reaction with aldehydes leads to the formation of acetal; with ketones it forms poly(vinyl ketal). Since it is an alcohol-like cellulose, it also participates in polymer analogous reactions.

Above 100 °C dehydration occurs, this process being accelerated by the acid or alkali traces existing in the polymer. In the meantime, physical-chemical properties are changed, e,g, the water solubility decreases, the brittleness and the glass transition temperature increases, double bonds form, and ring and oxygen bridges from as a result of intermolecular reactions. In principle, the degree of polymerization does not vary during hydrolysis; however, if the initial

PVAc has a very high degree of polymerization and the hydrolysis temperature is too high, PVA degradation occurs.

PVA is a white powder with a specific gravity in the range of 1.2-1.3 and a glass transition temperature of around 80 °C. PVA with 50-80 mol% acetyl groups is water stable and has better thermal stability and mechanical properties than PVAc. By drawing, the degree of crystallinity increases; orientation is necessary in the case of films and PVA fibers.

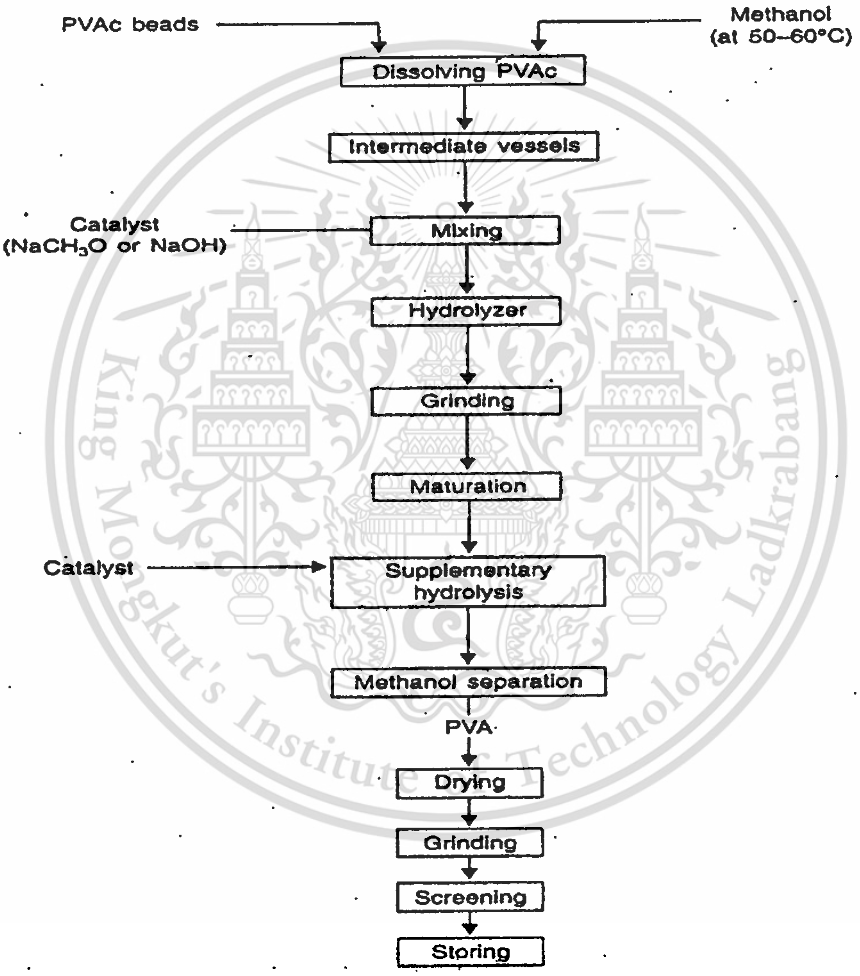


Figure 2.7 Poly(vinyl alcohol) production through PVAc hydrolysis.[28]

d) Applications

PVA is used for the production of fibers, artificial leather, tubing, gaskets with good stability to gasoline and other oil derivatives, rubber-like items, transportation belts, surgical fibers, emulsifiers, adhesives for paper and paperboard, and in general purpose adhesives for bonding paper, textiles, leather and porous ceramic surfaces.

PVA is an efficient binder for solid particles, pigment, ceramic products, plaster, cement building products, etc.. It is a good protective colloid for aqueous emulsion and is employed for this purpose in a large variety of emulsion and suspension systems. In the manufacture of textile fibers, the most important operation is to transform it into a product insoluble in water. This is obtained by treating PVA fiber with a concentrated aqueous solution of sodium sulfate containing certain amount of formaldehyde and sulfuric acid. Such a treatment will produce formal groups:

$$\cdots-\mathrm{CH_2}-\underset{\mathrm{OH}}{\underset{|}{\mathrm{CH}}}-\mathrm{CH_2}-\underset{\mathrm{OH}}{\underset{|}{\mathrm{CH}}}-\cdots+\mathrm{CH_2O}\cdots-\underset{\mathrm{O{-}CH_2{-}O}}{\underset{|\qquad\qquad|}{\mathrm{CH{-}CH_2{-}CH}}}-\mathrm{CH_2}-\cdots$$

Figure 2.8 Transform of PVA to formal group.[28]

Hydroxyl groups of neighboring PVA macromolecules may also participate in the formation of formal groups. After such treatment, PVA fibers show a higher rate of water absorption. Due to such characteristics, PVA fibers are used as substitutes for cotton fibers. These synthetic fibers have very good dimensional stability and tenacity. High strength and high modulus PVA fibers have been obtained by crosslinking, wet spinning and multistep drawing.

Light polarized films can be manufactured by casting a PVA solution on a surface, and drying and peeling the resulting film.

The useful relative humidity range and mechanical properties of starch were significantly extended by blending with PVA. The degree of hydrolysis and

MW of PVA significantly affects the water resistance and mechanical properties in blends with high amylose thermoplastic starch.

Poly(vinyl alcohol) (PVA) is a nontoxic water-soluble synthetic polymer, which is widely used in bio-chemical and biomedical applications. Water-soluble PVA is rendered insoluble by the introduction of crosslinks into the specimen. PVA has good film-forming, highly hydrophilic properties and has been studied as a membrane in various ways. PVA has recently been exploited as a substrate for enzyme immobilization in the form of photocrosslinkable PVA . PVA gels can be prepared from aqueous solutions by repetitive freezing and thawing [7]. PVA gels can be used in the medical field because they posses good biosuitability.

The film obtained by casting a PVA aqueous solution has a 100% amorphous· structure. PVA hydrogels prepared by freezing the concentrated aqueous PVA solution exhibit, however, a diffraction pattern from the spacing corresponding to 4.55 A^{o}. A recent report has also demonstrated that this hydrogel membrane was thermally stable up to 40.5 ^{o}C and that rejection increased with operation pressure [7].

2.4 Hydrogel

Hydrogels [29], or water-containing gels, are polymers characterized by hydrophilicity and insolubility in water. In water they swell to an equilibrium volume, but preserve their shapes. The hydrophilicity is due to the presence of water-solubilizing groups, such as -OH, -COOH, $-CONH_2$, -CONH-, SO_3H, etc. The insolubilizing and stability of shape are due to the presence of a three-dimensional network. The swollen state results from a balance between the dispersing forces acting on hydrated chains and cohesive forces that do not prevent the penetrating of water into the network. Cohesive forces are most often due to covalent cross-linking. Others are electrostatic, hydrophobic, or dipole-dipole in character. The degree and nature of cross-linking and the tacticity and crystallinity of the polymer are responsible for its characteristics in the swollen state. The ability to imbibe water and ions without the loss of shape or mechanical strength is valuable in many natural hydrogels, such as those found in muscle, tendons, cartilage, intestines, and blood.

Natural hydrogels are used in pulp and paper production, artificial silk, cellulosic membranes, and biomedical applications. Synthetic hydrogels are used in prosthetic materials, soft lenses, and membranes for controlled drug release because of their compatibility with living tissue.

The gels are fixed to supports by coating, grafting, or chemical modification. Ion-exchange and seperation membranes(qv) are hydrogels, although the term hydrogel is usually restricted to a synthetic, water-swelling polymer of soft, rubbery consistency. Its character is determined by the hydrophilic monomers and density of the polymer network.

Ionogenic or charged gels form a special group, with swelling and strength properties dependent on the pH of the environment.

Hydrogels have been classified in various ways. A limited number of monomers, copolymerized in various ways, have given rise to an immense variety of hydrogels.

Hydrogels can be designated by their chemical names, but the use of acronyms is more common.

2.4.1 Cross-linked Poly(vinyl alcohol) Gels

Poly(vinyl alcohol) (PVA) is a water-soluble film-forming polymer, with corresponding applications. To form a hydrogel, it must be cross-linked covalently; it contains no domains capable of hydrophobic interactions or dissociation. Nevertheless, because of its polar character, dissolved PVA can be stabilized by treatment with Cr^{3+}.

There are many possibilities for covalently cross-linking PVA. Some of the simplest methods use formaldehyde or glutaraldehyde.

2.4.2 Health and Safety Factors

In handling monomeric hydroxyalkyl acrylates, contact with eyes and skin should be avoided. If it occurs, washing with water usually prevents ill effects.

Acrylates are absorbed through the skin in toxic amounts. Polymerization and cross-linking suppress toxicity.Monomericacrylamide and *N,N*′- methylenebisacrylamide are eye and skin irritants. They are neurotoxic and should be handled in a ventilated area with rubber gloves and a dust respirator. Solutions in organic solvents are more hazardous than in water. Methacrylamides and substituted methacrylamides and acrylamides are less toxic. Acrylonitrile is a neuroxin and inhalation, body contact, and ingestion must be avoided. The effects of poisoning include headache, nausea, weakness, pallor, unconsciousness, and cessation of breathing; polyacrylonitrile does not present problems.

2.4.3 Uses

Hydrogels are employed principally in medicine. They are biomaterials, used in diagnostic or therapeutic devices and implants for short-term or long-term applications. Diagnostic devices include catheters, electrode catheters, carriers for enzyme immunoassay, gel-entrapped enzyme electrode probes, cell culture substrates, and electrophoresis gels. Therapeutic applications include adsorbent coatings for blood perfusion (blood detoxicants), hemodialysis membranes, blood oxygenators, degradable therapeutic systems, drug-derivery systems, and medicated and soft contact lenses(qv). The simplest method of drug delivery is the entrapment of the medicament into the hydrogel network. Controlled drug-release systems include noneroding (diffusion) and eroding reservior devices and osmotic, elastic, and vapour pressure pumping devices. Implant applications include intraocular lenses, artificial corneas, eye capillary drains, vitreous humor replacements, soft tissue substitutes, burn dressings, bone ingrowth sponges, urethral prosthese, artificial larynges, soft tissue replacements, plastic surgery, scleral buckling implants for retinal surgery, suture coatings, soft palate replacements, transdermal, drug-delivery patches; and adhesives. Hydrogels are used for fixation of herbicides, in chromatography, as enzyme carriers, in food processing, in separation membranes, in air treating, and as water retainers in sandy soils, known also as RAPG(reclaiming and ameliorating polymer gels) served as fillers in artificial leather.

2.5 Radiation

Radiation technique [30] that we usually use in preparation of hydrogel because the crosslinks must be composed of chemically stable C-C bonds. The water content can be varied over a wide range with the radiation dose and the hydrogel is not contaminated with foreign additives.

2.5.1 Types of Radiation

1) Gamma rays and X-rays

These are short-wavelength forms of photon or electromagnetic radiation. The different names derive from discovery at different times. A gamma ray has its origin in a nuclear interaction, whereas an X-ray originates from electronic or charged-particle collisions. The ways in which these photons interact with matter are identical. They are lightly ionizing and highly penetrating and leave no activity in the material irradiated.

2) Alpha particles

Alpha particles are the nuclei of helium atoms. They have a mass of 4 and a positive charge of 2 units. Normally of high energy (in the MeV range), they interact strongly with matter and are heavily ionizing. They have low penetrating power and travel in straight lines. A typical alpha particle energy is 5 MeV with a typical range of 50 mm in air and 23 μm in silicon.

3) Beta particles, electrons, positrons.

Beta particles have the same mass as an electron but may be either negatively or positively charged. With their small size and charge they penetrate matter more easily than alpha particles but are more easily deflected. Their high velocity, normally approaching that of light, means they are lightly ionizing.

4) Neutrons

A neutron has the same mass as a proton but has no charge and consequently is difficult to stop. The neutron can be slowed down by hydrogeneous material. The capture of a neutron results in the emission of a gamma ray. Neutrons are classified according to their energy: thermal(<1 eV); intermediate; and fast (>100 keV). Water is an especially effective shield for neutrons.

5) Protons

The proton is the nucleus of a hydrogen atom and carries a charge of 1 unit. The proton has a mass some 1800 times that of an electron, and consequently is more difficult to deflect, with a typical range of several centimetres in air and tens of micrometres in aluminium at energies in the MeV range.

2.5.2 Units [31]

Important quantities in radiation chemistry and radiation processing are the absorbed dose (the energy transferred from radiation to substrate) and the radiation-chemical yield. Radiation dose or absorbed dose mean the quantity of radiation energy absorbed by material as it passes through in processing. Usually measured by a unit called the gray(Gy) or rad , 1 gray mean 1 joule per 1 kg. of material (1 Gy = 100 rads). Absorbed dose has been reported in units of eV g^{-1}, eV cm-3, and rad (radiation absorbed dose), but for most purpose these units have been replaced by the System International or SI unit, the gray (1 Gy = 1 J kg^{-1}). Radiation-chemical yields were originally reported as ionic yields(M/N) and then as *G* values in units of molecules / 100 eV. The SI unit, which is the mol J^{-1} or μmol J^{-1} , is gradually coming into use. The relationships betweem absorbed dose, the chemical yield of a radiation-induced product, and the radiation-chemical yield of the product in terms of both *G* values and SI units are

$$G(\text{product})\ [\text{molecules} / 100\ \text{eV}] = \frac{9.648 \times 10^{6} \times \text{chemical yield (mol kg}^{-1})}{\text{absorbed dose(Gy)}} \tag{2.2}$$

and

$$\text{Radiation-chemical yield, } G(\text{product})(\mu\text{mol J}^{-1}) = \frac{10^{6} \times \text{chemical yield (mol kg}^{-1})}{\text{absorbed dose (Gy)}} \tag{2.3}$$

2.5.3 Advantages of Radiation Initiation

1. Radiation initiation may result in a purer product since contamination resulting from incomplete removal of catalyst is not a problem.
2. Variation in catalyst activity arising from poisoning or other sources, with consequent variation in product quality, is not a concern when radiation is used to initiate reaction.
3. Radiation-induced reactions can be carried out at relatively low temperatures, resulting in lower energy costs, less thermal damage to the system, and reduced danger of fire or explosion.
4. The intensity of ionizing radiation can be readily controlled, and can be reduced immediately if the reaction becomes too vigorous.
5. Compared with reactions initiated by ultraviolet light, ionizing radiation offers the advantage of greater penetrating power so that more uniform reaction takes place in a larger volume of reactant, avoiding the buildup of product on the walls of the reaction vessel. The use of metal rather than glass vessels is possible and reaction can be initiated in media that are not transparent to ultraviolet light. Selective decomposition of products having high molar extinction coefficients at wavelengths close to that of the photochemical source is also avoided.
6. The penetrating power of ionizing radiation makes it possible to irradiate materials in pressure vessels without the provision of special equipment.
7. Electron and charged-particle beams can be deflected by electric or magnetic fields so as to scan large areas, or particular areas, of the sample.(Electromagnetic radiation, i.e. γ or x radiation, does not carry an electric charge and cannot be deflected in this way.)

2.5.4 Disadvantages of Radiation Initiation

1. Operating and capital costs of ionizing radiation sources are higher than those for many chemical and photochemical processes.

2. Gamma-radiation sources must be replaced at intervals to maintain uniform source strength. The intervals depend on the half-life of the radioisotope used; for ^{60}Co sources, half-life 5.27 years, the source will probably need to be replenished every 2-3 years. With periodic maintenance, accelerators should maintain a constant level of radiation output.
3. Ionizing radiation is generally less specific in its action than chemical or photochemical initiation and, if radiolysis is prolonged, may bring about secondary changes in the product.
4. Radiation chemical yields of products are low unless the reaction is a chain reaction.
5. Dose-rate dependence, particularly with chain reactions, may make it difficult to increase output by simply increasing the radiation dose rate.
6. Safety concerns of the general public must be satisfied. In some jurisdictions this may be a lengthy and expensive process when sources of ionizing radiation are involved.

Ionization radiation is an expensive form of energy, and radiation processing will be economically viable only if the quantity of radiation energy used is small in relation to the value of the product, or if a rather modest amount of radiation energy can be used to treat a relatively large amount of product. Examples of these two situations are radiation sterilization of medical supplies and spices, whose value is likely to be high relative to the cost of the radiation processing required, and radiation-induced cross-linking of polymers, where the product is inexpensive but the quantity of radiation energy required is relatively low.

This experiment used Cobalt-60 source for gamma radiation of the hydrogels.

2.5.5 Cobalt-60 Source

Cobalt-60 is the most widely used source of γ radiation at the present time. It is produced by exposing natural cobalt-59 to neutrons in a nuclear reactor, where the $^{59}Co(n, \gamma)^{60}$ reaction gives radioactive cobalt with activities as high as 50-100 Ci g^{-1} . Co (1.9 - 3.7 TBq g^{-1}). Cobalt-59 is generaly irradiated in the form of pellets, small slugs, or thin disks of metal to give a uniformly active material, and these are placed in containers that are then assembled into radiation sources of the desired size. The walls of the metal containers serve to filter out β radiation emitted by the cobalt-60.

Gamma-emitted sources must be surrounded by relatively thick shields of dense material to protect the operating personnel. Many designs for cobalt-60 sources are available, but, in general, these fall into two groups. In the first the cobalt-60 encircles a cavity containing the sample to be irradiated and the whole is surrounded by a compact mass of shielding material, generally lead. Some forms of movable shielding are provided so that the sample can be introduced without exposing the radioisotope. In the Nordion International Inc."Gammacell" illustrated in Figure 2.5, the cobalt-60 is in the form of a hollow cylinder surrounded by lead-shielded, moving drawer. Cavity-type sources are compact and can be designed to give uniform high-intensity radiation throughout the sample volume, although the radiation intensity cannot be varied. The nature of the design limits the size of sample that can be irradiated to the volume of the cavity.

Gamma sources designed for commercial use must handle larger samples and are generally in the form of a small shielded chamber with provision to expose a high-activity cobalt-60 source, or sources, near the center of the chamber (Figure 2.6). Shielding is provided by massive concrete walls with a labyrinth entrance, and additional protection is afforded by the distance between the source and operating personnel. When not in use, the source is stored below floor level in a shielded container or a water-filled pit.

The chamber-type source can irradiate a much greater volume of material than a cavity source and can accommodate larger product-handling equipment. Radiation intensity can be varied by moving the sample closer to, or farther form, the source. A disadvantage is the greater area required and the bulk and weight of the concrete shielding; however, all very large cobalt-60 sources (over 10,000 curies) are of this type.

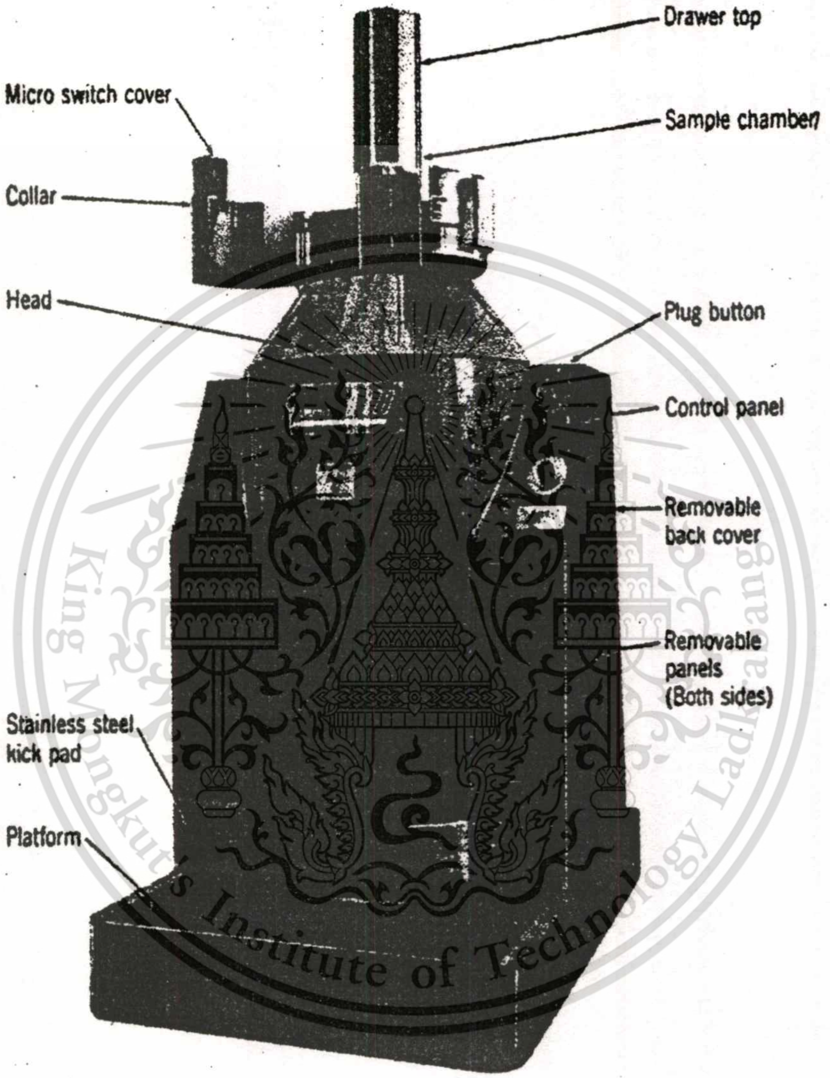


Figure 2.9 " Gammacell " cobalt-60 irradiation unit.[30]

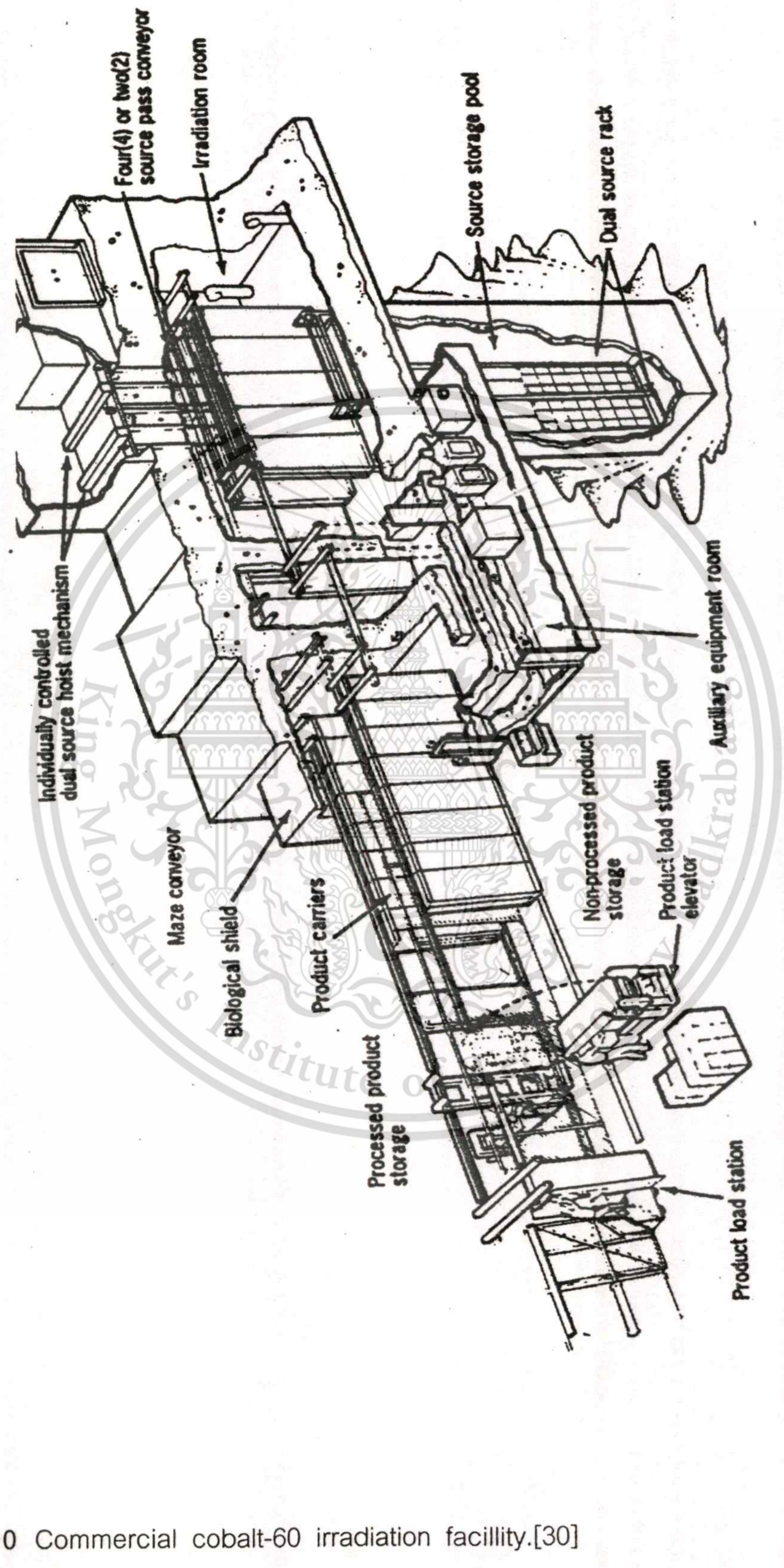


Figure 2.10 Commercial cobalt-60 irradiation facillity.[30]

2.5.6 Radiation Curing of Plastics

When polymers are irradiated, they can crosslinked and degraded. In the Table 2.2, there are courses of reactions in polymers exposed to radiation behaviour of the carbon backbone.

Table 2.2 Course of reaction in polymers exposed to radiation behaviour of the carbon backbone.

Cross-linking of chains	Degradation(scission) of chain
Polyethylene , Most elastomers	Polyisobutylene
Polypropylene	Polytetrafluoroethylene(Teflon)
Polystyrene	Polymethacrylates(Perspex,Lucite)
Poly(vinyl chloride)	Cellulose & derivatives Butyl rubber
Epoxy resins	
Silicones	
Poly(ethylene terephthalate)	

CHAPTER 3

EXPERIMENTAL DETAILS

3.1 Materials

1. Silk waste(SF)
2. Poly (vinyl alcohol)(PVA) MW ≈ 74,800 : Kuraray Co.Ltd , Japan.
3. Poly (vinyl alcohol)(PVA) MW ≈ 11,000 – 31,000 : Sigma Chamical Company
4. Hydrate Calcium chloride ($CaCl_2 \cdot 2H_2O$) : BDH Laboratory Supplies
6. Ethanol : Merck

3.2 Appparatus

1. Seamless Cellulose Tubing Small size 24 (molecular weight cut off 12000 – 14000 ca.)
2. Indicator papers
3. Parafilm
4. Aluminium foil
5. Beaker
6. Volumetric flask
7. Petri – dish
8. Test tube
9. Stirring rod
10. Forcept
11. Vacuum oven
12. Oven
13. Hot plate
14. Desiccator
15. Gamma cell 220
16. Gel strength tester : Shimadsu

3.3 Preparation of degummed silk fibroin

Silk waste 5 g. was boiled for 2 hours in 500 ml. of distilled water about 80-90 °C. The insoluble silk was then filtered, after that soaked in 200 ml. of methanol about 1 hr. The silk was filtered the methanol out and then soaked in 200 ml. of dilute detergent solution about 1 hr. After that, the silk was wrung out, and then soaked in 200 ml. of Na_2CO_3 0.5% solution, followed by stirred about 30 min.. The silk was filtered, and then rinsed throughly in distilled water until the distilled water became neutral. The cleaned silk was dried in the oven at 70 °C about 1 hr., after that, it was kept in desiccator.

3.4 Preparation of silk fibroin solution

Degummed silk fibroin 30 g. was dissolved in 100 ml. $CaCl_2$ solution (73 g. $CaCl_2 \cdot 2H_2O$ + 54 ml. H_2O + 47 ml. C_2H_5OH) at 100 °C. The silk fibroin solution was dialyzed using seamless cellulose tube with deionised water at least for three days. (MW $\approx$30,000) (Figure 3.1)

3.5 Preparation of PVA solution

PVA 10 g. was dissolved in deionised water with the autoclave at 121 °C for 25 min.

3.6 Preparation of silk fibroin / PVA hydrogel

Silk fibroin solution was mixed with PVA solution in the test tube. The test tube was flew by N_2 about 3 min. ,then closed the test tube with septum and sealed it with parafilm. Figure 3.2 show silk fibroin solution blend with PVA solution in the test tube. The test tubes were irradiated at 25 °C with γ-rays within the dosage range 10-50 kGy at dose rate 6 kGy/hr.

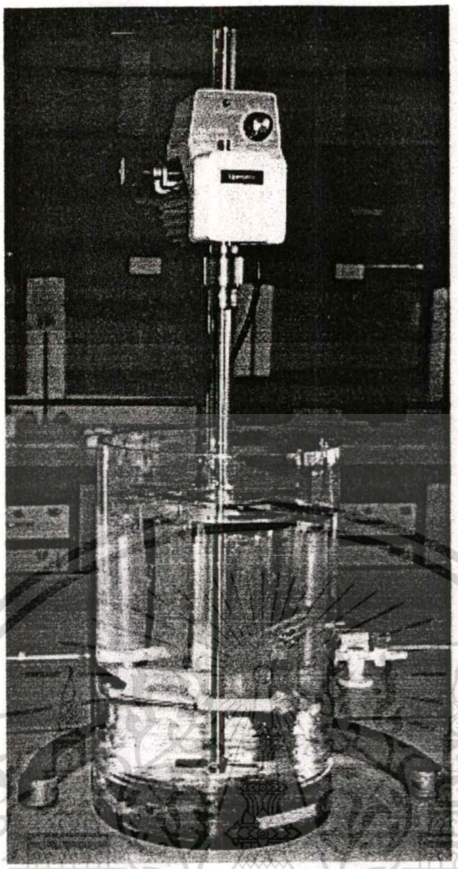


Figure 3.1 Dialysis of silk fibroin solution

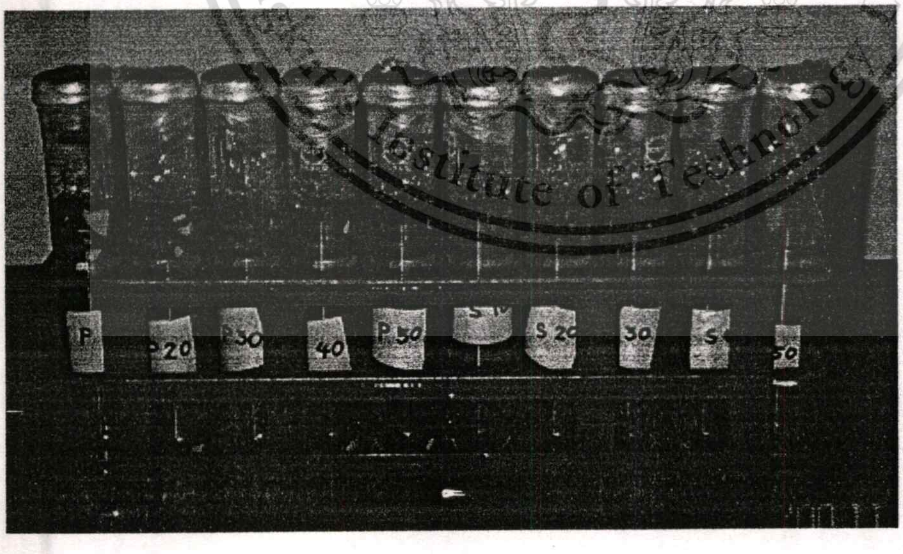


Figure 3.2 Silk fibroin solution blend with PVA solution in the test tube

3.7 Measurement

3.7.1 Physical Properties

3.7.1.1 %Gel Fraction

The resultant hydrogels were feed in the net (aluminium bag). The resultant net were immersed in water at room temperature for extraction of sol part, the water being occasionally changed. After that the sample were kept at 50 °C in vacuum oven for 2 hours.

$$\%\text{ Gel fraction} = \frac{\text{weight of dry sample after extraction}}{\text{weight of sample feed in the net}} \times 100 \qquad (3.1)$$

3.7.1.2 %Water Absorption

The samples from 3.7.1 were immersed in water at room temperature. The weight of the samples were measured after 1, 2, 3, 4, 5, 6, 7 and 8 hours.

$$\%\text{ Water absorption} = \frac{\text{weight of swollen gel - weight of dry gel}}{\text{weight of dry gel}} \times 100 \qquad (3.2)$$

3.7.2 Mechanical Property

3.7.2.1 Gel Strength

Strength of hydrogel was determined by compression method using Tensile tester(LLOYD LR 30) with a crosshead speed of 20 mm/min and force 1kN. Test sample was cut into 10 mm. Height and diameter of gel were measured. Gel strength was calculated in Mpa Unit. (Figures 3.3, 3.4)

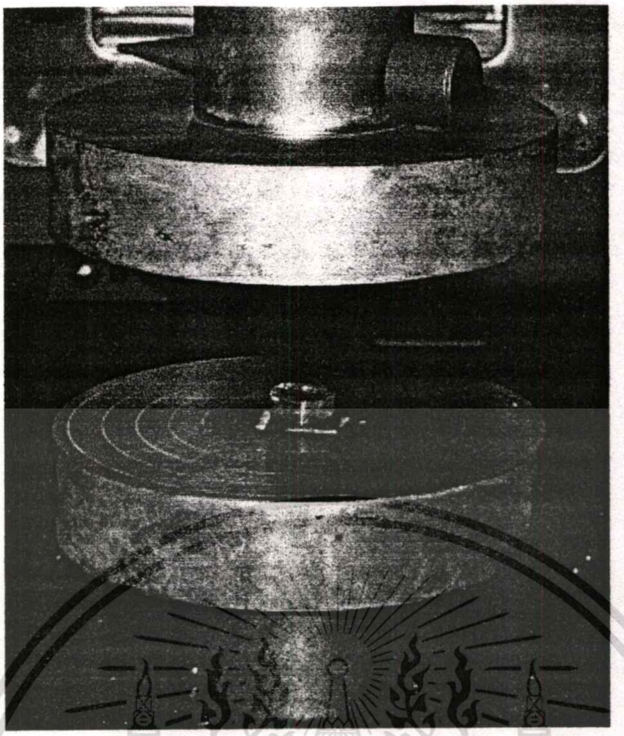


Figure 3.3 Compression test of hydrogel (before compression)

Figure 3.4 Compression test of hydrogel (after compression)

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physical Properties

Hydrogels were prepared from modified silk waste and poly(vinyl alcohol) by γ-irradiation technique. Then, the hydrogels were investigated for physical and mechanical properties.

The symbols used in this report were

PVA : Poly (vinyl alcohol)
S : Silk
P : PVA
kGy : kilogray

4.1.1 Physical Appearance

Physical appearance is an important characteristic of hydrogel for selection of the hydrogels which are suitable for biomedical application. Tables 4.1-4.6 show physical appearance of silk, PVA, and silk/PVA blend before and after irradiation.

Table 4.1 Physical appearance of silk solution and PVA solution, before and after irradiation.

Sample	Dose (kGy)	Before Irradiation	After Irradiation
Silk (1%,3%)	10 to 50	Pale yellow liquid	Pale yellow gel, unstable shape
PVA 3%	10	Transparent liquid	Transparent gel, stable shape, soft and sticky
	20	Transparent liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel
	30	Transparent liquid	Transparent gel, stable shape, more rigid, shrink much water outside gel
	40	Transparent liquid	Transparent gel, stable shape, more rigid, shrink much water outside gel

Table 4.1 (continued)

PVA 3%	50	Transparent liquid	Transparent gel, stable shape, most rigid, shrink very much water outside gel
PVA 10%	10	Transparent liquid	Transparent gel, unstable shape, soft
	20	Transparent liquid	Transparent gel, stable shape, rigid
	30	Transparent liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel
	40	Transparent liquid	Transparent gel, stable shape, more rigid, shrink much water outside gel
	50	Transparent liquid	Transparent gel, stable shape, more rigid, shrink very much water outside gel

Table 4.2 Physical appearance of hydrogel prepared from 1%silk solution blend with 3% low molecular weight PVA before and after irradiation. -

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:3	10	Pale white liquid	Transparent liquid
	20	Pale white liquid	Transparent liquid, little gel, unstable shape, soft
	30	Pale white liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel
	40	Pale white liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel
	50	Pale white liquid	Transparent gel, stable shape, more rigid, shrink much water outside gel
S/P 3:1	10	Pale white liquid	Pale white liquid
	20	Pale white liquid	Pale white liquid
	30	Pale white liquid	Pale white gel, stable shape, very soft
	40	Pale white liquid	Pale white gel, stable shape, more rigid but very soft
	50	Pale white liquid	Pale white gel, stable shape, more rigid but very soft

Table 4.3 Physical appearance of hydrogel prepared from 1%silk solution blend with 10% high molecular weight PVA before and after irradiation.

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:3	10	Pale white liquid	Transparent gel, unstable shape, very soft, sticky
	20	Pale white liquid	Transparent gel, stable shape, soft
	30	Pale white liquid	Transparent gel, stable shape, soft
	40	Pale white liquid	Transparent gel, stable shape, soft
	50	Pale white liquid	Transparent gel, stable shape, rigid
S/P 3:1	10	Pale white liquid	Pale white liquid
	20	Pale white liquid	Pale white gel, stable shape, soft
	30	Pale white liquid	Pale white gel, stable shape, soft
	40	Pale white liquid	Pale white gel, stable shape, soft, shrink little water outside gel
	50	Pale white liquid	Pale white gel, stable shape, rigid, shrink little water outside gel

Table 4.4 Physical appearance of hydrogel prepared from 3%silk solution blend with 10% high molecular weight PVA before and after irradiation.

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:1	10	Pale white liquid	Pale white gel, unstable shape, very soft
	20	Pale white liquid	Pale white gel, stable shape, soft
	30	Pale white liquid	Pale white gel, stable shape, rigid
	40	Pale white liquid	Pale white gel, stable shape, rigid, little shrink little water outside gel
	50	Pale white liquid	Pale white gel, stable shape, more rigid, shrink little water outside gel
S/P 1:3	10	Pale white liquid	Transparent gel, stable shape, soft
	20	Pale white liquid	Transparent gel, stable shape, soft, more tough
	30	Pale white liquid	Transparent gel, stable shape, rigid
	40	Pale white liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel
	50	Pale white liquid	Transparent gel, stable shape, more rigid, shrink little water outside gel

Table 4.4 (continued)

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 3:1	10	Pale white liquid	Pale white gel, unstable shape, soft
	20	Pale white liquid	Pale white gel, stable shape, soft
	30	Pale white liquid	Pale white gel, stable shape, rigid
	40	Pale white liquid	Pale white gel, stable shape, more rigid
	50	Pale white liquid	Pale white gel, stable shape, more rigid

Table 4.5 Physical appearance of hydrogel prepared from 1%silk solution blend with 3% high molecular weight PVA before and after irradiation.

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:3	10	Pale white liquid	Transparent gel, unstable shape, very soft
	20	Pale white liquid	Transparent gel, stable shape, very soft, sticky
	30	Pale white liquid	Transparent gel, stable shape, rigid
	40	Pale white liquid	Transparent gel, stable shape, more rigid
	50	Pale white liquid	Transparent gel, stable shape, more rigid
S/P 3:1	10	Pale white liquid	Pale white liquid
	20	Pale white liquid	Pale white gel, unstable shape, very soft
	30	Pale white liquid	Pale white gel, unstable shape, very soft
	40	Pale white liquid	Pale white gel, stable shape, soft
	50	Pale white liquid	Pale white gel, stable shape, soft

Table 4.6 Physical appearance of hydrogel prepared from 3%silk solution blend with 3% high molecular weight PVA before and after irradiation.

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:3	10	Pale white liquid	Pale white liquid
	20	Pale white liquid	Pale white gel, unstable shape, soft
	30	Pale white liquid	Pale white gel, stable shape, soft, little water
	40	Pale white liquid	Pale white gel, stable shape, rigid, little shrink little water outside gel
	50	Pale white liquid	Pale white gel, stable shape, more rigid, shrink little water outside gel

Table 4.6 (continued)

Sample	Dose (kGy)	Before Irradiation	After Irradiation
S/P 1:1	10	Pale white liquid	Pale white liquid
	20	Pale white liquid	Pale white gel, unstable shape, soft
	30	Pale white liquid	Pale white gel, stable shape, rigid little water outside gel
	40	Pale white liquid	Pale white gel, stable shape, more rigid, shrink little water outside gel
	50	Pale white liquid	Pale white gel, stable shape, more rigid, shrink little water outside gel

Figure 4.1 1%Silk solution blend with 3%high MW PVA at ratio 3:1 after irradiation at 10 kGy

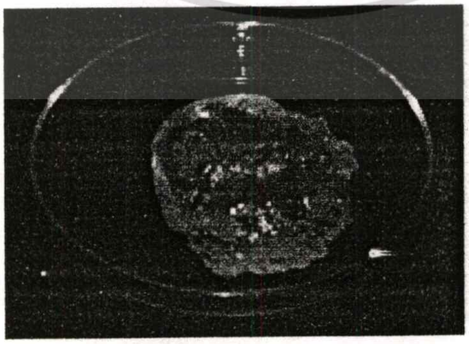


Figure 4.2 1%Silk solution blend with 3%high MW PVA at ratio 1:3 after irradiation at 10 kGy

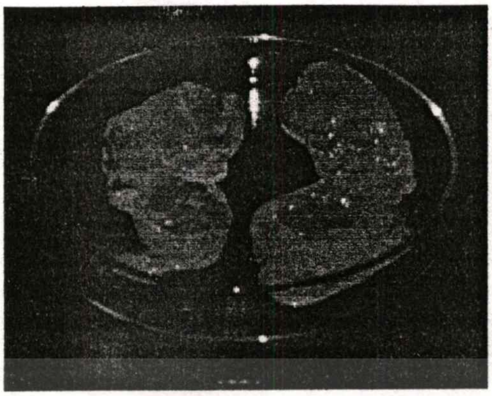


Figure 4.3 1%Silk solution blend with 3%high MW PVA at ratio 1:3 after irradiation at 20 kGy

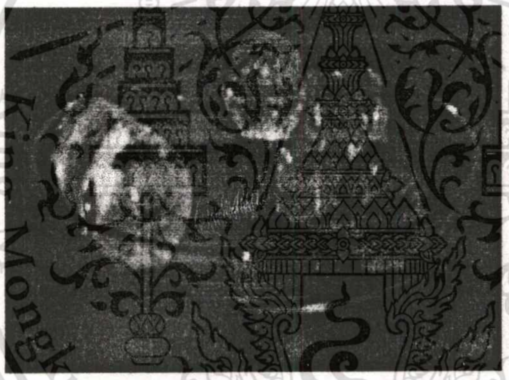


Figure 4.4 1%Silk solution blend with 3%high MW PVA at ratio 1:3 after irradiation at 30 kGy

4.1.2 %Gel Fraction

4.1.2.1 Influence of molecular weight of PVA

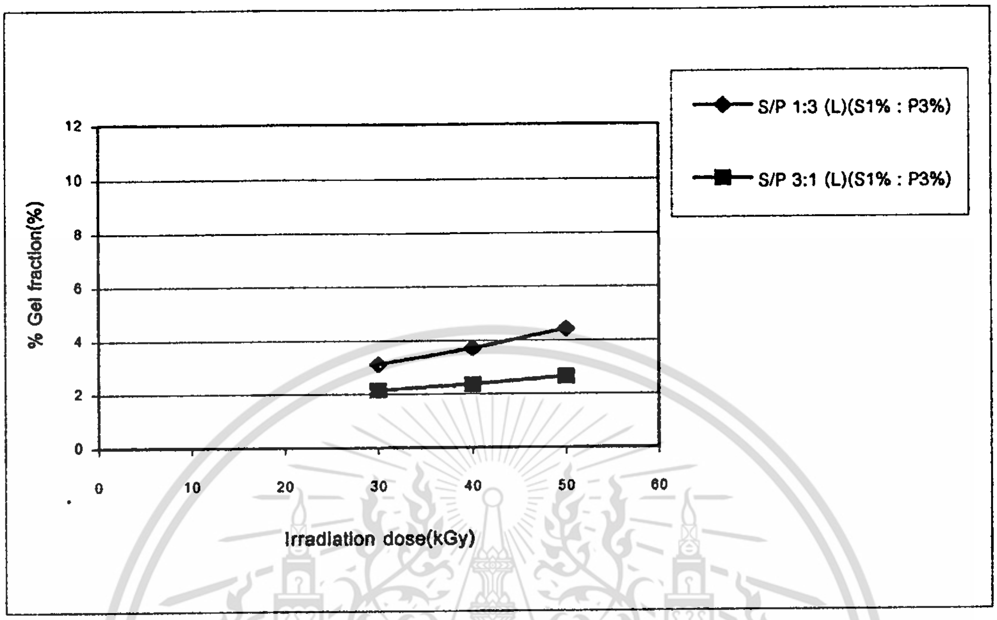


Figure 4.5 %Gel fraction of hydrogel prepared from 1% silk solution blend with 3% low molecular weight PVA solution at ratio of silk/PVA 1:3, 3:1

%Gel fraction determines %crosslink of the sample. It makes the samples to have stable shape and can absorb water.

From Figure 4.5, the results showed that the hydrogel prepared from low molecular weight PVA and irradiated at 10 and 20 kGy, din't give any gel fraction. The solution mixture became gel at 30 kGy. Percent gel fraction increased as the irradiation dose was increased, Thus the 50 kGy hydrogel showed the maximum %gel fraction. The hydrogels prepared from silk/PVA at ratio 1:3 provide the higher %gel fraction than the ratio of 3:1. The physical appearance of the hydrogels prepared from low molecular weight PVA din't suitable for the application. So high molecular weight PVA was used, instead.

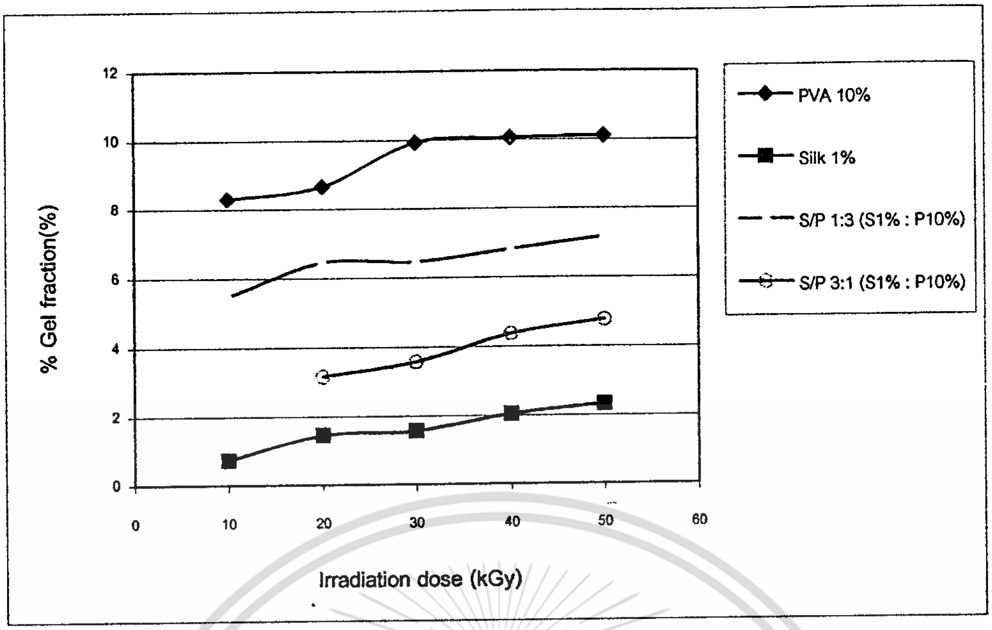


Figure 4.6 %Gel fraction of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3, 3:1 and 10% high MW PVA

From Figure 4.6, %gel fraction from high molecular weight PVA increased with irradiation dose increased and exhibited the same trend as low molecular weight PVA. The maximum %gel fraction received from 10% PVA and the minimum received from 1% pure silk. The hydrogel prepared from the solution of silk blend with PVA gave the amount of %gel fraction between those of PVA and silk. The S/P(1:3) hydrogel showed the %gel fraction higher than the %gel fraction of S/P(3:1) hydrogel. This meant that %gel fraction depended on PVA. According to the structure of the polymer, crosslinking and chain-scission reactions usually take place simultaneously. The Charlsby-Pinner equation was used for calculation the ratio of chain scission and crosslinking.

$$S + S^{0.5} = p_o / q_o + 1/ q_o P_{n,o} D_g \qquad [4.1]$$

S : soluble fraction (sol component)

p_o : ratio of main chain scissions per unit dose to chain units

q_o : proportion of chain units crosslinked per unit dose

D_g : the initial gel dose

Chaisupakitsin M. [20] found that p_o / q_o of silk fibroin was 0.34 and D_g was 1.41. On the contrary, p_o / q_o of PVA is 0.2 and D_g is 1.11. This meaned that silk solution undergo main chain scission faster than PVA solution at the same irradiation dose, however, D_g was slightly higher.

The blend hydrogels had %gel fraction between those of PVA and silk. It is presumably that radiation-induced radicals took place both in silk fibroin and PVA. Crosslinking or grafting is likely due to the combination of these radicals, resulting to gel formation. From Charlesby-Pinner equation, silk (the more easily degradable polymer)'s radicals and its fragmentations seem to retard or interupt crosslinking between PVA molecules in the blend hydrogel by crosslinking or grafting with PVA.

4.1.2.2 Influence of PVA Concentration

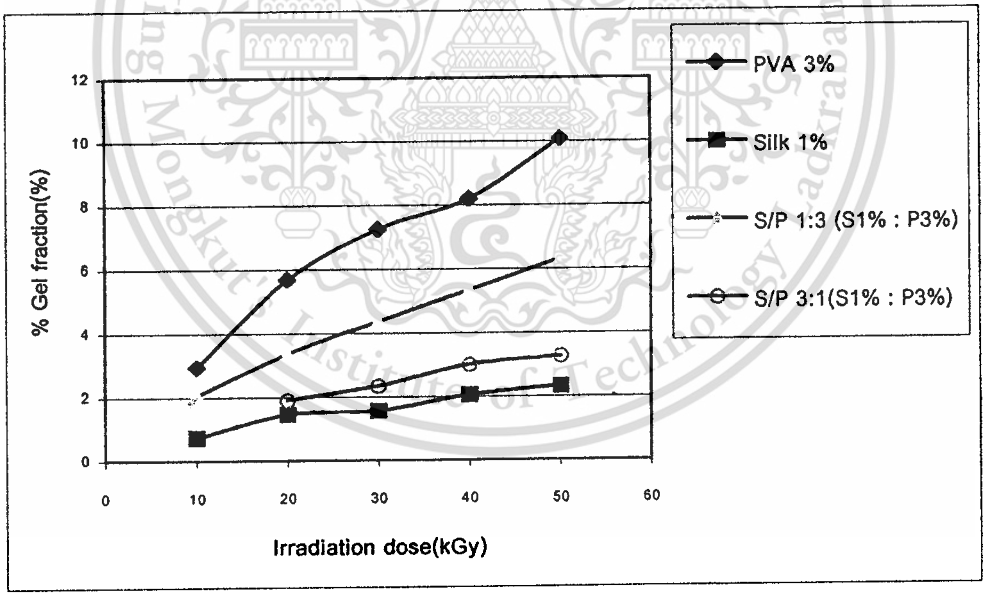


Figure 4.7 %Gel fraction of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3,3:1 and 3% high MW PVA

From Figure 4.7, the results showed that 3%PVA showed the highest %gel fraction. 1% silk showed the lowest gel fraction, and the hydrogels from silk/PVA blend had the %gel fraction between silk and PVA. Gel fraction of S/P(1:3) hydrogel was higher than S/P(3:1) hydrogel at the same irradiation dose. These results were the same as hydrogel prepared from 10% PVA as shown in Figure 4.7. As compared Figure 4.6 and Figure 4.7, gel fraction of 3%PVA blend hydrogel at low irradiation dose was less than that of 10%PVA, but at high irradiation dose, gel fractions were slightly different.

4.1.2.3 Influence of Silk Concentration

Figure 4.8 indicated the amount of gel fraction of blend hydrogel obtained from 3% silk. The results showed that increment of silk concentration affected on the amount of gel fraction. As compared Figure 4.8 with Figure 4.6, gel fraction of blend hydrogel in Figure 4.8 was similar to Figure 4.6.

In the case of high concentration of silk blended with low concentration of PVA, as shown in Figure 4.9, gel fractions of blend hydrogel were similar to those in Figure 4.7. Again, the result suggested that the amount of gel fraction depended on PVA concentration more than silk concentration.

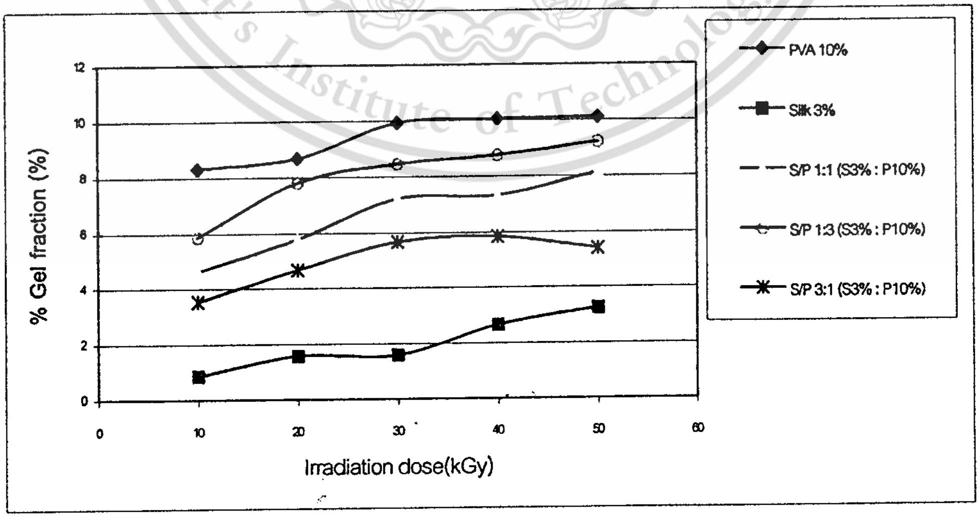


Figure 4.8 %Gel fraction of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:1, 1:3, 3:1 and high MW PVA10%

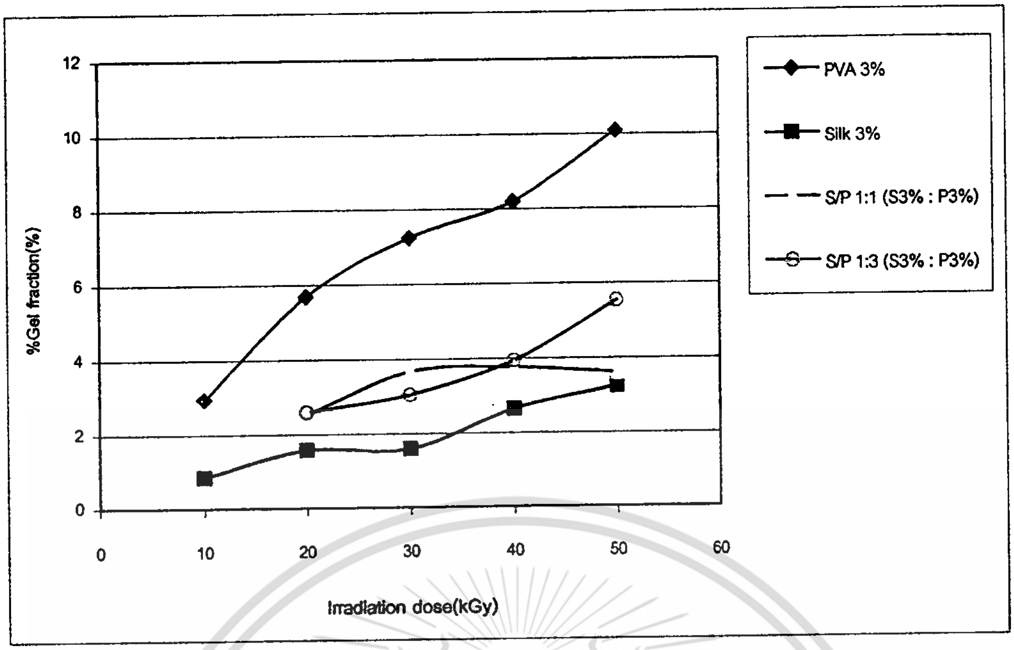


Figure 4.9 %Gel fraction of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:1,1:3 and high MW PVA3%

4.1.3 %Water absorption

4.1.3.1 %Water absorption of silk

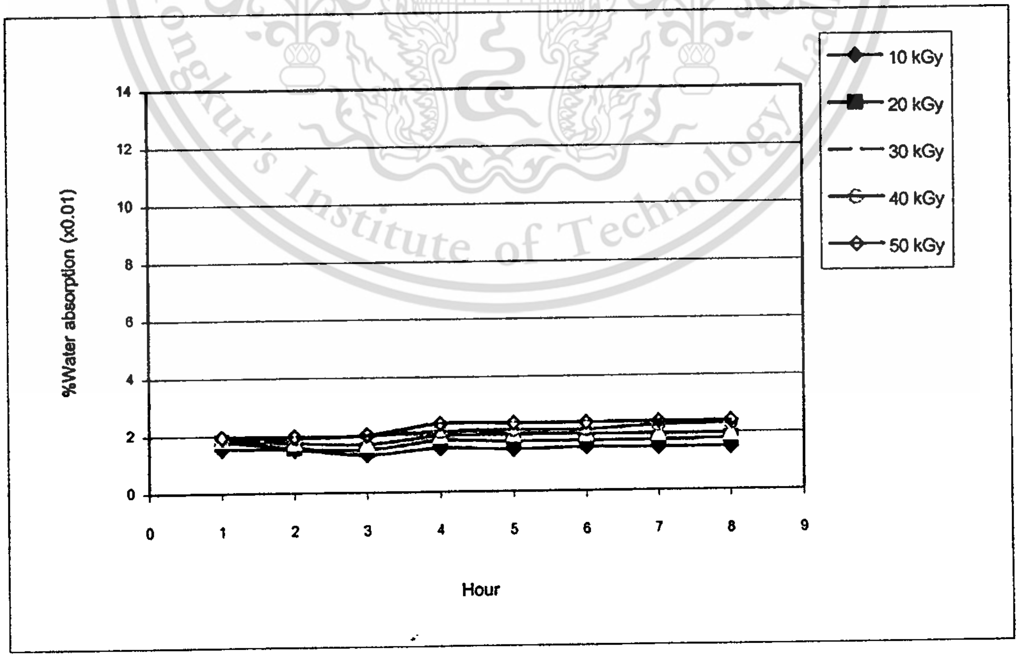


Figure 4.10 %Water absorption of aggregates received from 1% silk solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

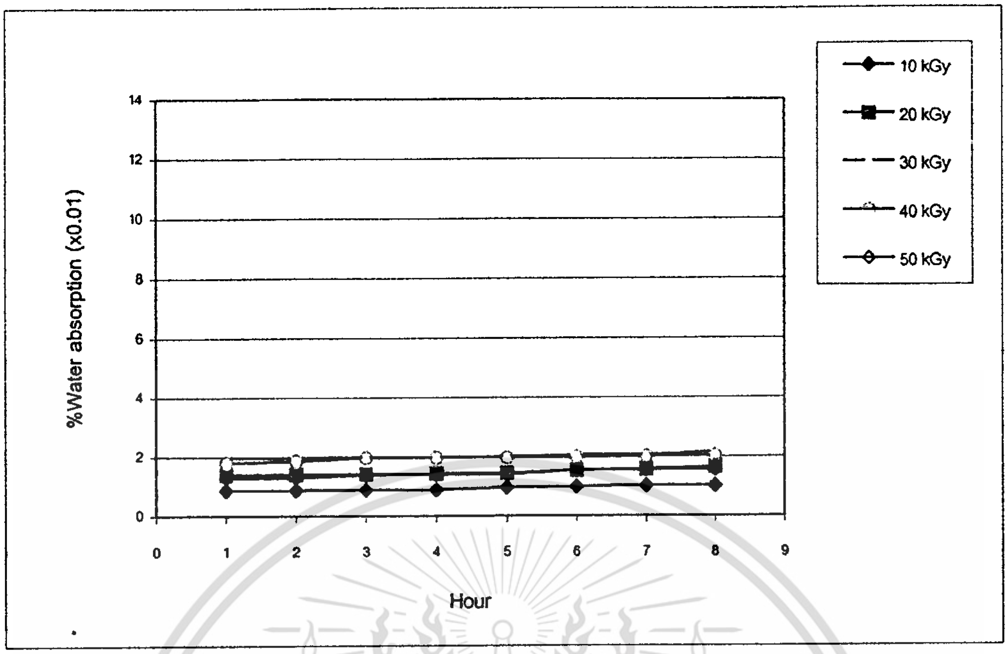


Figure 4.11 %Water absorption of aggregates receive from 3% silk solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

The unirradiated silk solution obtained from dialysis can be dissolved in water. In this case, conformation of silk solution is random coil or unfolded chain[5]. After irradiation, silk solution can aggregate. Figures 4.10 and 4.11 show %water absorption of aggregates received from 1%silk and 3%silk solution. Slightly increasing in water absorption with irradiation dose and soaking time was found in all samples.

4.1.3.2 %Water absorption of PVA

The water absorption of gel obtained from 3%PVA and 10%PVA solution was shown in Figures 4.12 and 4.13, respectively. The amount of absorbed water in irradiated samples was increased with increasing soaking time. The same trend was found in the case of 10%PVA. AS compared Figure 4.12 and Figure 4.13, 3%PVA hydrogel can absorb water more than 10%PVA hydrogel. This is due to smaller size of the intersics and higher molecule chain entanglement of 10%PVA. In the previous research [4], the more irradiation dose, the more crosslink and the less water absorption was observed.

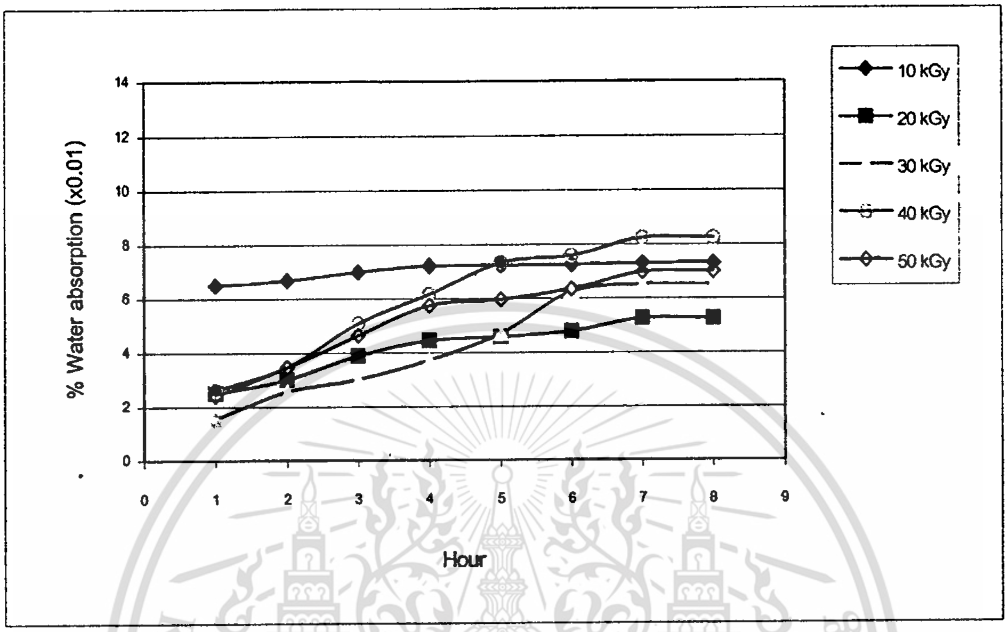


Figure 4.12 %Water absorption of 3% high MW PVA solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

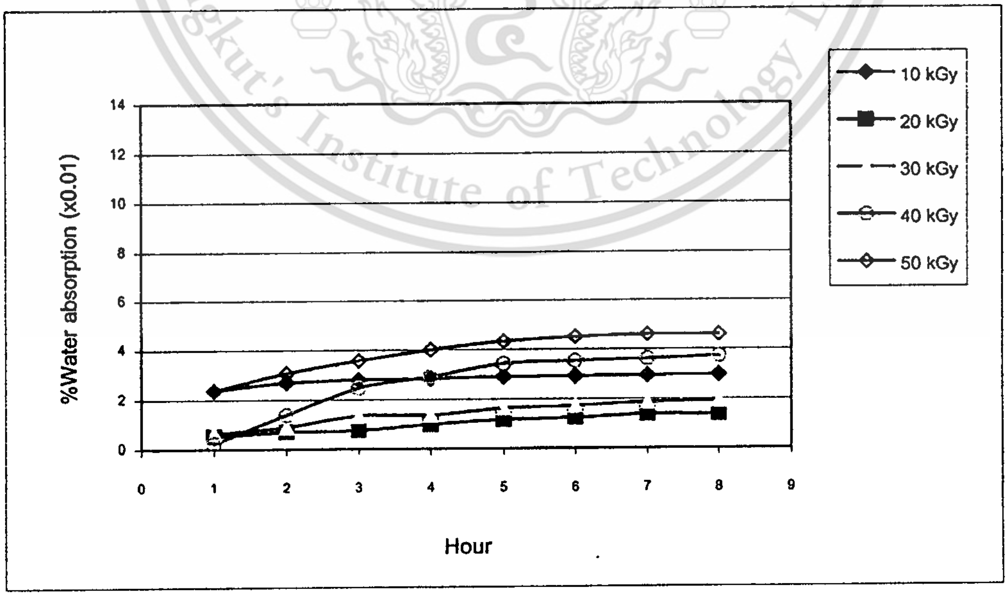


Figure 4.13 %Water absorption of 10% high MW PVA solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time.

4.1.3.3 %Water absorption of 1%silk and low MW PVA

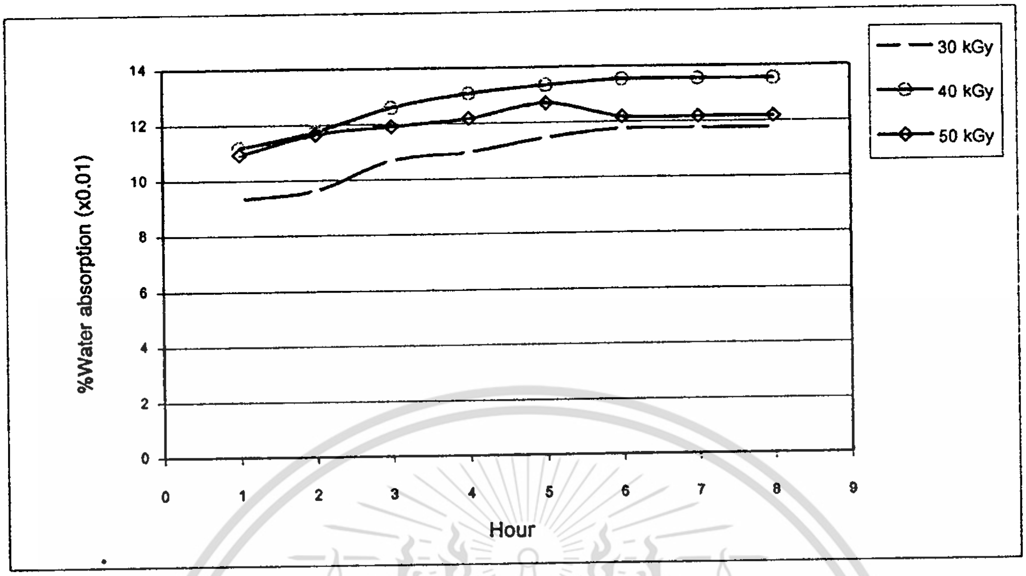


Figure 4.14 %Water absorption of blend hydrogel prepared from 1% silk solution and 3% low molecular weight PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

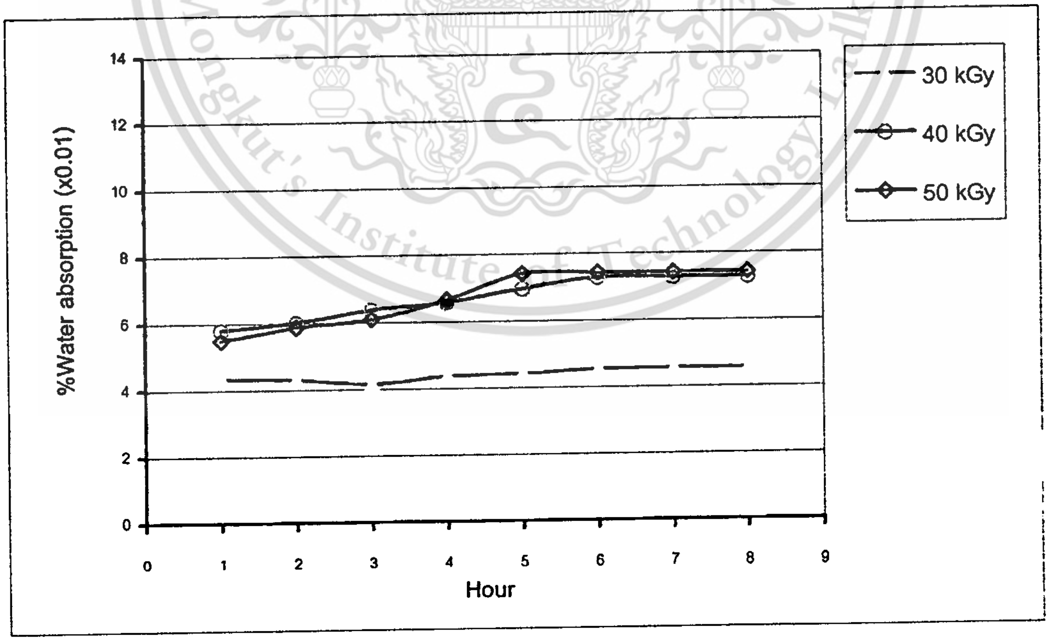


Figure 4.15 %Water absorption of hydrogel prepared from 1% silk solution and 3% low molecular weight PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as function of soaking time

Figures 4.14 and 4.15 showed %water absorption of hydrogel, that prepared from 1% silk blend with 3% low molecular weight PVA . The results showed that the more PVA in blend hydrogel (Figure 4.14) the more %water absorption. On the other hand, sample of higher silk ratio exhibited lower water absorption. The increment depended on irradiation dose and time.

4.1.3.4 %Water absorption of 1%silk and high MW PVA

Figures 4.16 and 4.17 showed %water absorption of hydrogel that prepared from 3% high molecular weight PVA and 1%silk. The results show that the %water absorption increased with the time increased until 5 hours, then it became constant. The silk/PVA ratio of 1:3 (Figure 4.16) gave higher %water absorption because the ratio of 1:3 had more PVA in hydrogel than the ratio of 3:1(Figure 4.17). Water absorption depended on PVA more than silk, the same as %gel fraction. In Figure 4.16, %water absorption increased when the irradiation dose increased until 30 kGy. This means that 30 kGy is an appropriate dose for this ratio. If the irradiation dose was higher than 30 kGy, it made too dense three-dimensional network, therefore water absorption decreased.

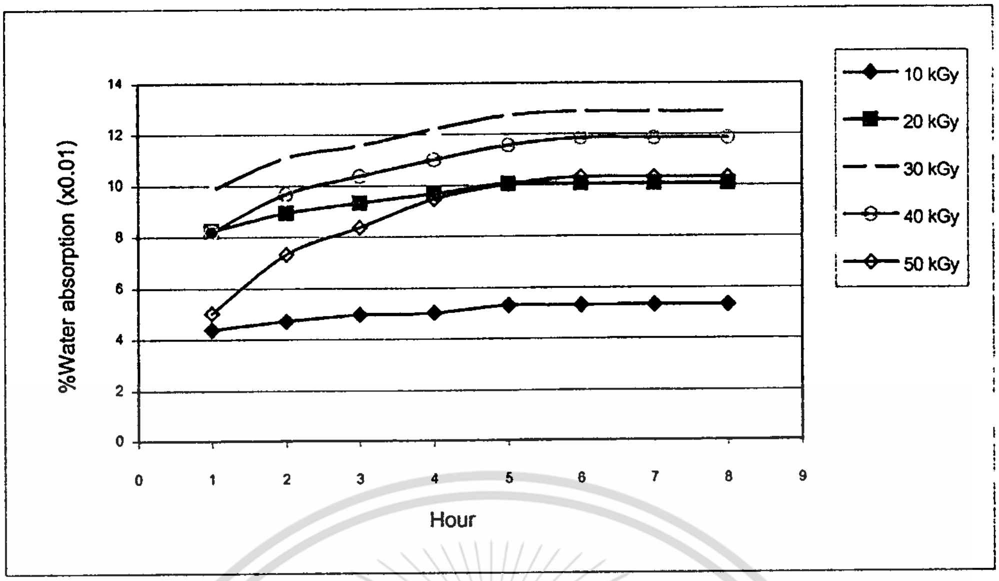


Figure 4.16 %Water absorption of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

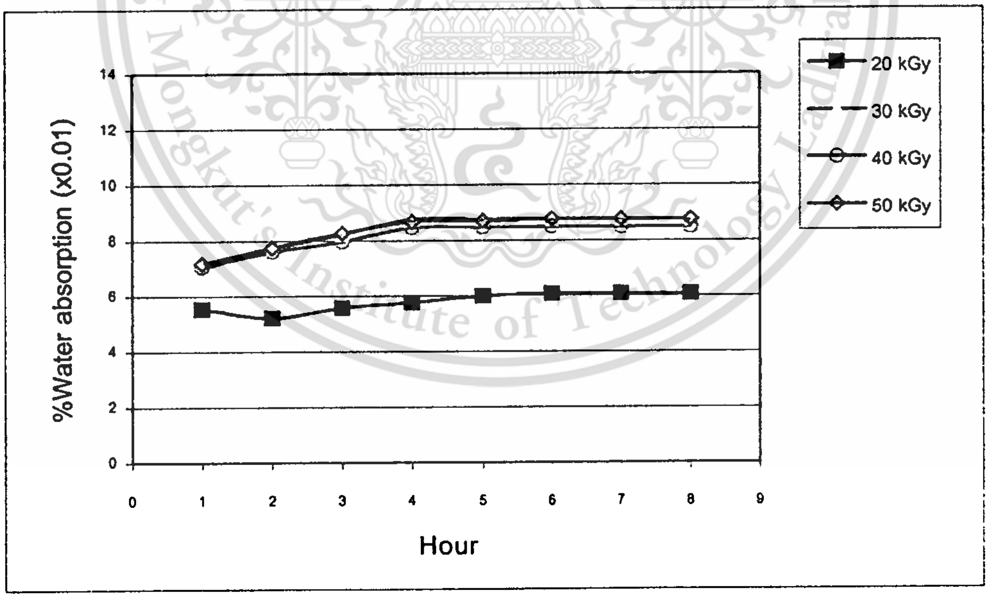


Figure 4.17 %Water absorption of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

4.1.3.5 %Water absorption of 3%silk and high MW PVA

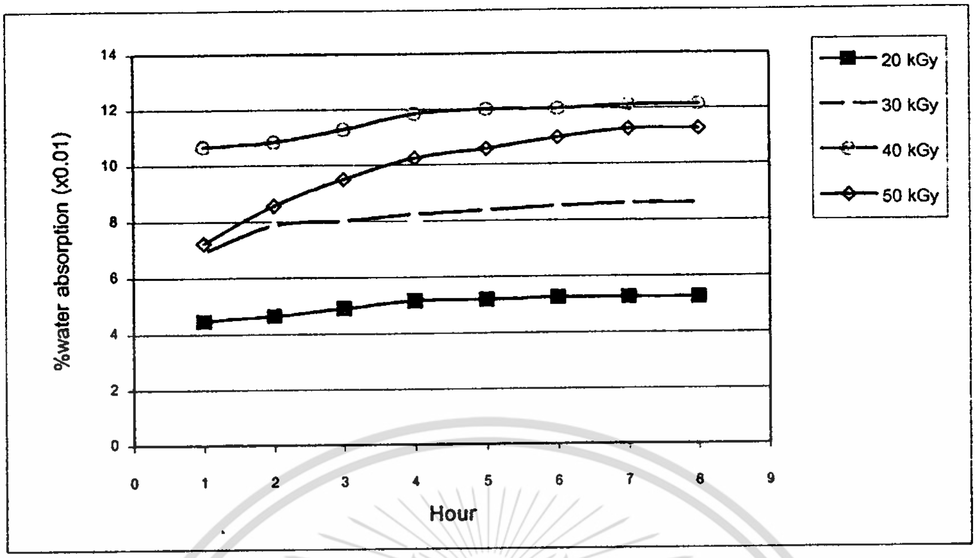


Figure 4.18 %Water absorption of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

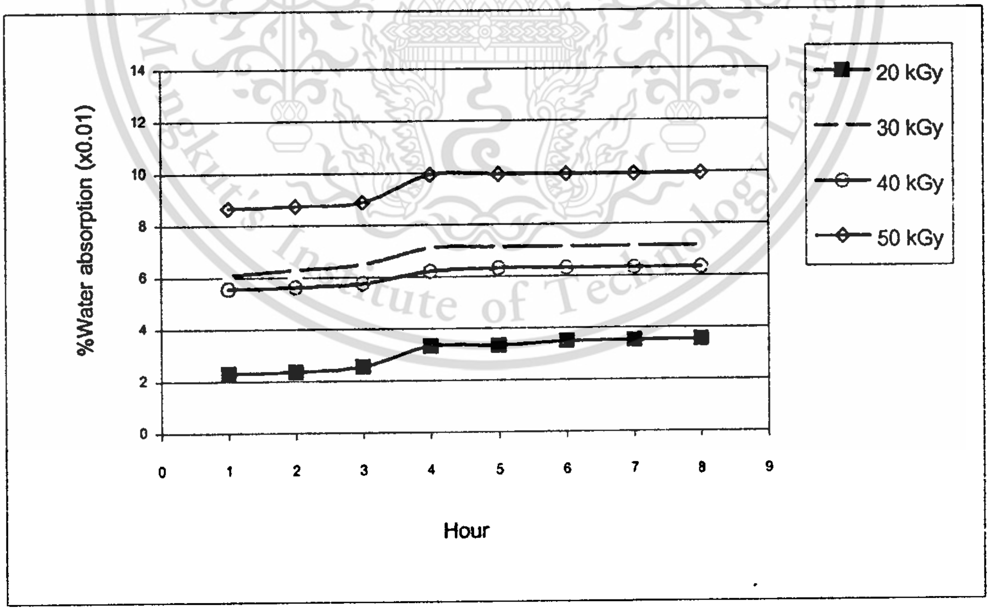


Figure 4.19 %Water absorption of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

%Water absorption of hydrogel prepared from 3%silk and 3%PVA were shown in Figures 4.18 and 4.19. In Figure 4.18, the ratio of silk/PVA at 1:3, %water absorption increased with the time increased until 5 hours. After that, it became constant. The maximum %water absorption was 40 kGy and the lowest was 20 kGy.

Figure 4.19 showed the ratio of silk/PVA at 1:1. %Water absorption increased with the soaking time increase until 4 hours, then it became constant. When compared between 1%silk and 3%silk blended with the same concentration of PVA (Figures 4.16, 4.18 and Figures 4.17, 4.19), the results indicated that both of them were similars.

4.1.3.6 %Water absorption of 1%silk and high concentration PVA

Figure 4.20 showed the result of 1%silk blend with 10%PVA. There was no significant difference of water absorption in all samples. It is interesting to note that %water absorption of silk/PVA(1:3) was less than %water absorption of hydrogel at ratio silk/PVA of 3:1 as shown in Figure 4.21. Lower swelling absorption of high concentration of PVA should be due to the phenomena like Thommdorf Norrish effect or gel effect.[32] High viscosity limits the diffusion of long-chain radicals as required for termination. By comparison, the diffusion of small molecules is less restricted. On the basis of the above findings, it is reasonable to consider that the %water absorption of 10%PVA hydrogels as shown in Figure 4.20 were less than 3%PVA hydrogels in Figure 4.16 when it was blended with 1%silk.

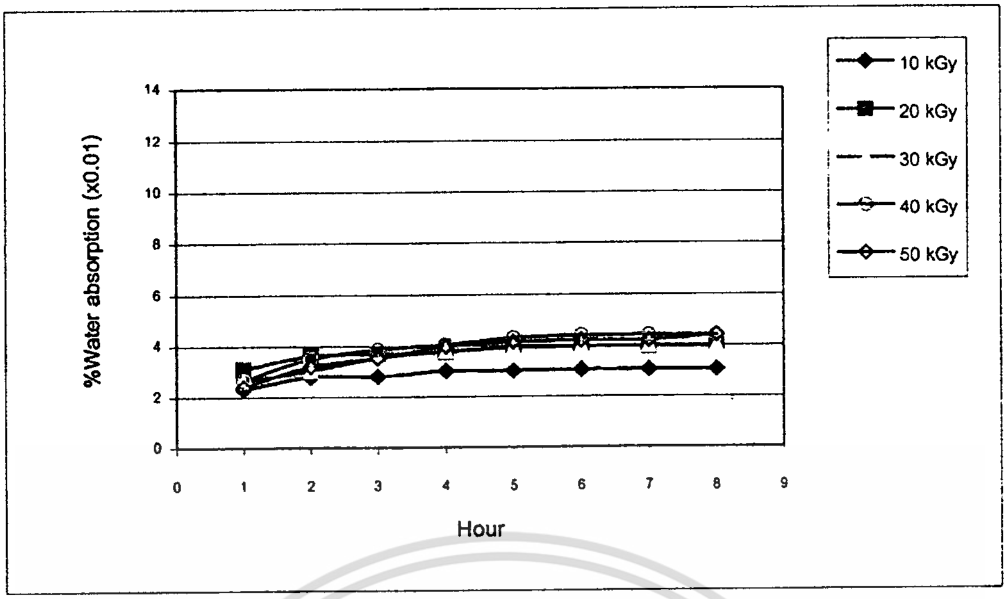


Figure 4.20 %Water absorption of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

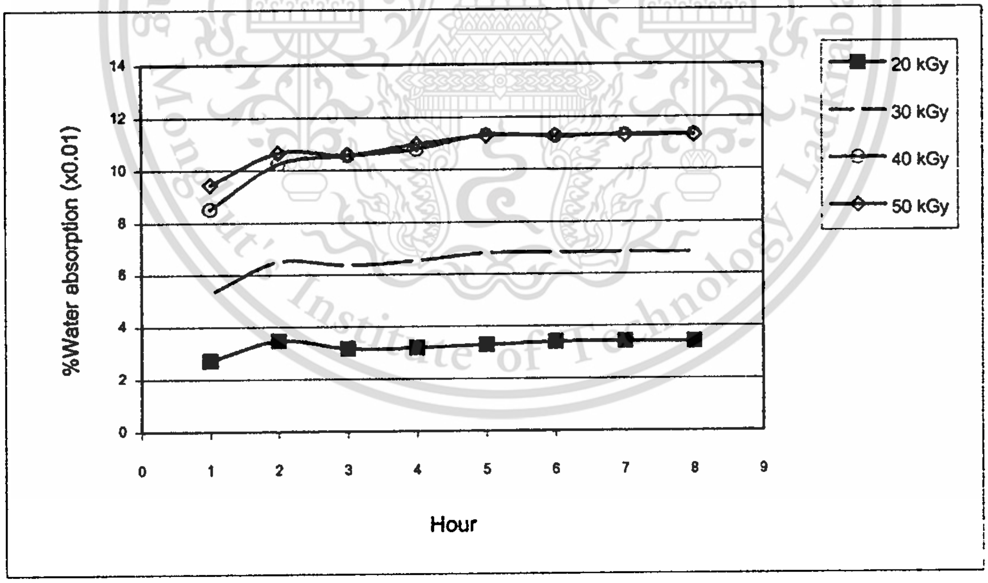


Figure 4.21 %Water absorption of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

4.1.3.7 %Water absorption of 3%silk and high concentration PVA

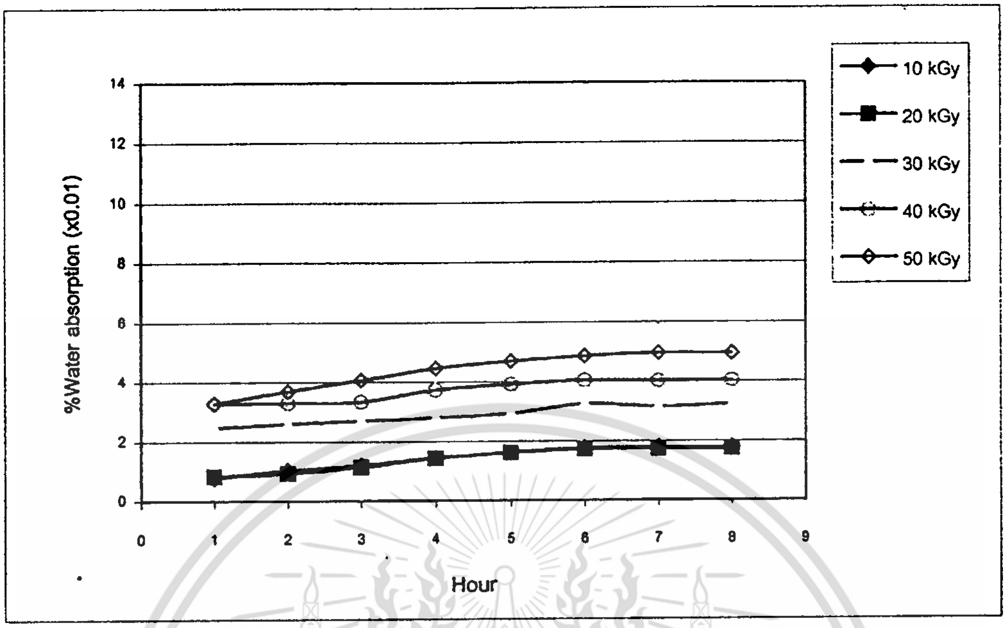


Figure 4.22 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

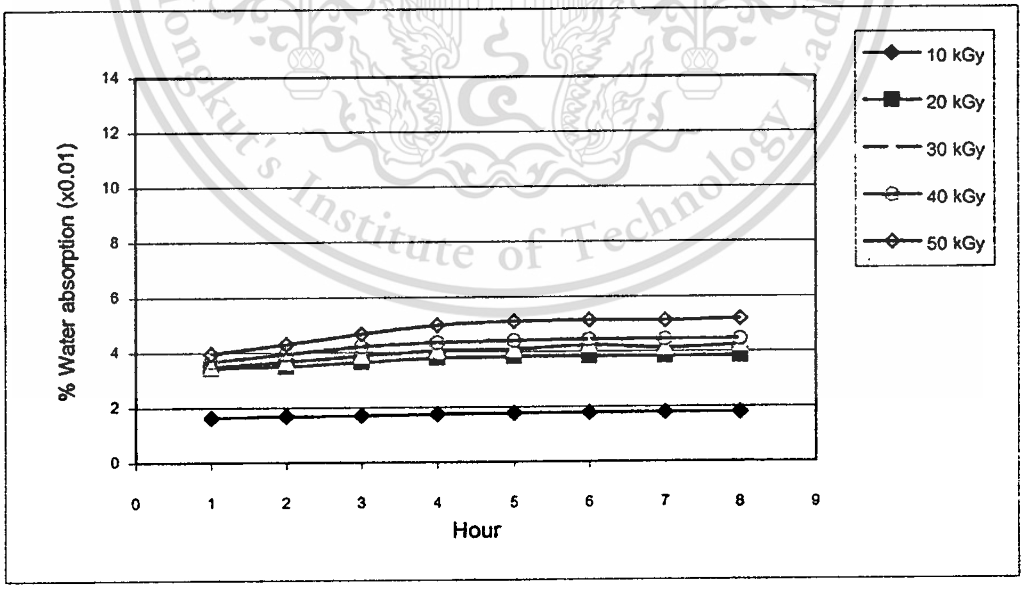


Figure 4.23 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

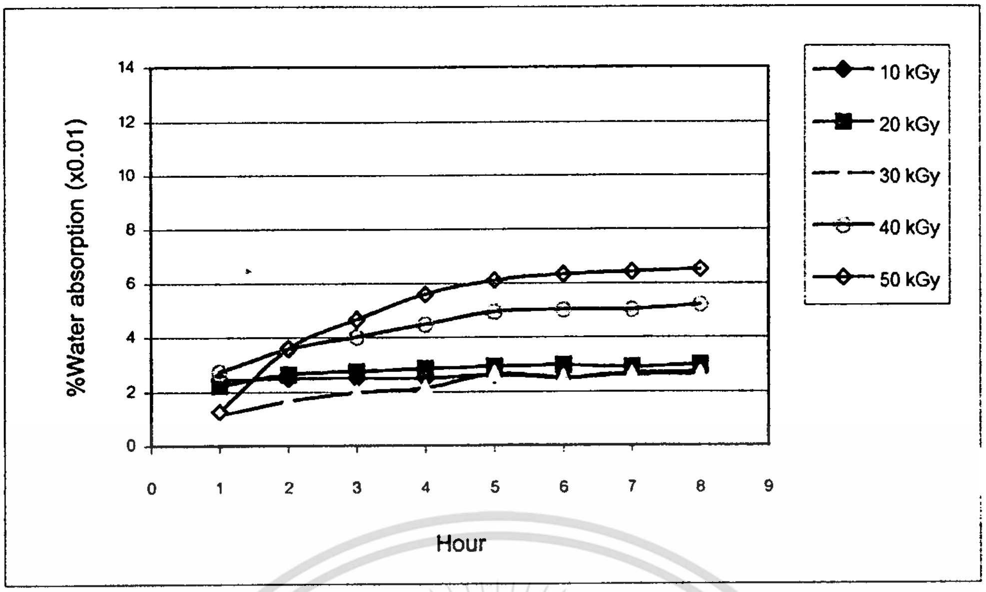


Figure 4.24 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Figures 4.22 and 4.23 showed %water absorption of hydrogel prepared from 3%silk and 10%PVA. %Water absorptions of both gels were similar. On the other hand, %water absorption increased with the time increase. The 50 kGy hydrogels had the maximum %water absorption, the same as shown in Figures 4.20 and Figure 4.21.

Figure 4.24 showed %water absorption of hydrogel prepared from 3%silk solution and 10%PVA solution at ratio of silk/PVA 1:1. The results showed that the %water absorption increased with soaking time and irradiation dose. The maximum %water absorption was at 50 kGy. At this ratio, %water absorption was slightly higher than the ratio of silk/PVA 1:3 and 3:1.

4.1.3.8 Relation between %water absorption and volume fraction

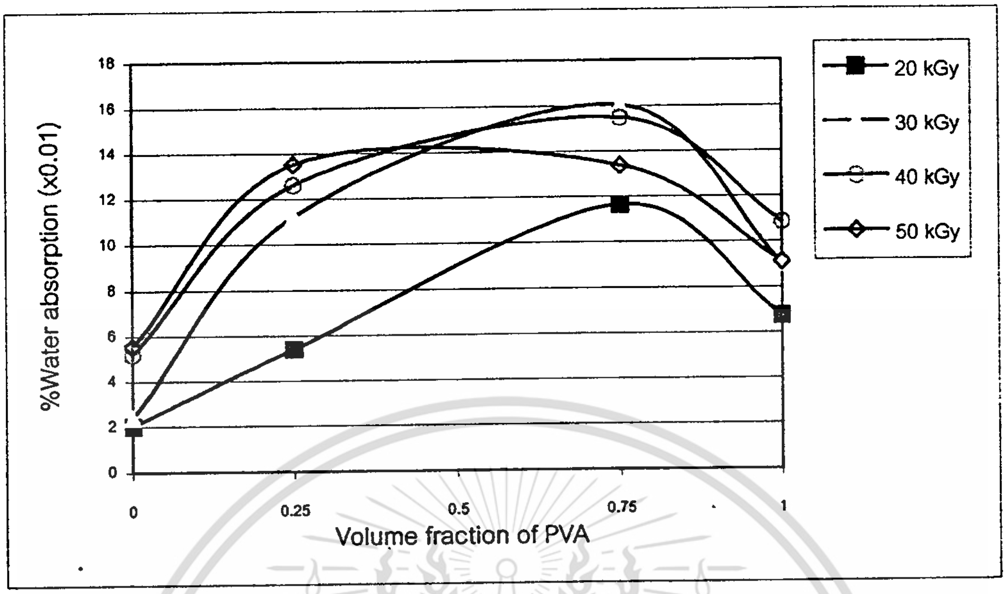


Figure 4.25 Relation between %water absorption and volume fraction of 1% silk solution 3% blend with high MW PVA after irradiated at 20,30,40 and 50 kGy.

The relationship between %water absorption and volume fraction of %PVA in hydrogel were plotted in Figures 4.25,4.26,4.27 and 4.28.

The results of hydrogels prepared from 1%silk and 3%PVA were shown in figure 4.25. %Water absorption of the hydrogels increased when the volume fraction of PVA increased. It was shown significantly that %water absorption depended on PVA. The blend hydrogels had %water absorption higher than pure PVA and pure silk. It is considered that the interaction between silk and PVA affect on the %water absorption. The results could be concluded that, in case of low concentration but high volume fraction of PVA, %water absorption depended on PVA more than silk.

%Water absorption of 1%silk and 10%PVA were shown in Figure 4.26. The results shown that %water absorption of the hydrogel at volume fraction of PVA 0.25 gave the maximum value. The results different from Figure 4.25. It is obviously seen that high concentration and high volume fraction of PVA induced high dense of crosslinking, therefore, it had low water absorption. The %water absorption of blend hydrogels was higher than silk and PVA pure, the same as the result from figure 4.25.

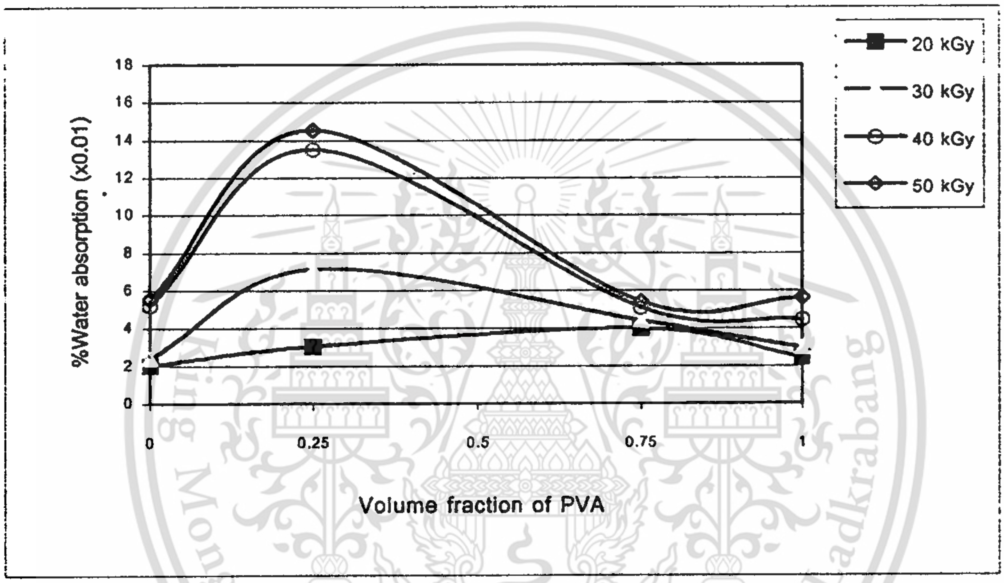


Figure 4.26 Relation between %water absorption and volume fraction of 1% silk solution blend with 10%high MW PVA after irradiated at 20,30,40 and 50 kGy.

When the experiment changed the concentration of silk in blended hydrogel to 3%, the results shown in Figure 4.27 and 4.28. It was found that, %water absorption of both volume fraction of PVA(0.25 and 0.75) were slightly different.

When compare Fig.4.25 with 4.27 and Fig.4.24 with Fig.4.28, the maximum %water absorption of 3%silk blend hydrogels were lower than 1%silk blend hydrogel. The results determined that high viscosity of silk affected the interaction between silk and PVA resulting in the ability of water absorption.

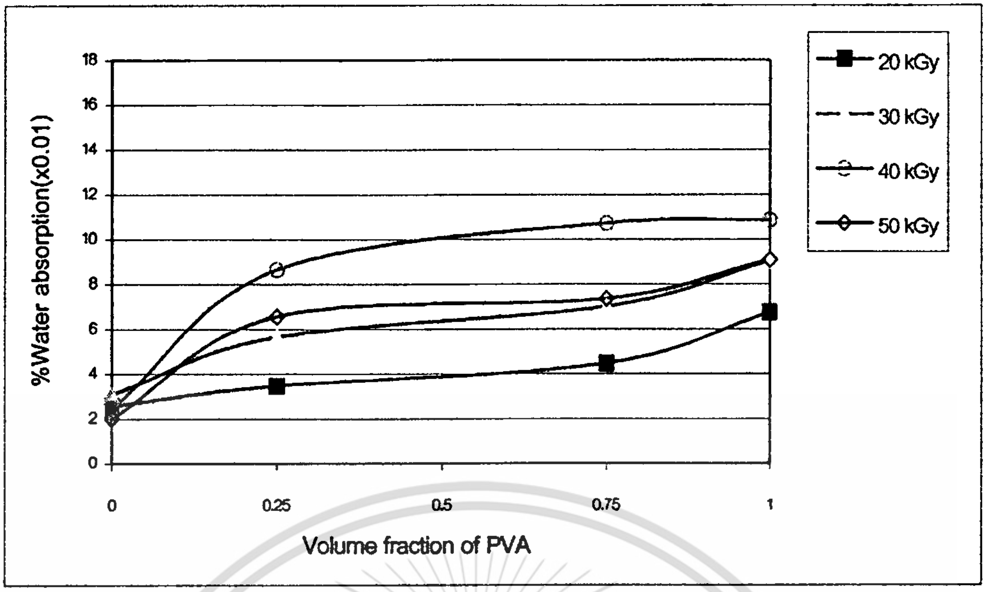


Figure 4.27 Relation between %water absorption and volume fraction of 3% silk solution blend with 3%high MW PVA after irradiated at 20,30,40 and 50 kGy.

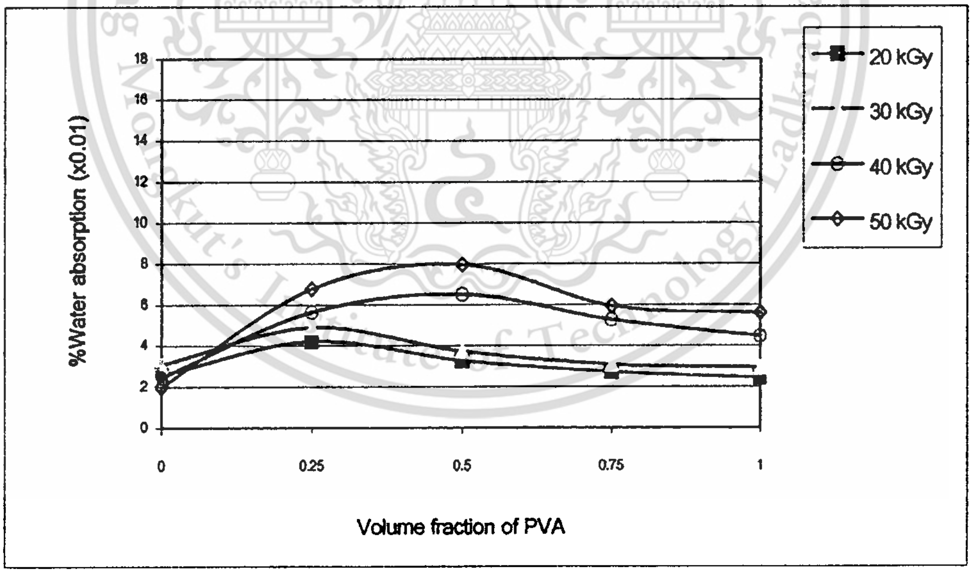


Figure 4.28 Relation between %water absorption and volume fraction of 3% silk solution blend with 10%high MW PVA after irradiated at 20,30,40 and 50 kGy.

4.2 Mechanical Properties

4.2.1 Gel Strength

Gel strength is an important property for select the appropriate hydrogel to use in various applications.

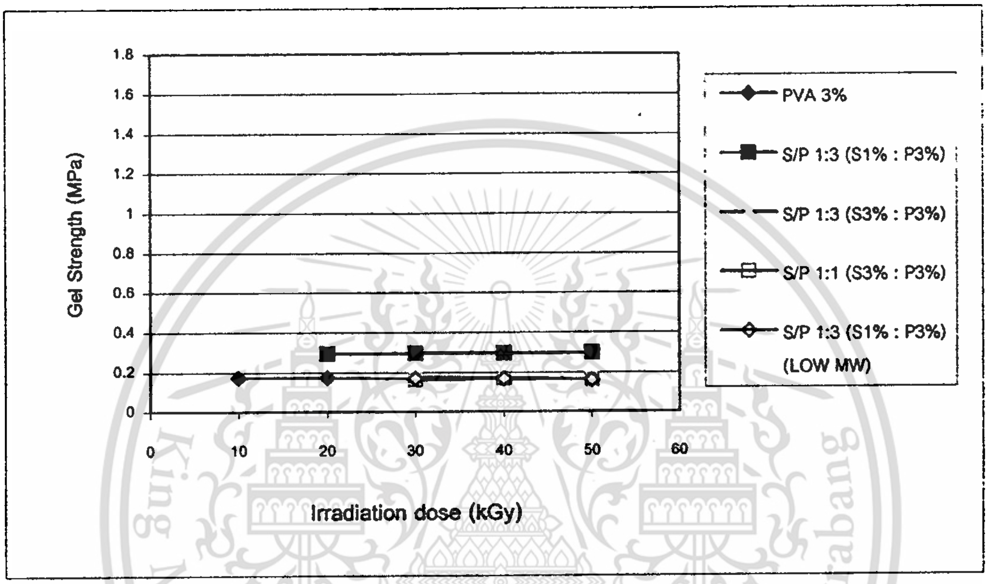


Figure 4.29 Gel strength of hydrogel prepared from 1%silk solution, 3%silk solution blend with 3%PVA solution at ratio of silk/PVA 3:1,1:3,1;1 and 3% high MW PVA

The gel strength of hydrogels prepared from 1%silk solution and 3% silk solution blend with 3%PVA solution were measured by compression test. In Figure 4.29, the results showed that gel strength of the hydrogel prepared from 1%silk and 3%PVA at ratio of silk/PVA 1:3 showed the highest gel strength when compare with the other samples. It is interesting to note that, gel strength of pure PVA and blend hydrogel that prepared from 3%silk and 3%PVA at ratio of silk/PVA 1:3 and 1:1 provided the same value as blend hydrogel that prepared from 1%silk and 3% low MW PVA at ratio of silk/PVA 1:3. It is considered that gel strength depended on ratio of PVA in the blend.

Gel strength of 10%PVA and blend hydrogel was plotted in Figure 4.30. The maximum gel strength was found from 3%silk and 10%PVA at ratio 1:3 of 20 kGy blend hydrogel. The second was found at 30 kGy for the same kind of blend hydrogel but at the silk/PVA ratio of 1:1.

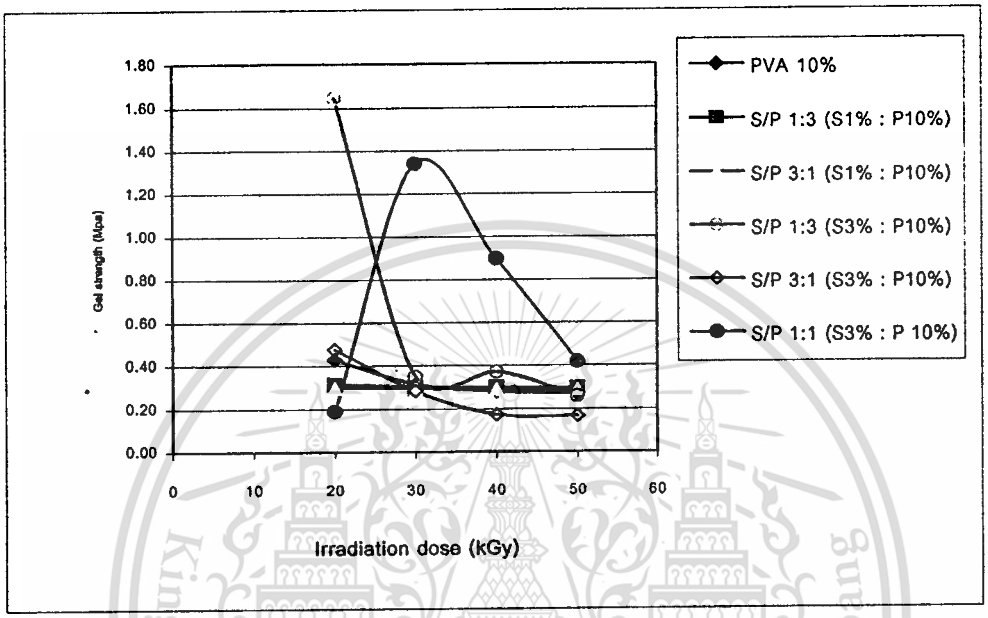


Figure 4.30 Gel strength of hydrogel prepared from 1%silk solution, 3%silk solution blend with 10%PVA solution at ratio of silk/PVA 3:1,1:3,1:1 and 10% high MW PVA

CHAPTER 5

CONCLUSIONS AND SUGGESTION

From the results, it is presumably that under gamma irradiation, both silk solution and PVA solution absorbed energy and then radical occurred. These radicals can recombine to form crosslink and/or undergo chain-scission to partially degrade. Our experimental data found that silk radicals trend to degrade more than crosslink. On the contrary, PVA became crosslink more than degrade. When silk solution mixed with PVA solution, the silk radicals and PVA radicals had opportunity to recombine and/or radicals of silk probably added into radicals of PVA resulting in grafted silk and co-crosslink. The typical reaction mechanisms were shown in Figure 5.1.

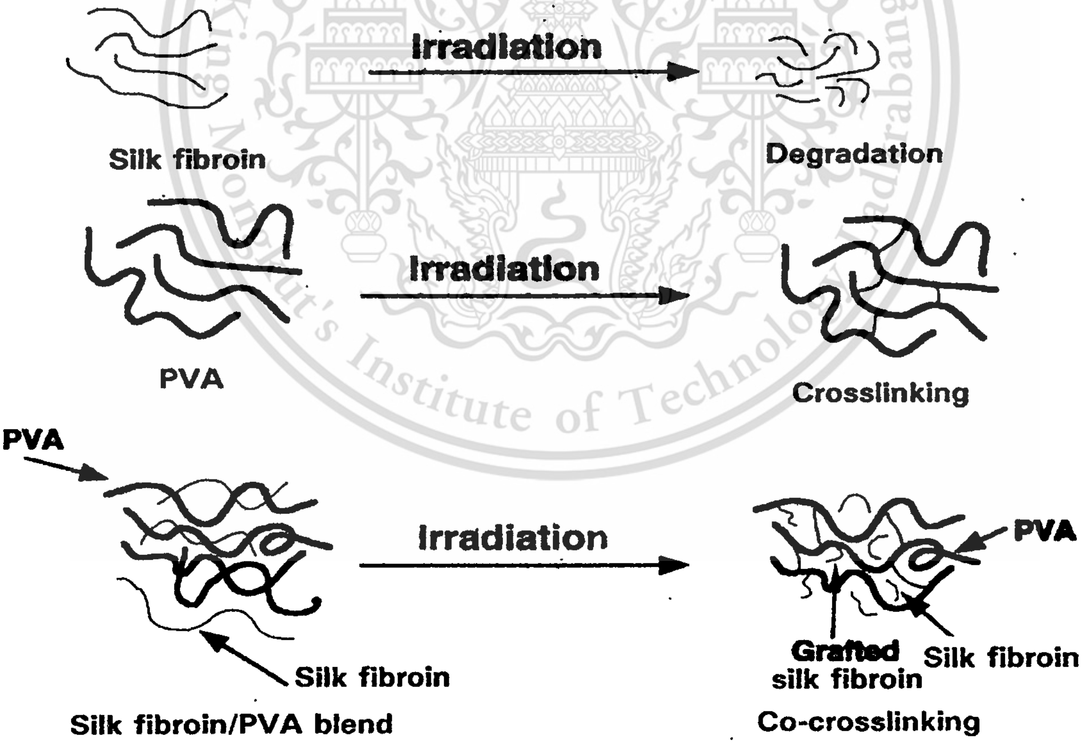


Figure 5.1 The proposed mechanism of silk solution and PVA solution after gamma-irradiation.

The results can be summarised that

1. Physical appearance of hydrogels depended on quantities of silk and PVA, ratio of silk/PVA, MW of PVA and irradiation dose.
    1.1 The low MW PVA gave less stable hydrogel than high MW PVA.
    1.2 The blend hydrogels that had more PVA gave more stable shape. When pure silk fibroin was irradiated, they had unstable shape but when it was mixed with PVA, the blend hydrogels became more stable.
    1.3 The blend hydrogels that had the ratio of silk more than PVA, gave a pale white color. If they had more PVA, they became transparent.
    1.4 At low irradiation dose, the blend hydrogels were soft and sticky but at high irradiation dose, they were more rigid.
2. %Gel fraction of hydrogels depended on PVA.
    2.1 The low MW PVA blend hydrogels gave less gel fraction than high MW PVA.
    2.2 At low irradiation dose, 10% high MW PVA blend hydrogels had %gel fraction higher than 3% high MW PVA blend hydrogels.
    2.3 At the same concentration of PVA, the 1%silk and 3%silk hydrogels had slightly different in %gel fractions.
3. %Water absorption of hydrogels depended on concentration and volume fraction of PVA.
    3.1 Silk solution after irradiation had a little %water absorption.
    3.2 PVA solution after irradiation had %water absorption higher than silk solution after irradiation.
    3.3 The low MW blend hydrogels had high %water absorption.
    3.4 The 3%high MW PVA blend with 1%silk and 3%silk at silk/PVA ratio of 1:3 had %water absorption higher than the ratio 3:1.
    3.5 The 10% high MW PVA blend with 1%silk and 3%silk at silk/PVA ratio of 3:1 had %water absorption higher than the ratio 1:3.
4. Addition of PVA into silk could increase %water absorption of hydrogel.
    4.1 The 3%high MW PVA blend with 1%silk and 3%silk at silk/PVA ratio of 1:3 and 3:1 had %water absorption higher than pure PVA and pure silk.

5. Gel strength of hydrogel depended on PVA.
    5.1 The blend hydrogels that had PVA more than silk gave the higher gel strength.
6. Low molecular weight PVA(11,000 – 31,000) was not suitable for preparing hydrogel.
    6.1 The hydrogel prepared from low MW PVA gave low gel strength, low gel fraction and unstable shape.
7. The appropriate formula was 1%silk / 3%PVA at ratio of 1:3 and irradiated at 30 kGy because they gave high water absorption, appropriate gel strength, and use appropriate irradiation dose.

Suggestions for future work

The results from this research informed the properties of hydrogels at different ratios and different radiation doses. It is important information to select the suitable condition for prepare hydrogel in the next research.

1. The 3%PVA blend with 1% silk solution hydrogel at silk/PVA ratio of 3:1 give high water absorption and appropriate gel strength, it is interesting for the next research.
2. Preparation of hydrogel in the film forming should be studied for biomedical application.

REFERENCES

[1] Demura M., Kitamura A., Shibamoto A., Asakura T. "Water Sorption, Membrane Potentials, and Ion Permeability of Styrene – Grafted Bombyx mori Silk Fibroin Membrane." J.Appl.Polym.Sci., vol. 27, 1988. pp. 535-543.

[2] Magashi J., Nakamura S. "Studies on Physical Properties and Structure of Silk. Glass Transition and Crystallization of Silk Fibroin." J.Appl.Polym.Sci., vol. 19, 1975. pp. 1013-1015.

[3] Magashi J., Magashi Y., Nakamura S., Kasai N., Kakudo M. "Physical Properties and Structure of Silk V. Thermal Behavior of Silk Fibroin in the Random-Coil Conformation." J.Appl.Polym.Sci., vol.15, 1977. pp.1675-1683.

[4] Ikada Y., Mita T.,et.al. "Preparation of Hydrogels by Radiation Technique." Radiat.Phys.Chem., vol. 9, 1977. pp. 633-645.

[5] Magoshi J., Mizuide M., Magoshi Y. "Physical Properties and Structure of Silk VI. Conformational Changes in Silk Fibroin Induced by Immersion in Water at 2 to 130 °C." J.Polym.Sci.:Polym.Phy.Ed., vol. 17, 1979. pp. 515-520.

[6] Demura M., Kitamura A., Shibamoto A., Asakura T. "Water Sorption, Membrane Potentials, and Ion Permeability of Styrene-Grafted Bombyx mori Silk Fibroin Membrane." J.Appl.Polym.Sci., vol. 36, 1988. pp. 535-543.

[7] Hirai T., Asada Y.,Suzuki T.,Hayashi S. "Studies on Elastic Hydrogel Membrane I.Effect of Preparation Conditions on The Membrane Performance." J.Appl.Polym.Sci., vol. 38, 1989. pp. 491-502.

[8] Yamaura K., Kirikawa S., Sakuma N., Tanigami T., Matsuzawa S. "Permeabilities of Water, Several Alcohols, and Water / Alcohol Mixtures Through Thin Films of Syndiotactic – Rich Poly(vinyl alcohol)." J.Appl.Polym.Sci., vol. 41, 1990. pp. 2453-2461.

[9] Yoshimizu H., Asakura T. "Preparation and Characterization of Silk Fibroin Powder and Its Application to Enzyme Immobilization." J.Appl.Polym.Sci., vol. 40, pp. 127-134.

[10] Minoura N., Tsukada M.,Nagura M. "Physico-Chemical Properties of Silk Fibroin Membrane as a Bimaterial." Biomaterials, vol. 11,1990. pp. 430-434.

[11] Yamaura K., Kuranuki N., Suzuki M., Tanigami T., Matsuzawa S. "Properties of Mixtures of Silk Fibroin / Syndiotactic-Rich Poly(vinyl alcohol)." J.Appl.Polym.Sci., vol. 41, 1990. pp. 2409-2425.

[12] Liang C.X., Hirabayashi K. "Improvements of The Physical Properties of the Fibroin Membrane with Sodium Alginate." J.Appl.Polym.Sci., vol. 45,1992. pp. 1937-1943.

[13] Kim J.H., Kim J.Y., Lee Y.M., Kim Y.Y. "Properties and Swelling Characteristics of Cross-Linked Poly(vinyl alcohol) / Chitosan Blend Membrane." J.Appl.Polym.Sci., vol. 45, 1992. pp. 1711-1717.

[14] Tsukada M., Freddi G.,Crighton J.S. "Structure and Compatibility of Poly(vinyl alcohol) – Silk Fibroin (PVA/SF) Blend Films." J.Appl.Polym.Sci., vol. 32, 1994. pp. 243-248.

[15] Zhang Z., Chu J. "Radiation Induced Graft of Acrylonitrile on Silk Protein in $Zncl_2$ Solution." JAERI-Conf., 1994. pp. 569-573.

[16] Kunioka M., Choi H.J. "Studies on Elastic Hydrogel Membrane. I.Effect of Preparation Conditions on the Membrane Performance." J.Appl.Polym.Sci., vol. 58, 1995. pp. 569-573.

[17] Liu Y., Liu H., Qian J.,Deng J., Yu T. "Structure and Properties of The Composite membrane of Regenerated Silk Fibroin and PVA and Its Application to Amperometric Tetrathiafulvalene – Mediating Glucose Sensor." Pure Appl. Chem., vol. 2, 1996. pp. 209-219.

[18] Chen X., Li W., Zhong W., Lu Y., Yu T. "pH Sensitivity and Ion Sensitivity of Hydrogels Based on Complex - Forming Chitosan / Silk Fibroin Interpenetrating Polymer Network" J.Appl.Polym.Sci., vol. 65, 1997. pp. 2257-2262.

[19] Jegal J., Lee K. "Nanofiltration Membranes Based on Poiy(vinyl alcohol) and Ionic Polymers" J.Appl.Polym.Sci., vol. 72, 1999. pp. 1755-1762.

[20] Chaisuppakitsin M. "Preparation of Hydrogel from Silk Fibroin by γ-Irradiation." Die Angewandte Makromoledulare Chemie., in pressed.

[21] Kasemchinun S., Kumta S. "Gamma Radiation Induced Hydrogel of Poly(vinyl alcohol) / Poly(acrylic acid) and Modified with Silk Protein" Senior Special Project of King Mongkut's Institute of Technology Ladkrabang. 2000.

[22] Mark H.F., Bikales N.M., Overberger C.G., Menges G., Kroschwitz J.I. **Encyclopedia of Polymer Science and Engineering.** Vol. 15, New York : A Wiley – Interscience Publication John Wiley & Sons. 1989. pp. 309-339.

[23] Kuchel P.W., Raiston G.B. **Theory and Problems of Biochemistry.** SCHAUM 'S GUTLINE SERIES : McGraw – Hill Book Company. 1988.

[24] Mathews C.K., Holde K.E.V. **Biochemistry.** 2nd Ed. The Benjamin : Cummings Publishing Company, INC. 1995.

[25] Rathore O.,Winningham M.J., Sogah D.Y. "A Novel Silk-Based Segmented Block Copolymer Containing GlyAlaGlyAla β-Sheets Templated by Phenoxathiin." J.Appl.Polym.Sci., vol. 38, 2000. pp. 1755-1762.

[26] Mark H.F., Bikales N.M., Overberger C.G., Menges G., Kroschwitz J.I. **Encyclopedia of Polymer Science and Engineering.** Vol. 13, New York : A Wiley – Interscience Publication John Wiley & Sons. 1988. pp. 673-680.

[27] Tsubouchi K. "New Applications of Silk" Farming Japan, vol. 30-5, 1996. pp. 36-41.

[28] Fredmand D., Barbalata A. **Synthetic Polymers.** New York : CHAPMAN & HALL. 1996.

[29] Mark H.F., Bikales N.M., Overberger C.G., Menges G., Kroschwitz J.I. **Encyclopedia of Polymer Science and Engineering.** Vol. 7, New York : A Wiley – Interscience Publication John Wiley & Sons. 1988. pp. 783-802.

[30] Siedle A.H., Adams L. **Handbook of Radiation Effects.** New York : Oxford University Press. 1993. pp. 280-283.

[31] Woods R.J., Pikaev A.K. **Applied Radiation Chemistry** : **Radiation Processing.** New York : John Wiley & Sons,Inc. 1994. pp.1-55.

[32] Fried J.R. Polymer Science and Technology. New Jersey : Prentice – Hall, Inc. 1995. pp. 49-50.

APPENDICES

Appendix A %Gel fraction

Table A1 %Gel fraction of hydrogel prepared from 1% silk solution blend with 3% low molecular weight PVA solution at ratio of silk/PVA 1:3, 3:1

Irradiation dose (kGy)	%Gel fraction (%)	
	S/P 1:3 (S1% : P3%)	S/P 3:1 (S1% : P3%)
30	3.1124	2.1486
40	3.7164	2.3591
50	4.4195	2.6413

Table A2 %Gel fraction of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3, 3:1 and 10% high MW PVA

Irradiation dose (kGy)	%Gel fraction (%)			
	PVA 10%	Silk 1%	S/P 1:3 (S1% : P10%)	S/P 3:1 (S1% : P10%)
10	8.3074	0.7360	5.4700	-
20	8.6649	1.4567	6.4519	3.1645
30	9.9347	1.5678	6.4632	3.5740
40	10.0550	2.0468	6.8083	4.3677
50	10.1052	2.3239	7.1617	4.7658

Table A3 %Gel fraction of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3, 3:1 and 3% high MW PVA

Irradiation dose (kGy)	%Gel fraction (%)			
	PVA 3%	Silk 1%	S/P 1:3 (S1% : P3%)	S/P 3:1 (S1% : P3%)
10	2.9450	0.7360	2.0514	-
20	5.6856	1.4567	3.3639	1.9104
30	7.2418	1.5678	4.3570	2.3366
40	8.1990	2.0468	5.3338	3.0046
50	10.0814	2.3239	6.3089	3.2705

Table A4 %Gel fraction of hydrogel prepared from 3 % silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:1, 1:3, 3:1 and high MW PVA10%

Irradiation dose (kGy)	%Gel fraction (%)				
	PVA 10%	Silk 3%	S/P 1:1 (S3% :P10%)	S/P 1:3 (S3% : P10%)	S/P 3:1 (S3% : P10%)
10	8.3074	0.8540	4.6163	5.8618	3.5367
20	8.6649	1.5678	5.7590	7.7871	4.6506
30	9.9347	1.5891	7.1971	8.4515	5.6515
40	10.0550	2.6401	7.3004	8.7519	5.8227
50	10.1052	3.2530	8.1251	9.2271	5.3925

Table A5 %Gel fraction of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:1,1:3 and high MW PVA3%

Irradiation dose (kGy)	%Gel fraction (%)			
	PVA 3%	Silk 3%	S/P 1:1 (S3% : P3%)	S/P 1:3 (S3% : P3%)
10	2.9450	0.8540	-	-
20	5.6856	1.5678	2.5196	2.5827
30	7.2418	1.5891	3.6591	3.0486
40	8.1990	2.6401	3.7654	3.9447
50	10.0814	3.2530	3.6027	5.5346

Appendix B %Water absorption

Table B1 %Water absorption of aggregates received from 1% silk solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (x 0.01)							
	Hour							
	1	2	3	4	5	6	7	8
10	1.5349	1.4986	1.3084	1.5264	1.4698	1.5236	1.4987	1.5021
20	1.8017	1.5628	1.4888	1.7933	1.7512	1.7456	1.7563	1.7968
30	1.8691	1.7456	1.6839	1.9616	1.9874	1.9845	1.9652	1.9645
40	1.8945	1.9017	1.9845	2.1045	2.1456	2.1364	2.2658	2.3147
50	1.9546	1.9623	1.9987	2.3849	2.3834	2.3845	2.3968	2.4165

Table B2 %Water absorption of aggregates receive from 3% silk solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (x0.01)							
	Hour							
	1	2	3	4	5	6	7	8
10	0.8783	0.8846	0.8900	0.8913	0.9779	0.9987	1.0235	1.0248
20	1.4028	1.4169	1.4258	1.4398	1.4583	1.5464	1.5787	1.6574
30	1.9687	1.9789	1.9876	1.9925	2.0136	2.0498	2.0598	2.1647
40	1.8142	1.8589	1.9674	1.9785	1.9825	1.9945	2.0145	2.0398
50	1.3039	1.3096	1.3891	1.4023	1.4189	1.5414	1.5571	1.5697

Table B3 %Water absorption of 3% high MW PVA solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (x0.01)							
	Hour							
	1	2	3	4	5	6	7	8
10	6.4989	6.685	7.0000	7.2254	7.2356	7.2677	7.3123	7.3233
20	2.5012	3.0157	3.8886	4.4501	4.5871	4.7895	5.2575	5.2564
30	1.5474	2.5694	3.0072	3.7299	4.6980	6.2456	6.5214	6.5216
40	2.6064	3.4785	5.0869	6.1628	7.3284	7.5984	8.2364	8.2366
50	2.4188	3.4698	4.6445	5.7519	5.9687	6.3548	6.9874	7.0156

Table B4 %Water absorption of 10% high MW PVA solution after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time.

Irradiation dose (kGy)	%Water absorption (x 0.01)							
	Hour							
	1	2	3	4	5	6	7	8
10	2.3667	2.696	2.7891	2.8372	2.9001	2.9139	2.9345	2.9598
20	0.5156	0.6852	0.7498	0.9695	1.1620	1.2299	1.3611	1.3687
30	0.6391	0.8874	1.3454	1.3471	1.6266	1.7212	1.8630	1.9298
40	0.2186	1.3800	2.4677	2.9078	3.4330	3.5349	3.6165	3.7149
50	2.3693	3.0661	3.5789	4.0088	4.3325	4.5161	4.6048	4.6052

Table B5 %Water absorption of blend hydrogel prepared from 1% silk solution and 3% low molecular weight PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
30	9.3278	9.6321	10.6888	10.9630	11.4592	11.7414	11.7462	11.7465
40	11.1846	11.7456	12.5862	13.0698	13.3404	13.5339	13.5364	13.5340
50	10.9408	11.6523	11.9152	12.1776	12.6848	12.1776	12.1775	12.1777

Table B6 %Water absorption of hydrogel prepared from 1% silk solution and 3% low molecular weight PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
30	4.3385	4.3096	4.1500	4.3674	4.4231	4.5308	4.5487	4.5480
40	5.7955	6.0147	6.3841	6.5874	6.9659	7.2841	7.2854	7.2850
50	5.4847	5.8725	6.0918	6.6584	7.4184	7.4256	7.4254	7.4369

Table B7 %Water absorption of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
10	4.3788	4.7103	4.9554	5.0236	5.2981	5.3014	5.3056	5.3057
20	8.2493	8.9265	9.3256	9.6800	10.0489	10.0539	10.0695	10.0610
30	9.8322	11.1058	11.5690	12.2097	12.7302	12.8847	12.8845	12.8865
40	8.1911	9.6804	10.3690	11.0153	11.5673	11.8379	11.8465	11.8479
50	5.0253	7.3208	8.3694	9.4633	10.0419	10.3051	10.3053	10.3052

Table B8 %Water absorption of hydrogel prepared from 1% silk solution and 3% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
20	5.5356	5.2164	5.5698	5.7546	5.9820	6.0686	6.0688	6.0687
30	7.0030	7.7302	8.2345	8.7556	8.7544	8.7524	8.7524	8.7546
40	7.0694	7.6065	7.9687	8.4653	8.4785	8.4864	8.4889	8.4888
50	7.1859	7.7644	8.2654	8.6728	8.6971	8.7696	8.7699	8.7698

Table B9 %Water absorption of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
20	4.4567	4.6325	4.9035	5.1535	5.1798	5.2569	5.2574	5.2598
30	6.9167	7.8546	8.0104	8.2479	8.3654	8.4965	8.5987	8.5999
40	10.6741	10.8457	11.2849	11.8295	11.9875	11.9987	12.1236	12.1298
50	7.2249	8.5647	9.5111	10.247	10.5647	10.9547	11.2547	11.2546

Table B10 %Water absorption of hydrogel prepared from 3% silk solution and 3% high MW PVA solution at ratio of silk/PVA 1:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
20	2.2980	2.3640	2.5397	3.3245	3.3385	3.4857	3.5120	3.5410
30	6.0932	6.2854	6.4986	7.1014	7.1256	7.1254	7.1369	7.1365
40	5.5698	5.6398	5.7687	6.2095	6.3257	6.3216	6.3266	6.3345
50	8.6516	8.7412	8.8728	9.9335	9.9354	9.9334	9.9345	9.9452

Table B11 %Water absorption of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
10	2.3043	2.7950	2.8015	3.0124	3.0131	3.0522	3.0532	3.0546
20	3.1023	3.6201	3.6789	3.7584	3.9565	3.9672	3.9600	3.9671
30	2.6328	3.0339	3.5329	3.7846	3.9081	3.9601	3.9781	3.9782
40	2.6584	3.4998	3.8560	4.0285	4.2908	4.3896	4.3890	4.3845
50	2.4089	3.1944	3.5334	3.9654	4.1342	4.2024	4.2030	4.3965

Table B12 %Water absorption of hydrogel prepared from 1% silk solution and 10% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
20	2.7144	3.4736	3.1697	3.1987	3.2889	3.4083	3.4156	3.4196
30	5.2794	6.4903	6.3506	6.5284	6.8021	6.8145	6.8263	6.8264
40	8.5232	10.2408	10.5423	10.7856	11.3250	11.262	11.324	11.3269
50	9.4378	10.6526	10.5848	10.9632	11.2949	11.2965	11.295	11.2951

Table B13 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:3 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
10	0.7839	1.0404	1.2089	1.4482	1.6215	1.7709	1.7856	1.7895
20	0.8432	0.9436	1.1437	1.4422	1.6099	1.7261	1.7325	1.7368
30	2.4698	2.5987	2.6945	2.7894	2.9258	3.2412	3.1456	3.2356
40	3.2789	3.3057	3.3396	3.7269	3.9125	4.0428	4.0254	4.0362
50	3.2824	3.6868	4.0639	4.4647	4.7044	4.8682	4.9586	4.9547

Table B14 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 3:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time.

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
10	1.6342	1.6838	1.6985	1.7428	1.7802	1.7811	1.7955	1.7958
20	3.4343	3.496	3.6295	3.7902	3.8145	3.8195	3.8247	3.8365
30	3.4712	3.6613	3.8707	4.0454	4.0823	4.2190	4.1269	4.2258
40	3.6572	3.9314	4.1898	4.3355	4.3889	4.4225	4.4365	4.4358
50	3.9428	4.2841	4.6533	4.9757	5.0991	5.1369	5.1205	5.1873

Table B15 %Water absorption of hydrogel prepared from 3% silk solution and 10% high MW PVA solution at ratio of silk/PVA 1:1 after irradiated at 10,20,30,40 and 50 kGy as a function of soaking time

Irradiation dose (kGy)	%Water absorption (%)							
	Hour							
	1	2	3	4	5	6	7	8
10	2.4385	2.4987	2.5113	2.5013	2.6111	2.5127	2.6833	2.7211
20	2.2439	2.6369	2.7444	2.8551	2.9243	2.9738	2.9000	2.9900
30	1.1495	1.6772	1.9812	2.1415	2.6620	2.5078	2.6265	2.6539
40	2.7438	3.5669	4.0103	4.4922	4.9340	5.0135	5.0097	5.2000
50	1.2847	3.5938	4.6566	5.5928	6.1100	6.3335	6.4201	6.5200

Appendix C Gel Strength

Table C1 Gel strength of hydrogel prepared from 1%silk solution, 3%silk solution blend with 3%PVA solution at ratio of silk/PVA 3:1,1:3,1;1 and 3% high MW PVA

Irradiation dose (kGy)	Gel streng (MPa)				
	PVA 3%	S/P 1:3 (S1% : P3%)	S/P 1:3 (S3% : P3%)	S/P 1:1 (S3% : P3%)	S/P 1:3 (L) (S1% : P3%)
10	0.1702	-	-	-	-
20	0.1702	0.2939	-	-	-
30	0.1665	0.2949	0.1737	0.1592	0.1665
40	0.1650	0.2958	0.1687	0.1665	0.1657
50	0.1607	0.2967	0.1607	0.1592	0.1578

Table C2 Gel strength of hydrogel prepared from 1%silk solution blend with 10%PVA solution at ratio of silk/PVA 3:1,1:3 and 10% high MW PVA

Irradiation dose (kGy)	Gel strength (MPa)		
	PVA 10%	S/P 1:3 (S1% : P10%)	S/P 3:1 (S1% : P10%)
20	0.4282	0.3136	0.2973
30	0.3155	0.3039	0.2962
40	0.2817	0.3002	0.2912
50	0.2711	0.2956	0.2909

Table C3 Gel strength of hydrogel prepared from 3%silk solution blend with 10%PVA solution at ratio of silk/PVA 3:1,1:3,1:1 and 10% high MW PVA

Irradiation dose (kGy)	Gel strength (MPa)			
	PVA 10%	S/P 1:3 (S3% : P10%)	S/P 3:1 (S3% : P10%)	S/P 1:1 (S3% : P10%)
20	0.4282	1.6446	0.4768	0.1890
30	0.3155	0.3516	0.2844	1.3405
40	0.2817	0.3722	0.1723	0.8974
50	0.2711	0.2589	0.1661	0.4183

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