# THESIS APPROVAL

## GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Chemistry)
**DEGREE**

Chemistry
**FIELD**

Chemistry
**DEPARTMENT**

**TITLE:** Sericin Recovery from Silk Cocoon Degumming Wastewater by Membrane Process

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THESIS

# SERICIN RECOVERY FROM SILK COCOON DEGUMMING WASTEWATER BY MEMBRANE PROCESS

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Chemistry)
Graduate School, Kasetsart University
2009

Tatcha Sonjui 2009: Sericin Recovery from Silk Cocoon Degumming Wastewater by Membrane Process. Master of Science (Chemistry), Major Field: Chemistry, Department of Chemistry. Thesis Advisor: Associate Professor Cholticha Noomhorm, Ph.D. 91 pages.

Flat sheet asymmetric polymeric membranes were prepared from the homogeneous solution of polysulfone (PSF) in N-methyl pyrrolidone by phase inversion process with the various concentration and molecular weights of poly(dioxolane) (PDXL) ($\overline{M}_n$ = 10,000 Da and 200,000Da) and polyethylene glycol (PEG) ($\overline{M}_n$ = 300, 2000, 4600 and 10,000 Da) as the polymeric additives. Surface morphology of membranes was investigated. The performance of all the prepared PSF membranes was used to recover sericin from the silk cocoon degumming wastewater. The effect of polymeric additives and transmembrane pressure (200, 300 and 400 kPa) was studied in term of permeate flux (PF), protein retention, solid retention and molecular weight cut-off (MWCO) of sericin retention. The results showed that permeate flux and molecular weight cut off (MWCO) of the permeate increases but protein retention and solid retention decreases with increasing concentration, molecular weight and transmembrane pressure of both polymeric additives. At 200 kPa with the same 2% concentration of PEG and PDXL, the steady state permeate flux was increased in PSF/NMP/PEG300 to PSF/NMP/PEG10,000 from 9.7 $l/m^2h$ to 19.5 $l/m^2h$ and in PSF/NMP/PDXL10,000 to PSF/NMP/PDXL200,000 from 22.4 $l/m^2h$ to 28.5 $l/m^2$ h but the percent protein retention was decreased from 75.4% to 60.8% and from 60.6% to 52.4%, respectively. Hence it clearly revealed that the morphological parameters and flux performance of the membranes have a significant inter-relationship with the concentration, molecular weight and transmembrane pressure of both polymeric additives.

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# ACKNOWLEDGEMENTS

I would like to gratefully thank and give appreciation to my advisor, Associate Professor Cholticha Noomhorm, Ph.D., for her valuable recommendations, helpful discussions, encouragement and kindness throughout the course of my graduate study at Kasetsart University. I would also like to thank Assistant Professor Amornrat Promboon, Ph.D., my co-advisor; Assistant Professor Nantana Jiratumnukul, Ph.D. and Pinsuda Viravathana, Ph.D. from the representative Graduate School; for their valuable comments and suggestion.

I wish to acknowledge the Department of Chemistry, Faculty of Science, Kasetsart University for making this research program possible. I would like to thank Kasetsart University Research and Development Institute for financial support of this research.

I would like to thank my friends and staffs at the Department of Chemistry and Biochemistry, Faculty of Science, Kasetsart University for their help during this study.

Finally, I am especially appreciative to my family for their support and encouragement throughout my graduate career. Their love and companionship have been an essential source of strength to me during this course.

Tatcha Sonjui
May 2009

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# LIST OF ABBREVIATIONS

APS = Ammonium persulfate
BSA = Bovine serum albumin
DW = Distilled water
IFM = Infinite focus microscopy
MWCO = Molecular weight cut off
NMP = N-methyl-2-pyrrolidone
PDXL = Poly(dioxolane)
PEG = Poly(ethylene glycol)
PF = Permeate flux
PSF = Polysulfone
SDS = Sodium dodecyl sulfate
SDS-PAGE = Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM = Scanning electron microscopy
TEMED = Tetramethylethylene diamine
Tris = Tris(hydroxymethyl)aminomethane

# SERICIN RECOVERY FROM SILK COCOON DEGUMMING WASTEWATER BY MEMBRANE PROCESS

## INTRODUCTION

Thailand is well known for the traditional production of silk which has been carried on for generations, making Thai silk a prestigious symbol of the country internationally. Silk is a natural protein fiber produced from silkworm (*Bombyx mori*) cocoon. It consists of 70-80% of two bundles fibrous protein called fibroin and 20–30% of amorphous matrix of water soluble globular protein called sericin which functions as a gum to bond the two fibroin filaments together. In silk production industry, sericin must be removed and discarded as waste from cocoon cooking process during drawing silk fiber from the cocoon or reeling and silk degumming process or scouring after the silk yarns are produced prior to dyeing to gain shiny aspect and soft characteristic. During the last decade, sericin has emerged as a valuable commercial resource in many industries such as in cosmetics and pharmaceuticals, in food, and in production of many functional biomaterials. The cosmetics industry is using sericin in skin care products such as skin moisturizing and antiwrinkle action possibly due to its high content of hydroxyl-amino acid, serine, for water-binding capacity that keeps the skin moist and its composition of amino acids is very similar to that of skin natural moisturizing factor (Voegeli *et al*., 1993). It has been reported that consumption of sericin elevated intestinal absorption of zinc, iron, magnesium and calcium in rats; therefore it can be a valuable natural ingredient in food industry (Sasaki *et al*., 2000). Dietary sericin also suppresses the development of colon tumorigenesis by significantly reducing the number of colon adenomas (Zhaorigetu *et al*., 2001). Immunohistochemical analyses showed that sericin possesses photoprotective effect against UVB-induced skin damage and tumor promotion by reducing oxidative stress (Zhaorigetu *et al*., 2003). Studies on the mechanism of antioxidant action of sericin showed that sericin can suppress lipid peroxidation and inhibiting the tyrosinase activity which is responsible for the browning reactions of various foods and promotes biosynthesis of melanin (Kato *et* *al*., 1998). Sericin can be cross-linked, copolymerized and blended with other polymers to produce a new range of biodegradable materials with improved properties (Zhang, 2002; Kongdee and Chinthawan, 2007). In addition, sericin can also be used as improving reagent or a coating material for natural and synthetic fibers, fabrics. Moreover, sericin has also been found to be useful as a degradable biomaterial, biomedical material and polymers for forming articles, functional membranes (Zhang 2002; Altman *et al*., 2003; Cho *et al*., 2003; Gimenes *et al*., 2007). Use of sericin as a finishing agent for natural or man made textiles enhances moisture absorption, antistatic properties, softness and comfort (Lee *et al*., 2004; Miyake *et al*., 2004). Filters made of polyamide or polyester fibers coated with sericin have antioxidation and antimicrobial activity, suggesting their potential use as indoor air filters to reduce free radicals and fungi or bacteria contamination (Sarovart *et al*., 2003). Hence, recovery of sericin not only can reduce the environmental impact but also can generate revenue as a natural value added material in silk production.

The total annual worldwide cocoon production was about 1 million metric tons, corresponding to around 125,000 metric tons of raw silk (Baker, 2004). This was equivalent to about 400,000 metric tons of dry cocoon and about 50,000 metric tons of sericin mainly from the degumming processing, were discarded as waste each year (Zhang, 2002). At present, most of sericin has been recovered extensively from silk degumming process (Vaithanomsat and Kitpreechavanich, 2008), little attention has been directed toward the recovery of sericin from the dry cocoon wastewater although it accounts for a lot more of sericin (Capar *et al*., 2008). Various methods which are based on adsorption, precipitation, coagulation, evaporation, chromatography and membrane separation have been proposed to recover sericin from degumming wastewater. Simple extraction sericin from silk wastewater by ethanol was suggested by Wu *et al.,* (2007) where 90% ethanol concentration provided 71% extraction yield for high molecular weight sericin. The method of ethanol extraction has been widely used for extracting sericin from cocoon in lab-scale applications. However it does not seem to be an environmentally friendly method at industrial scale, since a final ethanol concentration of 75% is required for precipitating sericin.

Membrane process is a technique that permits concentration and separation without use of heat and has emerged as one of the fastest growing process in recovery of valuable materials from industrial wastewater today. Particles are separated on the basis of their molecular size and shape with the use of pressure and specially designed semi-permeable membranes. Membrane can be defined essentially as a barrier, which separates two phases and restricts transport of various chemicals in a selective manner. A membrane can be homogenous or heterogeneous, symmetric or asymmetric in structure, can be solid or liquid, can carry a positive or negative charge, and can be neutral or bipolar. Transport through a membrane can be affected by convection or by diffusion of individual molecules, induced by an electric field or concentration, pressure or temperature gradient. The membrane thickness may vary from as small as hundred microns to several millimeters.

Phase inversion process is the most widely used process for preparing asymmetric polymeric membranes, in which a homogeneous polymer solution of two or more components was cast onto a suitable support as a thin film and then immersing into a coagulation bath containing a suitable nonsolvent (usually miscible with the solvent but immiscible with the polymer) and solvent in the casting solution film is exchanged with non-solvent and phase separation occurs in the casting film (Lloyd, 1985; Mulder, 1996; Zemand and Zydney, 1996; Cheryan, 1998). Morphology of the asymmetric membranes prepared via the phase inversion process generally shows a characteristic of a dense top layer and microporous sublayer. These membranes are widely used today in various applications such as microfiltration, ultrafiltration, and reverse osmosis (Munari *et al*, 1988; Mulder, 1996) because the thin top layer plays a role of selective barrier film for solute while the porous sublayer that includes macrovoids and microporous offers excellent mechanical strength to membrane (Kim *et al.*, 2005). Membranes prepared by phase inversion process involve formation of a two phase system: one solid polymer rich phase and the other liquid polymer poor phase. The polymer rich phase forms the membrane structure while the polymer poor phase forms the membrane pores. Membrane formation is best described by mass transfer phenomena occurring during the immersion step (Reuvers *et al.*, 1987a). Two types of demixing are taking place during the phase inversion process: instantaneous demixing and delayed demixing. Membranes formed by instantaneous demixing generally show a highly porous structure (with macrovoids) and a finely porous, thin skin layer. Membranes formed by delayed demixing show a porous (often closed-cell, macrovoid-free) substructure with a dense, relatively thick skin layer. As a result, structure and properties of membranes prepared by phase inversion method depend upon many factors. Addition of small amount of additive into the casting solution is one of these major factors. It is reported that additives can affect the final membrane characteristics either by changing solvent capacity or by changing phase separation kinetic and thermodynamic properties. Some additives have the tendency to enlarge macrovoids, others can suppress the formation of macrovoids improving interconnectivity of the pores and resulting in higher porosities in the top layer and sublayer. Thus, by variation of the additive concentration or additive molecular weight, optimal membrane structure can be obtained through enlargement or suppression of macrovoids in the membrane. A number of works on the roll of additives in the membrane structures have been reported in the literatures. For examples, addition of LiCl causes larger macrovoids, and the higher the amount of LiCl is added, the larger macrovoid is formed (Lee *et al*., 2002). It is found that the macrovoids are suppressed when organic acids such as propionic acid is added, because the acid form acid-base complex with the basic polar solvents such as N-methyl pyrrolidone (NMP), N-methylformamide (DMF), N-methylacetamide (DMAc) (Han, 1999). Polymer is another important additive for controlling the structure of membranes. Poly(vinyl pyrrolidone), PVP, and poly(ethylene glycol), PEG, are the two most important polymeric additives used in study the membrane performances because they are miscible with most of the membrane materials and are soluble in both aqueous and many organic media. It has been concluded that adding polymeric additive on the formation of phase inversion membrane is accounted for the formation of micropores, porosity increasing and the macrovoid formation disappearance (Jung *et al*., 2004). For examples, using PVP as additive in the formation of poly(ether sulfone), PES, membrane in NMP as solvent showed that PVP suppresses the formation of macrovoids by reducing the possibility of delayed demixing (Boom *et al*., 1992), while adding PVP in the casting solution of PES and DMF indicated the enlargement of the macrovoids structure (Yeo *et al.*, 2000). It was also found that higher molecular weight of PVP was added on polyetherimide hollow fiber membrane; the bigger pore was made (Xu *et al*., 1999), etc.

Therefore, selection of materials, such as polymers, additives, and solvent included in the casting solution and nonsolvent, is important in the fabrication of membrane having desired morphology. Polysulfone (PSF) is one of the most widely used materials for fabrication of membranes having selectivity suitable for many applications because of the high thermal resistance, high chemical inertness, excellent mechanical strength, and hydrolytic and biological stabilities (Harris *et al*., 1988). The effects of PEG on the formation of polysulfone membranes have been extensively studied. The effect of various molecular weights of PEG as additives on the thermodynamics and kinetics (Kim and Lee, 2004), on rheology and thermodynamics (Zheng *et al.*, 2006b), and on the morphology and the structure (Chakrabarty *et al*., 2008a, 2008b and 2008c) of polysulfone (PSF) casting solution on membrane formation by induced phase-inversion process were reported. Results showed that molecular weights of PEG do play a significant role on morphological as well as flux properties of the membranes prepared. It showed that with increase in molecular weight of PEG, the pore number as well as pore area in membrane increases.

PEG solubility pattern can be altered by including hydrophobic comonomer in the polymer backbone. Poly(dioxolane), PDXL, is a perfect alternate copolymer of poly(ethylene oxide) and poly(methylene oxide). The solubility of PDXL is much limited than of PEG due to the extra methylene group. At room temperature, PDXL is generally considered as insoluble in water while aqueous PEG solution is transparent. Solubility of PDXL is increased upon heating to destroy the polymer aggregations until it reaches its lower critical solubility temperature (LCST) or cloud point. Phase separation of PDXL and PEG occur at $70^{o}$C (LCST = $70^{o}$C) and $100^{o}$C (LCST = $100^{o}$C), respectively in pure aqueous solutions (Benkhira *et al.*, 1992 and 1994).

In this paper, the asymmetric polysulfone (PSF) membranes were prepared by phase inversion process to recover sericin from the cocoon degumming wastewater. The concentration of two different additives, PDXL and PEG with various molecular weights ($\overline{M}_n$) ranging from 300, 2000, 4,600 to 10,000 Da for PEG and 10,000 to 200,000 Da for PDXL were used to prepare PSF membranes, separately with the NMP solvent. The solvent and additive concentration are varied from 83 and 86 wt%, and 2 and 5 wt% respectively, while the polymer concentration was held constant at 12 wt% which are normally being used for preparation of PSF membranes as reported in the literatures. Effects of molecular weight and concentration of additives, PEG and PDXL, on morphology and the permeation characteristics of the prepared membranes were investigated. Morphology of each membrane was characterized by scanning electron microscopy (SEM). Finally, the membrane performances for recovery of sericin from cocoon cooking wastewater was studied by permeate flux, solid retention, protein retention and molecular weight cut-off (MWCO) of sericin to evaluate the most suitable membrane for sericin recovery. Molecular weight distribution of sericin is between 10,000 and 300,000 Da (Fabiani *et al.*, 1996; Zhang *et al.*, 2004). It has been reported that end use of the sericin depends on the molecular weight. For example, sericin with molecular weight greater than 20 kDa is suitable in making biomaterials and membranes, whereas sericin with molecular weight lower than 20 kDa is suitable for cosmetics, skincare products and medications (Zhang, 2002). Silk production in Thailand is approximately 1,500 metric tons per year (Baker, 2004), which means that at least 300 metric tons of sericin is discarded. The selling price of sericin in Thailand is around 100 Baht per gram. Therefore, development of suitable membranes for recovery of sericin from silk production industry would definitely provide significant benefits for Thailand from the available sericin as a whole.

# OBJECTIVES

1. To prepare membrane by phase inversion method.

2. To study the effect of concentration and molecular weight of polymeric additives on membrane morphology and permeate flux.

3. To recover the sericin protein from silk cocoon degumming wastewater by membrane process.

# LITERATURE REVIEW

## 1. Sericin

### 1.1 Introduction

Silk derived from silkworm *Bombyx mori* is a natural protein that is mainly made of sericin (30%) and fibroin (69%) proteins; and others (1%). Sericin constitutes 25–30% of silk protein and it envelops the fibroin fiber with successive sticky layers that help in the formation of a cocoon. Sericin ensures the cohesion of the cocoon by gluing silk threads together. Most of the sericin must be removed during raw silk production at the reeling mill and the other stages of silk processing.

Like fibroin, sericin is a macromolecular protein. Its molecular weight ranges widely from about 10 kDa to over 300 kDa. The sericin protein is made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups. In addition, the amino acids serine and aspartic acid constitute approximately 33.4% and 16.7% of sericin, respectively. Sericin is a water-soluble protein. Sericin can be degraded when sericin is dissolved in a polar solvent, hydrolyzed in acid or alkaline solutions, or degraded by a protease. The size of the resulting sericin molecules depends on factors such as temperature, pH, and the processing time. Lower molecular weight sericin peptides (≤20 kDa) or sericin hydrolysates are used in cosmetics including skincare and haircare products, health products, and medications. High-molecular weight sericin peptides (≥20 kDa) are mostly used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics. The small sericin peptides are soluble in cold water and can be recovered at early stages of raw silk production. The larger sericin peptides are soluble in hot water and can be obtained at the latter stages of silk processing or from processes for silk degumming. Because of its properties, sericin is particularly useful for improving artificial polymers such as polyesters, polyamide, polyolefin, and polyacrylonitrile. Sericin is also used as a coating or blending material for natural and artificial fibers, fabrics, and polymer articles. This review details the many applications of sericin.

## 1.2 Applications of sericin

### 1.2.1 Biodegradable materials

Environment-friendly biodegradable polymers can be produced by blending sericin with other resins. Polyurethane foams incorporating sericin are said to have excellent moisture-absorbing and -desorbing properties (Nomura *et al*., 1995). Polymer films, foams, molding resins, and fibers containing sericin (0.01–50% w/w) can be produced by reacting a composition comprising a polyol (e.g., a polyether polyol obtained by addition polymerization of glycerol, propylene oxide, and ethylene oxide), tolylene diisocyanate, dibutyltin dilaurate (a catalyst), and trichloromonofluoromethane (a blowing agent) in the presence of sericin. The moisture absorption/desorption rates of the sericin-containing polyurethane foam was two- to fivefold greater than that of control.

Other procedures were also reported for producing sericin-containing polyurethane with excellent mechanical and thermal properties (Hatakeyama, 1996). In one process, sericin powder was first dissolved in an organic solvent such as tetrahydrofuran or dioxane which may contain a polyether or polyester polyol compound. Sericin in the solution was then reacted with a polyisocyanate (including aliphatic, alicyclic, or aromatic polyisocyanate). The polyurethane produced containing biodegradable sericin segments and it was biodegradable. The resulting polyurethane can be made into film, fibers, and molded objects. The polyurethane produced was inexpensive because it contains a significant amount of waste sericin.

Synthetic resin pumice made by mixing an aqueous sericin solution and a synthetic resin were reported (Kabayama, 2000). The resin pumice was prepared as follows: The polyol stock solution was first formed by mixing a foaming agent, a foam-shaping agent, the polyol, a catalyst, a fire retardant and the aqueous sericin solution or sericin powder. The mixture was kneaded. Next, a polyisocyanate stock solution was mixed with the kneaded sericin to initiate a polyaddition reaction. The reaction generated heat and the gas released from the stock solution produced cells characteristic of the foam. Eventually, the foamed fluid solidified into the three-dimensional structure of a rigid urethane foam. The foam may be molded during formation.

In 2007, Wu *et al.* developed a new effective technology for the extraction of sericin from silk wastewater. Sericin was extracted with 75% (v/v) ethanol to obtain crude powder. The chemical composition of sericin powder, including protein, sugar, ash, and amino acid, was assayed in detail. The molecular weight distribution of sericin was also investigated by gel filtration chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and high performance liquid chromatography (HPLC) analytical methods. The results suggested that sericin represented a family of proteins with wide-ranging molecular weight distribution. The conformation changed in the course of ethanol precipitation was studied by circular dichroism (CD) analysis. Data implied that the major conformation of sericin protein was random coil, which decreased slightly after being treated with ethanol, and the appearance of β-turn conformation maybe associated with the packing of molecular chains induced by ethanol. Furthermore, sericin was found to inhibit tyrosinase activity when chlorogenic acid was used as a substrate, and had obvious radical scavenging effects with the 2.2-diphenyl-1-picryl-hydrazil (DPPH) assay. Result suggested that sericin might be a valuable ingredient for food.

After that in 2008, Wu *et al.* reported that bioactive peptides were produced from sericin, a by-product from silk industry wastewater, by using protease P. The optimal hydrolysis conditions (pH of 8.40, temperature of 43.97$^{o}$C and the enzyme/substrate ratio of 3: 100) were estimated by using response surface methodology with a maximum degree of hydrolysis of 14.43% and the minimum of half maximal inhibitory concentration ($IC_{50}$) value for the ferrous ion-chelating activity of 0.128 mg/ml. The freeze dried sericin hydrolysate (SH), produced by using protease P and under the above optimal conditions, contained 82% of crude protein, 13% of moisture and 5% of ash. The physiochemical analysis showed that the amino acids with hydroxyl groups (Serine and Threonine) of SH accounted for 30% or so, and SH was a mixture of hydrophilic peptides mostly distributed among 250–4000 Da. SH exhibited excellent antioxidant activities in two measurements, including the ferrous ion-chelating activity and the reducing power. Moreover, SH also exhibited a notable tyrosinase-inhibitory effect in a dose-dependent manner.

Non-catalytic hydrothermal decomposition of sericin and fibroin from silk waste into useful protein and amino acids was examined in a closed batch reactor at various temperatures, reaction times, and silk to water ratios to examine their effects on protein and amino acid yields (Lamoolphak *et al.*, 2008). For the decomposition of sericin, the highest protein yield was found to be 0.466 mg protein/mg raw silk, obtained after 10 min hydrothermal reaction of silk waste at 1: 100 silk to water ratio at 120$^{o}$C. The highest amino acid yield was found to be 0.203 mg amino acids/mg raw silk, obtained after 60 min of hydrothermal reaction of silk waste at 1: 20 silk to water ratio at 160$^{o}$C. For the hydrothermal decomposition of fibroin, the highest protein yield was 0.455 mg protein/mg silk fibroin (1: 100, 220$^{o}$C, 10 min) and that of amino acids was 0.755 mg amino acids/mg silk fibroin (1:50, 220$^{o}$C, 60 min). The rate of silk fibroin decomposition could be described by surface reaction kinetics. The soluble reaction products were freeze-dried to obtain sericin and fibroin particles, whose conformation and crystal structure of the particles were shown to differ from the original silk materials, particularly in the case of fibroin, in which the change from b-sheet conformation to a-helix/random coil was observed.

### 1.2.2 Membrane materials

Membrane-based separations (e.g., reverse osmosis, dialysis, ultrafiltration, microfiltration) are widely used in processes such as desalination of water, production of extremely pure water, the bioprocessing industry and some chemical processes. Sericin and fibroin can be used to make membranes for use in separation processes. Mizoguchi *et al.* (1991) described a cross-linked thin film made of sericin for use as a separating membrane for water and ethanol. The membrane was made by mixing hydrochloric acid, aqueous solution of a cross-linking agent such as formaldehyde, aqueous solution of thermally reactive water-soluble urethane resin copolymer, and aqueous sericin. The resulting solution was spread on a smooth plate such as a glass plate at room temperature and allowed to stand at room temperature to obtain the cross-linking with formaldehyde. The resulting film was treated with hot air at ~120°C for 10 min to cross-link the urethane. A cross-linked thin film (about 130 mm thick) was formed and used to separate a mixture of water and alcohol. The sericin membrane could effectively separate the alcohol from the mixture. The membrane could be reused.

Acrylonitrile used in making certain synthetic polymers can be copolymerized with sericin to prepare a protein-containing synthetic polymer film for separating water from organics (Yamada and Fuwa, 1993a). To make the membrane, 30 ml of 10% aqueous sericin solution was added to a mixture of acrylonitrile (10 g), calcium peroxydisulfide (3 g), sodium lauryl sulfate (0.15 g), and distilled water (300 ml). The reaction mixture was maintained at 60°C for 4 h. The copolymer precipitate formed was collected by filtration, washed repeatedly with water, and dried. The copolymer was then dissolved in 5 ml of dimethyl sulfoxide at 80°C. The solution was used to cast a membrane (30 mm thick) on a plate held at 80°C for 2 h.

Yamada and Fuwa (1993b) also prepared a membrane from sericin. This membrane was capable of resolving racemic mixtures. The filter membrane had a three-dimensional reticulated structure that was obtained by mutually bonding and cross-linking sericin, a water-soluble epoxy compound, and a cross-linking agent. Three kinds of reaction were involved in forming the membrane. First, the epoxy compound copolymerized with the hydroxyl, amino, and carboxyl groups of sericin. Second, the epoxy compound copolymerized with the carboxyl group of sericin in the presence of glutaraldehyde. Third, hydroxyl, amino, and carboxyl groups of sericin were copolymerized with the glutaraldehyde. Sericin solution (10% sericin) was mixed with diglycidyl ether (0.38 M), diethylenetriamine (0.14 M), and distilled water, and the mixture was cast on a plate and allowed to stand for 48 h at room temperature. Then the temperature was raised to 85°C for 2 h. The sericin membrane obtained was immersed in a mixture of glutaraldehyde (0.1%), sulfuric acid (1%), and sodium sulfate (20%) for 24 h. The resulting cross-linked filter membrane could resolve racemic mixtures. This ability was apparently associated with the chiral conformation of the amino acids residues in sericin.

Hirotsu and Nakajima (1998) reported that an insolubilized silk fibroin membrane could be used to preferentially remove water from a mixture of water and alcohol. Pure sericin is not easily made into membranes, but membranes of sericin cross-linked, blended, or copolymerized with other substances are made readily. Because sericin contains a large amount of amino acids with neutral polar functional groups, sericin-containing membranes are quite hydrophilic. Sericin composite membranes are permselective for water in an aqueous-organic liquid mixture.

The gel material produced by mixing agar or agarose with sericin of 20 kDa average molecular weight can separate ether–alcohol mixtures (Yoshikawa *et al.*, 2001a). To form the gel, sericin powder is mixed with agar or agarose, sodium azide, and deionized water at 60°C, and the solution is cast on a plate for 24 h at room temperature. The agar/agarose-sericin film is a porous gel film that absorbs water. The film contains from 0.1% to 60% sericin and can withstand pressures of 0.01–2 kgf/cm$^2$. The agar/agarose-sericin gel film can be used for separating methyl butylether (MTBE) from a mixture of MTBE and alcohol. Blended gel films made of agar/agarose and sericin or polyoxyethylene and hydroxyalkyl cellulose are also good separating materials for ether–alcohol mixtures, especially mixtures of MTBE and methanol (Yoshikawa *et al.*, 2001b).

Hydrophilic membranes were prepared from sericin and poly(vinyl alcohol) (PVA) for applications in alcohol dehydration by pervaporation (Marcelino *et al.,* 2007). Sericin/PVA blend membranes were prepared by blending sericin and PVA, followed by chemical crosslinking with dimethylolurea. The permeation of water/ethanol mixtures through the membranes was investigated. It was shown that the blend membranes are preferentially permeable to water. In the temperature range of 50–70°C, a permeate water concentration of 93.1–94.1 wt% was achieved at 8.5 wt.% water in the feed, which corresponded to a separation factor of ca. 145–172. As a comparison, membranes were also fabricated from pure sericin and PVA alone, and tested for pervaporation separation under the same conditions. In addition, to elucidate the permselectivity of the membrane, sorption/desorption studies were carried out as well as to determine the sorption isotherms for both permeating components. It was shown that membrane permselectivity ethanol to water was primarily derived from its sorption selectivity, and there was a strong coupling effect for the permeation and sorption of permeate in the membranes.

### 1.2.3 Functional biomaterials

In 1978, Miyairi and Sugiura reported a cross-linked sericin film for enzyme immobilization with glutaraldehyde as the cross- linking agent. The heat stability, the electroosmosis resistance, and the stability of the immobilized b-glucosidase on the cross-linked sericin film were higher than for the free enzyme. However, the activity of the immobilized preparation was low. Several other authors have since used cross-linked sericin film for enzyme immobilization.

Sericin blends well with water-soluble polymers, especially with polyvinyl alcohol (PVA). Ishikawa *et al.* (1987) investigated the fine structure and the physical properties of blended films made of sericin and PVA. The high-molecular weight sericin was extracted by boiling the cocoon shell (silk protein) in water for 30 min. The extracted sericin was mixed with PVA (91 kDa) and the mixture was cast on a plastic plate and dried at room temperature for 24 h. Thermal analysis, X-ray diffraction, and electronic microscopy showed that the membrane (50 mm thickness) formed had a microphase-separated structure. The interfacial region between the two phases consisted of PVA–sericin complex. The membrane had good fracture strain and showed little elongation at elevated temperature. The film with 10–30% sericin had good thermal and mechanical properties.

Asakura *et al.* (1992) and Demura *et al.* (1992) reported coating glucose oxidase (GOD) on non-woven fibroin fabrics using sericin and/or fibroin aqueous solutions. When sericin alone was used for the GOD coating, a high activity of the immobilized enzyme was obtained but some leakage of sericin from the coated layer occurred. On the other hand, when GOD was coated on non-woven silk fibroin fabric using a mixture of sericin and fibroin and treated with 80% methanol, the insolubilization of the coated layer was markedly improved compared with the use of sericin alone.

Nakajima (1994) has found that sericin film located on the layer of a liquid crystal can uniformly orient the liquid crystal molecules to provide distortion-free high-quality liquid crystal displays. The sericin film was prepared as follows: Sericin powder which was recovered by extraction of silkworm cocoon, silk fiber, or raw silk in boiling water for 2 h; was washed with benzene and ethanol (2:1 by volume) to remove lipid compounds. The purified sericin powder was dissolved in water and filtered. The filtered solution was smeared on a transparent plate and dried while spinning the plate at 100–5000 rpm.

Iwamoto *et al.* (1995) prepared mixed membranes of sericin and syndiotactic PVA, and investigated their physical properties and ability to immobilize an enzyme. The two components of the mixed membranes seemed to be well blended when the sericin content was 30% or less. The coil-to-β transition of sericin occurred when the blended film was soaked in methanol or stretched (especially in the latter case), and this caused the separation of the two components. The blend films had enough mechanical strength for use in immobilizing GOD. The immobilization of the enzyme was the result of wrapping up of the enzyme molecules by sericin; however, the immobilized GOD and sericin slowly leaked from the carrier membrane, especially in the stretched membrane. This leakage could be prevented by coating the membranes with the PVA. The enzyme activity immobilized in the mixed membrane could be maintained for long periods; the decrease of activity in a coated membrane was less than 20% after about 8 months of storage.

Sericin protein can be coated on surfaces of various durable materials to enhance functionality (Li, 1996). Sericin can be used in preparation of art pigments and for surface protection of articles. The materials coated with sericin have excellent weatherability, good permeability, and do not warp on drying.

A blended hydrogel made of sericin or fibroin and PVA is said to have excellent moisture adsorbing and desorbing properties and elasticity. The gel was produced by dissolving 15 g PVA in 70 ml water at elevated temperature. The solution was cooled to room temperature and mixed with 15 g sericin powder with stirring. The resulting solution was irradiated at 40 kGy to form the hydrogel. The hydrogel can be used to culture seeds, as a soil conditioner, and in medical materials and wound dressings. Wang *et al*. (1998) investigated the structures and physical properties of PVA/sericin blended hydrogel membranes. Also, a recent patent reported on a PVA/sericin cross-linked hydrogel membrane produced by using dimethyl urea as the cross-linking agent (Nakamura and Koga, 2001). The polymeric hydrogel had a high strength, high moisture content, and durability for use as a functional film.

Also, sericin-coated film is used on the surfaces of refrigeration equipment because of its antifrosting action (Tanaka, 2001). Use of the coated sericin film is an effective antifrosting method that can be widely applied to refrigerators, deep freezers, and refrigerated trucks and ships. Moreover, use of the coated film on roads and roofs can prevent frost damage and ease snow removal.

### 1.2.4 Medical biomaterials

Film made of sericin and fibroin has excellent oxygen permeability and is similar to human cornea in its functional properties. It is hoped that the sericin–fibroin blend film can be used to form artificial corneas (Murase, 1994). For making the film, the silk protein is dissolved in a haloacetic acid such as trifluoroacetic acid, $CF_3COOH$. Fully dissolving 1 g cocoon shell (sericin and fibroin) in 3 ml of 98% $CF_3COOH$ produces a gel-like substance that is poured into molds or formed into a film. The solidified molding is washed repeatedly with water. Similar methods can be used to produce contact lenses, highly elastic artificial blood vessels, and other prostheses.

Silk protein can be made into a biomaterial with anticoagulant properties, by a sulfonation treatment of sericin and fibroin (Tamada, 1997). The product was preferably obtained by adding concentrated sulfuric acid at 10–90% concentration in an amount of 0.5 to 500 folds to the extracted sericin or fibroin and carrying out the sulfonation at a temperature of 20–100$^{o}$C for up to several hours. The resulting anticoagulant is a potential substitute for heparin. The anticoagulant can be used to treat surfaces of medical devices. The sulfonated silk protein anticoagulant has been claimed to interfere with the attachment of the human immunodeficiency virus to immunocytes. Consequently, the anticoagulant can be used in toothpaste and shaving creams to prevent the spread of HIV.

Tsubouchi (1999a) developed a silk fibroin-based wound dressing that could accelerate healing and could be peeled off without damaging the newly formed skin. The non-crystalline fibroin film of the wound dressing had a water content of 3–16% and a thickness of 10–100 mm. Subsequently, the wound dressing was made with a mixture of both fibroin and sericin (Tsubouchi, 1999b). The non-crystalline fibroin–sericin film had a degree of crystallization of less than 10%. The film had a thickness of 10–130 mm and a density of 1100–1400 kg m$^{-3}$. The occlusive dressing had a 10% or greater solubility in water at room temperature and a water absorptivity of 100% or more at room temperature.

A membrane composed of sericin and fibroin is an effective substrate for the proliferation of adherent animal cells and can be used as a substitute for collagen. Tsukada *et al.* (1999) investigated the attachment and growth of animal cells on films made of sericin and fibroin. Cell attachment and growth were dependent on maintaining a minimum of around 90% sericin in the composite membrane. Films of pure component proteins (i.e., fibroin or sericin) permitted cell attachment and growth comparable to that on collagen, a widely used substrate for mammalian cell culture.

A novel mucoadhesive polymer had been prepared by template polymerization of acrylic acid in the presence of silk sericin (Ahn *et al.,* 2001). FT-infrared data indicated that the polymer was a hydrogen-bonded complex of poly(acrylic acid) (PAA) and sericin. The glass transition temperatures of sericin and PAA in the PAA/sericin polymer complex were inner shifted compared with that of sericin and PAA separately. This could be due to the increased miscibility of PAA with sericin because of hydrogen bonding. The dissolution rate of the PAA/sericin interpolymer complex depended on the pH. The mucoadhesive force of the PAA/sericin polymer complex was similar to that of a commercial product, Carbopol 971P NF. Potentially, the PAA/sericin polymer can be used in transmucosal drug delivery (TMD) system.

Kazuhisa *et al*., (2001) reported on the biologically functional properties of sericin at the molecular level. They have found that the sericin-rich repetitive sequence in silk sericin and natural sericin hydrolysate can protect both cells and proteins from freezing stresses. To study the biological functions of sericin, they focused on the sericin-rich sericin peptide consisting of 38 amino acids, which is a highly conserved and internally repetitive sequence of a sericin protein. The corresponding gene was chemically synthesized, and the PCR-amplified gene was ligated to oligomerize sericin peptide and fused at the amino terminus to a His-tagged and proteolytic cleavage sequence in an inducible expression vector. When the dimers of sericin peptides were overexpressed in *Escherichia coli*, the transformants showed increased resistance to damage by freezing. Further, the purified dimeric sericin peptide from *E. coli* was found to be effective in protecting lactate dehydrogenase from denaturation caused by freeze thaw cycles. These protective effects against freezing stress in cells and proteins were also observed with natural sericin hydrolysate. These results indicated that sericin and sericin hydrolysates have important cryoprotective activity and are valuable in numerous applications.

Zhaorigetu *et al.,* (2003) was conducted to assess protective effect of an antioxidant protein, sericin, on ultraviolet b-light (UVB) induced acute damage and tumor promotion in mouse skin. In experiment 1, HR-1 hairless mice were treated with 180 mJ/cm$^2$ of UVB once daily for 1 and 7 days. The treatment for 7 days caused red sunburn lesions of the skin. The intensity of red color and area of these lesions were inhibited by the topical application of sericin at the dose of 5mg after UVB treatment. Immunohistochemical analyses showed that the application of sericin significantly suppressed UVB-induced elevations in 4-hydroxynonenal (4-HNE), expression of cyclooxygenase-2 (COX-2) protein, and proliferating cell nuclear antigen (PCNA)-labeling index in the UVB-exposed epidermis. In experiment 2, HR-1 hairless mice were treated with 200 nmol of 7,12-dimethylbenz [α] anthracene (DMBA) followed 1 week later by irradiation with 180 mJ/cm$^2$ of UVB twice weekly for 22 weeks. The protective effect of sericin was evident in terms of significant reduction in tumor incidence and tumor multiplicity at the dose of 5 mg. The results suggest that sericin possesses photoprotective effect against UVB-induced acute damage and tumor promotion by reducing oxidative stress, COX-2 and cell proliferation in mouse skin.

### 1.2.5 Functional fibers, fabrics and articles

Functional properties of some synthetic fibers can be improved by coating with natural macromolecules such as chitin, chitosan, fibroin, and sericin. Synthetic polyester fibers have micropores of 0.001–10 mm diameter. Sericin molecule can be introduced into these micropores and cross-linked. Yamada and Matsunaga (1994) reported a sericin-modified polyester fiber obtained by cross-linking with glyceryl polyglycidyl ether and diethylene triamine. Wakabayashi and Sugioka (1994) have also prepared sericin-modified polyester fibers. The sericin-modified polyester fiber can be more than five times as hygroscopic as untreated polyesters and more than 85% of initial hygroscopicity remains after 50 washes.

According to Yamada and Nomura (1998), sericin-coated fibers can prevent abrasive skin injuries and the development of rashes. In one study, synthetic and other fibers were coated by sericin by immersing in a 3% aqueous solution of sericin for a given time and drying at 100°C for 3 min. The fabrics woven from the sericin-coated fibers were tested in products such as diapers, diaper liners, and wound dressing. Takai (1999) also prepared a hydrophilic fiber by attaching 0.1–5% sericin on surfaces of a thermoplastic fiber (rayon) and a cellulose fiber (cotton). These sericin-coated fibers were absorbent and did not cause skin rash.

A rubber can be made more biocompatible by blending with sericin. A blend of hydrolyzed sericin (5–50 kDa molecular weight, 0.01–10.0% w/w) in rubber produces a product with reduced irritability to skin than native rubber. This modified rubber can be made into articles such as rubber gloves, bicycle handle grips, and handles for various sport equipment. Powdered sericin with particles smaller than 20 mm in diameter can be blended with a compound rubber (e.g., butadiene or olefin rubber) and thermoplastics (e.g., vinyl acetate resin), and the mixture can be made into an artificial leather product (Ueda and Makita, 2000).

Cotton fibers were modified with silk sericin in a pad-dry-cure process was investigated by Kongdee and Chinthawan (2007). By using glutaraldehyde and dimethyloldihydroxyethylene urea as crosslinking agents, sericin was successfully bound to cotton fibers as seen from IR absorption band at 1515 and 1546 cm$^{-1}$, and 1687 cm$^{-1}$ which corresponded to –NH and –C=O of sericin respectively, and the band at 1747, 1789 and 1750 cm$^{-1}$ indicated –C=O of the regenerated functional groups. The examination of the modified fibers by Scanning electron microscope showed round fibers and deposited sericin on cotton fiber surfaces. The modification of cotton fibers with sericin using these crosslinking agents was clearly proved by dyeing with an acid dye. In sample treated with sericin, the increase in the color strength and b-values, and the decrease in L-values were the proof of the reaction between cellulose and sericin using glutaraldehyde and dimethyloldihydroxyethylene urea as crosslinking agents.

### 1.3 Recovery of sericin by membrane process

Large volumes of waste water containing proteins (mainly sericin), salts and tensioactives are produced in the degumming process (washing) of both yarn and tissues made from natural silk. This waste water bearing a COD above 6000 mg/l are generally treated in biological plants with increasing costs. Sericin represents, on the contrary, a valuable by-product that can be used in cosmetics and pharmaceutical products. To reduce treatment costs and at the same time to recover sericin and reuse process water, hybrid membrane processes were studied by Fabiani *et al.,* (1996). Laboratory and field experiments showed that ultrafiltration of the degumming waste solution with different membranes (with molecular weight cut-off in the 20-30,000 dalton range) allowed recovery of more than 97% of the sericin but the permeate COD level exceeds 800 mg/l whose value was still too high for water reuse. A final reverse osmosis treatment on the ultrafiltration permeates allowed reaching a low COD level (50 mg/L) and recovery of more than 70% of the wastewater for process reuse. Alternative process flow sheets for the silk degumming solution treatment are discussed on the basis of the obtained results.

In 2008, Vaithanomsat and Kitpreechavanich studied to recover of sericin protein produced during silk degumming process. Sericin waste solution from conventional degumming process in Thailand contained approximately 4840 mg/L of BOD, 8870 mg/L of COD and 0.11% of nitrogen content. This indicated protein contamination and required high cost for wastewater treatment. To reduce the treatment costs as well as to recover valuable sericin protein, membrane filtration and enzymatic hydrolysis of recovered sericin were studied. Results showed that the quality improvement of wastewater (BOD, 158 mg/L; COD, 260 mg/L) as well as that an amount of sericin protein with molecular weight 2427–9863 Da were recovered after membrane filtration process. The recovered sericin was further enzymatically hydrolyzed to obtain sericin hydrolysate (average molecular weight 1046–2795 Da), which is mostly suitable for further cosmetics application.

Myung *et al.* (2008) reported the synthesis of the ultrafiltration membranes prepared by an interfacial polymerization and the separation of silk proteins and silk oligopeptides by the membrane. Silk protein is produced in the degumming process from natural silk and it is discarded in silk processing wastewater even though it is a useful biopolymer. The silk proteins are degraded to a small molecular oligopeptide in an enzymatic hydrolysis reaction. Enzymes are immobilized on silica particles. And then, the enzyme-immobilized silica particles were embedded onto chitosan beads. The ultrafiltration membrane with a molecular weight cut-off in the range of 2000–3000 was synthesized by the interfacial polymerization on a porous polysulfone support. The ultrafiltration of the peptide solution allows separation of the small molecular silk oligopeptides from mixtures of high molecular weight silk proteins and low molecular weight oligopeptides. Molecular weight of the separated low molecular silk oligopeptides is lower than 3000 Da. It was observed that by in a membrane-coupled packed bed enzyme reactor, a valuable natural biopolymer could be recovered.

In 2008, Capar *et al.,* described determination of a membrane-based process for sericin recovery from cocoon cooking wastewaters (CCW) that will enable value-added utilization of waste sericin. The iso-electric point (p*I*) of sericin was found as 5–6, whose molecular weight (MW) was distributed as 180–200, 70–80, 30–40 and 10–25 kDa. Prior to membrane filtration, sericin was separated from other impurities via centrifugation (CFG) followed by microfiltration (MF) in the pre-treatment stage, which also helped minimize post membrane fouling. Ultrafiltration (UF) and nanofiltration (NF) were adopted at a pH equal to p*I* of sericin. UF achieved partial recovery of sericin polypeptides at 37–60%, which was attributed to increased transmission of uncharged sericin polypeptides at their p*I*. On the other hand, NF achieved sericin recovery as high as 94–95%, containing all MW fractions. Severe flux decline was the major problem due to protein–membrane interactions and high sericin concentrations, where concentration polarization mainly had a dominant effect. Although flux declines were as high as 58–88% in UF and 70–75% in NF, flux recovery by at least 83% was achieved by chemical cleaning using NaOH and free chlorine.

# 2. Membrane

## 2.1 Introduction

Materials used for the membrane cover a wide range, from organic polymeric materials to inorganic materials. Normally they are solid. Membrane are prepared from these materials and used for various separation processes, such as membrane gas separation, pervaporation, reverse osmosis, ultrafiltration, and microfiltration, depending on the pore size at the surface of the membrane.

There are different methods to prepared membranes. The principle underlying the membrane preparation is, however, always the same; i.e., it is to control the pore size and the pore size distribution at the surface layer and to decrease the thickness of the surface layer.

Membrane can be categorized according to their (i) geometry, (ii) bulk structure, (iii) production method, (iv) separation regime, and (v) application. This basic classification scheme is shown in Figure 1. Membranes can be produced in flat-sheet or tubular (hollow-fiber) geometry. Flat-sheet membranes are package either in plate-and-frame or spiral-wound modules, whereas tubular membranes are packaged in hollow-follow modules. Although hollow-fiber modules have the highest membrane packing density per module volume, spiral-wound and plate-and-frame modules are also commonly used in large-scale separation processes. (Pinnau and Freeman, 2000).

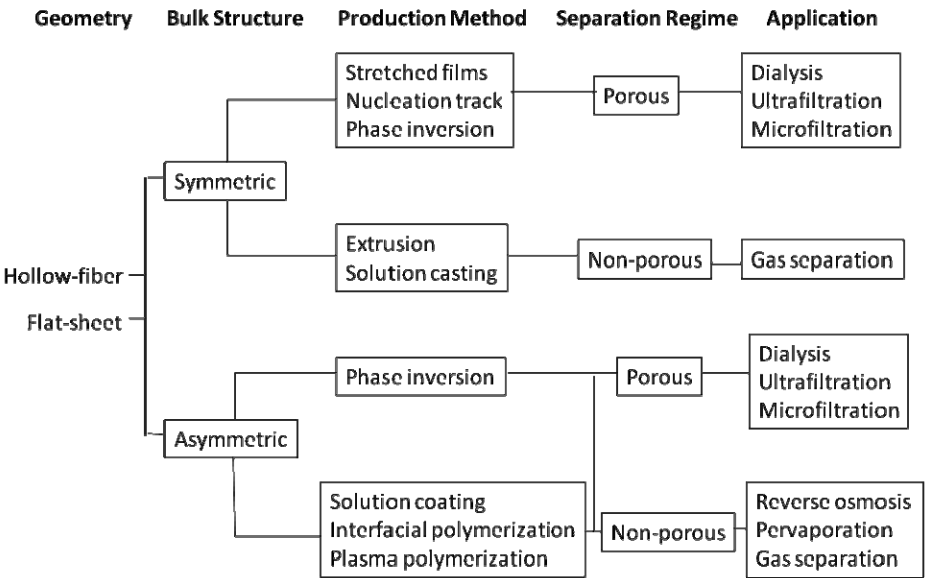


**Figure 1** Classification scheme of synthetic membranes based on their geometry, bulk structure, production method, separation regime, and application

**Souces:** Pinnau and Freeman (2000)

## 2.2 Asymmetric Membrane

Asymmetic membranes can be categorized into three basic structures: (i) integral-asymmetric with a porous skin layer, (ii) integral-asymmetric with a dense skin layer, and (iii) thin-film composite membrane. Porous integral-asymmetric membranes are made by the phase inversion process and are applied in dialysis, ultrafiltration, and microfiltration applications, whereas integral-asymmetric membranes with a dense skin layer are applied in reverse osmosis and gas separation applications (Koros *et al.,* 1994).

Membranes either have a symmetric (isotropic) or an asymmetric (anisotropic) structure. Symmetric membranes have a uniform structure throughout the entire membrane thickness, whereas asymmetric membranes have a gradient in structure. The separation properties of asymmetric membranes are determined by their entire structure. On the other hand, the separation properties of asymmetric membrane are determined primarily by the densest region in the membrane. The most common symmetric and asymmetric membrane types are shown in Figure 2.

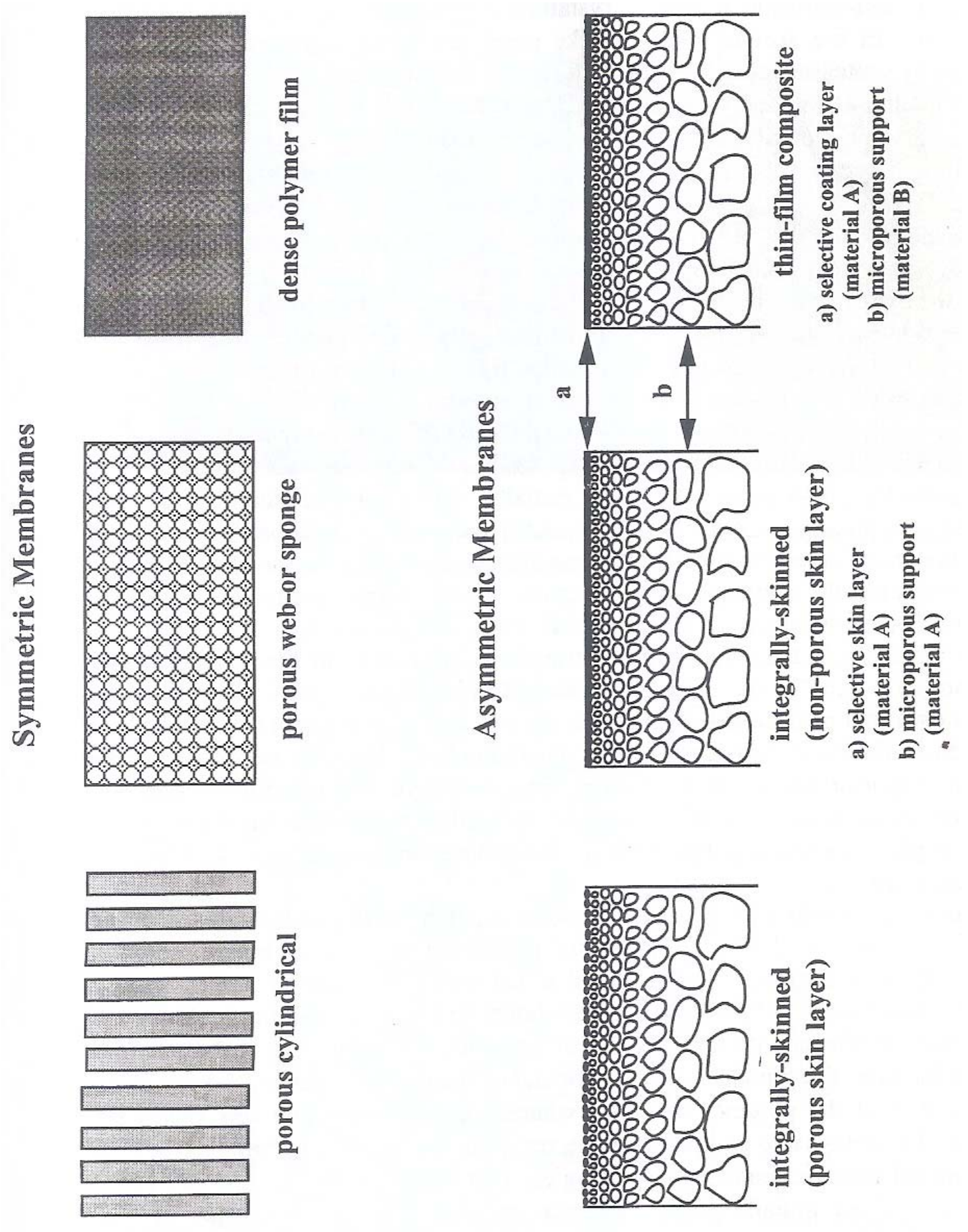


**Figure 2** Schematic representation of symmetric and asymmetric membrane structures

**Source:** Pinnau and Freeman (2000)

Membrane structure depends on the kinetics of phase separation process and the local distribution of the polymer-rich phase at the point of vitrification. Solidification of a binary polymer/solvent system occurs when the binodal line intersects the curve defining the glass transition, as shown in Figure 3. The location of the vitrification point, VP, is very important for the formation of membranes. If liquid-liquid phase separation occurs during the course of cooling an initially stable solution, containing phase separation and/or coarsening of the resulting phases will be arrested at the temperature where the tie-line intersects the vitrification point. Typical morphologies of membranes made from solution A, B and C, respectively at various stage of phase separation is schematically shown in Figure 4 . (Pinnau and Freeman, 2000).

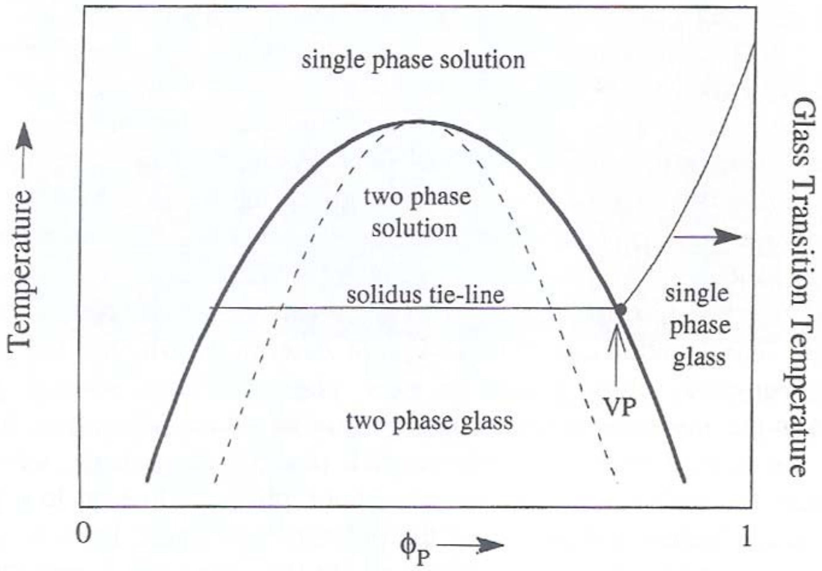


**Figure 3** Thermodynamic equilibrium and glass transition temperature of a binary polymer-solvent system as a function of solution composition. VP = vitrification point

**Source:** Pinnau and Freeman (2000)

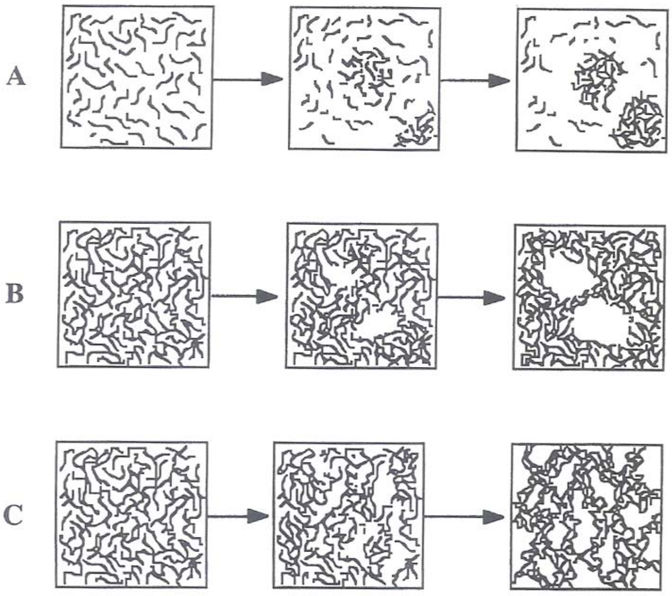


**Figure 4** Structures of membranes resulting from (A) nucleation and growth of a polymer-rich phase, (B) nucleation and growth of a polymer-poor phase, and (C) spinodal decomposition

**Source:** Pinnau and Freeman (2000)

## 2.3 Membrane preparation by polysulfone

### 2.3.1 Introduction

Polysulfone has overall thermal and chemical stability combined with good mechanical and membrane making qualities because its highly resistant to mineral acid, alkali, and electrolytes, in pH ranging from 2 to 13. It is resistant to oxidizing agents, therefore it can be cleaned by bleaches. It is also resistant to surfactants and hydrocarbon oils but not resistant to low-polar organic solvents (eg. ketones and chlorinated hydrocarbons), and aromatic hydrocarbons. Mechanically, polysulfone has high compaction resistance, recommending its use under high pressures. It is also stable in aqueous acids and bases and many non-polar solvents; however it is soluble in dichloromethane and methylpyrrolidone. Polysulfone allows easy manufacturing of membranes, with reproducible properties and controllable size of pores. Such membranes can be used in applications like hemodialysis, waste water recovery, food and beverage processing, and gas separation. These polymers are also used in the automotive and electronic industries.

### 2.3.2 Polysulfone membranes prepared by phase inversion process

Kim and Lee (1998) investigated the effect of PEG additive as a pore-former on the structure formation of membranes and their permeation properties connected with the changes of thermodynamic and kinetic properties in phase inversion process. The membranes were prepared by using polysulfone (PSF)/N-methyl-2-pyrrolidone (NMP)/poly(ethylene glycol) (PEG) casting solution and water coagulant. The resulting membranes prepared by changing the molecular weight of PEG additive and the ratio of PEG to NMP were characterized by scanning electron microscope observations, measurements of water flux and PEG rejection. The thermodynamic and kinetic properties of membrane-forming system were studied through coagulation value, light transmittance and viscosity. The correlations between the final membrane structure/permeation properties and thermodynamic/kinetic properties of membrane forming system were discussed extensively.

Yamasaki *et al*. (2000) studied the addition of a surfactant, sodium dodecyl sulfate (SDS), to the casting solution increased the oxygen permeance through asymmetric polysulfone membranes significantly, while maintaining relatively high selectivity of oxygen over nitrogen. A phase inversion technique with partial solvent evaporation (0 to 15 min at 95$^{o}$C) and a dual coagulation step using 2-propanol and water was used to prepare the asymmetric polysulfone membranes. The content of SDS in the solution was varied from 1 to 2.0 wt%. The oxygen permeance showed a maximum at 1.0 wt% of SDS for a given solvent evaporation time. Scanning electron microscopy (SEM) indicated that the skin layer growth was very limited with an increase in the solvent evaporation time for the membranes prepared with SDS.

In 2002, Han and Nam reported the effect of thermodynamic enhancement for membrane prepared by phase separation. The membranes were prepared by using polysulfone (PSF)/ N-methyl-2-pyrrolidone (NMP)/ polyvinylpyrrolidone (PVP) casting solution with water as non-solvent. In demixing of cast solutions through the vapor exposure, the thermodynamic enhancement and the kinetic hindrance work simultaneously showing a trade-off relationship against one another. At low concentration (5 wt %) of PVP, the variation in thermodynamic property controls the demixing process to result in the enhanced phase separation of the solution. With further increment of PVP, however, the solution demixing is delayed and the kinetic hindrance due to viscosity buildup overwhelms the thermodynamic factor: the enhancement in thermodynamic immiscibility could not contribute to the increase in demixing rate. When prepared via immersion process, the membranes show the similar trend in the water permeability as the phase separation data in the vapor exposure. That is, at low PVP concentration of 5 wt %, the flux increases with the enhancement in the phase separation, but it drops with further increase of PVP in the casting solution rather than improved.

In order to clarify the mechanism through which poly(ethylene glycol) (PEG) can affect the kinetics of membrane formation, phase-inversion process was used in membrane preparation from polysulfone (PSF) casting solution with PEG introduction. The rheological and thermodynamic variation in PSF solution by PEG introduction was investigated by using the method of viscosity measurement and triangle phase diagram. Zheng *et al.*, (2006a) found that PEG addition could work in favor of enhancement thermodynamically and hindrance rheologically in PSF solution demixing. With the increase of temperature, the viscosity of PSF casting solution decreased and the miscibility of PSF solution with non-solvent increased. On the basis of the relationship between wet membrane thickness and coagulation time, a new method used to calculate kinetics parameter ($D_a$ ) of membrane formation was founded and an equation of $D_a = d^2/t$ was established (where $d$ = wet membrane thickness, and $t$ = coagulation time). With the increase of PEG concentration, it was revealed that $D_a$ increased firstly and then decreased at certain temperature (20°C). With the increase of coagulation bath temperature, $D_a$ increased continuously when the composition of casting solution was constant. $D_a$ was mainly influenced by the surface morphology of membrane and the thermodynamic, rheological properties of casting solution. Zheng *et al.*, (2006b) further studied the relationship between the porosity of membrane and the kinetics parameter of membrane formation. It was found that there was substantial relationship between $D_a$ and porosity in PSF ultrafiltration membrane, and that the linear relationship between $D_a$ and porosity in PSF ultrafiltration membrane could be described as $P(\%) = a + bD_a$ (where $P$ = porosity of membrane, $a$ and b = constant variables following the correlation). The faster the coagulation process the higher the membrane porosity, which accords with the conclusion of instantaneous demixing tend to form macrovoids (finger-like pore with higher porosity) and delayed demixing tend to form microvoids (sponge-like pore with lower porosity).

Flat sheet asymmetric polymeric membranes were prepared from homogeneous solution of polysulfone (PSF) by phase inversion method. N-methyl-2-pyrrolidone (NMP) and dimethylacetamide (DMAc) were used as solvents separately. Polyethylene glycol (PEG) of three different molecular weights (400 Da, 6000 Da and 20000 Da), were used as the polymeric additives in the casting solution were studied by Chakrabarty *et al.* (2008a). The morphology and structure of the resulting membranes were observed by scanning electron microscope (SEM). The pore number, pore permeability and their distribution and average pore size of the membranes were determined by the liquid displacement method. The permeation performances of the membranes were evaluated in terms of pure water flux (PWF), equilibrium water content (EWC), hydraulic permeability, and solute rejection. Solution of bovine serum albumin (BSA) of molecular weight 68,000 Da was used to study the permeation performance of prepared membranes using a batch membrane cell of 100 ml capacity. Results showed that increaseing molecular weight of PEG, the pore number as well as pore area in membranes increases. Membranes with PEG of higher molecular weights have higher PWF and higher hydraulic permeability due to higher porosity. With increase in molecular weight of PEG from 400 to 20000, the PWF increases from 15.3 to 2713.4 L/m$^2$.h with NMP as solvent while with DMAc as solvent, the PWF increases from 24.5 to 555.6 L/m$^2$.h. Similarly, EWC increases from 56.8% for PEG 400 to 78.8% for PEG 20000 for PSF/NMP/PEG membranes. Similar trend is observed for PSF/DMAc/PEG membranes. The BSA rejection data is maximum with PEG 6000 for both the solvents and the values are 56.4% for NMP (at pH 4.8) and 42.4% for DMAc (at pH 9.5). Finally, results from both techniques viz. scanning electron microscopy (SEM) and gas permeation tests were compared with those calculated from pure water permeation tests using Hagen–Poiseuille equation (Chakrabarty *et al.*, 2008c). It is found that though the values obtained from all the techniques vary from each other; their trends with increase in molecular weight of PEG seem to be the same. It is seen that when molecular weight of PEG increases from 400 to 20,000 Da, the mean pore size of the prepared membranes decreases from 0.11 to 0.44 μm for PSF/NMP membranes and 0.08-0.04 μm for PSF/DMAc membranes, while the porosity and pore density showed an increasing trend; the pressure normalized gas flux rises significantly and the thickness of the top layer of the prepared membrane sheet increases.

At the same time, effect of polyvinyl pyrrolidone (PVP) of different molecular weights (24,000, 40,000 and 360,000 Da) on the structure and permeation properties of polysulfone (PSF) membranes was also investigated by Chakrabarty *et al.,* (2008b). The membranes were prepared at the same condition and test at the same way as uses PEG at three different molecular weights as the polymeric additives in the casting solution. Results showed that the morphological parameters and flux performance of the membranes have a significant inter-relationship with the molecular weight of PVP. The membrane pore number and pore area are seen to increase with molecular weight of PVP. However, at a constant pressure, the PWF is found to decrease while BSA rejection increases significantly for the membranes prepared with both the solvents. For the membrane composition considered in this study, the solvent DMAc is found to be more suitable than NMP, in terms of BSA rejection, irrespective of the pH of the BSA solution. The maximum rejection found in this study is 76% with PSF/DMAc membrane with PVP 360,000 (at pH 9.3).

# MATERIALS AND METHODS

## Materials

### 1. Cocoon cooking wastewater

The *Bombyx mori* silk cocoons were obtained from Faculty of Biochemistry, Kasetsart University, Thailand. The silkworms were carefully separated and the cocoons were cut into small pieces. About 25 g of cut silk cocoon and 2 g of bromelain were mixed with 1 L of distilled water (0.025% w/v, silk cocoon 1kg : distilled water 40 Liter) and was boiled at 55$^{o}$C for 1 h in the controlled temperature water bath. Bromelain was used to enhance the digestion of sericin from the cocoons. The silk cocoon degumming wastewater obtained was centrifuged at 10,733 x g for 5 min using the Hermle Z323 Labortechnik (Germany) centrifugation equipment to settle the suspended solids.

### 2. Chemicals

The following chemicals were obtained from commercial sources and used directly without further purification unless noted:

2.1 Chemicals for preparing membranes

- Polysulfone -[-$C_6H_4$-4-$C(CH_3)_2$-$C_6H_4$-4-O-$C_6H_4$-4-$SO_2$-$C_6H_4$-4-O-]$_n$- , $\overline{M}_n = 22{,}000$ (AR grade, Sigma-Aldrich, USA)
- Poly(ethylene glycol) -[-O-$C_2H_4$-]$_n$- , $\overline{M}_n$ = 300, 2000, 4600 and 10,000 (AR grade, Sigma-Aldrich, USA)
- Poly(dioxolane) -[-O-$CH_2$-O-$C_2H_4$-]$_n$- , $\overline{M}_n$ = 10,000 and 200,000 (Nippon Shokubai Japan )
- N-methyl-2-pyrrolidone, NMP (AR grade, Fluka, Switzerland)

- Deionized water used for all the experiments was produced by a water purifier ELGASTAT optima 60 model.

### 2.2 Chemicals for SDS-PAGE analysis

- 30% Acrylamide/*N,N′*-methylenebis acrylamide solution, 19: 1 (AR grade, Bio-Rad Laboratory Inc., German)
- Ammonium persulfate (Reagent grade, Amersham Life Science, UK)
- Bromophenolblue (AR grade, Merck, Germany)
- Sodium dodecyl sulfate, SDS (AR grade, Bio Basic Inc., Canada)
- *N,N,N′,N′*-tetramethylethylene diamine, TEMED (AR grade, Pharmacia Biotech, Sweden)
- Tris(hydroxymethyl)aminomethane, Tris (Molecular Biology Grade, Pacific science, USA.)

### 2.3 Chemicals for protein determination (Lowry's method)

- Bovine serum albumin, BSA (AR Grade, Fluka, Switzerland)
- Sodium carbonate (AR Grade, VolChem, Italy)
- Sodium hydroxide (AR Grade, Merck, Germany)
- Copper sulfate, $CuSO_4.5H_2O$ (ACS-For Analysis, Carlo Erba, Italy)
- Potassium sodium tartrate (AR Grade, Ajax Finechem, Australia)
- Folin-Ciocalteau's phenol reagent (AR Grade, Carlo Erba, Italy)

## 3. Equipment

- Membrane test cell (Nitto Denko Corporation, Japan)
- Hotplate/stirrer (IKAMAG, IKA-Labortechnik, Malaysia)
- UV/visible spectrophotometer (Perkin Elmer, USA)
- Scanning electron microscopy (SEM) (Electron Microscopy Ltd, Cambridge, England)

- Infinite focus microscopy (IFM) (Hi-Tech Instruments Sdn. Bhd., Alicona, Australia).
- Electrophoresis equipment
- Power supply (E-C Apparatus Corp., USA)
- Centrifuge (Hermle Z323 Labortechnik, Germany)
- Autopipette (Pipetteman, Gilson, France)

## Methods

### 1. Membrane Preparation

Thin film flat sheet membranes with various weight ratios of additives such as poly(ethyleneglycol) and poly(dioxolane) were cast by phase inversion process as shown in Figure 5 Then, the morphology of the prepared membranes was investigated by scanning electron microscopy (SEM), infinite focus microscopy (IFM) and the feed water and permeate water will be analyzed in term of permeate flux (PF), protein retention, solid retention and molecular weight cut-off of sericin.

#### 1.1 Casting solution

Polysulfone (PSF) casting solutions were prepared by dissolving 12 wt% of polymer (PSF) in 83 wt% or 86 wt% of N-methyl-2-pyrrolidone (NMP) at room temperature (≈ 25°C) and about 72% relative humidity. Each mixture was then mixed with 2 wt% or 5 wt% of PDXL or PEG with different molecular weight, separately. In order to get homogeneous solutions, the PSF solution was stirred on magnetic stirrer for about 24 h at room temperature. After the solution (consisting of polymer, solvent and additive) became homogeneous, it was kept constant at room temperature for 24 h.

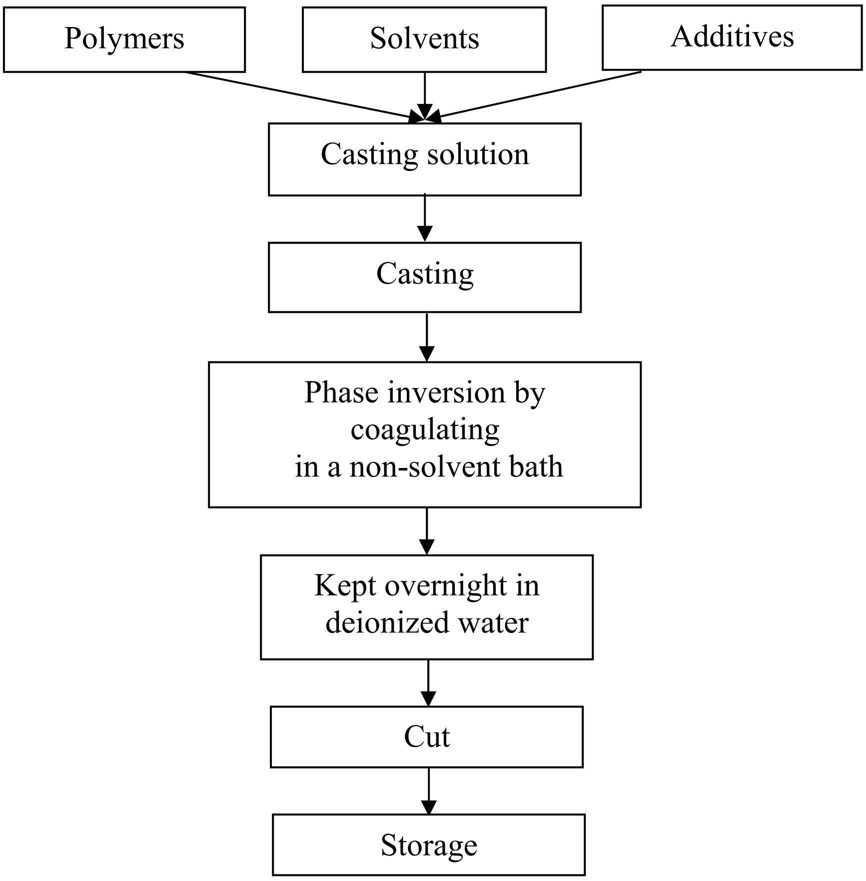


**Figure 5** Schematic of general procedure for production of membranes by phase inversion process

### 1.2 Membrane casting

The prepared casting solution was spread uniformly on a glass plate (0.10m × 0.10m) with the help of glass rod to maintain the sheet and required thickness (approximately 200 μm) was controlled with the aid of an appropriate spacers provided on both ends of the glass plate. The resulting film was then immersed into coagulation bath containing deionized water at room temperature for PEG and at 60°C for PDXL in which the solvent (NMP) was removed by miscible with the deionized water. The cast films changed their color from transparent to white immediately after immersion into the coagulation bath. The prepared membrane sheets were washed under running deionized water to remove the additional amount of additives and then kept overnight in a deionized water bath. Finally, the sheets were cut in the form of circular disks of diameter 0.09 m to place inside the membrane cell for filtration experiments. Membrane with different composition was shown in Table 1.

**Table 1** Composition of the casting solution

| Membrane | (wt%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PSF | NMP | PEG 300 | PEG 2000 | PEG 4600 | PEG 10,000 | PDXL 10,000 | PDXL 200,000 |
| PSF | 12 | 88 | - | - | - | - | - | - |
| PEG300-2 | 12 | 86 | 2 | - | - | - | - | - |
| PEG2000-2 | 12 | 86 | - | 2 | - | - | - | - |
| PEG4600-2 | 12 | 86 | - | - | 2 | - | - | - |
| PEG10,000-2 | 12 | 86 | - | - | - | 2 | - | - |
| PDXL10,000-2 | 12 | 86 | - | - | - | - | 2 | - |
| PDXL200,000-2 | 12 | 86 | - | - | - | - | - | 2 |
| PEG300-5 | 12 | 83 | 5 | - | - | - | - | - |
| PEG2000-5 | 12 | 83 | - | 5 | - | - | - | - |
| PEG4600-5 | 12 | 83 | - | - | 5 | - | - | - |
| PEG10,000-5 | 12 | 83 | - | - | - | 5 | - | - |
| PDXL10,000-5 | 12 | 83 | - | - | - | - | 5 | - |
| PDXL200,000-5 | 12 | 83 | - | - | - | - | - | 5 |

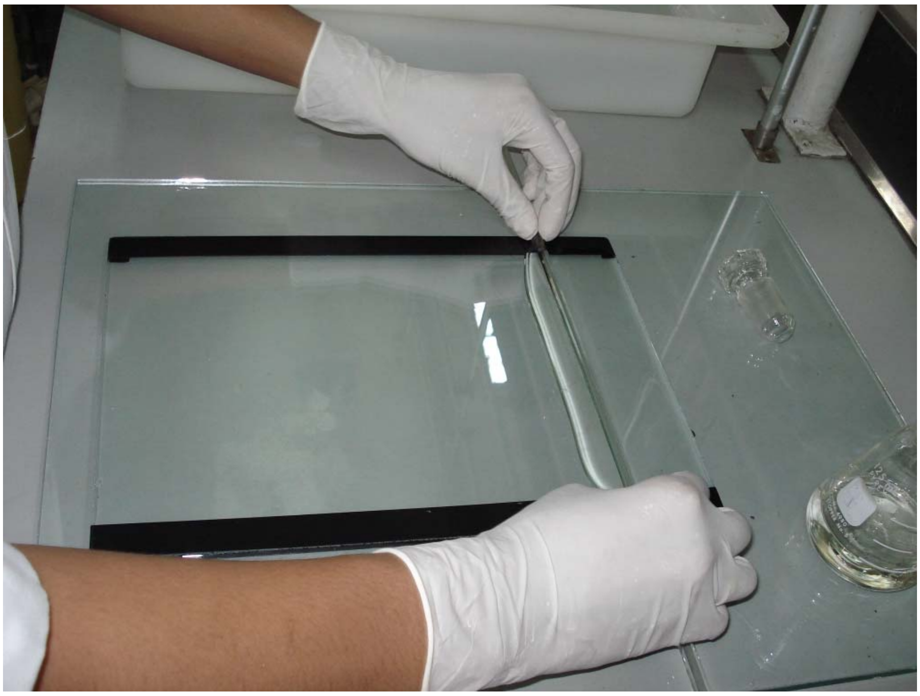


**Figure 6** Membrane casting

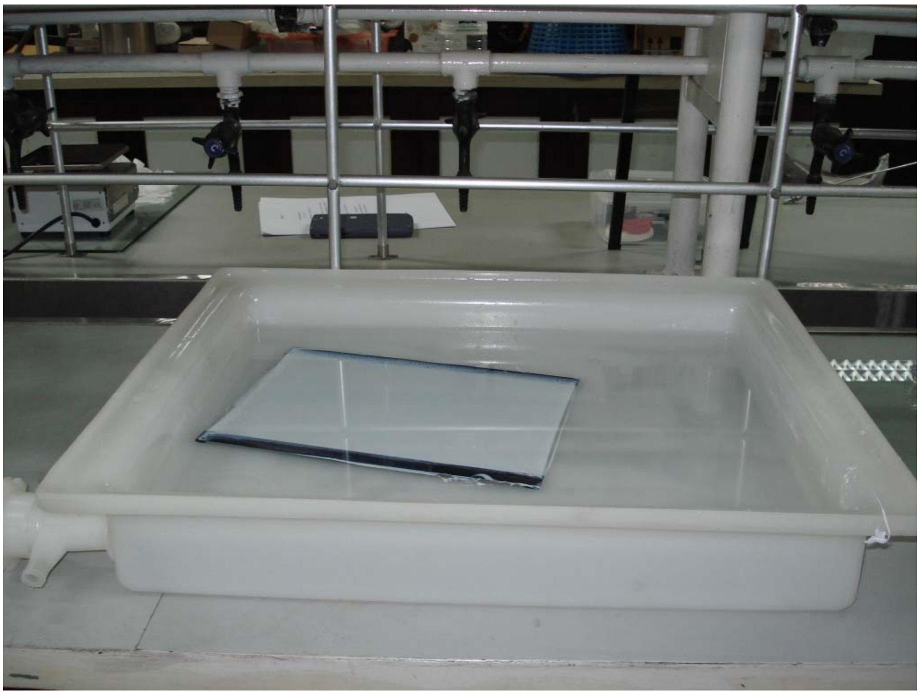
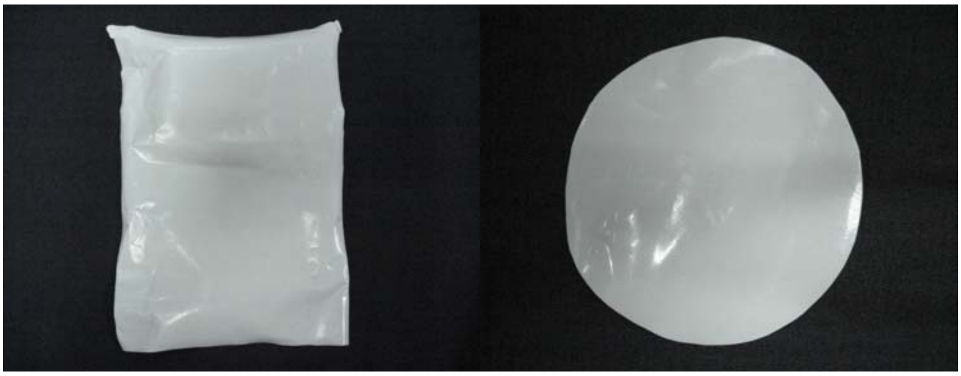


**Figure 7** Membrane formation by phase inversion method



(a) (b)

**Figure 8** (a) Membrane formation and (b) prepared PSF membrane

## 2. Filtration experiments

### 2.1 Membrane test cell

The batch performance of the prepared membranes was studied with a stirred batch experiment with RO/UF flat sheet stainless steel membrane test cell having the maximum volume of 380 ml from Nitto Denko Corporation. A schematic diagram of the set up was shown in Figure 9 and Figure 10. A flat circular sheet membrane having diameter of 0.09 m with the effective area of 0.0064 $m^2$ was placed over a base support inside the cell and the permeating solution was collected from the bottom of the cell (Figure 11). The cell was pressurized using a nitrogen cylinder.

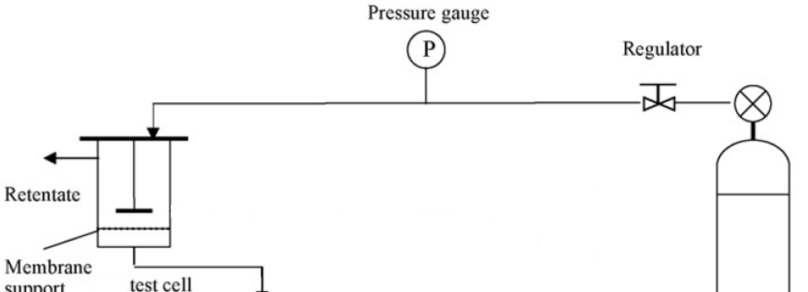


**Figure 9** Schematic diagram of the experimental set-up

**Figure 10** Membrane module set up for recovery sericin from silk cocoon degumming wastewater in this study

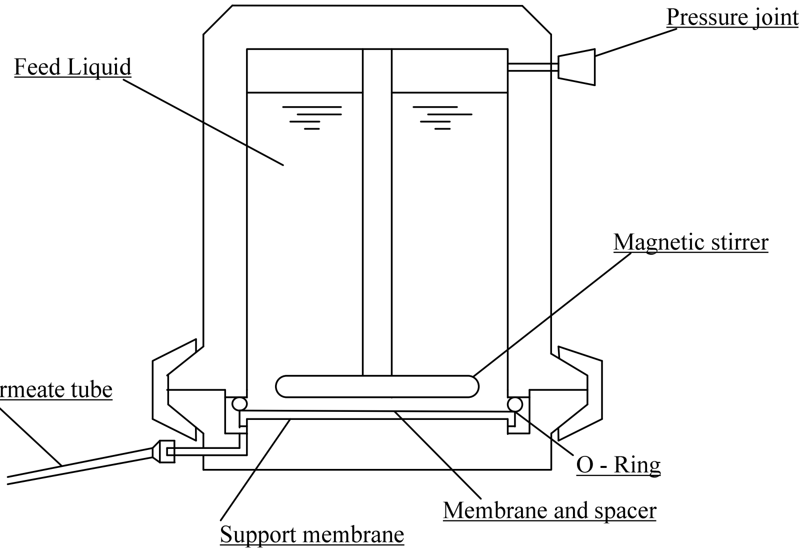


**Figure 11** Assembly of the flat sheet stainless membrane test cell used in this study

### 2.2 Sericin recovery by membrane process

The flat sheet membranes were put into a stainless steel test cell. A 200 ml of silk cocoon degumming wastewater was fed into the test cell and maintained at a constant temperature of 30$^{o}$C (Room temperature). The flow rate was varying at different pressure as 200, 300 and 400 kPa.

## 3. Analytical method

### 3.1 Membrane characterization

The top surface and cross-sectional morphologies of the prepared membranes were characterized by scanning electron microscopy (SEM) (1455VP, Electron Microscopy Ltd, Cambridge, England). The samples were fractured in liquid nitrogen and coated with a thin gold layer. While the membranes cross-sectional morphology after filtration was characterized by infinite focus microscopy (IFM) (Hi-Tech Instruments Sdn. Bhd., Alicona, Australia).

### 3.2 Permeate flux (PF)

The performance of the prepared membranes were evaluated to recover sericin from silk cocoon degumming wastewater in terms of their permeability as permeate flux by allowing silk cocoon degumming wastewater feed to pass through the compact membrane. Permeate flux values at different transmembrane pressure (200, 300 and 400 kPa) were measured under steady state condition using the equation:

$$J_W = \frac{Q}{A\Delta T}$$ (Chakrabarty *et al.*, 2008a)

where, $J_W$ is permeate flux (l/m$^2$ h)

$Q$ is volume of permeated water (l)

$A$ is effective membrane area (m$^2$)

$\Delta T$ is sampling time (h)

### 3.3 Protein retention

The Lowry protein assay method (Lowry *et al.*, 1951) combines the reactions of cupric ions with the peptide bonds under alkaline conditions (the Biuret method) with the oxidation of aromatic protein residues. The Lowry method is best used with protein concentrations of 20-200 µg/ml. The method is based on the reaction of $Cu^+$, produced by the oxidation of peptide bonds, with Folin's reagent (a mixture of phosphotungstric acid and phosphomolybdic acid in phenol) in the Folin-Ciocalteau's phenol reaction. The reaction involves reduction of the Folin reagent and oxidation of aromatic residues (mainly tryptophan, also tyrosine). The concentration of the reduced Folin reagent is measured by absorbance at 650 nm.

Mixed the 100 µl of cocoon cooking wastewater in the feed or in the permeate samples with 2.5 ml potassium sodium tartrate-copper sulphate solution (Appendix A1) in the different test tubes. Mix the solution well. This solution was incubated at room temperature for 10 min. Then add 0.25 ml of reagent Folin-Ciocalteau's phenol solution (Folin:DW; 1:1) to each tube and . Zero the colorimeter with blank and take the optical density (measure the absorbance) at 650 nm. Check the absorbance of unknown sample and determine the concentration of the unknown sample using the Bovine serum albumin (BSA) standard curve plotted.

Different dilutions of BSA solutions range 20-200 µg/ml were prepared by mixing stock BSA solution (2 mg/ml) with distilled water in the test tubes. The 100 µl of BSA solution were mixed with the same reagent as used for the unknown sample. Plot the absorbance against protein concentration to get a standard calibration curve.

The percentage of protein retention was calculated as followed:

$$protein\ retention\,(\%) = \left( \frac{C_f - C_p}{C_f} \right) x100 \qquad \text{(Chakrabarty } et\ al.\text{, 2008b)}$$

where $C_f$ and $C_p$ are the concentration of protein in the silk cocoon degumming wastewater feed and permeate in mg $L^{-1}$, respectively.

### 3.4 Solid retention

The amount of percentage solid retention (%$R$) in the retentate was determined by gravimetric analysis via evaporation of 50 ml of silk cocoon degumming wastewater feed or permeate sample, separately at 105°C for 24 h. The percentage of solid retention was calculated as followed:

$$R\,(\%) = \left( \frac{W_f - W_p}{W_f} \right) x\ 100$$

where $W_f$ and $W_p$ are the weight of silk cocoon degumming wastewater feed and permeate in mg, respectively.

### 3.5 Molecular weight cut-off of sericin

Polyacrylamide gel electrophoresis (PAGE) is a widely used technique for separating proteins. The most widely used method was developed by Laemmli (1970) using the denaturing (SDS) discontinuous method. SDS (sodium dodecyl sulfate) and β-mercaptoethanol was denatured the proteins, dissociate the proteins into subunits, and to coat them with negative charges (SDS). This relatively uniform charge to mass ratio allows the proteins to migrate in an electric field and separate according to mass/size.

Laemmli system uses buffers of different pH and composition to generate a voltage gradient and a discontinuous pH between the stacking and the resolving gel. A 4% acrylamide stacking gel (pH 6.8) (Appendix A2) is poured on top of a 15% acrylamide resolving gel (pH 8.8) (Appendix A3). The stacking gel (with a large pore size) serves to concentrate all of the proteins (the large ones can catch up with the small ones) on top of the resolving gel (SE 245 Dual Gel Caster, Amersham Bioscience). After entering the resolving gel (which has a smaller pore size) the proteins are separated according to relative molecular size.

Protein samples are diluted 1:1 in sample buffer (Appendix A4) and are boiled at 100°C for 10 minutes. The β-mercaptoethanol in the sample buffer reduces the protein's disulfide bonds and the SDS denatures the proteins. The sample buffer contains glycerol to increase the density so that when the sample is loaded it sinks to the bottom of the well. Bromophenol blue dye was in the sample buffer to monitor the electrophoresis process. The gel electrode assembly is placed in the SE 260 mini-vertical gel electrophoresis unit (Amersham Bioscience), reservoir buffer (Appendix A5) was added to upper and lower chambers. The system was run at 80 V for about 2 h. Following electrophoresis, the gel were stained with silver stain (Page Silver™ Silver Stain Kit Fermentas, Life Science), allowing visualization of the separated proteins. Standard proteins consisting of synthesis protein having molecular weight ranging from 250 kDa to10 kDa were used as molecular weight marker s (Precision Plus Protein™ Standard BIO-RAD), in order to calibrate the gel and determine the weight of unknown proteins by comparing the distance traveled relative to the marker.

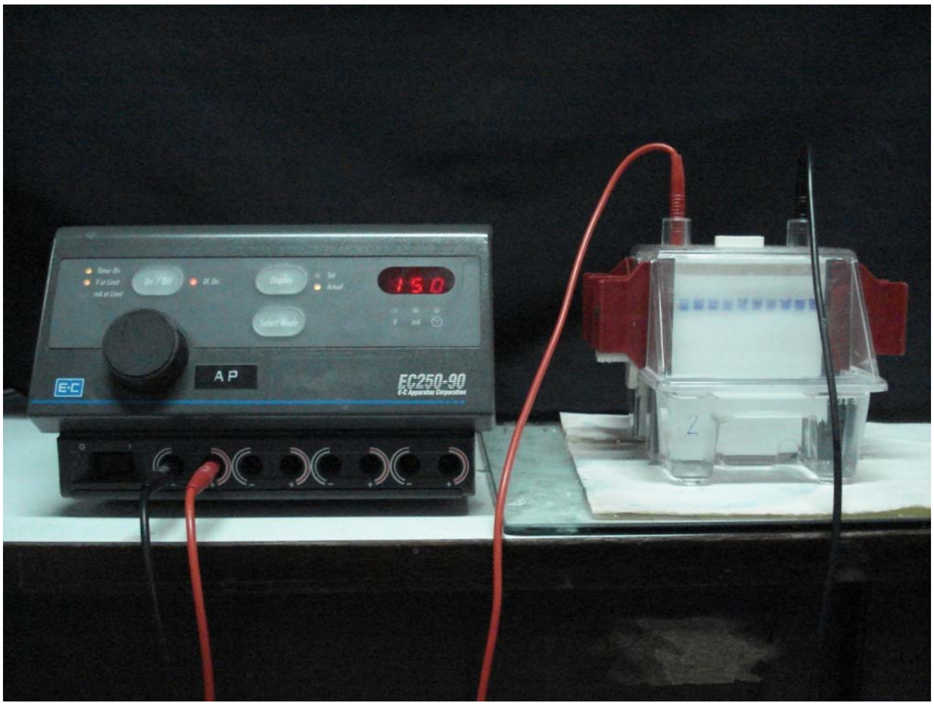


**Figure 12** SDS-PAGE set up used in this study

# RESULTS AND DISCUSSION

## 1. Membrane morphology

Morphology of the prepared polysulfone (PSF) membranes by phase inversion process was investigated by scanning electron microscopy (SEM) and their cross sectional SEM morphologies are shown in Figure 13 and Figure 14. All the prepared PSF membranes shows asymmetric structures consisting of a dense top-layer and a porous with finger-like cavities having macrovoid enclosed in the PSF matrix sub-layer the same as most of polysulfones membranes formed when using NMP/water as the solvent/nonsolvent pair due to the high mutual affinity of NMP in PSF casting solution with water during the phase inversion (Reuvers *et al*., 1987a; Mulder, 1996; Kim *et al*., 2005). Addition of PEG or PDXL into the casting solution clearly plays a significant role on morphology of the membranes prepared. During phase inversion process, micropores are formed both on the sites where PEG or PDXL and NMP exist via exchanging of water inflow and NMP, PEG or PDXL outflow (Kesting, 1985; Munari *et al*., 1988). The morphologies of PSF membrane prepared from different pore forming PEG and PDXL additives concentration and molecular weight indicate that the pore size and porosity of the top-layer in the prepared membranes increase as the concentration and molecular weight of PEG or PDXL increase. Because of the miscibility of casting solution (PSF/NMP/PEG or PSF/NMP/PDXL) with water is inversely proportional to the concentration and molecular weight of PEG and PDXL, the casting solution becomes less thermodynamically stable when containing more concentration and higher molecular weight of PEG and PDXL. The thermodynamically less stable membrane casting solution then enhances the precipitation rate (increases the ratio of water inflow to NMP, PEG or PDXL outflow) and more porous membranes are formed (Young and Chen, 1995; Kim and Lee, 1998; Zheng *et al*., 2006a-b). In addition, it is observed that the thickness of the top-layer and the structure of sub-layer also depend on solubility and diffusivity of the PEG and PDXL. The thickness of top-layer is of important in membrane filtration, because the permeate flux decreases as the thickness of the top-layer increases (Jung *et al*., 2004).

The top-layer is found thicker as higher concentration and molecular weight PEG is added. Because of lower diffusivity of higher molecular weight of PEG due to the higher viscosity relative to the NMP, PSF molecules takes more time to come to the top surface giving the PSF molecules enough time to agglomerate and rearrange and thus a comparatively denser and thicker top-layer is formed (Kim and Lee, 1998; Jung *et al.*, 2004; Chakrabarty *et al.*, 2008c). However, it is seen that the thickness of the top-layer does not depend on the concentration and molecular weight of PDXL. This may be due to the relatively comparable high diffusivity of both PDXL10,000 and PDXL200,000. When keeping the temperature in the precipitation bath at 60$^{o}$C, PDXL gives very less time for PSF molecules to agglomerate on the top surface. Therefore, a thin top-layer is observed. Finally, both PEG and PDXL additives caused significant suppression of the finger-like cavity with macrovoids sub-layer, because the low molecular weight additives can easily be washed out quickly together with the NMP into the water precipitation bath during the formation of membrane. In fact, the top-layer porosity is closely related to the macrovoid formation. When more porous top-layer is obtained as in the case of PSF/NMP/PDXL200,000; the macrovoids formation is largely suppressed. The more porous top-layer needs the larger distance from the top surface to the staring point of macrovoids to limit the large nonsolvent inflow to the sub-layer because a large nonsolvent inflow induce many nuclei formation in the sub-layer and thus, prevent the macrovoids formation. Therefore, a porous sponge-type membrane with no macrovoids is formed under the highly less stable system, because the top-layer of the membrane is too porous to form the resistance layer which can limit many nuclei formation in the sub-layer (Kim and Lee, 1998).

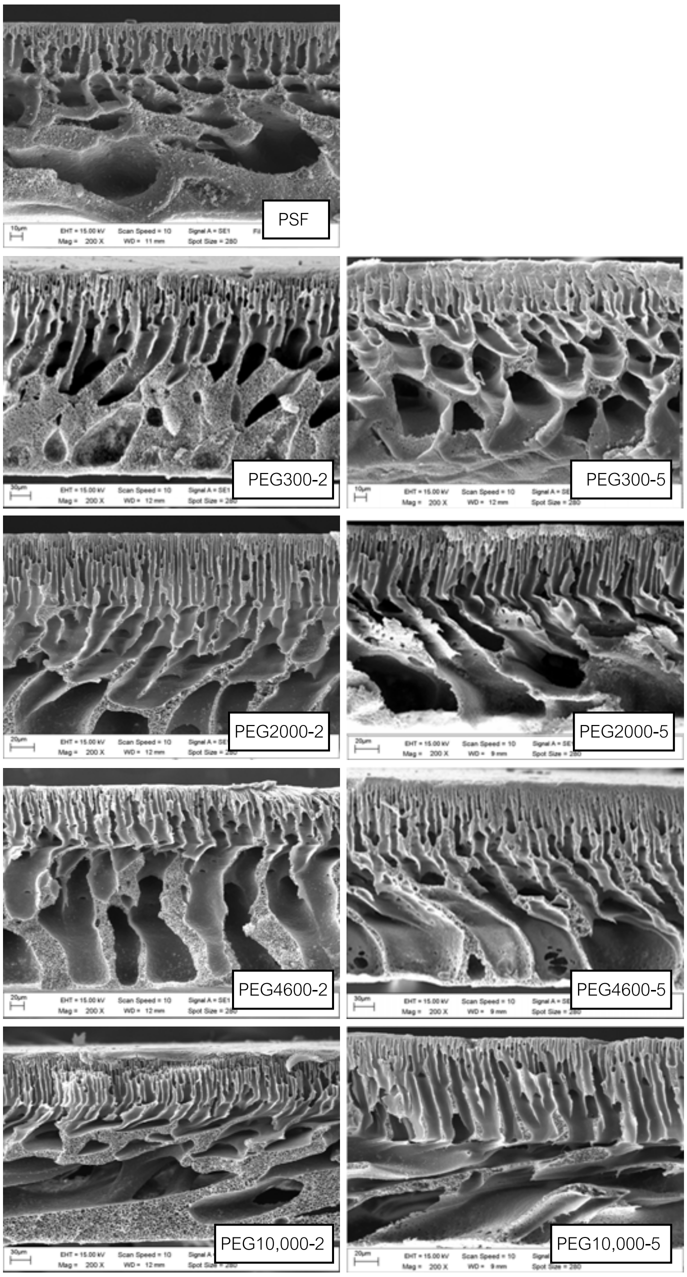


**Figure 13** SEM cross-sectional morphologies of asymmetric membranes prepared with various PEG concentration and molecular weight as additives and coagulation bath temperature is 25$^{o}$C: PSF (PSF/NMP = 12/88), PEG300-2 (PSF/NMP/PEG300 = 12/86/2), PEG2000-2 (PSF/NMP/PEG2000 = 12/86/2), PEG4600-2 (PSF/NMP/PEG4600 = 12/86/2), PEG10,000-2 (PSF/NMP/PEG10000 =12/86/2), PEG300-5 (PSF/NMP/PEG300 = 12/83/5), PEG2000-5 (PSF/NMP/PEG2000 =12/83/5), PEG4600-5 (PSF/NMP/PEG4600 =12/83/5) and PEG10,000-5 (PSF/NMP/PEG10000 = 12/83/5) wt%

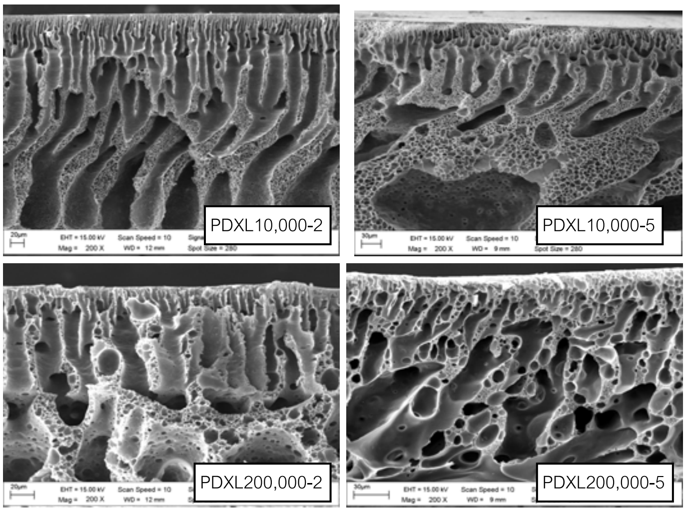


**Figure 14** SEM cross-sectional morphologies of asymmetric membranes prepared with various PDXL concentration and molecular weight as additives and coagulation bath temperature is 60°C: PDXL10,000-2 (PSF/NMP/PDXL10000 = 12/86/2), PDXL200,000-2 (PSF/NMP/PDXL200000 =12/86/2), PDXL10,000-5 (PSF/NMP/PDXL10000 = 12/83/5) and PDXL200,000-5 (PSF/NMP/PDXL200000 = 12/83/5) wt%

Figure 15 and Figure 16 (see also Appendix Figure B1 to Appendix Figure B13) show the top surface (air side) SEM images of all membranes prepared in this study. Surface morphology may be explained from the concept of quaternary phase diagram of membrane formation (Chakrabarty *et al.*, 2008a). The formation of the top surface is possibly due to demixing of the casting solution by means of nucleation and growth of the solid polymer rich phase (Reuvers and Smolders, 1987b; Kimmerle and Strathmann, 1990) forming nodule/aggregate on the surface that leads to much better interconnected pores. The nodular structure of the membrane top layer became more prominent when increasing the molecular weight of both PEG and PDXL due to their low mobility in the initial stage of immersion in the coagulation bath as can be seen in PDXL10,000-5 (PSF/NMP/PDXL = 12/83/5 wt%) and PDXL200,000-5 (PSF/NMP/PDXL200,000 = 12/83/5 wt%).

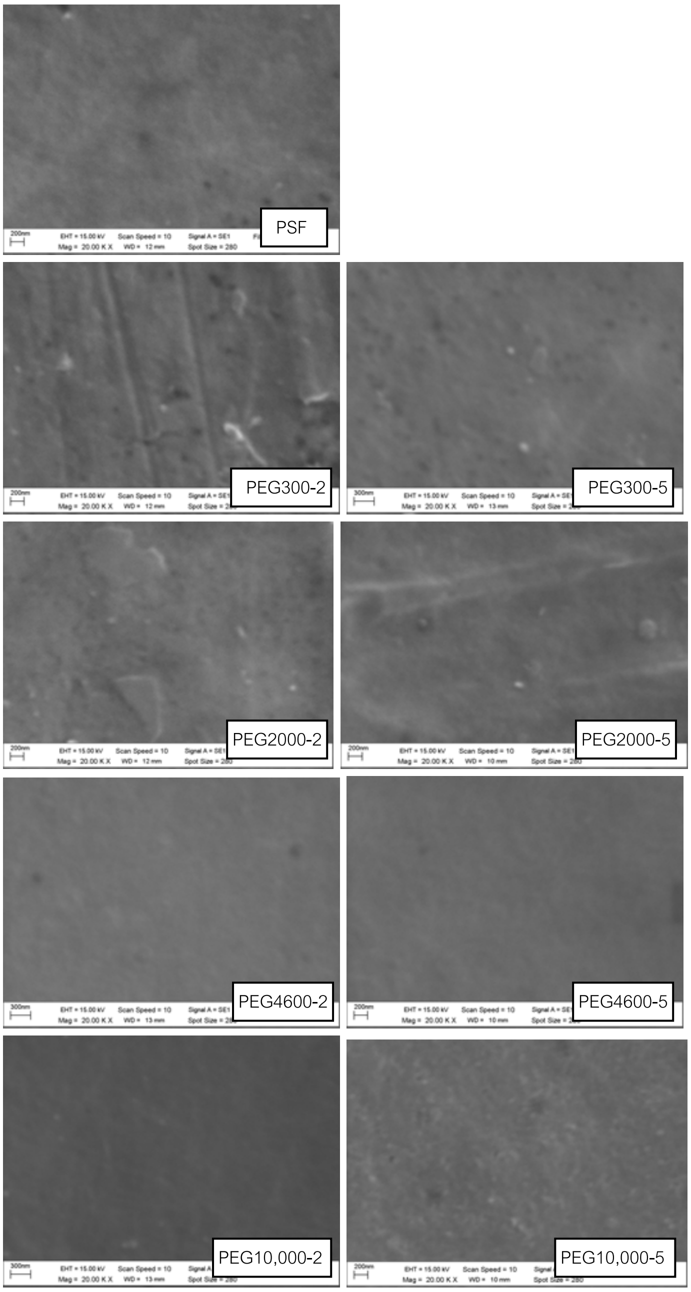


**Figure 15** SEM top-surface (air side) morphologies of asymmetric membranes prepared with various PEG concentration and molecular weight as additives and coagulation bath temperature is 25°C: PSF (PSF/NMP = 12/88), PEG300-2 (PSF/NMP/PEG300 = 12/86/2), PEG2000-2 (PSF/NMP/PEG2000 = 12/86/2), PEG4600 (PSF/NMP/PEG4600 = 12/86/2), PEG10,000-2 (PSF/NMP/PEG10000 =12/86/2), PEG300-5 (PSF/NMP/PEG300 = 12/83/5), PEG2000-5 (PSF/NMP/PEG2000 =12/83/5), PEG4600-5 (PSF/NMP/PEG4600 =12/83/5) and PEG10,000-5 (PSF/NMP/PEG10000 = 12/83/5) wt%

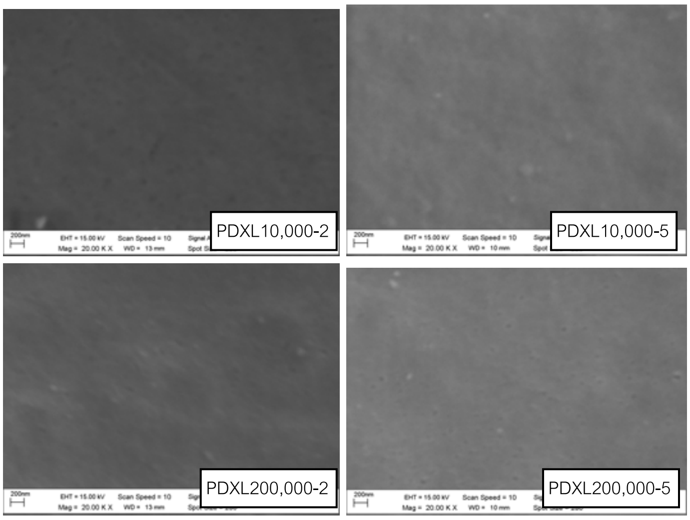


**Figure 16** SEM top-surface (air side) morphologies of asymmetric membranes prepared with various PDXL concentration and molecular weight as additives and coagulation bath temperature is 60$^{o}$C: PDXL10,000-2 (PSF/NMP/PDXL10000 = 12/86/2), PDXL200,000-2 (PSF/NMP/PDXL200000 =12/86/2), PDXL10,000-5 (PSF/NMP/PDXL10000 = 12/83/5) and PDXL200,000-5 (PSF/NMP/PDXL200000 = 12/83/5) wt%

## 2. Permeate flux (PF)

The performance of all the prepared membranes was used to recover sericin from the silk cocoon degumming wastewater. All the prepared membranes were subjected to hydraulic compaction at the different of transmembrane pressures of 200, 300 and 400 kPa. The effect of permeate flux on compaction time for all the PSF membranes prepared is shown in Figure 17 to Figure 22 and the effect of transmembrane pressure on PF for all membranes prepared with 2 and 5 wt% of PEG and PDXL is also shown in Figures 23 and 24. It clearly shows that PF declined gradually due to compaction with time and after about 2 h of compaction; it reaches a steady-state value. This is due to the reduction in pore size on the membrane surface from the physical adsorption of the gummy sericin protein on the membrane surface causing the walls of the pores became closer and denser (Mulder, 1996; Capar *et al.*, 2008). Additional of PEG and PDXL additives in the prepared membranes, PSF/NMP/PEG and PSF/NMP/PDXL, exhibites better PF than that prepared directly only from PSF/NMP. The PF for each membrane is found to increase with increasing concentration, molecular weight of both additives and transmembrane pressures (200, 300 and 400 kPa). This is due to the increase in effective driving force (transmembrane pressure) required for permeation. For example, at 200 kPa and 2 wt% of additives (Figure 17), the steady state permeate flux is increased in PEG300-2 (PSF/NMP/PEG300 = 12/86/2 wt%) to PEG10,00-2 (PSF/NMP/PEG10,000 = 12/86/2 wt%) from 9.7 L/m$^2$h to 19.5 L/m$^2$h and in PDXL10,000-2 (PSF/NMP/PDXL10,000 = 12/86/2 wt%) to PDXL200,000-2 (PSF/NMP/PDXL200,000 = 12/86/2 wt%) from 22.4 L/m$^2$h to 28.5 L/m$^2$ h. In addition, PF is higher when using PDXL as additive (PSF/NMP/PDXL10,000, 22.4 L/m$^2$h) than using PEG as additives (PSF/NMP/PEG10,000, 19.5 L/m$^2$h) as shown in Figures 23 and 24. These PF results agreed well with the enlargement of pore size and porosity of the top-layer in the PSF membranes prepared as the concentration and molecular weight of PEG or PDXL increase from the SEM observations. It is also found that the top-layer was thicker as higher concentration and molecular weight of PEG is added. However it has been widely accepted that the permeate flux decreases as the thickness of the top-layer increases (Jung *et al.*, 2004). Therefore, it can be concluded that PF is dependent on the pore size and porosity rather than on the thickness of the top-layer for the PSF membrane prepared.

The cross-sectional morphology of membranes after filtration was characterized by infinite focus microscopy (IFM) as depicted in Figure 25. The IFM pictures were taking from PSF (PSF/NMP = 12/88 wt%) and PDXL200,000-5 (PSF/NMP/PDXL200000 = 12/83/5 wt%). As expected, a heavy sericin protein cake layer was developed on the membrane surface.

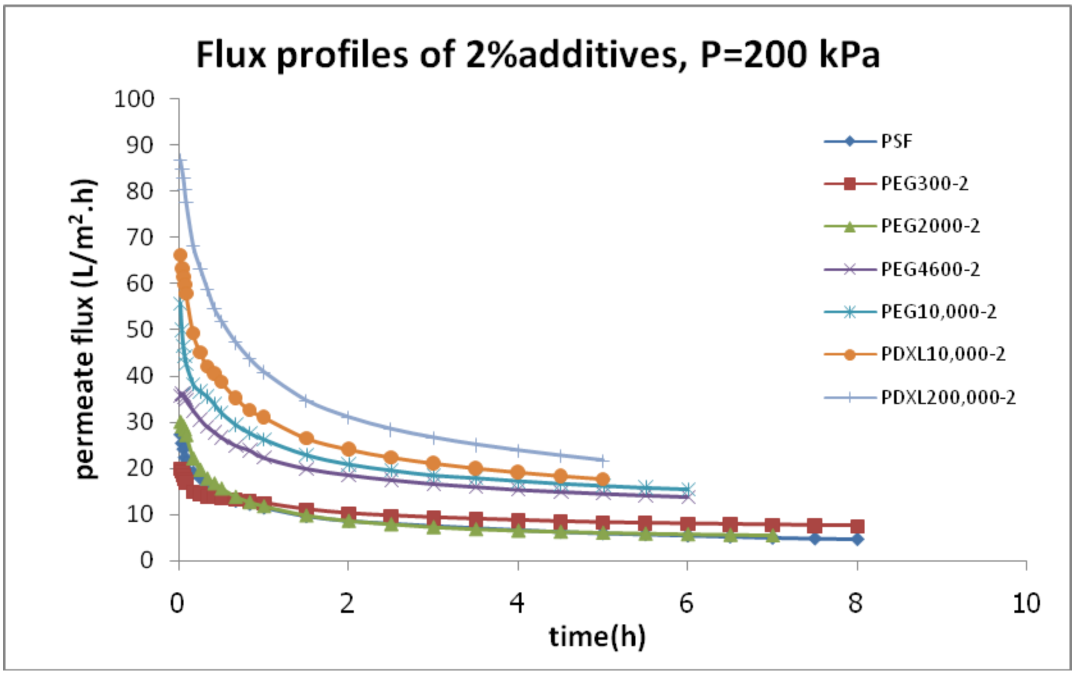


**Figure 17** Flux profile during compaction of membranes prepared from 2 wt% of PEG and PDXL at transmembrane pressure of 200 kPa

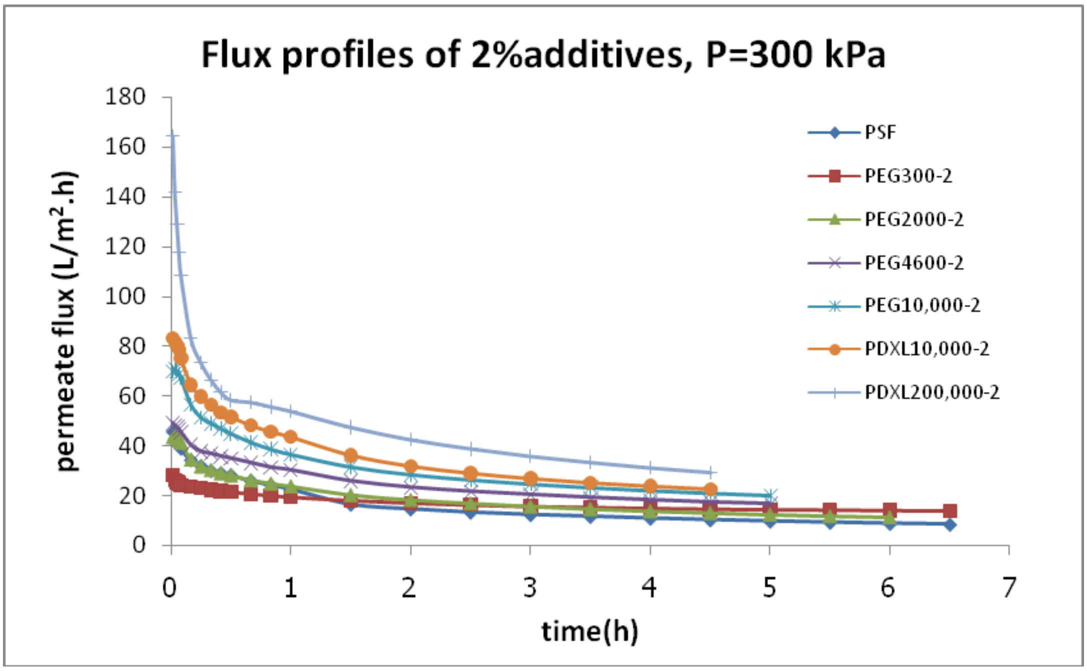


**Figure 18** Flux profile during compaction of membranes prepared from 2 wt% of PEG and PDXL at transmembrane pressure of 300 kPa

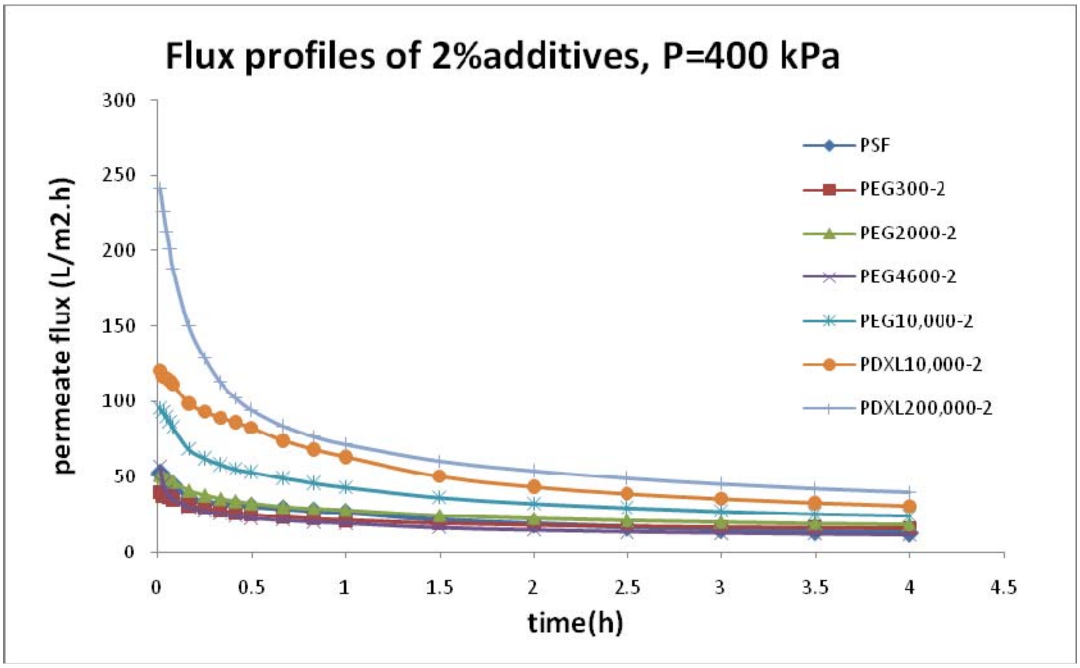


**Figure 19** Flux profile during compaction of membranes prepared from 2 wt% of PEG and PDXL at transmembrane pressure of 400 kPa

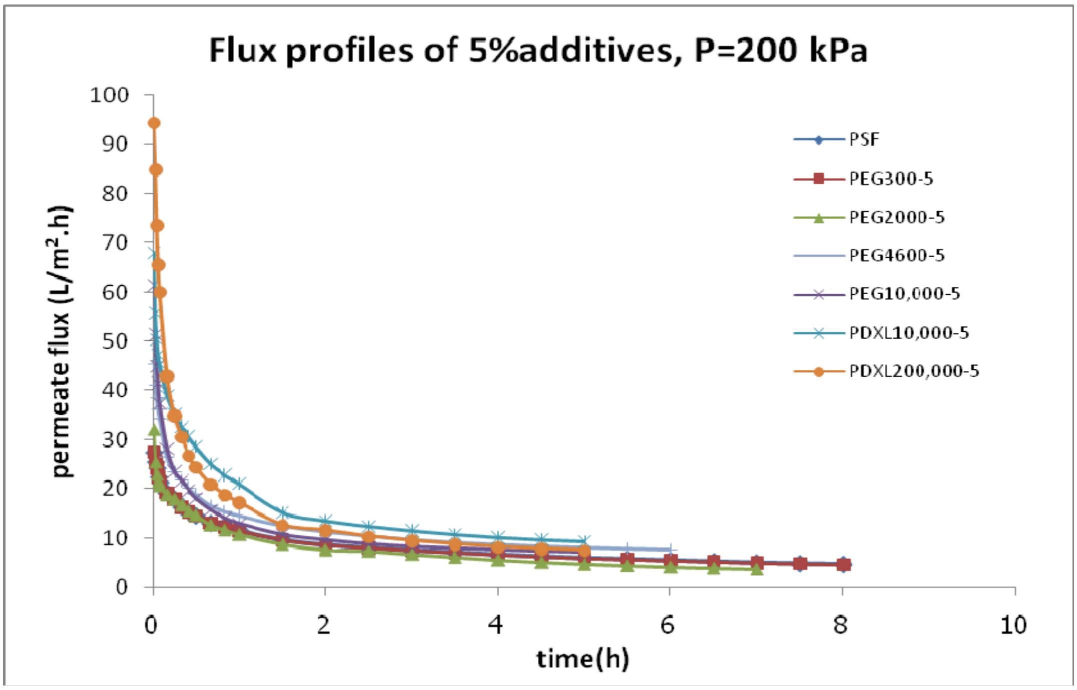


**Figure 20** Flux profile during compaction of membranes prepared from 5 wt% of PEG and PDXL at transmembrane pressure of 200 kPa

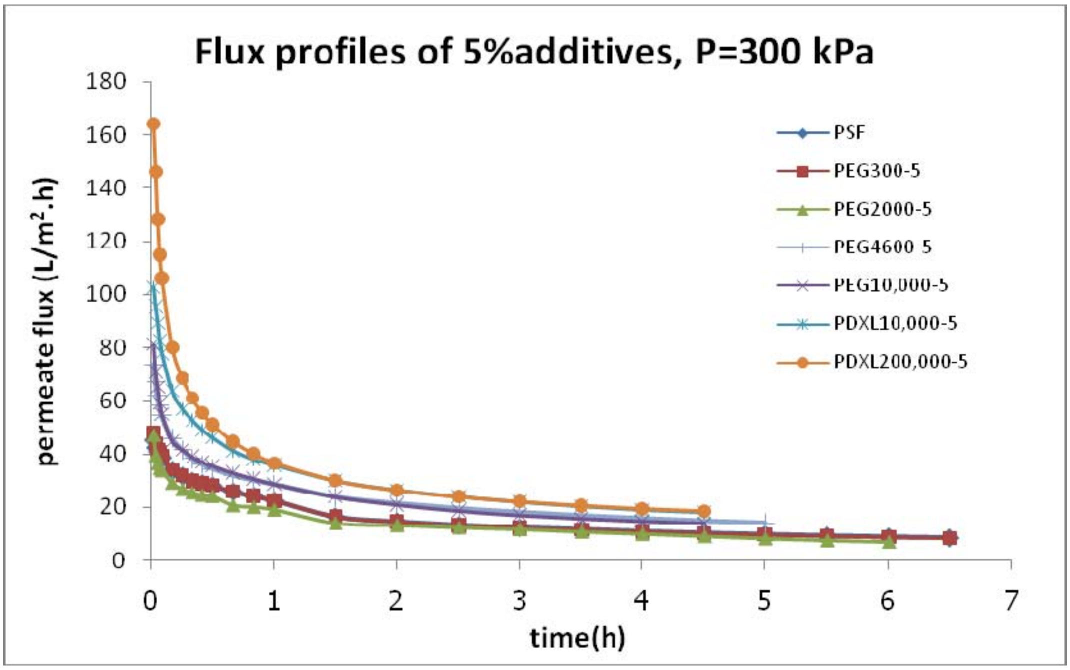


**Figure 21** Flux profile during compaction of membranes prepared from 5 wt% of PEG and PDXL at transmembrane pressure of 300 kPa

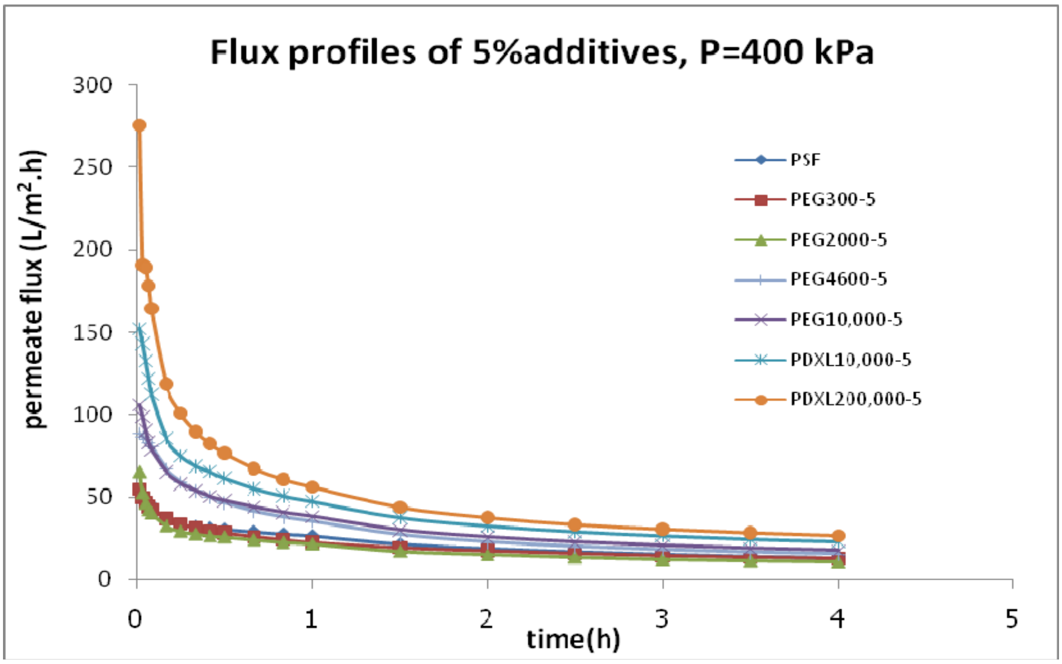


**Figure 22** Flux profile during compaction of membranes prepared from 5 wt% of PEG and PDXL at transmembrane pressure of 400 kPa

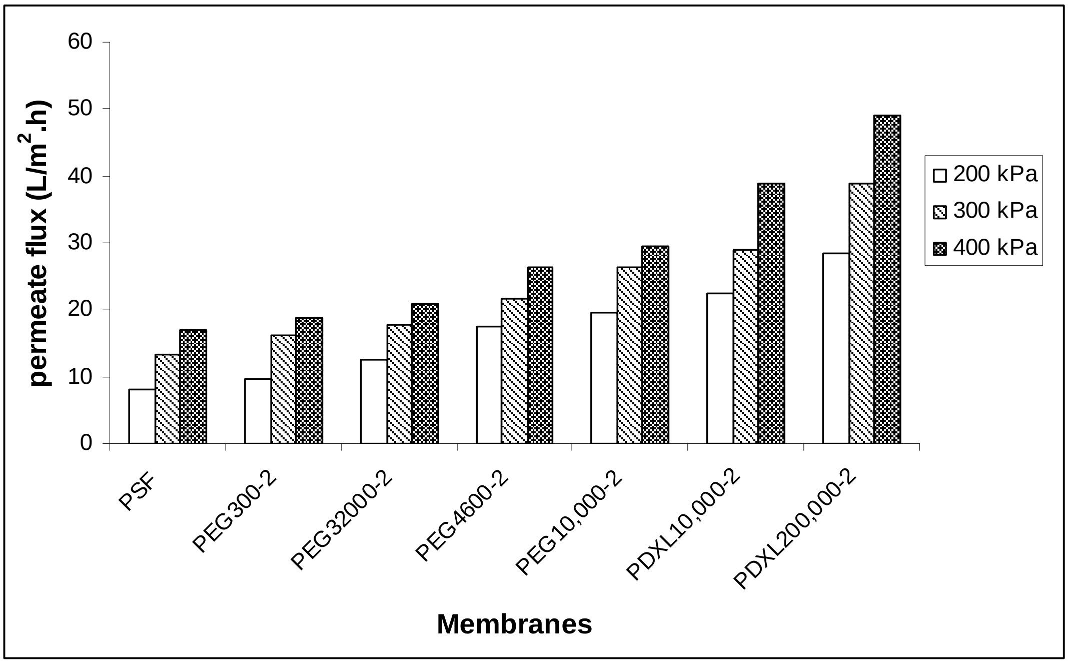


**Figure 23** Effect of transmembrane pressure on permeate flux for PSF membranes prepared with 2 wt% of PEG and PDXL

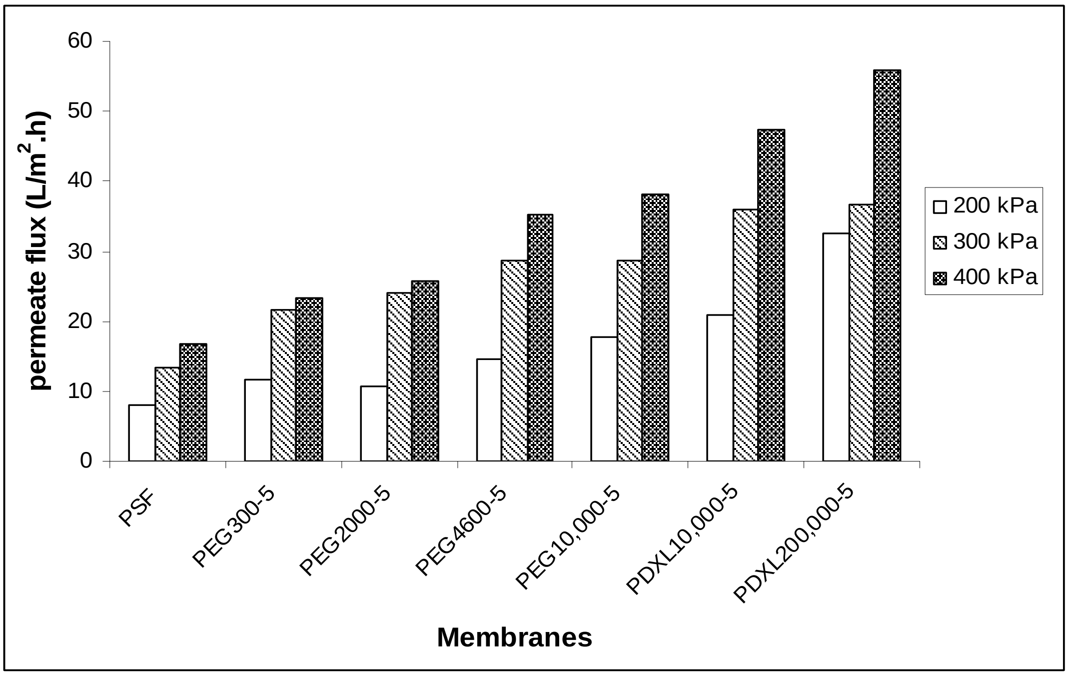


**Figure 24** Effect of transmembrane pressure on permeate flux for PSF membranes prepared with 5 wt% of PEG and PDXL

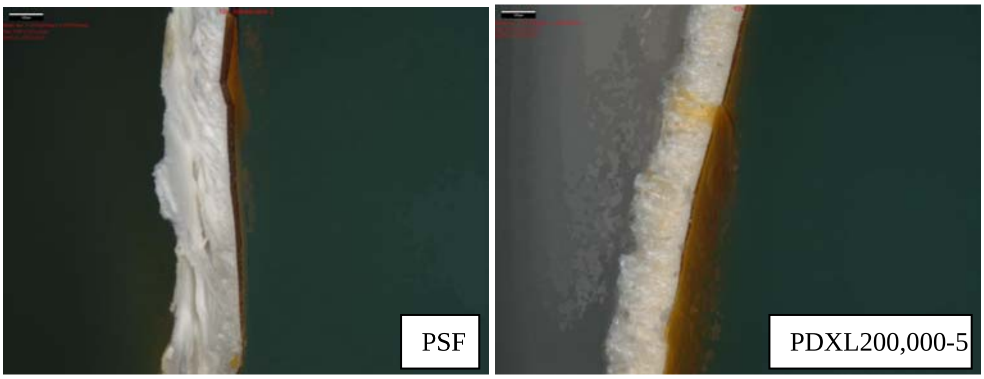


**Figure 25** IFM images of PSF and PDXL200,000-5 membranes cross-section after filtration process

## 3. Protein retention

Table 2 showed the concentration and percentage of protein retention obtained from silk cocoon degumming wastewater as feed. The effect of concentration and molecular weight of PEG and PDXL additives on the protein retention at various transmembrane pressures is shown in Figure 26 and Figure 27. The results showed that with increasing transmembrane pressures from 200 kPa to 400 kPa, the concentration and percentage of protein retention are found to slightly decrease. For examples, at transmembrane pressure of 200 kPa with the same 2% concentration of PEG and PDXL, the percent protein retention was decreased from 75.4% of PEG300-2 to 60.8% of PEG10,000-2 and from 60.6% of PDXL10,000-2 to 52.4% of PDXL200,000-2 when increasing the molecular weight of PEG from 300 to 10,000 Da and PDXL from 10,000 to 200,000 Da, respectively. At the same 300 Da molecular weight of the PEG used and transmembrane pressured at 200 kPa, the percent protein retention was also decreased from 75.4% of PEG300-2 to 66.4% of PEG300-5 when increasing the concentration of PEG from 2 to 5 wt%. This protein retention corresponded with the permeate flux and also agreed well with the enlargement of pore size and porosity of the top-layer in the PSF membranes prepared as the concentration and molecular weight of PEG or PDXL increased as seen from the SEM observations.

**Table 2** Percentage of protein retention from membranes prepared in this study.

| membrane | Protein retention (%) | | |
|---|---|---|---|
| | 200 kPa | 300 kPa | 400 kPa |
| PSF | 71.9 | 70.2 | 68.2 |
| PEG300-2 | 75.4 | 69.7 | 67.8 |
| PEG2000-2 | 70.0 | 68.3 | 64.9 |
| PEG4600-2 | 65.6 | 60.6 | 61.3 |
| PEG10,000-2 | 60.8 | 57.9 | 48.8 |
| PDXL10,000-2 | 60.6 | 56.7 | 47.6 |
| PDXL200,000-2 | 52.4 | 43.3 | 42.3 |
| PEG300-5 | 66.4 | 64.7 | 63.5 |
| PEG2000-5 | 62.0 | 59.1 | 55.5 |
| PEG4600-5 | 59.1 | 47.1 | 48.6 |
| PEG10,000-5 | 46.4 | 39.2 | 36.1 |
| PDXL10,000-5 | 36.5 | 37.0 | 33.4 |
| PDXL200,000-5 | 33.2 | 29.3 | 25.5 |

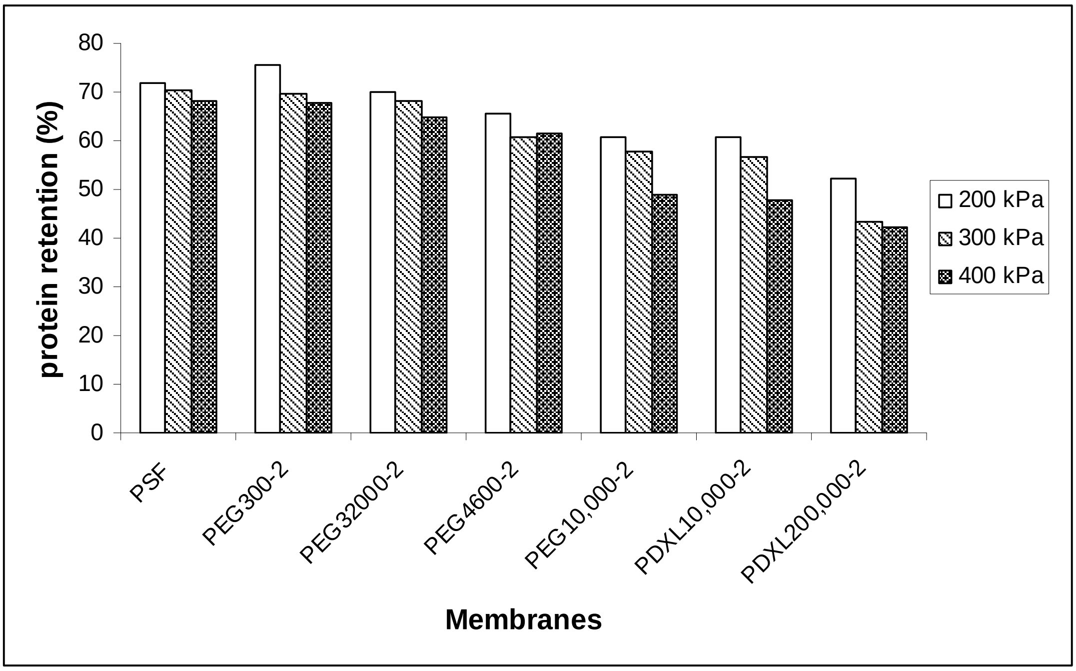


**Figure 26** Effect of transmembrane pressure on protein retention for PSF membranes prepared with 2 wt% of PEG and PDXL

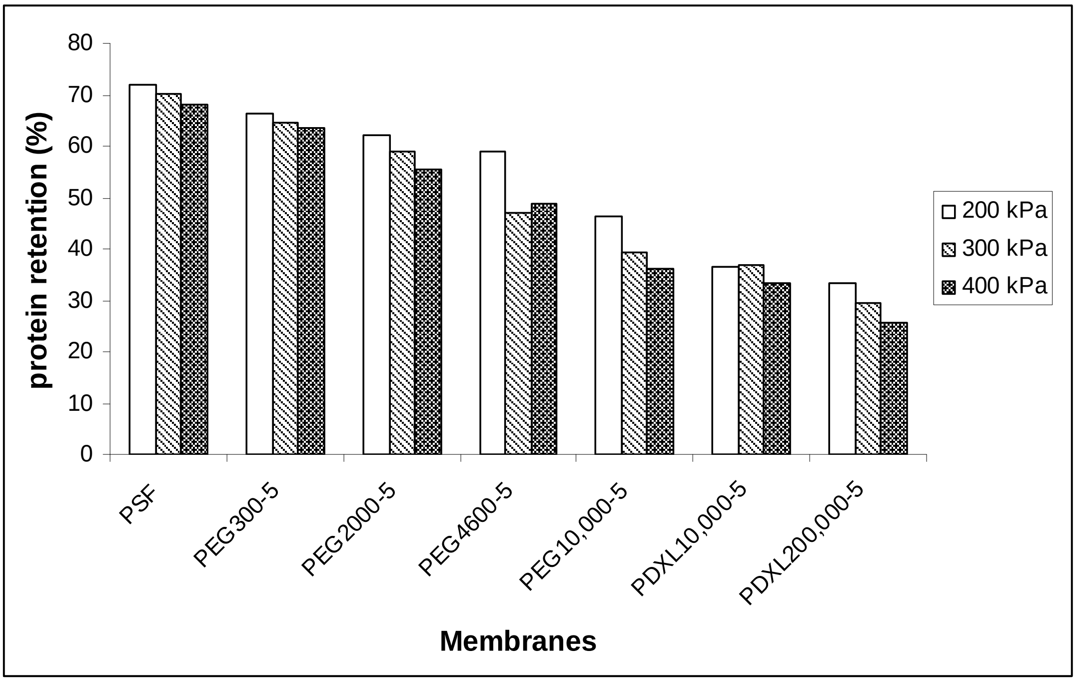


**Figure 27** Effect of transmembrane pressure on protein retention for PSF membranes prepared with 5 wt% of PEG and PDXL

### 4. Solid retention

Table 3 showed the results of percentage solid retention. It seemed that PEG membranes showed higher percentage of solid retention than PDXL membranes. The percentage solid retention was in agreement with the percentage protein retention. The high retention and comparatively low permeate flux in PEG membranes may also be understood from the morphology of their cross section as discussed earlier. The thin asymmetric layer probably explained for the improvement in the solid retention while the thick sponge finger-like sublayer took part for the rather low permeate flux. Therefore, membranes with PEG as additive showed higher solid retention than PDXL membranes. Membranes with high transmembrane pressure showed lower solid retention than the lower transmembrane pressure for both additives (Figure 28 and Figure 29). For examples, at transmembrane pressure of 200 kPa with the same 2 wt% of PEG and PDXL, the percent solid retention was decreased from 51.4% of PEG300-2 to 34.7% of PEG10,000-2 and from 25.9% of PDXL10,000-2 to 22.2% of PDXL200,000-2 when increasing the molecular weight of PEG from 300 to 10,000 Da and PDXL from 10,000 to 200,000 Da, respectively. At the same 300 Da molecular weight of the PEG used and transmembrane pressured at 200 kPa, the percent protein retention was also decreased from 51.4% of PEG300-2 to 47.7% of PEG300-5 when increasing the concentration of PEG from 2 wt% to 5 wt%, respectively. The effect of different additives at the same concentration and molecular weight also shown that when using PEG as additive percent solid retention was higher (PEG10,000-2, 34.7% and PDXL10,000-2, 25.9%) than using PDXL as additives (PEG10,000-5, 36.6% and PDXL10,00-5, 21.6%) at 200 kPa as shown in Figures 28 and 29.

**Table 3** Percentage of solid retention from membranes prepared in this study

| membrane | Solid retention (%) | | |
|---|---|---|---|
| | 200 kPa | 300 kPa | 400 kPa |
| PSF | 41.7 | 45.9 | 42.1 |
| PEG300-2 | 51.4 | 45.7 | 49.1 |
| PEG2000-2 | 39.0 | 35.0 | 41.9 |
| PEG4600-2 | 39.9 | 36.1 | 35.4 |
| PEG10,000-2 | 34.7 | 28.5 | 31.8 |
| PDXL10,000-2 | 25.9 | 21.1 | 21.5 |
| PDXL200,000-2 | 22.2 | 20.5 | 16.8 |
| PEG300-5 | 47.7 | 40.9 | 40.5 |
| PEG2000-5 | 42.7 | 41.6 | 41.8 |
| PEG4600-5 | 38.6 | 31.2 | 29.5 |
| PEG10,000-5 | 36.6 | 30.0 | 30.0 |
| PDXL10,000-5 | 21.6 | 19.4 | 18.5 |
| PDXL200,000-5 | 19.1 | 20.9 | 14.4 |

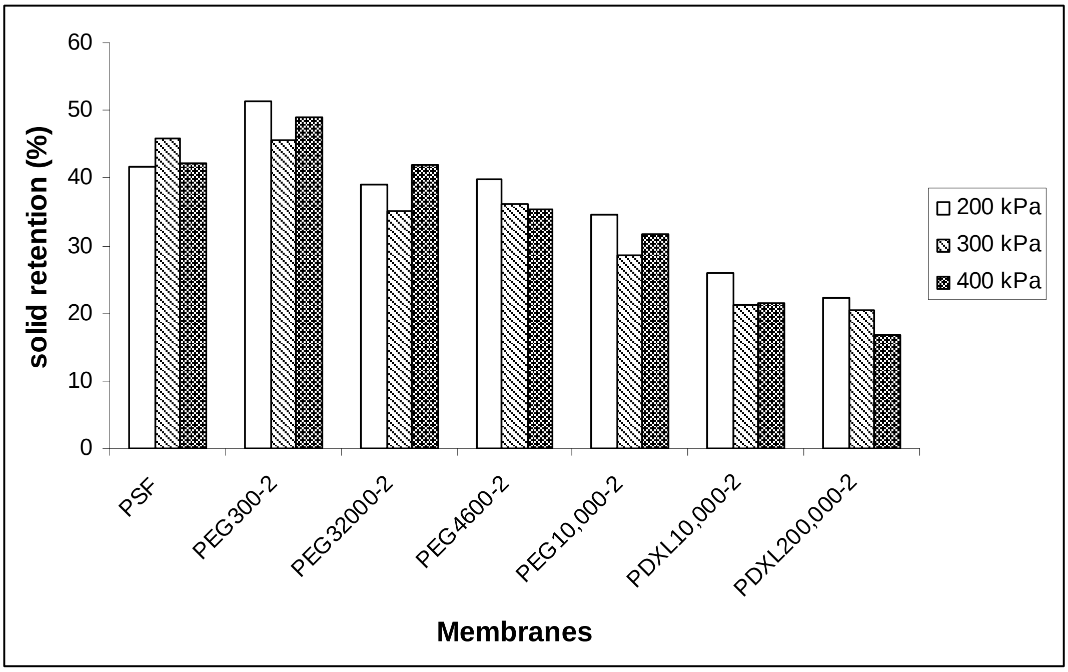


**Figure 28** Effect of transmembrane pressure on solid retention for PSF membranes prepared with 2 wt% of PEG and PDXL

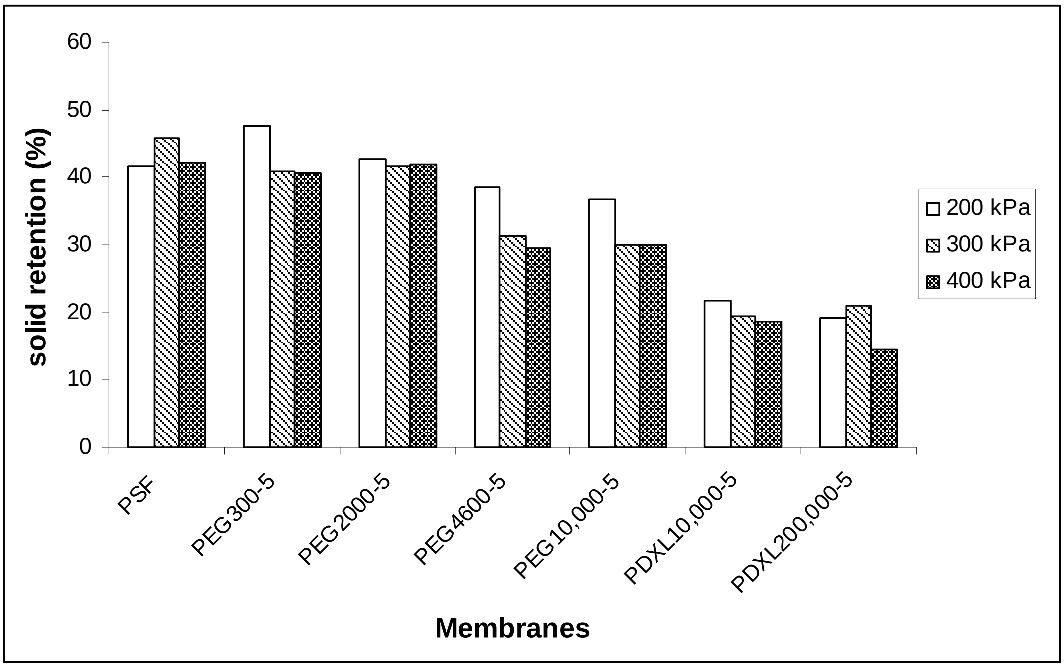


**Figure 29** Effect of transmembrane pressure on solid retention for PSF membranes prepared with 5 wt% of PEG and PDXL

## 5. Molecular weight cut-off of sericin

The molecular weight cut off (MWCO) in the silk cocoon degumming wastewater feed prior to membrane filtration and the permeate were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as shown in Table 4 and the profile of their molecular weight distribution was shown in Figure 30. Electrophoresis results indicated that the molecular weight distribution of sericin in the silk cocoon degumming wastewater feed obtained was 10 kDa to 250 kDa (Figure 30). Molecular weight of sericin is commonly ranging from 10 kDa to over 300 kDa, thus sericin might be somewhat degraded by bromelain into lower molecular weight during the preparation. The results showed that molecular weight cut off (MWCO) of the permeate increased with increasing concentration, molecular weight of both additives and transmembrane pressures (200, 300 and 400 kPa) according to the pore size in the top-layer of membrane prepared as showm in Figure 31 and Figure 32. The bigger the pore size, the higher molecular weight permeated. Thus, the PSF membrane prepared can be used for sericin recovery from silk cocoon degumming wastewater with obtained molecular weight suitable for properly end use. For example, PEG300-2 (PSF/NMP/PEG300 = 12/86/2 wt%) can only be used to concentrate silk cocoon degumming wastewater feed, PDXL200,000-2 can be used to separate sericin with molecular weight greater than 50,000 Da at 200 kPa and PDXL200,000-5 can be used to separate sericin with molecular weight greater than 75,000 Da at 200 kPa.

**Table 4** Molecular weight cut-off (MWCO) of the prepared membranes obtained in this study

| membrane | MWCO (Da) of the permeate | | |
|---|---|---|---|
| | 200 kPa | 300 kPa | 400 kPa |
| PSF | 20,000 | 25,000 | 20,000 |
| PEG300-2 | 10,000 | 17,000 | 15,000 |
| PEG2000-2 | 15,000 | 25,000 | 15,000 |
| PEG4600-2 | 20,000 | 25,000 | 20,000 |
| PEG10,000-2 | 37,000 | 40,000 | 50,000 |
| PDXL10,000-2 | 40,000 | 40,000 | 45,000 |
| PDXL200,000-2 | 50,000 | 60,000 | 75,000 |
| PEG300-5 | 20,000 | 27,000 | 25,000 |
| PEG2000-5 | 25,000 | 30,000 | 30,000 |
| PEG4600-5 | 35,000 | 37,000 | 40,000 |
| PEG10,000-5 | 45,000 | 50,000 | 50,000 |
| PDXL10,000-5 | 50,000 | 75,000 | 80,000 |
| PDXL200,000-5 | 75,000 | 80,000 | 100,000 |

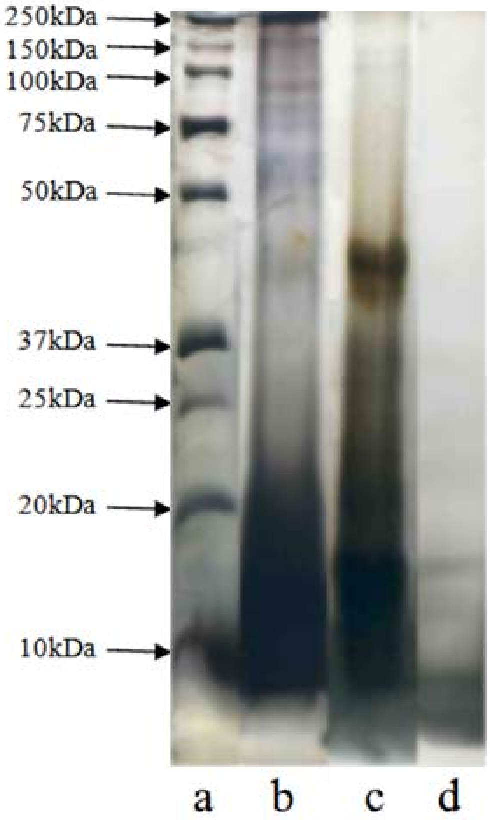


**Figure 30** SDS-PAGE of sericin (a) markers consisted of synthesis protein from 10 kDa to 250 kDa. (b) silk cocoon degumming wastewater feed (c) and (d) permeate sample from PDXL200,000-5 and PEG300-2 at transmembrane pressure 200 kPa, respectively

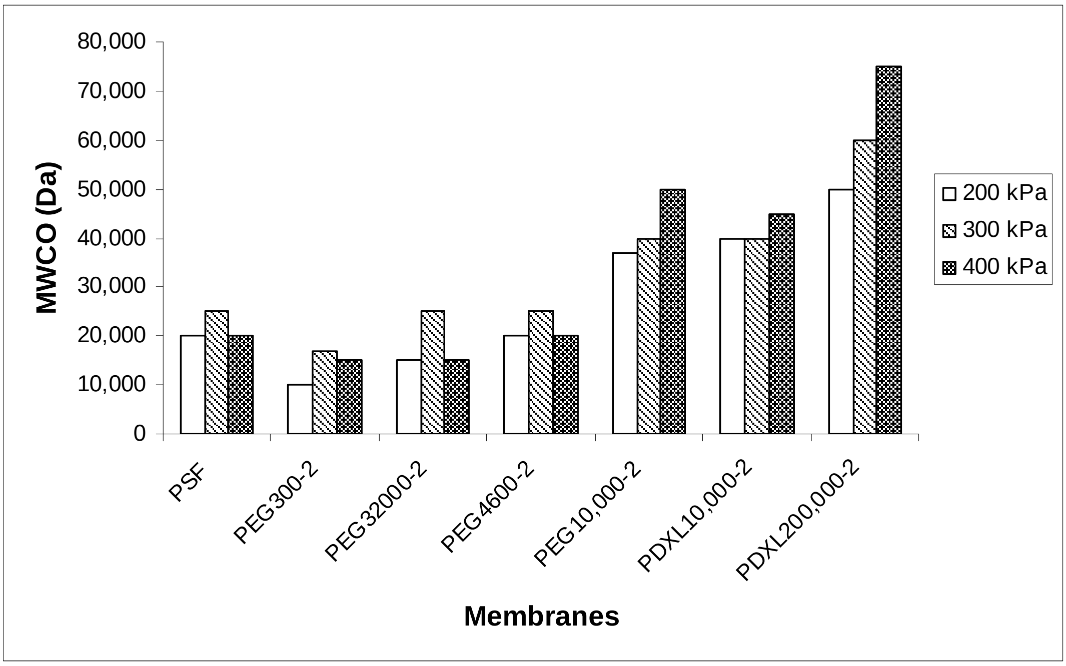


**Figure 31** Effect of transmembrane pressure on MWCO for PSF membranes prepared with 2 wt% of PEG and PDXL

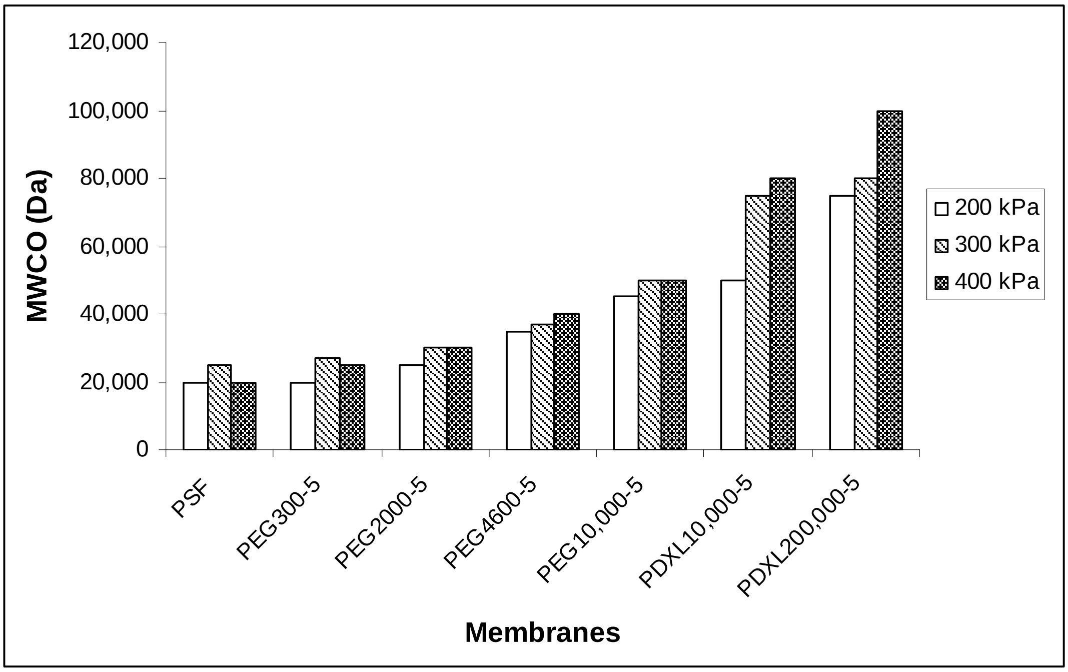


**Figure 32** Effect of transmembrane pressure on MWCO for PSF membranes prepared with 5 wt% of PEG and PDXL

# CONCLUSION

Flat sheet polysulfone (PSF) membranes were successfully prepared by phase inversion process from a homogeneous casting solution having solvent and additive concentration varying from 83 to 86 wt%, and 2 to 5 wt%, respectively, while the polymer concentration was held constant at 12 wt%. The concentration of two different additives, PDXL and PEG with various molecular weights ($\overline{M}_n$) ranging from 300, 2000, 4600 to 10,000 Da for PEG and 10,000 to 200,000 Da for PDXL were used in the membrane prepared.

Cross sectional SEM morphologies showed that all PSF membranes prepared with PEG or PDXL as additives were having asymmetric structures consisting of a dense top-layer and a porous with finger-like cavities sub-layer. Pore size and porosity of the top-layer increased as the concentration and molecular weight of PEG or PDXL increased. Top-layer of the prepared membranes was thicker as more concentration and higher molecular weight of PEG was used, but it did not thicker when PDXL was used as additive. Increasing the concentration and molecular weight of both additives caused significant suppression of the finger-like cavities in the sub-layer.

Addition of PEG and PDXL additives exhibited better permeate flux (PF) than membrane prepared from only PSF/NMP, however, PF of all the PSF membrane prepared was dependent on the pore size and porosity rather than on the thickness of the top-layer for the PSF membrane. PF declined gradually due to compaction with time while the steady-state value of PF for each membrane was found to increase with increasing concentration, molecular weight of both additives and transmembrane pressures (200, 300 and 400 kPa).

The percentage protein retention and percentage solid retention decreased with increasing the concentration, molecular weight of PEG and PDXL in casting solution and transmembrane pressures. Molecular weight distribution of sericin in the silk cocoon degumming wastewater feed obtained was 10 kDa to 250 kDa. SDS-PAGE resulted show that molecular weight cut off (MWCO) of the permeate increased with increasing the concentration and molecular weight of both PEG and PDXL additives and with increasing the transmembrane pressure.

The PSF membrane prepared can be used for sericin recovery from silk cocoon degumming wastewater with obtained molecular weight suitable for proper end use.

# LITERATURE CITED

Ahn, J.S., H.K. Choi, H.K. Lee, J.H. Nahm and C.S. Cho. 2001. Novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of silk sericin. **J. Appl. Polym. Sci.** 80: 274–280.

Altman, G.H., F. Diaz, C. Jakuba, T. Calabro, R.L. Horan, J. Chen, H. Lu, J. Richmond and D.L. Kaplan. 2003. Silk-based biomaterials. **Biomaterials** 24: 401-416.

Asakura, T., H. Sakai, K. Komatsu and S. Kurioka. 1992. **Carrier supporting immobilized physiologically active substance and production thereof.** Japan Patent 04-053490A.

Baker, F. 2004. **Selected Indicators of Food and Agriculture Development in Asia-Pacific Region 1993-2003.** FAO Regional Office for Asia and the Pacific, Bangkok, Thailand.

Benkhira, A., E. Franta and J. Francois. 1992. Polydioxolane in aqueous solutions 1. Phase diagram. **Macromolecules** 25: 5697-5704.

_____, _____, M. Rawiso and _____. 1994. Conformation of poly(1,3-dioxolane) in dilute and semidilute aqueous solutions. **Macromolecules** 27 (14): 3963-3972**.**

Boom, R.M., I.M Wienk, Th. Van den Boomgaard and C.A. Smolders. 1992. Microstructures in phase inversion membranes. Part 2. The role of a polymeric additive. **J. Membr. Sci.** 73: 277–292.

Capar, C., S. S. Aygun and M.R. Gecit. 2008. Treatment of silk production wastewater by membrane processes for sericin recovery. **J. Membr. Sci.** 325: 920-931.

Chakrabarty, B., A.K. Ghoshal and M.K. Purkait. 2008a. Effect of molecular weight of PEG on membrane morphology and transport properties. **J. Membr. Sci.** 309: 209-221.

_____, _____ and _____. 2008b. Preparation, characterization and performance studies of polysulfone membranes using PVP as an additive. **J. Membr. Sci.** 315: 36-47.

_____, _____ and _____. 2008c. SEM analysis and gas permeability test to characterize polysulfone membrane prepared with polyethylene glycol as additive. **J. Colloid Interf. Sci.** 320: 245-253.

Cheryan, M. 1998. **Ultrfiltration and Microfiltration Handbook.** Technomic Publishing, Co., Lancaster, PA.

Cho, K.Y., J.Y. Moon, Y.W. Lee, K.G. Lee, J.H. Yeo, H.Y Kweon, K.H. Kim, and C.S. Kim. 2003. Preparation of self-assembled silk sericin nanoparticles. **Int. J. Biol. Macromol.** 32: 36-42.

Demura, M., H. Takenoshita, T. Asakura, H. Sakai, A. Kurioka, K. Komatsu and M. Kaneko. 1992. **J Seric Sci Jpn.** 61(1): 66–72.

Fabiani, C., M. Pizzichini, M. Spadoni and G. Zeddita. 1996. Treatment of waste water from silk degumming processes for protein recovery and water reuse. **Desalination.** 105: 1–9.

Gimenes, M.L., L. Liu, and X. Feng. 2007. Sericin/poly(vinyl alcohol) blend membranes for pervaporation separation of ethanol/water mixtures. **J. Membr. Sci.** 295: 71-79.

Han, M.J. 1999. Effect of propionic acid in the casting solution on the characteristics of phase inversion polysulfone membranes. **Desalination** 121: 31-39.

_____ and S.T. Nam. 2002. Thermodynamic and rheological variation in polysulfone solution by PVP and its effect in the preparation of phase inversion membrane. **J. Membr. Sci.** 202: 55-61.

Harris, J.E. and R.N. Johnson 1988. Polysulfones. *In* H.F. Mark, N.M. Bikales, C.G. Overberger and G. Menges (eds.). **Encyclopedia of Polymer Science and Engineering** Vol.13 2nd ed. New York. 196-211.

Hatakeyama, H. 1996. **Biodegradable sericin-containing polyurethane and its production.** Japan Patent 08-012738A.

Hirotsu, T. and S. Nakajima. 1998. Water–alcohol separation by pervaporation through silk fibroin membranes. **Sen'I Gakkaishi.** 44(2): 70–77.

Ishikawa, H., M. Nagura and Y. Tsuchiya. 1987. Fine structure and physical properties of blend film compose of silk sericin and poly(vinyl alcohol). **Sen'i Gakkaishi.** 43(6): 283–287.

Iwamoto, K., T. Noguchi, A. Yeramoto and E. Iizuka. 1995. Studies on physical properties of mixed membranes of silk sericin and syniotactic polyvinyl alcohol, and their ability to immobilize an enzyme. **J. Seric. Sci. Jpn.** 65(4): 427–434.

Jung, B., J.K. Yoon, B. Kim and H.W. Rhee. 2004. Effects of molecular weight of polymeric additives on formation, permeation properties and hypochlorite treatment of asymmetric polyacrylonitrile membranes. **J. Membr. Sci.** 243: 45-47.

Kabayama, M. 2000. **Synthetic resin pumice and its production.** Japan Patent 2000-014592A.

Kato, N., S. Sato, A. Yamanaka, H. Yamada, N. Fuwa and M. Nomura. 1998. Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. **Biosci. Biotechnol. Biochem.** 62: 145-147.

Kazuhisa, T., H. Takagi, M. Takahashi, H. Yamada and S. Nakamori. 2001. Cryoprotective effect of the serine-rich repetitive sequence in silk protein sericin. **J. Biochem.** 129(6): 979–986.

Kesting, R.E. 1985. **Synthetic Polymeric Membranes: A Structure Perspective**. 2nd ed. Wiley-Interscience. New York.

Kim, J.H. and K.H.Lee. 1998. Effect of PEG additive on membrane formation by phase inversion. **J. Membr. Sci.** 138: 153-163.

_____ and _____. 2004. Effect of poly(ethylene glycol) 200 on the formation of polyetherimide asymmetric membrane and its performance in aqueous solvent mixture permeation. **J. Membr. Sci.** 230: 183-188.

_____, M.S. Kang and C.K. Kim. 2005. Fabrication of membranes for liquid separation. Part 1. Ultrafiltration membranes prepared from novel miscible blends of polysulfone and poly(1-vinylpyrrolidone-co-acrylonitrile) copolymers. **J. Membr. Sci.** 265: 167-175.

Kimmerle, K. and H. Strathmann. 1990. Analysis of the structure-determining process of phase inversion membranes. **Desalination** 79: 283–302.

Kongdee, A. and N. Chinthawan. 2007. Modification of cotton fibers with sericin using non-formaldehyde released crosslinking agents. **RJTA.** 11(3): 18-26.

Koros, W.J. and I. Pinnau. 1994. **Polymeric Gas Separation Membranes**. D.R. Paul, D.R and S. Yampol. eds. CRC Press. Boca Raton. 209-271.

Lamoolphak, W., D. Wanchai and S. Artiwan. 2008. Hydrothermal production and characterization of protein and amino acids from silk waste. **Bioresour. Technol.** 99: 7678–7685.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature.** 227: 680–685.

Lee, H.J., J. Won, H. Lee and Y.S. Kang. 2002. Solution properties of poly(amic acid)-NMP containing LiCl and their effects on membrane morphologies. **J. Membr. Sci.** 196: 267-277.

Lee, S.R., K. Miyazaki, K. Hisada and T. Hori. 2004. Application of silk sericin to finishing of synthetic fabrics. **Sen'i Gakkaishi** (The society of fiber science and technology , Japan). 60(1): 9-15.

Li, X. 1996. **Usage of sericin in durable material.** China Patent 1116227A.

Lloyd, D.R. 1985. **Materials science for synthetic membranes.** ACS Symp. Ser. 269: ACS, Washington DC. 273-294.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. **J. Biol. Chem.** 193: 265-275.

Marcelino, L.G., L. Li and F. Xianshe. 2007. Sericin/poly(vinyl alcohol) blend membranes for pervaporation separation of ethanol/water mixtures. **J. Membr. Sci.** 295: 71–79.

Miyairi, S. and M. Sugiura. 1978. Properties of b-glucosidase immobilized in sericin membrane. **J. Ferment. Technol.** 56(4): 303–308.

Miyake, H., S. Yamashita, H. Wakisaka, Y. Shimizu and M. Nagura. 2004. Fiber processing by high molecular weight sericin and its basic properties. **Sen'i Gakkaishi.** 60(9): 271-275.

Mizoguchi, K., T. Iwatsubo and N. Aisaka. 1991. **Separating membrane made of cross-linked thin film of sericin and production thereof.** Japan Patent 03-284337A.

Mulder, M. 1996. **Basic Principles of Membrane Technology**. 2nd ed. Kluwar Academic Publishers. Boston. MA.

Munari, S., A. Bottino, G. Capannelli, P. Moretti and P.Petit Bon. 1988. Preparation and characterization of polysulfone-polyvinylpyrrolidone based membranes. **Desalination** 70: 265-275.

Murase, M. 1994. **Method for solubilizing and molding cocoon silk, artificial organ made of cocoon silk, and medical element made of cocoon silk.** Japan Patent 06-166850A.

Myung, S.W., I.H. Choi, S.M. Lee, J.Y. Park, I.C. Kim and K.H. Lee. 2008. Separation of silk proteins and silk oligopeptides by thin film composite ultrafiltration membrane. Desalination 234: 158–165.

Nakajima, Y. 1994. **Liquid crystal element.** Japan Patent 06-018892A.

Nakamura, K. and Y. Koga. 2001. **Sericin-containing polymeric hydrous gel and method for producing the same.** Japan Patent 2001-106794A.

Nomura, M., Y. Iwasa and H. Araya. 1995. **Moisture absorbing and desorbing polyurethane foam and its production.** Japan Patent 07-292240A.

Pinnau, I. and B.D. Freeman. 2000. Formation and modification of polymeric membranes: overview. I. Pinnau and B.D. Freeman. eds. **Membrane formation and modification.** ACS Symp. Ser. 744. Washington, DC. 1-22

Reuvers, A.J. and C.A. Smolders. 1987b. Formation of membranes by means of immersion precipitation: Part II. The mechanism of formation of membranes prepared from the system cellulose acetate–acetone water. **J. Membr. Sci.** 34: 67–86.

_____, W.A. van der Berg and C.A. Smolders. 1987a. Formation of membranes by means of immersion precipitation. Part I. A model to describe mass transfer during immersion precipitation. **J. Membr. Sci.** 34: 45-65.

Sarovart, S., B. Sudatis, P. Meesilpa, B. P. Grady and R. Magaraphan. 2003. The use of sericin as an antioxidant and antimicrobial for polluted air treatment. **Rev. Adv. Mater. Sci.** 5: 193-198.

Sasaki, M., H. Yamada and N. Kato. 2000. Consumption of silk protein, sericin elevates intestinal absorption of zinc, iron, magnesium and calcium in rats. **Nutr. Res.** 20: 1505-1511.

Takai, Y. 1999. **Hydrophilic fiber and aggregate of the same and production thereof.** Japan Patent 11-350352A.

Tamada, Y. 1997. **Anticoagulant and its production.** Japan Patent 09-227402A.

Tanaka, T. 2001. **Antifrosting method, antifrosting agent and snow melting agent.** Japan Patent 2001-055562A.

Tsubouchi, K. 1999a. **Wound covering material.** U.S. Patent US5951506.

_____. 1999b. **Occlusive dressing consisting essentially of silk fibroin and silk sericin and its production.** Japan Patent 11-070160A.

Tsukada, M., S. Hayasaka, K. Inoue, S. Nishikawa and S. Yamamoto. 1999. **Cell culture bed substrate for proliferation of animal cell and its preparation.** Japan Patent 11-243948A.

Ueda, K. and M. Makita. 2000. **Rubber molding having durable skincare property.** Japan Patent 2000-169595A.

Vaithanomsat, P. and V. Kitpreechavanich. 2008. Sericin separation from silk degumming waste water. **Sep. Purif. Technol.** 59: 129-133.

Voegeli, R., J. Meier and R. Blust. 1993. Sericin silk protein: unique structure and properties. **Cosmetics Toiletries** 108: 101-108.

Wakabayashi, S. and M. Sugioka. 1994. **Synthetic fiber improved in hygroscopicity.** Japan Patent 06-017372A.

Wang, S., Y. Goto, Y. Ohkoshi and M. Nagura. 1998. Structures and physical properties of poly (vinyl alcohol)/sericin blend hydrogel membranes. **J. Seric. Sci. Jpn.** 67(4): 295–302.

Wu, J. H., Z. Wang and S.Y. Xu. 2007. Preparation and characterization of sericin powder extracted from silk industry wastewater. **Food Chem.** 103: 1255-1262.

_____, _____ and _____. 2008. Enzymatic production of bioactive peptides from sericin recovered from silk industry wastewater. **Process Biochemistry** 43: 480–487.

Xu, Z.L., T.S. Chung, and Y. Huang. 1999. Effect of polyvinylpyrrolidone molecular weights on morphology, oil/water separation, mechanical and thermal properties of polyetherimide/polyvinylpyrrolidone hollow fiber membranes. **J. Appl. Polym. Sci.** 74: 2220-2233.

Yamada, H. and A. Matsunaga. 1994. **Synthetic fiber woven or knitted fabric improved in hygroscopicity.** Japan Patent 06-017373A.

_____ and M. Nomura. 1998. **Fibrous article for contact with skin.** Japan Patent 10-001872A.

_____ and Y. Fuwa. 1993b. **Filter membrane and production thereof.** Japan Patent 05-345117A.

_____ and _____ 1993a. **Pervaporation membrane and production thereof.** Japan Patent 05-345118A.

Yamasaki, A., R. K. Tyagi, A. E. Fouda, K. Jonnason and T. Matsuura. 2000. Effect of surfactant as an additive on the formation of asymmetric polysulfone membranes for gas Separation. I. Pinnau and B.D. Freeman. eds. **Membrane formation and modification.** ACS Symposium series 744. Washington, DC. 87-95

Yeo, H.T., S.T. Lee and M.J. Han. 2000. Role of polymer additive in casting solution in preparation of phase inversion polysulfone membranes. **J. Chem. Eng. Jpn.** 33: 180-185.

Yoshikawa, M., A. Murakami and Y. Okushita. 2001a. **A blend film containing agar or/and agarose, and sericin and production thereof.** Japan Patent 2001-129371A.

_____,_____ and _____. 2001b. **Separating membrane and separating method thereof.** Japan Patent 2001-129373.

Young, T.H. and L.W. Chen. 1995. Pore formation mechanism of membranes from phase inversion process. **Desalination** 103: 233-247.

Zemand, L.J. and A.L. Zydney. 1996. **Microfiltration and Ultrafiltration: Principles and Applications.** Marcel Dekker. New York.

Zhang, Y.Q. 2002. Applications of natural silk protein sericin in biomaterials. **Biotechnol. Adv.** 20(2): 91–101.

_____, M.L. Tao, W.D. Shen, Y.Z. Zhou, Y. Ding, Y. Ma and W.L. Zhou. 2004. Immobilization of l-asparaginase on the microparticles of the natural silk sericin protein and its characters. **Biomaterials** 25: 3751–3759.

Zhaorigetu, S., M. Sasaki, H. Watanabe and N. Kato. 2001. Supplemental silk protein, sericin, suppresses colon tumorigenesis in 1,2-dimethylhydrazine-treated mice by reducing oxidative stress and cell proliferation. **Biosci. Biotechnol. Biochem.** 65(10): 2181-2186.

_____, N. Yanaka, _____, _____ and _____. 2003. Inhibitory effects of silk protein, sericin on UVB-induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse. **J. Photochem. Photobiol. B: Biol.** 71: 11-17.

Zheng, Q.Z., P. Wang and Y.N. Yang. 2006a. Rheological and thermodynamic variation in polysulfone solution by PEG introduction and its effect on kinetics of membrane formation via phase-inversion process. **J. Membr. Sci.** 279: 230-237.

_____, _____, _____ and D.J. Cui. 2006b. The relationship between porosity and kinetics parameter of membrane formation in PSF ultrafiltration membrane. **J. Membr. Sci.** 286: 7-11.

# APPENDICES

# Appendix A

## Solution preparation

## 1. Potassium sodium tartrate – copper sulfate solution

Solution A: Dissolved sodium carbonate 20 g in 960 ml distilled water (DW), then add 3M sodium hydroxide 35 ml. Mix the solution well.

Solution B: Dissolved 1 g of $CuSO_4.5H_2O$ in 100 ml DW.

Solution C: Dissolved 2 g of potassium sodium tartrate in 100 ml DW.

Mix solutions A, B and C in 100: 1: 1 (v/v/v) by mixing solution B and C first and then add solution A before use.

## 2. Preparation of a 4% stacking gel

| | |
|---|---|
| 30% Acrylamide/Bis Solution, 19: 1 | 0.83 ml |
| 4X Tris-HCl/SDS, pH 6.8 | 1.67 ml |
| Distilled water | 3.77 ml |
| 10% SDS | 0.067 ml |
| 15% APS | 0.33 ml |
| TEMED | 0.02 ml |

## 3. Preparation of a 15% Resolving/separating gel

| | |
|---|---|
| 30% Acrylamide/Bis Solution, 19: 1 | 5 ml |
| 4X Tris-HCl/SDS, pH 8.8 | 1.875 ml |
| Distilled water | 3.15 ml |
| 10% SDS | 0.1 ml |
| 15% APS | 0.3 ml |
| TEMED | 0.02 ml |

### 4. Preparing 5x sample buffer for SDS-PAGE

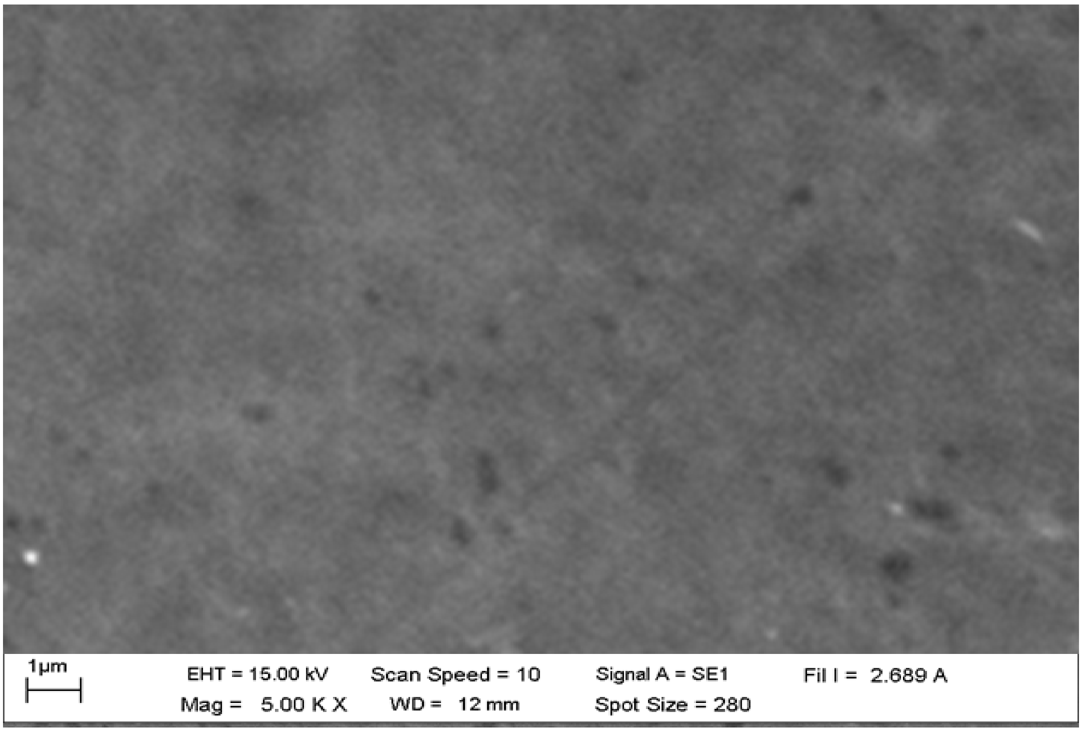
Mix 0.6 ml of 1M Tris-HCl pH 6.8 with 5 ml of 50% glycerol, 10% SDS 2 ml, β-mercaptoethanol 0.5 ml, 1% bromphenolblue 1 ml and DW 0.9 ml.

### 5. Preparing electrophoresis buffer for SDS-PAGE

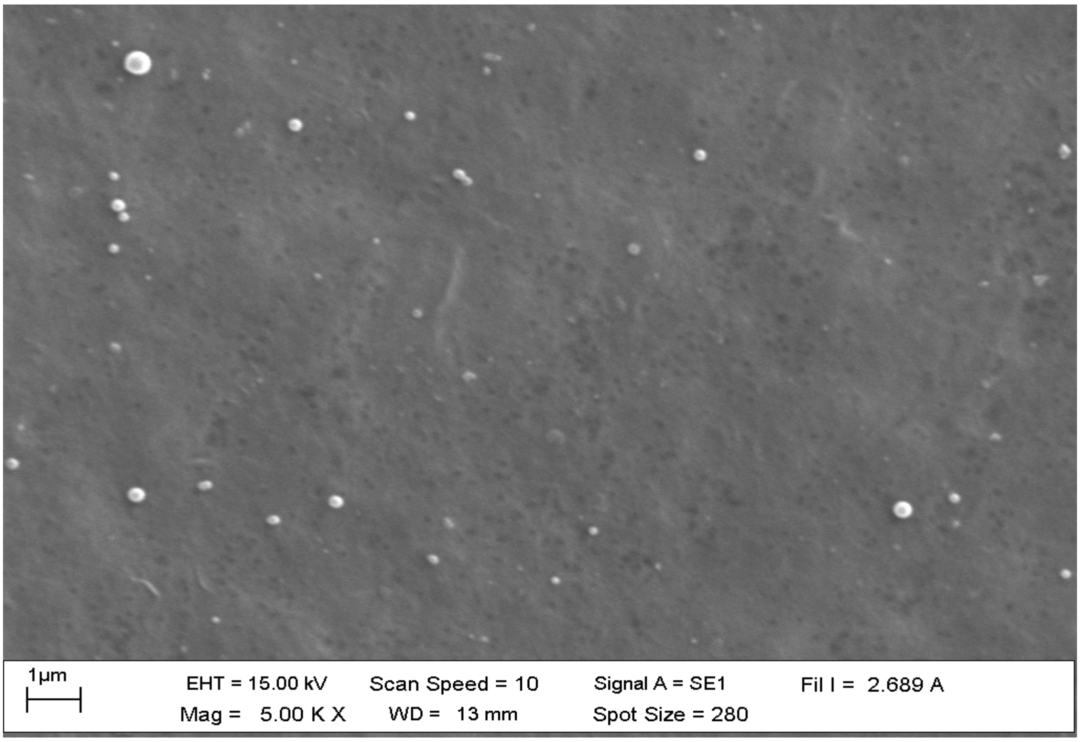
Dissolved tris(hydroxymethyl)-aminomethane 3.02 g, glycine 14.40 g and SDS 1 g in DW, adjusted volume by adding DW to reach 1 Liter.

# Appendix B

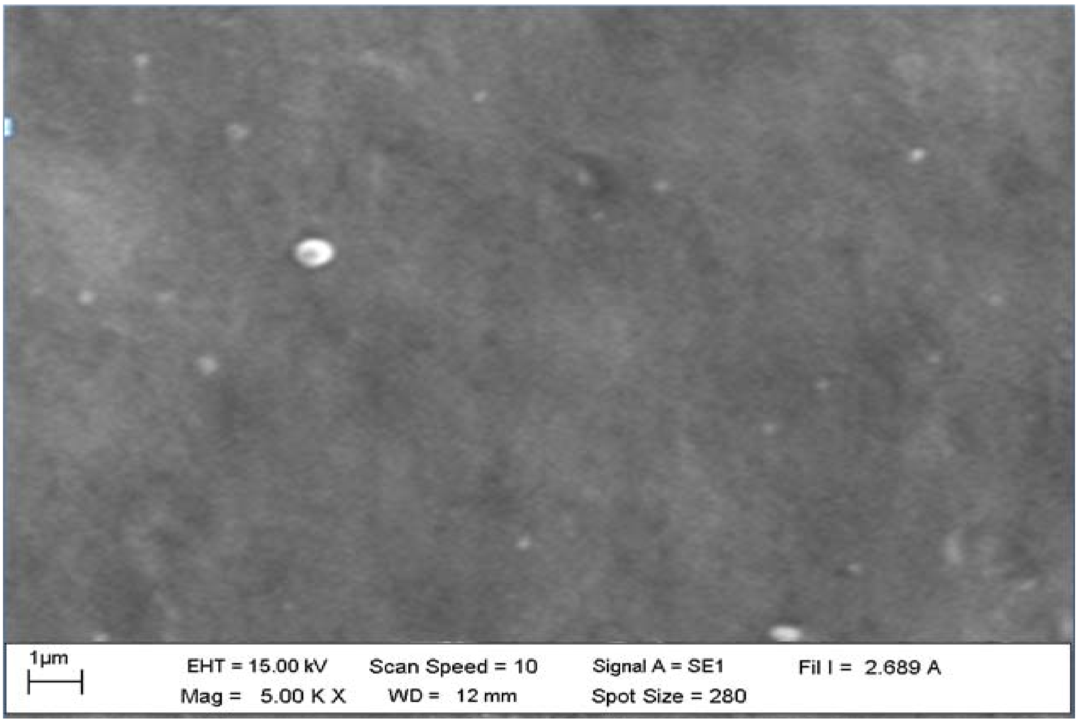
SEM top-surface (air-side) morphologies of asymmetric membranes prepared



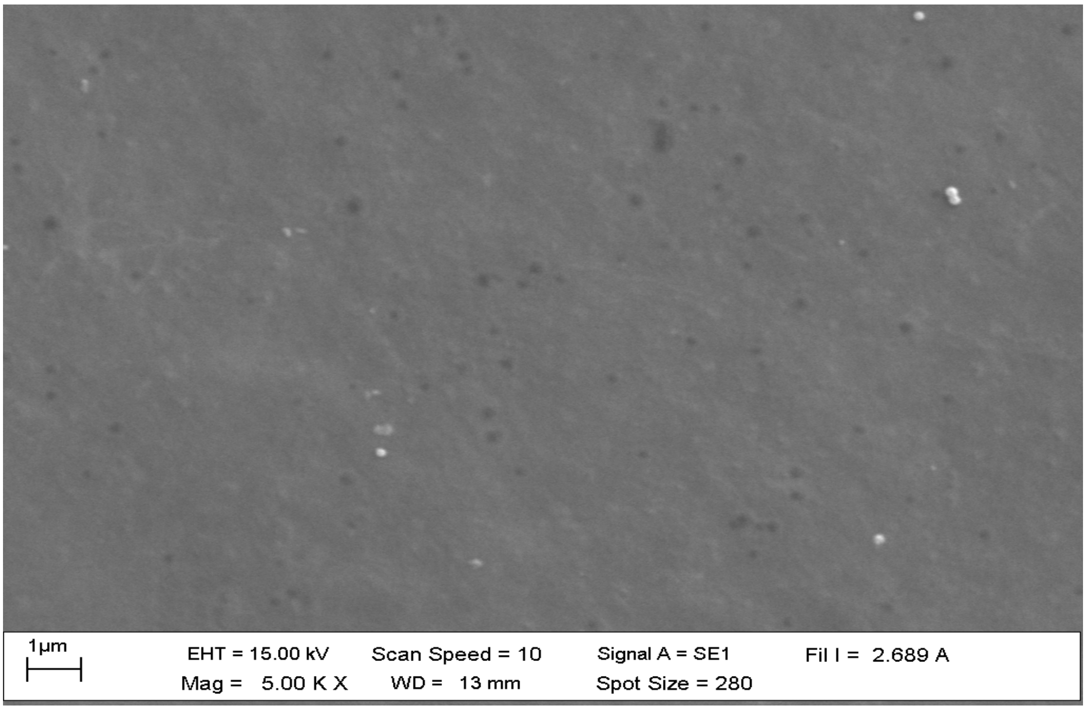
**Appendix Figure B1** (PSF) PSF/NMP = 12/88



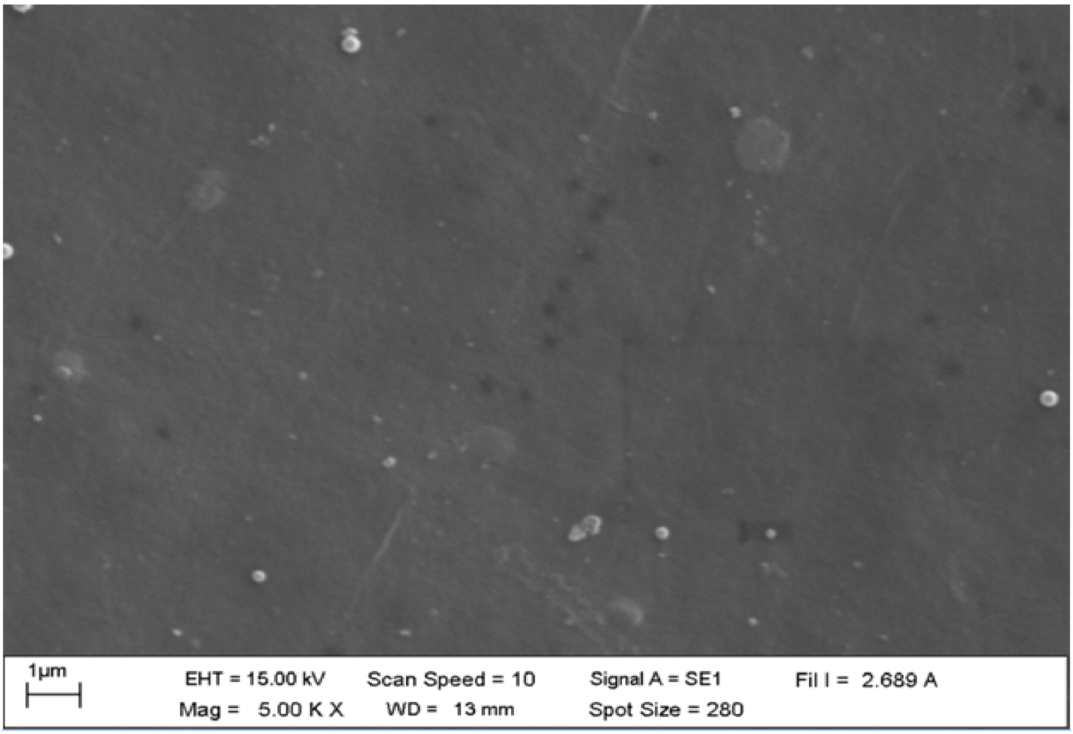
**Appendix Figure B2** (PEG300-2) PSF/NMP/PEG300 = 12/86/2



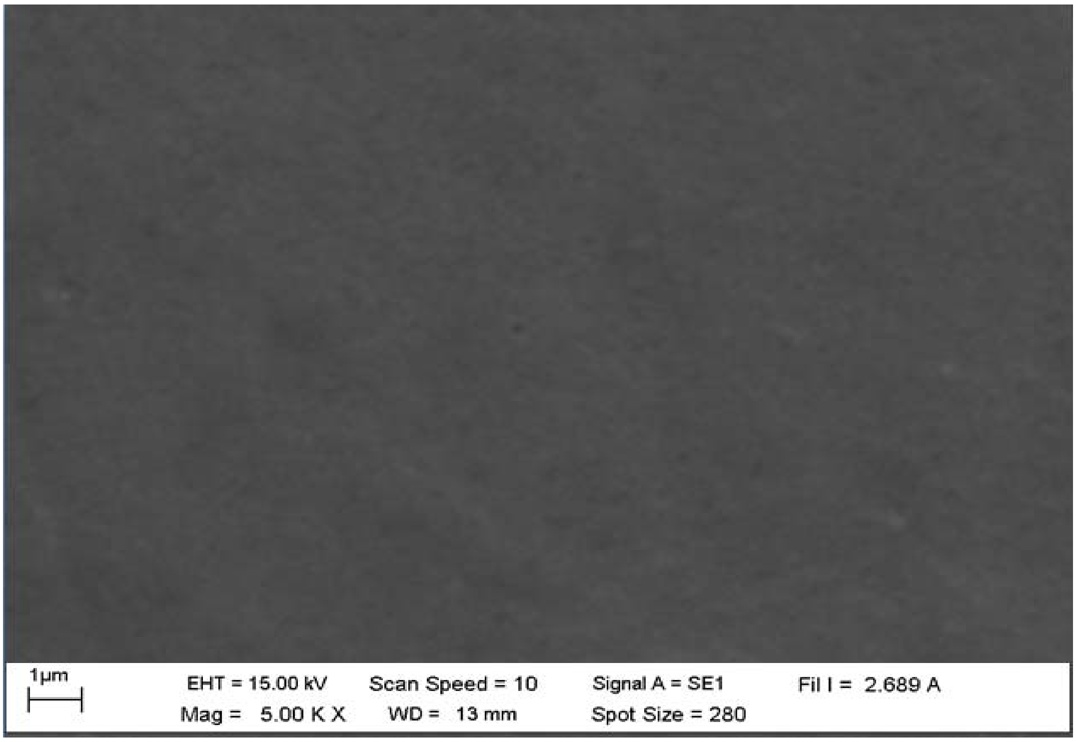
**Appendix Figure B3** (PEG2000-2) PSF/NMP/PEG2000 = 12/86/2



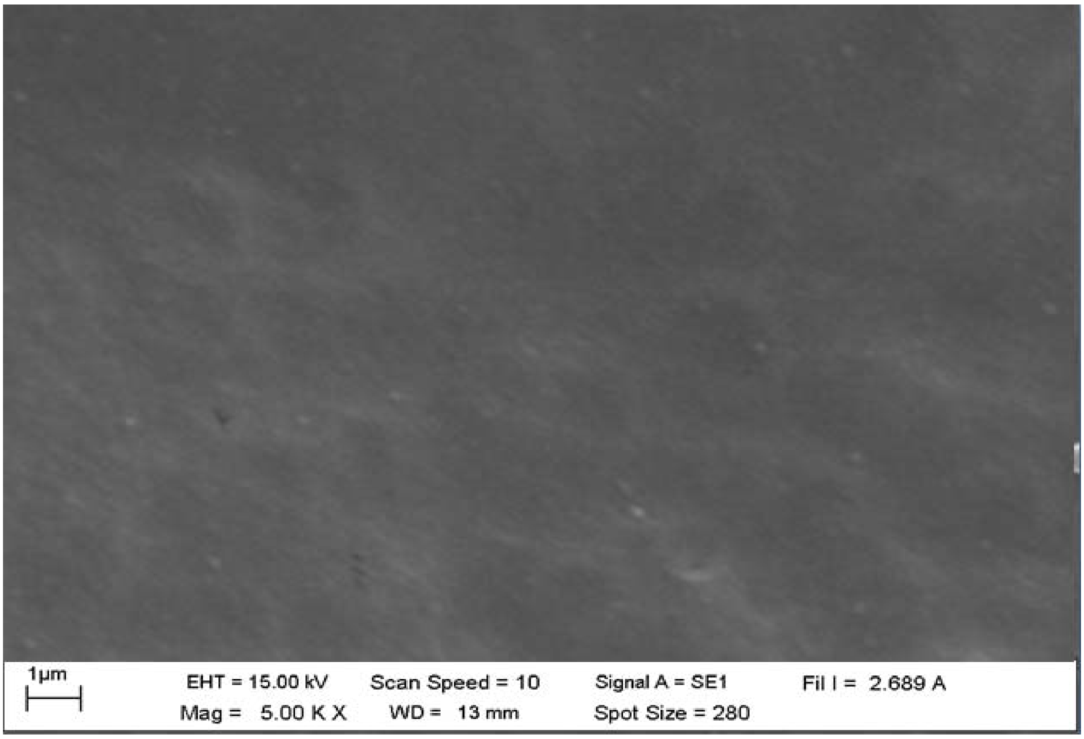
**Appendix Figure B4** (PEG4600-2) PSF/NMP/PEG4600 = 12/86/2



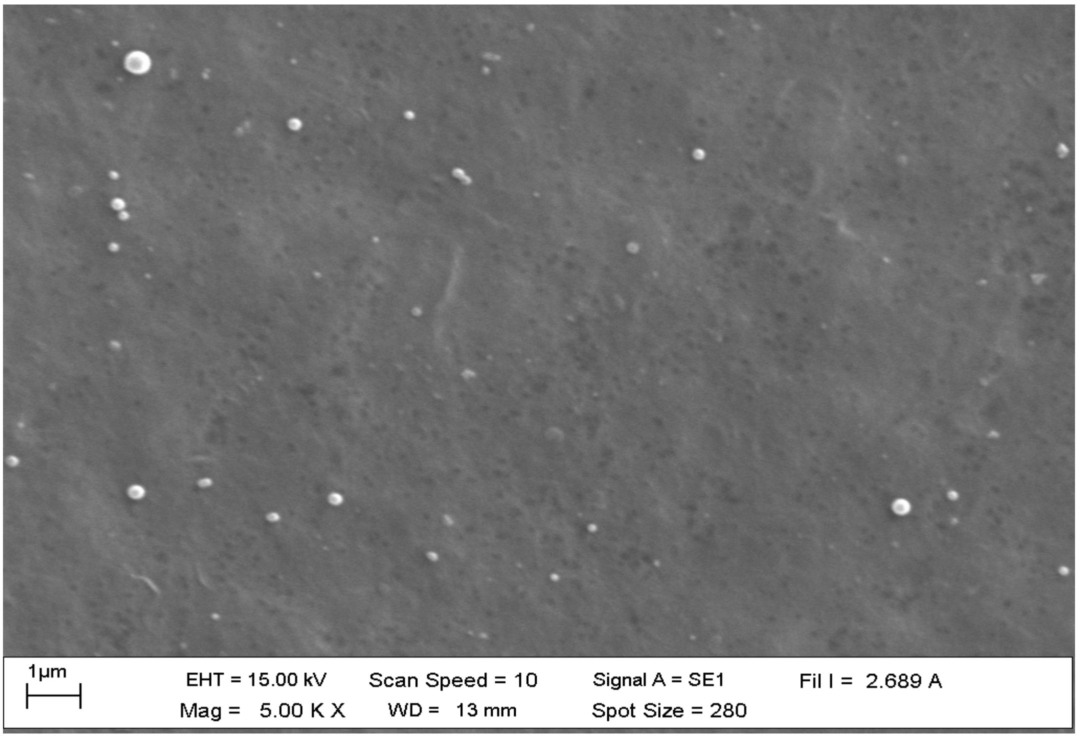
**Appendix Figure B5** (PEG10,000-2) PSF/NMP/PEG10,000 = 12/86/2



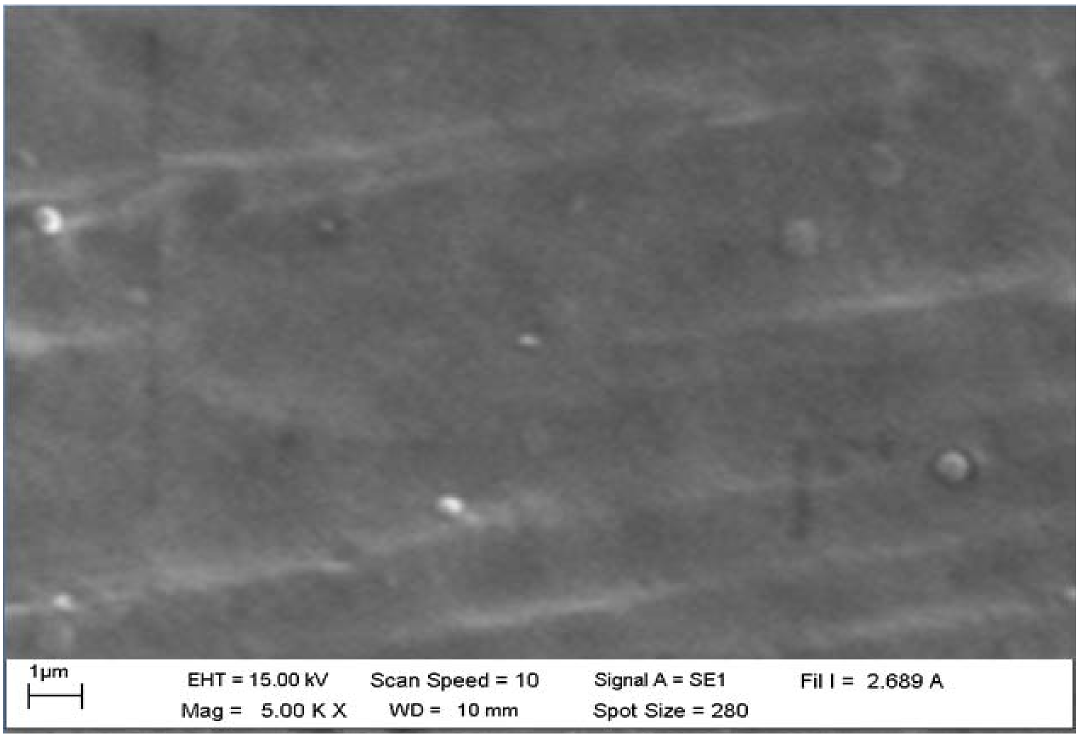
**Appendix Figure B6** (PDXL200,000-2) PSF/NMP/PDXL10,000 = 12/86/2



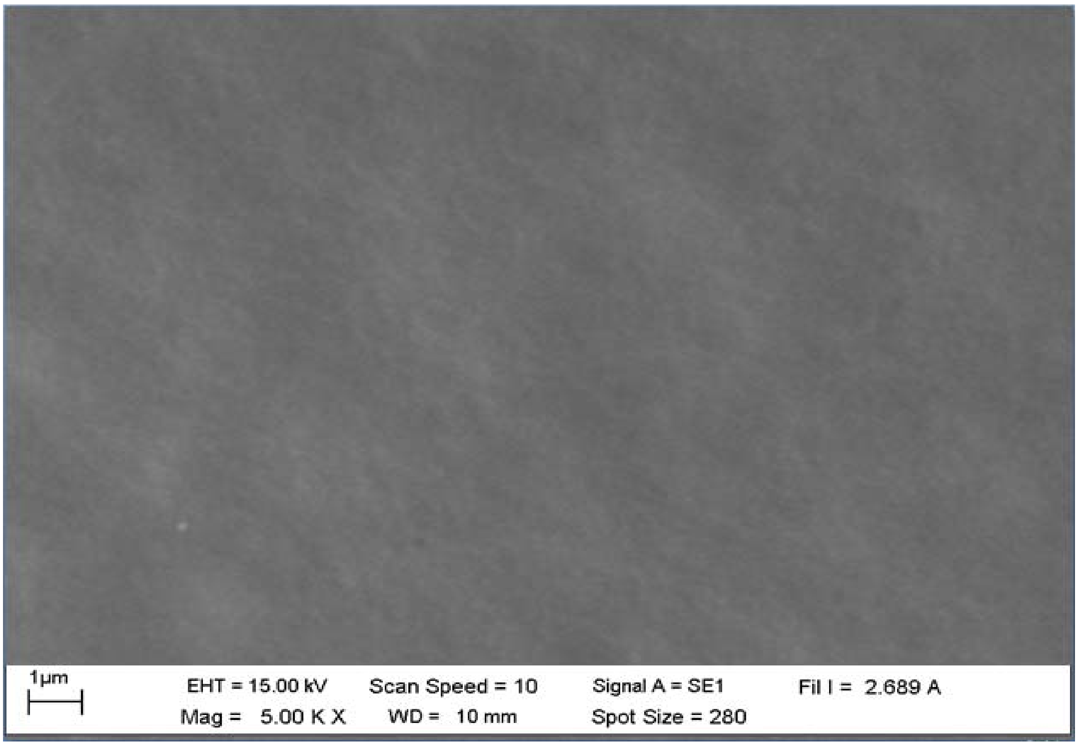
**Appendix Figure B7** (PDXL200,000-2) PSF/NMP/PDXL200,000 =12/86/2



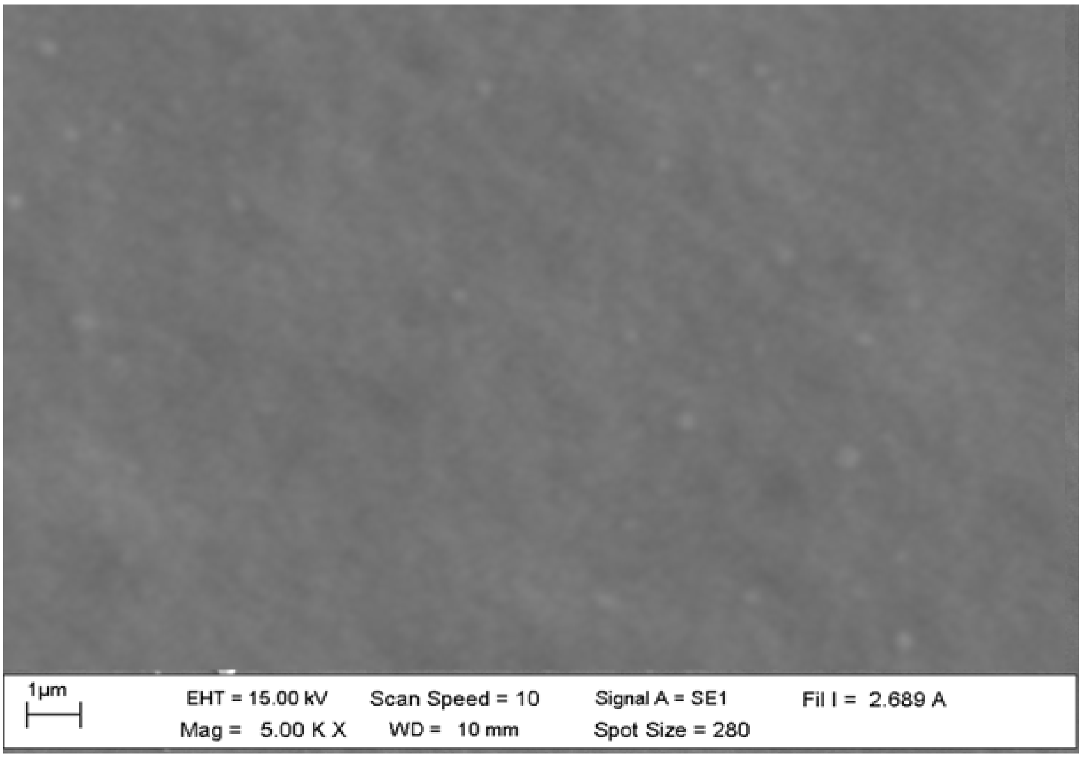
**Appendix Figure B8** (PEG300-5) PSF/NMP/PEG300 = 12/83/5



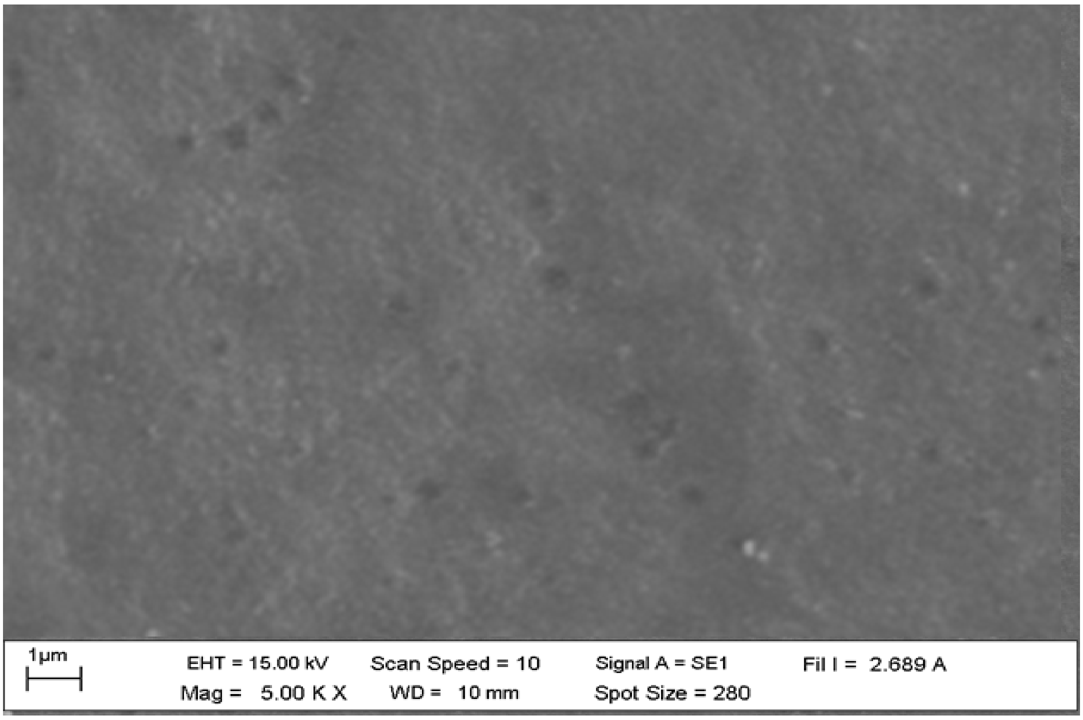
**Appendix Figure B9** (PEG2000-5) PSF/NMP/PEG2000 = 12/83/5



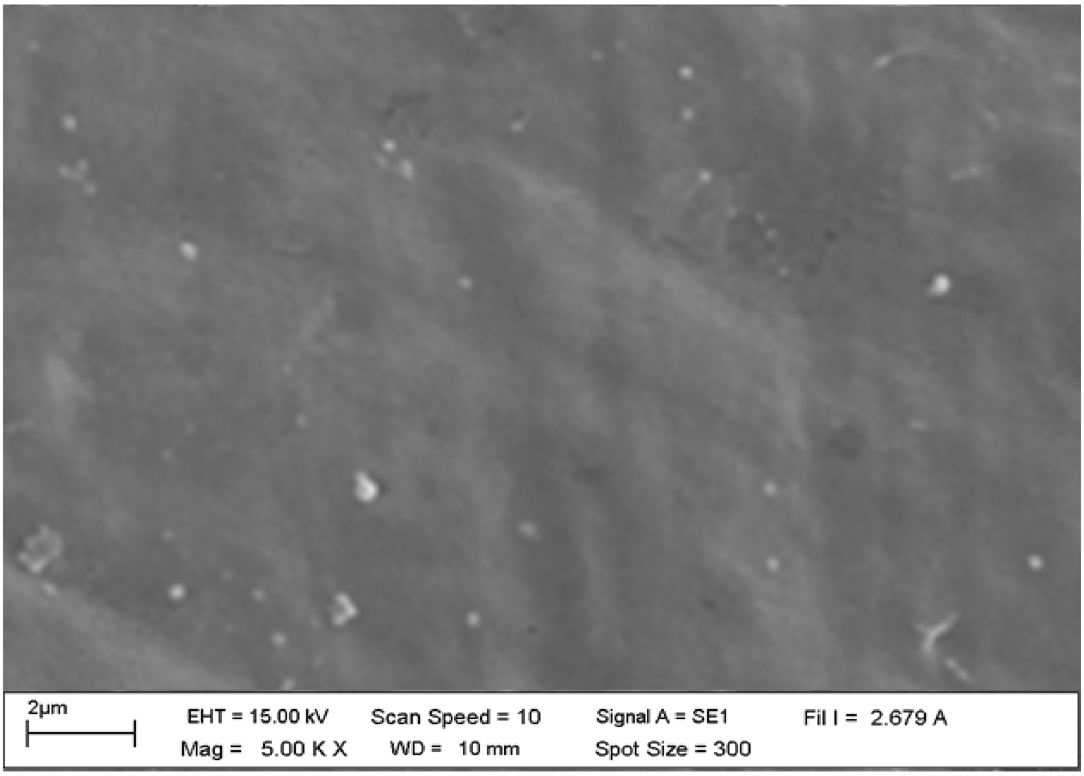
**Appendix Figure B10** (PEG4600-5) PSF/NMP/PEG4600 = 12/83/5



**Appendix Figure B11** (PEG10,000-5) PSF/NMP/PEG10,000 = 12/83/5



**Appendix Figure B12** (PDXL10,000-5) PSF/NMP/PDXL10,000 = 12/83/5



**Appendix Figure B13** (PDXL200,000-5) PSF/NMP/PDXL200,000 = 12/83/5

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