Heat-treated poly(vinyl alcohol) membranes for bioseparations

by

Aurelia Amanda

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Chemical Engineering

Major Professor: Surya K. Mallapragada

Iowa State University

Ames, Iowa

2000

Graduate College

Iowa State University

This is to certify that the Master's thesis of

Aurelia Amanda

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

TABLE OF CONTENTS

ABSTRACT vi

CHAPTER 1. GENERAL INTRODUCTION 1

Thesis Organization 1

Literature Review 2

Bioseparations Overview 2

Membrane Technology in Bioseparations 5

Poly(vinyl alcohol) 10

Literature Cited 15

CHAPTER 2. SEMICRYSTALLINE POLY(VINYL ALCOHOL) ULTRAFILTRATION MEMBRANES FOR BIOSEPARATIONS 22

Abstract 22

Introduction 23

Experimental 25

Membrane Preparation 25

PVA Membranes 25

PVA/Pluronic® Membranes 26

Membrane Characterization 26

Selectivity Studies 27

Results and Discussion 29

Conclusions 33

Nomenclature 34

Acknowledgments 35

References 35

CHAPTER 3. PERMEABILITY STUDIES AND MAPPING OF PROTEIN FOULING ON HEAT-TREATED POLY(VINYL ALCOHOL), POLYETHERSULFONE, AND REGENERATED CELLULOSE MEMBRANES USING DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPY 51

Abstract 51

Introduction 52

Experimental 55

Membrane Preparation 55

Membrane Characterization 55

Permeability Studies 56

Diffuse Reflectance Infrared Fourier Transform Spectroscopy 57

Results and Discussion 58

Conclusions 63

Acknowledgments 64

Literature Cited 64

CHAPTER 4. FABRICATION AND PERMEABILITY STUDIES OF ASYMMETRIC HEAT-TREATED POLY(VINYL ALCOHOL) MEMBRANES 83

Introduction 83

Experimental 85

Membrane Preparation 85

Membrane Characterization 86

Permeability Studies 86

Results and Discussion 87

Conclusions 88

Acknowledgments 88

Literature Cited 89

CHAPTER 5. GENERAL CONCLUSIONS 93

Research Overview 93

Future Directions 95

ACKNOWLEDGMENTS 98

ABSTRACT

Heat-treated poly(vinyl alcohol) (PVA) membranes were fabricated for potential use in bioseparations. Poly(vinyl alcohol) is a hydrophilic polymer and therefore PVA membranes are expected to have high resistance to membrane fouling. Moreover, its semicrystalline structure provides good mechanical strength. The membranes were used to separate model macromolecules, FITC dextran and myoglobin, from a model small molecular weight solute, L-tryptophan. The membranes were heat-treated in an oven at 100°C for 1 hour to increase their crystallinity. The crystallinity of the membranes was measured using differential scanning calorimetry (DSC). The mechanical strength of the membranes was evaluated by measuring their compressive and tensile moduli using dynamic mechanical analyzer (DMA). The effects of heat-treatment, average molecular weight of PVA, stirring speed, membrane thickness, and addition of Pluronic® on the permeabilities of small molecular weight solutes across the PVA membranes were studied. The permeability studies were carried out using side-by-side diffusion cells.

The mechanical strength and extent of fouling on these heat-treated PVA membranes were compared to commercial polyethersulfone (PeS) and regenerated cellulose membranes with molecular weight cut off of 10,000. It was found that the compressive moduli of swollen PVA membranes were comparable to PeS membranes, but it was higher than regenerated cellulose membranes. However, the tensile moduli of swollen PVA membranes with no backing supports were lower than the tensile moduli of PeS and regenerated cellulose membranes. Mapping of protein fouling on these membranes was measured qualitatively using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Regenerated

cellulose membranes had significantly lower selectivities than PeS and PVA membranes. Both permeability and fouling studies indicated that PVA membranes had better fouling resistance than the commercial PeS and regenerated cellulose membranes.

Asymmetric PVA membranes, prepared by utilizing the solubilities of PVA with two different degree of hydrolysis in water, did not give higher solute flux compared to the regular PVA membranes. However, their selectivities are comparable to the regular heat-treated PVA membranes.

CHAPTER 1

GENERAL INTRODUCTION

Thesis Organization

The rapid growth of biotechnology calls for researchers and engineers to look for the most efficient processes that are feasible economically and are scalable for manufacturing of valuable biological products. Applications of membranes in bioseparations are attractive primarily because of the energy savings and the mild conditions of separations. Poly(vinyl alcohol) (PVA) membranes are particularly interesting because they are hydrophilic polymers thus they have high fouling resistance, and they also have good mechanical properties. PVA hydrogels have been widely used as biomaterials because of their biocompatibility and low protein adsorption. PVA hydrogel membranes prepared by chemical crosslinking techniques are commonly used in biomedical applications. But the unreacted crosslinking agents can be hazardous to biomaterials when they leach out. An alternative technique to increase mechanical strength of PVA membranes is by heat-treatment technique. Besides increasing the mechanical strength of PVA membranes, heat-treatment technique is simple and avoids the use of crosslinking agents.

In this work, heat-treatment technique was used to fabricate PVA membranes to separate macromolecules, such as proteins from small molecular weight solutes. This chapter details prior work done in the fabrication of surface modified membranes and PVA membranes using different techniques. The thesis consists of two chapters submitted for publication: CHAPTER 2 is a paper published in the Journal of Membrane Science, and

CHAPTER 3 is a paper submitted to the Biotechnology Progress. Chapter 4 covers additional work in improving solute diffusion by using asymmetric membranes.

In CHAPTER 2, the effects of heat-treatment, membrane thickness, average molecular weight of PVA, stirring speed, and addition of surfactants, Pluronic®, on the selectivity of separation of large and small molecular weight solutes were analyzed. The degrees of crystallinity of untreated and heat-treated PVA membranes were examined using differential scanning calorimetry. The mechanical strength of untreated and heat-treated PVA membranes was measured using dynamic mechanical analysis and compared to commercial polyethersulfone (PeS) membranes. The permeabilities of small molecular weight solutes across heat-treated PVA membranes were compared to commercial PeS and regenerated cellulose membranes in CHAPTER 3. Fouling on these membranes were analyzed qualitatively using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Fabrication of asymmetric heat-treated PVA membranes made by utilizing the solubilities of two different degree of hydrolysis of PVA in water is discussed in CHAPTER 4. The mechanical strength and selectivity of asymmetric heat-treated PVA membranes were compared to regular heat-treated PVA membranes. CHAPTER 5 gives the summary of this work and some potential projects that can be done in the future.

Literature Review

Bioseparations Overview

Biotechnology has grown very rapidly in recent years. According to a recent study conducted by a consulting firm, Ernst and Young, biotechnology industry has doubled its size from 1993 to 1999 (1). Biotechnology is defined as the development of useful products

derived from biological systems (2, 3). Biotechnology covers a wide range of areas, such as: genetically engineered crop products, artificial organs, engineered microorganisms for waste treatment, and recovery of therapeutic molecules from fermentation broths (3-5).

Downstream processing or bioseparations are the common terms used in the separations of biological products obtained from fermentation processes (6). There are hundreds of different products that can be extracted from biological systems, ranging from simple molecules such as: alcohols, amino and organic acids, vitamins, and antibiotics to much more complex molecules such as: therapeutic proteins, enzymes, hormones, and polysaccharides (5). Because of the diluteness and complexity of the fermentation broths, several separation steps involving different types of separation technology are often required to isolate the desired products making the separations of these products remain as the biggest challenge in biotechnology. The efficiency of downstream processing largely determines the feasibility of biotechnology production since the separation processes involved represent a significant economic portion of the total manufacturing costs, especially for the manufacture of very high purity products where several steps are often required to achieve the desired purity (6). High-value products are present in the fermentation broths in very low concentrations, and the isolation and purification of these usually delicate products are very sophisticated and expensive (6).

Most bioseparations processes are characterized by the following four steps (2, 7):

1. Removal of insolubles

   Fermentation broths contain insolubles such as cell debris and suspended solids, and the first step usually involves the separations of these substances. The common separation

techniques used are conventional filtration using rotary vacuum filters, sedimentation, centrifugation, and microfiltration. For intracellular products, cell disruption step is necessary prior to isolation of the products.

2. Recovery and isolation of products

   The desired products are isolated from other substances present in the broths to significantly increase their concentrations. The concentrated solution is usually obtained by extraction, adsorption, precipitation, and ultrafiltration.

3. Purification

   In this step, the impurities are removed and the desired products are further concentrated. Some techniques commonly used include affinity chromatography, electrophoresis, and precipitation.

4. Polishing

   Crystallization is the most common technique used to obtain the final products with a very high degree of purity.

As mentioned earlier, the feed solutions from the fermentation process consist of hundreds of different molecules with different properties, and isolating the desired products is a very difficult and challenging task. Membrane separation is a relatively new technology applied in the bioseparations industry, and its use to remove solid particulates and fractionate proteins is starting to gain popularity among biotechnologists (5). Kalyanpur claims that membrane separations are an effective method for virus removal in the final purification of biotechnology products (8).

Membrane Technology in Bioseparations

Prior to 1960, membrane separations were mostly used in the laboratories, and not until Loeb and Sourirajan synthesized high-flux asymmetric membranes for reverse osmosis in 1960 that membrane technology found its place in the separations industry (9). Since then, membrane separations have been widely used in the food industry, waste treatment plants, water purification, biomedical applications, and biotechnology.

Membrane separations in down-stream processing offer several advantages over the more conventional separations (10). Membrane separations can be carried out under mild conditions in which no organic solvents are involved (11). They can be operated at low temperature, if necessary, which is suitable for heat sensitive materials (5). Membrane separations also offer energy savings compared to distillation, evaporation, or crystallization because they usually do not involve phase changes (12). The compactness of membrane modules makes them easy to integrate with other downstream processes (13), and they also have large areas available for separations (10). The design and operation of membrane separations are also relatively simple and easy to scale up (5).

Membrane separations can be catagorized into seven different processes: microfiltration, ultrafiltration, reverse osmosis, electrodialysis, gas separation, pervaporation, and facilated transport (13). Microfiltration and ultrafiltration are used in downstream processing to remove suspended solid particulates, isolate antibiotics, or fractionate proteins from fermentation broths. Ultrafiltration separates molecules based on their size (0.01 - 0.02 μm; molecular weight 10^3-10^6). Ultrafiltration membranes retain macromolecules such as proteins, while allowing small molecular weight solutes such as antibiotics and vitamins to pass. Some major applications of ultrafiltration at the industrial scale include: waste water

treatment in chemical industries, concentration and clarification of juices in food and dairy industries, and recovery of antibiotics, vaccines, enzymes, and therapeutic proteins in pharmaceutical industries (3, 12, 14).

A major problem in the membrane separations industry is membrane fouling, and reduction of membrane fouling is among the top priorities in the research and development of membrane technology (15). Fouling results in the loss of productivity due to flux decline and change in membrane selectivity (5, 11). Membrane fouling is caused by adsorption of biomolecules on the membrane surface or inside the membrane pores. Fouling can be seen as a two-step process: first is the adsorption of proteins onto the membrane, followed by subsequent attachment of protein aggregates via disulfide bonds onto the protein layer that has been formed on the surface of the membrane (16). Unlike concentration polarization where the flux decline can be recovered by back-pressure or flow reversion, fouling is often irreversible (17). Chemical agents are often required to remove the foulants. Unfortunately, even after membrane cleaning, it is often very difficult to return the membrane performance to its original state, such that maintaining its selectivity while recovering the flux to an acceptable level at the same time. Membrane cleaning makes up a significant portion of the cost of membrane separation processes: for example, 47% of total separation cost was spent on membrane cleaning and replacements in producing a 35% whey concentrate (15). In addition to the high cost of cleaning agents and the loss of production time spent on cleaning, long and regular exposure of the membranes under these cleaning agents can shorten the membrane lifetime. Therefore, it is essential to minimize membrane cleaning (18), and cleaning can be reduced by minimizing membrane fouling. Another disadvantage of the use of membranes in bioseparations is that many commercial membranes are not manufactured

specifically for bioseparations industry, and thus the properties of the membranes are often not suitable for separations of biological substances (5). Moreover, since fermentation broths consist of biological products such as proteins and lipids, membrane fouling in bioseparations industry is more severe than in other membrane applications (5, 18).

Polymers are the most common materials used to fabricate membranes. Besides polymer membranes, membranes made of ceramics and metals are also available commercially, although their expensive cost has limited their applications (17). The most popular polymers used in the industry are cellulose acetate, polyamide, polysulfone, sulphonated polysulfone, polyethersulfone, and polyvinylidenedifluoride (19, 20). Cellulosic (cellulose acetate and regenerated cellulose), polysulfone, and polyethersulfone membranes are the most common membranes used in the bioseparations industry (3, 21). Fouling of human serum albumin and polyethyleneglycol was evaluated on hydrophobic (polyethersulfone and polyvinylidenedifluoride) and hydrophilic (cellulose acetate and polyacrylonitrile) membranes by measuring their free energy of adhesion. The studies indicated that hydrophilic membranes showed significantly higher free energy of adhesion compared to hydrophobic membranes indicating that hydrophilic membranes had higher fouling resistance than hydrophobic membranes (22). Cellulose acetate membranes are made of hydrophilic polymers, and they have good resistance to membrane fouling. However, cellulose acetate membranes are sensitive to heat and extreme pH and have poor mechanical properties compared to the hydrophobic membranes commonly used (11, 19). Polyamides, another class of hydrophilic membranes, have better heat and pH stabilities, but they are not compatible with chlorine, which is usually present in many cleaning agents (19, 23). Polysulfones membranes are able to overcome the thermal, chemical, and mechanical

problems encountered with cellulose acetate and polyamide membranes, however, these membranes are susceptible to fouling because of their hydrophobic nature (11, 19, 23).

One approach to reduce protein fouling is by adjusting the pH of the process solution in which the protein is most soluble, but since the solution contains many different types of proteins, this technique becomes impractical (5). Other approaches have been directed toward changing the membrane properties (11). Hydrophobic membranes are more prone to fouling because of the interactions of the hydrophobic parts of the membranes with the hydrophobic parts of the proteins (5). Because of these interactions, hydrophobic membranes are harder to clean than hydrophilic membranes (24). Therefore, there have been many attempts to render hydrophobic membranes or hydrophobic biomaterials more hydrophilic by modifying their surface chemistry (5, 11).

Poly(ethylene terephthalate) (PET) films were modified with a hydrophilic polymer, poly(ethylene oxide) (PEO) of varying molecular weights using cyanuric chloride chemistry (25). The modified films exhibited significant decrease in protein adsorption, and modified films of high molecular weight PEO showed less protein adsorption compared to modified films of low molecular weight of PEO. Bergstrom and co-workers immobilized linear and branched low molecular weight PEO on polystyrene surfaces (26). The studies showed that polystyrene surfaces modified with linear PEO showed less protein adsorption compared to surfaces modified with branched PEO because the linear PEO chains have more freedom with their spatial movement than the branched PEO chains. Another study also showed that PEO modified carboxylated polystyrene beads had lower protein adsorption than unmodified beads although the PEO modified beads did not exhibit complete protein rejection (27).

Many studies used surfactants, which consist of both hydrophilic and hydrophobic polymer chains, to modify the membrane surfaces. Low density polyethylene films were modified by adsorption of PEO containing surfactants: PEO/poly(propylene oxide) (PPO) and PEO/poly(butylene oxide) (PBO) (28). The hydrophobic chains of the surfactants, PPO and PBO, interacted with the hydrophobic films, while the hydrophilic chains, PEO, prevented protein adsorption because of their high mobility in the aqueous solution and strong interactions with water. The studies found that the modified films had a higher resistance toward albumin adsorption. Amiji and Park (29) modified low-density polyethylene and dimethyldichlorosilane-treated glass with surfactants called Pluronic®. Pluronic® is a triblock copolymers of poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO). They claimed that protein rejection by Pluronic® modified surfaces depended mainly on the length of PPO chains; thus an effective protein rejection could be achieved if there is sufficient amount of PPO anchored tightly to the hydrophobic surface. Meanwhile, the PEO chains extended freely in the aqueous solution were used to prevent protein adsorption by steric repulsion. In another study by Ueda and co-workers (30), PET membranes were modified with hydrophilic polymers: poly[2-methacryloyloxyethyl phosphorylcholine (MPC)–*co*–*n*–butyl methacrylate (BMA)] and poly[2-hydroxyethyl methacrylate (HEMA)]. It was shown from dynamic contact angle studies that protein adsorbed on poly(MPC-*co*-BMA) modified PET membranes was easier to desorb upon cleaning than poly(HEMA) modified PET membranes and unmodified PET membranes.

Polysulfone ultrafiltration membranes were modified with Pluronic® to separate 1-naphthol and phenol in wastewater treatment (31). The Pluronic® modified membranes were employed by utilizing the formations of micelles. In aqueous solution, the hydrophobic chains of Pluronic® form inner cores of the micelles while the hydrophilic chains are freely extended into the aqueous solution. The hydrophobic solutes were trapped inside the core of the micelles when the solution was heated at a temperature range of 35 °C to 45°C, and they were released at 15°C when these solutes became insoluble in Pluronic®. After the solutes were released, the Pluronic® was regenerated and reused again. Hester and co-workers fabricated 'self-healing' protein resistant membranes made of poly(vinylidene fluoride) (PVDF) modified with poly(methyl methacrylate) (PMMA) backbones and PEO side chains using a two-steps phase inversion method (32). The hydrophilic PEO side chains, which could be washed away during separation process or cleaning, were regenerated by heat-treatment in water, and the regenerated membranes showed partial rejection of protein adsorption.

Poly(vinyl alcohol)

The main disadvantage of surface modification is the difficulties to obtain good stability of the chemical groups incorporated onto the membrane surfaces. These groups can be easily swept away during the separation process or membrane cleaning. Another alternative to reduce fouling is by using hydrogels as the membrane materials. Poly(vinyl alcohol) (PVA) hydrogels are hydrophilic and have good mechanical strength due to their semicrystalline nature, which make them suitable for use in biomedical and bioseparations

applications (33, 34). Poly(vinyl alcohol) (PVA; $-CH_2-CH(OH)_n-$) is commonly synthesized by hydrolysis of vinyl acetate groups in poly(vinyl acetate) (35). The solubility of PVA in water depends largely on its degree of hydrolysis (36). PVA with degree of hydrolysis higher than 98% becomes soluble in water at a temperature above its glass transition temperature ($T_g = 85^oC$) whereas PVA with lower degree of hydrolysis (87 - 89% and approximately 80%) dissolves in water at room temperature (37). The crystallites in PVA behave as physical cross-links that contribute to its mechanical strength (11). The presence of hydroxyl groups forms strong intermolecular hydrogen bonds, which contribute to insolubilities of PVA in water (35).

Besides the applications of PVA as textile fibers, adhesive, and emulsifying agents (36), there have been great interests in the use of PVA as biomaterials. PVA hydrogels have been studied extensively for their use as biomaterials primarily because of their biocompatibility and low level of cell adhesion and protein adsorption. Because of their low protein adsorption and biocompatibility, PVA hydrogels were found to be a potential candidate to replace conventional materials for soft contact lenses (38). *In vivo* studies of PVA hydrogels as artificial articular cartilage showed that PVA hydrogels had good biocompatibility and the mechanical strength necessary for articular cartilage (39). PVA matrices are also widely used as drug delivery devices, and different preparation techniques were studied to control the rate of drug diffusion (40-44). Anticoagulant heparin was immobilized onto PVA hydrogels to prevent blood clotting in cardiovascular systems (45, 46). In another study, heparin/PVA hydrogels were used as sensor membranes (47). Burczak and co-workers (48, 49) studied the potential use of irradiated and chemical crosslinked PVA membranes for artificial pancreas by evaluating the permeation of proteins

as well as the biocompatibility of PVA hydrogels in long-term *in vivo* studies. Asymmetric PVA membranes made by addition of hydrophilic polymer, poly(ethylene glycol) (PEG) were also investigated for their use as artificial pancreas (50). The studies found that compared to the homogeneous PVA membranes, the asymmetric membranes allowed higher permeation of glucose and insulin while they were still capable of rejecting macromolecules from immune system such as immunoglobulin. Nylon 4 membranes for hemodialysis exhibited enhanced biocompatibility after the membranes were plasma coated with PVA (51). Hemodialysis membranes made of acetylsalicylic acid loaded PVA membranes also showed improved blood compatibility (52). Other studies also exploited the use of PVA due to its biocompatibility for glucose sensors (53) and contraceptive devices (54). Baker and co-workers fabricated immunoisolation membranes by coating microfiltration membranes made of polyethersulfone with PVA hydrogels crosslinked *in situ* with glutaraldehyde (55). The membranes exhibited high permeabilities of microsolutes and good selectivities showed by the low permeabilities of macromolecules with molecular weight higher than 10^3 Da. These membranes were also sterilized using autoclaving, ethylene oxide, and electron beam sterilization techniques, and the membranes showed no significant changes on their permeabilities after sterilization.

Different techniques have been employed to increase the mechanical strength of PVA membranes: chemical crosslinking, irradiation, freeze-thaw, and heat-treatment. Drug diffusion across chemically crosslinked PVA membranes were controlled by varying the amount of crosslinking agents (33, 34, 42). Copolymers of PVA and poly(acrylic acid) (PAA) hydrogels prepared by chemical crosslinking technique were studied by Peppas and co-workers. The mesh size of the hydrogels that determines the rate of drug diffusion was

controlled by changing the pH of the environment and ionic content of PAA (33, 34). Benign techniques become necessary for biomedical applications and separations of biological products because toxic crosslinking agents can be harmful to biomolecules when they leach out (43, 56). These unreacted crosslinking agents are also difficult to remove, and chemically crosslinked hydrogels do not have the mechanical strength necessary for biomedical devices (57). Physical crosslinking techniques using irradiation, freeze-thaw, and heat-treatment are examples of benign techniques. Peppas and Merrill (58) showed that heat-treatment augmented the tensile strength of PVA hydrogels after the gels were crosslinked with irradiation. Heat-treated PVA membranes have higher water-salt selectivity but lower permeabilities than irradiated PVA membranes (59, 60). PVA membranes made by cycles of freezing and thawing processes followed by heat-treatment showed lower microsolute permeabilities than PVA membranes prepared by heat-treatment (56, 57).

Heat-treatment technique is particularly interesting because of its simplicity. The degree of crystallinity of PVA films increases with temperature (37), and it can be easily controlled by varying the temperature and the time of heat-treatment. However, the temperature and time of heat-treatment should not exceed 130°C and should not be longer than 2 hours respectively to avoid PVA degradation (61). The wettability of the PVA films also changes with the degree of heat-treatment. The amount of water on the PVA hydrogels decreases as the temperature and time of heat-treatment increased causing a decline of the blood compatibility of PVA (62). PVA films prepared by addition of glycerol showed an enhanced resistance to protein adsorption and platelet adhesion (63).

Weissenborn and co-workers (64) fabricated dye-affinity membranes by covalently bound hydrophilic polymers: dextran, hydroxyethylcellulose, and PVA onto bisoxirane-nylon

membranes, and subsequently anchored the dye ligands, Cibacron Blue F3G-A. The membranes were used to isolate L-alanine dehydrogenase produced by *Mycobacterium tuberculosis* in fermentation broth. The water flux across the membranes was hindered due to the coating layer of hydrophilic polymers on the surface of the membranes. Cell debris from the fermentation broth formed concentration polarization layer causing a decline in permeabilities and 50% product loss.

Li and Barbari (65) fabricated thin-gel composite PVA membranes using interfacial crosslinking technique and claimed that the biocompatibility of the membranes made them ideal for bioseparations. PVA hydrogels with varying amounts of crosslinking agents were spin-coated on top of regenerated cellulose support membranes (66). The modified membranes were used as ultrafiltration membranes to retain bovine serum albumin, and the recovery of water flux after membrane cleaning showed that the thin-gel composite membranes had a significant fouling decline compared to the unmodified membranes. PVA hydrogels were impregnated onto the pores of microfiltration membranes, and the composite membranes were interfacially modified with crosslinking agents, toluene diisocyanate, to form asymmetric membranes (67). The modified thin-gel composite membranes had higher permeabilities and better mechanical stability. Dai and Barbari (68) compared symmetric and asymmetric PVA membranes. The asymmetric membranes were prepared by interfacially modifying the symmetric membranes with glutraldehyde as crosslinking agents. The studies showed that the modified membranes had higher microsolute flux and the desired selectivities for size-selective macroencapsulation membranes. However, one of the main drawbacks of this technique of fabricating PVA membranes is the use of toxic crosslinking agents.

In summary, membrane separations seem to offer an efficient way to separate macromolecules from small molecular weight solutes. PVA membranes are hydrophilic with low protein adsorption, and they have good mechanical properties. PVA membranes produced by heat-treatment technique are easy to fabricate, strong, and benign. Therefore, the biocompatibility and fouling resistance of heat-treated PVA membranes make them ideal candidates for the bioseparations industry. However, PVA heat-treated ultrafiltration membranes have not been fabricated and tested for bioseparations. In this work, we outline the fabrication process of PVA membranes by heat-treatment and conduct protein fouling and selectivity studies to compare the fouling characteristics and selectivity of separation with commercial hydrophobic polyethersulfone and hydrophilic regenerated cellulose membranes.

Literature Cited

(1) Thayer, A. *Chem. Eng. News*, **2000**, *July 31*, 26.

(2) Belter, P. A.; Cussler, E. L; Hu, W. S. *Bioseparations – Downstream Processing for Biotechnology*; John Wiley & Sons, Inc.: New York, **1988**; pp. 1-12.

(3) Shuler, M. L.; Kargi, F. *Bioprocess Engineering – Basic Concepts*; Prentice Hall: New Jersey, **1992**; pp. 311-362.

(4) McGregor, W. C. *Membrane Separations in Biotechnology*; McGregor, W. C., Ed.; Marcel Dekker, Inc.: New York, **1986**.

(5) Matson, S. L. Membrane Bioseparations. In *Membrane Science and Technology Series 2 – Membrane Separations Technology Principles and Applications*; Noble, R. D.; Stern, S. A., Eds.; Elsevier: Amsterdam, **1995**, pp. 353-413.

(6) Liddell, J. M. Introduction to Downstream Processing. In *Engineering Processes for Bioseparations*; Weatherley, L. R., Ed.; Butterworth-Heinemann Ltd.: Oxford, **1994**, pp. 5-34.

(7) Bailey, J. E.; Ollis, D. F. *Biochemical Engineering Fundamentals*; McGraw-Hill, Inc.: New York, **1986**, pp. 726-797.

(8) Kalyanpur, M. Downstream Processing in the Biotechnology Industry – An Overview. In *Downstream Processing of Proteins – Methods and Protocols*; Desai, M. A., Ed.; Humana Press, Inc.: New Jersey, **2000**, pp. 1-21.

(9) Matsuura, T. *Synthetic Membranes and Membrane Separation Processes*; CRC Press Inc.: Boca Raton, **1994**, pp. 383-445.

(10) Belfort, G. Membrane Processes in Biotechnology: An Introduction. In *Membrane Processes in Separation and Purification*; Crespo, J. G.; Boddeker, K. W., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, **1994**, pp. 3-7.

(11) Mulder, M. Nature of Membranes. In *Membranes in Bioprocessing Theory and Applications*; Howell, J.A.; Sanchez, V.; Field R.W., Eds.; Blackie Academic and Professional: London, **1993**, pp. 13-32.

(12) Bell, G.; Cousins, R. B. Membrane Separations Processes. In *Engineering Processes for Bioseparations*; Weatherley, L. R., Ed.; Butterworth-Heinemann Ltd.: Oxford, **1994**, pp. 135-165.

(13) Baker, R. W.; Cussler, E. L.; Eykamp, W.; Koros, W. J.; Riley, R. L.; Strathmann, H. Introduction to Volume II. In *Membrane Separation Systems – Recent Development and Future Directions*; Baker, R. W.; Cussler, E. L.; Eykamp, W.; Koros, W. J.; Riley,

R. L.; Strathmann, H., Eds.; Noyes Data Corporation: Park Ridge, New Jersey, **1991**, pp. 96-99.

(14) Scott, K. Overview of the Application of Synthetic Membrane Processes. In *Industrial Membrane Separation Technology*; Scott, K.; Hughes, R., Eds.; Blackie Academic and Professional: London; New York, **1996**, pp. 8-31.

(15) Eykamp, W. Ultrafiltration. In *Membrane Separation Systems – Recent Development and Future Directions*; Baker, R. W.; Cussler, E. L.; Eykamp, W.; Koros, W. J.; Riley, R. L.; Strathmann, H., Eds.; Noyes Data Corporation: Park Ridge, New Jersey, **1991**, pp. 360-395.

(16) Kelly, S. T.; Zydney, A. L. Protein Fouling during Microfiltration: Comparative Behavior of Different Model Proteins. *Biotechnol. Bioeng.*, **1997**, *55*, 91-100.

(17) Eykamp, W. Membrane Separations in Downstream Processing. In *Handbook of Downstream Processing*; Goldberg, E., Ed.; Blackie Academic and Professional: London, **1997**, pp. 90-139.

(18) Cook, M. A. Membrane Processing in Biotechnology: A Case History. In *Downstream Processing of Natural Products*; Verrall, M. S., Ed.; John Wiley & Sons, Ltd.: England, **1996**, pp. 139-146.

(19) Merry, A. J. Membrane Equipment and Plant Design. In *Industrial Membrane Separation Technology*; Scott, K.; Hughes, R., Eds.; Blackie Academic and Professional: London; New York, **1996**, pp. 32-66.

(20) Mulder, M. *Basic Principles of Membrane Technology*; Kluwer Academic Publishers: **1991**, Netherlands, pp. 281-311.

(21) Zydney, A. L.; Kuriyel, R. Protein Concentration and Buffer Exchange Using Ultrafiltration. In *Downstream Processing of Proteins – Methods and Protocols*; Desai, M. A., Ed.; Humana Press, Inc.: New Jersey, **2000**, pp. 23-34.

(22) Cornelissen, E. R.; van den Boomgaard, Th.; Strathmann, H. *Colloids Surf. Physicochem. Eng. Aspects*, **1998**, *138*, 283-289.

(23) Kesting, R. E. *Synthetic Polymeric Membranes – A Structural Perpective*; John Wiley & Sons: Toronto; **1985**, pp. 22-81.

(24) Howell, J. A.; Nystrom, M. Fouling Phenomena. In *Membranes in Bioprocessing Theory and Applications*; Howell, J.A.; Sanchez, V.; Field, R.W., Eds.; Blackie Academic and Professional: London, **1993**, pp. 203-241.

(25) Gombotz, W. R.; Guanghui, W.; Horbett, T. A.; Hoffman, A. S. *J. Biomed. Mater. Res.*, **1991**, *25*, 1547-1562.

(26) Bergstrom, K.; Osterberg, E.; Holmberg, K.; Hoffman, A. S.; Schuman, T. P.; Kozlowski, A.; Harris, J. M.. *J. Biomater. Sci. Polym. Edn.*, **1994**, *6*, 123-132.

(27) van Delden, C. J.; Bezemer, J. M.; Engbers, G. H. M.; Feijen, J. *J. Biomater. Sci. Polymer Edn.*, **1996**, *8*, 251-268.

(28) Lee, J. H.; Kopecek, J.; Andrade, J. D. *J. Biomed. Mater. Res.*, **1989**, *23*, 351-368.

(29) Amiji, M.; Park, K. *Biomaterials*, **1992**, *13*, 682-692.

(30) Ueda, T.; Ishihara, K.; Nakabayashi, N. *J. Biomed. Mater. Res.*, **1995**, *29*, 381-387.

(31) Choi, Y. K.; Lee, S. B.; Lee, D. J.; Ishigami, Y.; Kajiuchi, T. *J. Membr. Sci.*, **1998**, *148*, 185-194.

(32) Hester, J. F.; Banerjee, P.; Mayes, A. M. *Macromolecules*, **1999**, *32*, 1643-1650.

(33) Gudeman, L. F.; Peppas, N. A. *J. Appl. Polym. Sci.* **1995**, *55*, 919-928.

(34) Gudeman, L. F.; Peppas, N. A. *J. Membr. Sci.* **1995**, *107*, 239-248.

(35) Hodge, R. M.; Edward, G. H.; Simon, G. P. *Polymer*, **1996**, *37*, 1371-1376.

(36) Toyoshima, K. General Properties of Polyvinyl Alcohol in Relation to its Applications. In *Polyvinyl Alcohol*; Finch, C. A., Ed.; John Wiley & Sons: Great Britain, **1973**, pp.17-65.

(37) Toyoshima, K. Properties of Polyvinyl Alcohol Films. In *Polyvinyl Alcohol*; Finch, C. A., Ed.; John Wiley & Sons: Great Britain, **1973**, pp.339-389.

(38) Hyon, S. H.; Cha, W. I.; Ikada, Y.; Kita, M.; Ogura, Y.; Honda, Y. *J. Biomater. Sci. Polymer Edn.*, **1994**, *5*, 397-406.

(39) Noguchi, T.; Yamamuro, T.; Oka, M.; Kumar, P.; Kotoura, Y.; Hyon, S. H.; Ikada, Y. *J. Appl. Biomater.*, **1991**, *2*, 101-107.

(40) Byron, P. R.; Dalby, R. N. *J. Pharm. Sci.*, **1987**, *76*, 65-67.

(41) Wan, L. S. C.; Lim, L. Y. *Drug Develop. Ind. Pharm.*, **1992**, *18*, 1895-1906.

(42) Kim ,C. J.; Lee, P. I. *Pharm. Res.*, **1992**, *9*, 10-16.

(43) Mallapragada, S. K.; Peppas, N. A.; Colombo, P. *J. Biomed. Mater. Res.*, **1997**, *36*, 125-130.

(44) Peppas, N. A.; Wright, S. L. *Eur. J. Pharm. Biopharm.*, **1998**, *46*, 15-29.

(45) Smith, B. A. H; Sefton, M. V. *J. Biomed. Mater. Res.*, **1988**, *22*, 673-685.

(46) Sefton, M. A. *Can. J. Chem. Eng.*, **1989**, *67*, 705-712.

(47) Brinkman, E.; van der Does, L.; Bantjes, A. *Biomaterials*, **1991**, *12,* 63-70.

(48) Burczack, K.; Fujisato, T.; Hatada, M.; Ikada Y. *Biomaterials*, **1994**, *15*, 231-238.

(49) Burczack, K.; Gamian, E.; Kochman, A. *Biomaterials*, **1996**, *17*, 2351-2356.

(50) Young, T. H.; Yao, N. K.; Chang, R. F.; Chen, L. W. *Biomaterials*, **1996**, *17*, 2139-2145.

(51) Shih, C. Y.; Lai, J. Y. *J. Biomed. Mater. Res.*, **1993**, *27*, 983-989.

(52) Paul, W.; Sharma, C. P. *J. Biomater. Sci. Polymer. Edn.*, **1997**, *8*, 755-764.

(53) Liu, Y. C.; Chen, X.; Qian, J. H.; Liu, H. Y.; Shao, Z. Z.; Deng, J. Q.; Yu, T. Y. *Appl. Biochem. Biotechnol.*, **1997**, *62*, 105-117.

(54) Sanders, J. M; Matthews, H. B. *Hum. Exp. Toxicol.*, **1990**, *9*, 71-77.

(55) Baker, R.; Fournier, R. L.; Sarver, J. G.; Long, J. L.; Goldblatt, P. J.; Horner, J. M.; Selman, S. H. *Cell Transplant.*, **1997**, *6*, 585-595.

(56) Hickey, A. S.; Peppas, N. A. *J. Membr. Sci.* **1995**, *107*, 229-237.

(57) Takamura, A.; Ishii, F.; Hidaka, H. *J. Control. Release*, **1992**, *20*, 21-28.

(58) Peppas, N. A.; Merrill, E. W. *J. Polym. Sci.*, **1976**, *14*, 441-457.

(59) Katz, M. G.; Wydeven, T. *J. Appl. Polym. Sci.*, **1981**, *26*, 2935-2946.

(60) Katz, M.G.; Wydeven, T. *J. Appl. Polym. Sci.*, **1982**, *27*, 79-87.

(61) Mallapragada, S. K.; Chin, S. F. Multilayered Semicrystalline Polymeric Controlled Release Systems. In *Tailored Polymeric Materials for Controlled Delivery Systems*; McCulloch, I.; Shalaby, S. W., Eds.; ACS Symposium Series, **1998**, pp. 176-184.

(62) Ko, J. H; Ericson, D.; Tucker, J.; Walker, R. Effect of Annealing on the Physical Properties and Blood Compatibility of Poly(vinyl alcohol) Hydrogels. *ANTEC*, **1994**, 2651-2655.

(63) Fujimoto, K.; Minato, M.; Ikada, Y. Poly(vinyl alcohol) Hydrogels Prepared under Different Annealing Conditions and Their Interactions with Blood Components. In

Polymers of Biological and Biomedical Significance; Shalaby, S. W.; Ikada, Y.; Langer, R.; Williams, J. Eds.; ACS Symposium Series 540, **1994**, pp. 228-241.

(64) Weissenborn, M.; Hutter, B.; Singh, M.; Beeskow, T. C.; Anspach, F. B. *Biotechnol. Appl. Biochem.*, **1997**, *25*, 159-168.

(65) Li, R. H.; Barbari, T. A. Size-Selective Bioseparations using Interfacially-Crosslinked Poly(vinyl alcohol) Gel Membranes. In *New Developments in Bioseparation*, AIChE Symp. Ser. 290, 1992, pp. 65-71.

(66) Li, R. H.; Barbari, T. A. *J. Membr. Sci.*, **1995**, *105*, 71-78.

(67) Li, R. H.; Barbari, T. A. *J. Membr. Sci.*, **1996**, *111*, 115-122.

(68) Dai, W. S.; Barbari, T. A. *J. Membr. Sci.*, **1999**, *156*, 67-79.

CHAPTER 2

SEMICRYSTALLINE POLY(VINYL ALCOHOL) ULTRAFILTRATION MEMBRANES FOR BIOSEPARATIONS

A paper published in the Journal of Membrane Science[1]

Aurelia Amanda, Ames Kulprathipanja, Merethe Toennesen, and

Surya K. Mallapragada[2]

Abstract

Semicrystalline poly(vinyl alcohol) (PVA) ultrafiltration (UF) membranes were fabricated for use in bioseparations to separate small molecular weight solutes from macromolecules. PVA was chosen because of its hydrophilic nature, and these membranes are expected to minimize membrane fouling due to protein adsorption. The membranes were annealed at temperatures above the polymer's glass transition temperature to crystallize them in order to improve their mechanical properties. The degree of crystallinity of these membranes was measured using differential scanning calorimetry. Studies were conducted to measure the selectivity of separation of two different sized solutes using these membranes. These studies showed that heat-treated PVA membranes had a higher selectivity compared to the untreated PVA membranes. The effects of heat treatment, stirring speed, membrane thickness, average molecular weight of PVA, and addition of Pluronic® F127 and Pluronic® F68 on the separation of fluorescein isothiocyanate-dextran and myoglobin from L-tryptophan were studied. The mechanical strength of heat-treated PVA membranes, and the

---

[1] Reprinted from J. Membr. Sci., *76*, 87-95 Copyright (2000) , with permission from Elsevier Science

[2] Corresponding author

selectivity of separation of these membranes were compared with commercial polyethersulfone (PeS) membranes. Mechanical studies using dynamic mechanical analysis showed that the tensile modulus of commercial PeS membranes was significantly higher than the heat-treated PVA membranes that were swollen in deionized water for 1 and 5 hours. However, the compressive moduli of PVA membranes were found to be of the same order as the compressive moduli of PeS membranes. The tensile and compressive moduli of heat-treated PVA/1%(w/v) Pluronic® F127 membranes were considerably lower than PeS and PVA membranes. It was found that the PVA membranes were more resistant to fouling than the PeS membranes.

Keywords: poly(vinyl alcohol), bioseparations, heat-treated membranes, hydrophilic membranes, Pluronic®

1. Introduction

One of the purification methods with increasing popularity in the bioseparations industry is membrane separations. Using membranes, separations can be carried out under mild conditions with no organic solvents required. Membrane separations are also attractive because the energy consumption is relatively low, they are simple to scale up, and the membrane properties can be easily controlled [1]. One major problem with membrane separations is the formation of a fouling layer on the surface and even inside the pores of the membrane. Polysulfone membranes are currently widely used in the bioseparations industry. Because of the relative hydrophobic nature of polysulfone membranes, hydrophobic interactions between the membrane and proteins tend to foul the membrane easily. This

fouling layer varies from system to system and makes it difficult to characterize the membranes and control the flux across them. Mass transfer inside the fouling layer is diffusion controlled, and protein fractionation can occur because of this layer [2]. Much research has been done to reduce membrane fouling. One approach to reduce fouling is by using hydrophilic polymers, such as cellulose acetate [1]. Although cellulose acetate membranes have outstanding properties in reducing membrane fouling, these membranes do not exhibit long term chemical, thermal, and biological stability [1]. Other approaches involving surface modification of hydrophobic membranes with polyethyleneoxide or Pluronics® (trademark of BASF) have met with mixed success [3-5].

In this study, ultrafiltration (UF) membranes were fabricated from heat-treated poly(vinyl alcohol) (PVA). The UF membranes were used to separate macromolecules from small molecular weight solutes. PVA membranes are suitable for bioseparations because they are hydrophilic, and can thus minimize membrane fouling [2, 6-7]. Moreover, PVA has good thermal and pH stability, is non-toxic, and is convenient to use because it is easily processed. Many studies have been performed by chemically cross-linking the PVA membranes [2, 6-9]. By heat-treating the membranes, the use of toxic cross-linking agents that can be harmful when they leach out can be avoided. Some other researchers have tried to cross-link PVA membranes using irradiation [9-10]. However, these membranes did not have good long-term stability. Hickey and Peppas [11] tried to increase the mechanical strength of PVA membranes by a freezing and thawing technique. Katz and Wydeven [10, 12] studied the permeability of water and salts in heat-treated and irradiated PVA membranes, and they found that the heat-treated PVA membranes have a higher salt selectivity than the irradiated PVA membranes. Therefore in this study, we have used heat-

treated membranes for bioseparations. The semicrystalline nature of PVA imparts better mechanical properties to the polymer. These membranes were annealed to increase their crystallinity, and thereby their mechanical strength. The degree of crystallinity of the UF membranes can easily be controlled by varying the conditions of heat-treatment.

The degree of crystallinity of UF membranes was measured using differential scanning calorimetry (DSC). The strength of UF membranes was characterized by measuring the compressive and tensile moduli using dynamic mechanical analysis (DMA). The membrane selectivity was evaluated using side-by-side diffusion cells. The effects of membrane crystallinity, stirring speed, membrane thickness, average molecular weight of PVA, and addition of Pluronic® F127 and Pluronic® F68 on membrane selectivities were analyzed. Pluronics® are block copolymers of polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO). Pluronics® were added to increase the flux and decrease protein adsorption further due to the presence of the PEO chains. The selectivity and flux through PVA membranes was compared to PeS membranes to study whether PVA membranes were more resistant to fouling than the PeS membranes.

2. Experimental

2.1 Membrane Preparation

2.1.1 PVA Membranes

UF membranes were prepared by dissolving PVA with different molecular weights (Elvanol®, E.I. duPont de Nemours and Co., Wilmington, DE; $\overline{M_n}$=17,600 and $\overline{M_n}$=35,420 and degrees of hydrolysis of >99%) in deionized water at 90°C for 6 hours (10%w/v). The solutions were cast into petri dishes and dried slowly for five days at approximately 12°C for

obtaining uniform membranes. Some of the membranes were heat-treated at 100±1°C for 1 hour. The membranes used for the selectivity studies had thicknesses ranging from 100 to 250 μm. The membrane thickness was measured using a caliper, and an average was taken for each membrane. The average thickness of membranes used for selectivity studies was 149 ± 4.25 and 203 ± 4.62 μm.

2.1.2 PVA/Pluronic® Membranes

Pluronic® F127 and F68 were obtained from Sigma Chemical Company, St. Louis, MO. The PEO content in Pluronic® F127, which contributes to the hydrophilic property in Pluronic®, is 70% of the total weight, whereas the PEO content of Pluronic® F68 is 80% of the total weight. A mixture of PVA (10%w/v; $\overline{M_n}$=35,420; Elvanol®, E.I. duPont de Nemours and Co., Wilmington, DE) and 1% (w/v) Pluronic® was prepared by dissolving the PVA in deionized water (90°C for 6 hours) and Pluronic® in deionized water (12°C for 1 hour). The solutions were then mixed, cast into petri dishes, and dried at 12°C. The membranes formed were heated at 100°C for 1 hour and cooled slowly.

2.2 Membrane Characterization

The crystallinity of the PVA membranes was measured using a differential scanning calorimeter (DSC7, Perkin Elmer, Norwalk, CT). The temperature was varied from 25 to 250°C at a rate of 20°C/min. The degree of crystallinity was obtained from the ratio of heat of melting of the PVA membranes to the heat of melting of a 100% crystalline PVA sample [13]. The heat-treated membranes were not expected to undergo degradation as long as the

crystallization times were not greater than 2 hours or the crystallization temperatures were not greater than 130°C [14].

The compressive and tensile moduli of heat-treated PVA membranes without and with Pluronic®F127 were measured using a dynamic mechanical analyzer (DMA7, Perkin Elmer, Norwalk, CT) and compared to the properties of commercial PeS membranes (MWCO=5,000; Millipore Corporation, Bedford, MA). The static force was increased from 100 to 1000 mN at a constant rate of 100 mN/min with one exception. For the compressive modulus measurement of heat-treated PVA membranes swollen for 1 hour, the static force was increased from 300 to 1200 mN at a constant rate of 100 mN/min. For this study, the heat-treated PVA membranes with dry thicknesses ranging from 270-300 μm were swollen in deionized water for 1 and 5 hours, and PVA/Pluronic® membranes were swollen for 1 hour. These measurements were conducted under swollen conditions to simulate the membrane performance under actual conditions of use.

2.3 Selectivity studies

The selectivity of UF membranes was analyzed using side-by-side diffusion cells (Figure 1). The diffusion cells were custom-made by the glass blowing shop at Iowa State University. The membrane area exposed for separation was 4.9 cm^2, and the volume of each cell was 18.5 mL. The model macromolecules were fluorescein isothiocyanate-dextran (FITC-dextran; MW=4,400, Sigma Chemical Company, St. Louis, MO) and myoglobin from horse skeletal muscle (MW~18,800, Sigma Chemical Company, St. Louis, MO). Since myoglobin is more hydrophobic than FITC-dextran, it was used to study the extent of fouling for PVA and PeS membranes. The model small molecular weight solute was L-tryptophan

(MW=204.2, Sigma Chemical Company, St. Louis, MO). The donor cell was filled with a mixture of FITC-dextran and L-tryptophan (0.05 mg/mL each), or a mixture of myoglobin and L-tryptophan (0.1 mg/mL each). A higher concentration was used for the myoglobin and L-tryptophan mixtures so that the effect of fouling could be observed more clearly. The receptor cell was initially filled with deionized water. Magnetic stirrers are used in both cells to keep uniform concentrations throughout. The concentrations of FITC-dextran/myoglobin and L-tryptophan were obtained by taking samples regularly from the sample port in the receptor cell for 4 hours and measuring their absorbances using a UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD).

The diffusion coefficients of solutes through these membranes were evaluated by first determining the permeability coefficients of the membranes from selectivity studies [7] using the relation

$$\ln\left[1-\frac{2c_t}{c_o}\right]=-\left(\frac{2A}{V}\cdot P\cdot t\right) \qquad [1]$$

where c_t is the concentration of solute in the receptor cell at time t, c_o is the initial concentration in the donor cell, A is the membrane area exposed to diffusion, V is the volume of half diffusion cell, and P is the permeability coefficient. The permeability coefficients of solutes through these membranes were found from the slope of $-(V/2A) \cdot \ln [1-2c_t/c_o]$ versus time.

The partition coefficients were evaluated for each solute by immersing 1 cm^2 of the membranes in deionized water at room temperature. Next, these membranes were put in 25

mL of each solute with concentration of 0.1 mg/mL. The concentrations of the solutes in the solutions were measured after 7 days. The solute concentrations inside the membranes were calculated using a mass balance. The partition coefficients were evaluated using the following equation

$$K_d = \frac{c_m}{c_s} = \frac{V_s \cdot (c_o - c_e)}{V_m \cdot c_o} \qquad [2]$$

where K_d is the partition coefficient, c_m is the solute concentration incorporated in the membranes, c_s is the solute concentration at equilibrium, V_s is the volume of solute in the solution, c_e is the solute concentration at equilibrium, and V_m is the volume of solute in the membranes assuming that it is equal to the membrane volume.

Finally, the effective diffusion coefficients (D_m) of solutes across the membranes were determined from the permeability (P), membrane thickness (l), and partition coefficients (K_d)

$$D_m = P \cdot l / K_d \qquad [3]$$

3. Results and Discussion

Uncrystallized PVA membranes were subject to rupture upon prolonged use. Therefore, the mechanical strength of the membranes was increased by heat-treating the PVA membranes at 100°C for 1 hour. Table 1 shows that the crystallinity of heat-treated PVA membranes was increased significantly which indicated that the mechanical properties of these heat-treated membranes were also enhanced. It can also be seen from Table 1 that the degree of crystallinity of PVA with higher average molecular weight ($\overline{M_n}$=35,420) was slightly lower than PVA with lower average molecular weight ($\overline{M_n}$=17,600). This is because

the PVA chains in the polymer with the larger molecular weight have more entanglements and cannot move as freely as the chains of the polymer with smaller molecular weight to rearrange and form crystals.

Table 2 shows that the compressive moduli of heat-treated PVA membranes that were swollen for 1 hour and 5 hours were comparable to those of PeS membranes. The compressive modulus of PVA membranes swollen for 1 hour was approximately the same as that for PVA swollen for 5 hours. The tensile moduli of heat-treated PVA membranes that were swollen for 1 hour and 5 hours however were an order of magnitude smaller than PeS membranes. It should be noted that the commercial PeS membranes tested have backing supports whereas the PVA membranes fabricated in the laboratory do not. Also, the tensile modulus of PVA swollen for 5 hours was significantly lower than the tensile modulus of PVA swollen for 1 hour. Both the tensile and compressive moduli of PVA blends with Pluronic® F127 membranes were much lower than those for PeS and PVA without Pluronic® membranes. The hydrophilic parts of Pluronic® caused the membranes to be weaker due to extensive water uptake.

As can be seen in Figure 2, the untreated membranes had a relatively high selectivity and flux. The y-axis depicts the weight fraction of the original solute in the donor cell that has diffused across the membrane into the receptor cell. However, untreated PVA membranes were subject to rupture upon prolonged use. As stated earlier, the crystallinity of PVA membranes increased significantly after heat-treating the membranes at 100°C for 1 hour. The diffusion of L-tryptophan was found to be slower for heat-treated membranes than for untreated membranes (Figure 3). This can also be seen in Table 3 where the diffusion coefficient of L-tryptophan across heat-treated PVA membranes was smaller than the

diffusion coefficient across untreated membranes. However, the mechanical strength of the heat-treated membranes improved significantly.

The permeability coefficient of L-tryptophan across heat-treated PVA membranes was 55% lower than across untreated membranes. Previous studies have showed that NaCl permeability coefficients decreased by about 99.5% for PVA membranes heat-treated at 175°C for 1hour [12]. The decrease was much more significant for the latter since the membranes were heat-treated at much higher temperatures. It should be noted that the flux of FITC-dextran was not shown in Figure 3 because for heat-treated membranes, the flux of FITC-dextran was essentially zero. All results are at least in triplicate, and the error bars depict standard deviation in almost all cases.

It was found that the solute flux was slightly higher for PVA membranes with the larger molecular weight ($\overline{M_n}$=35,420) than PVA membranes with the smaller molecular weight ($\overline{M_n}$=17,600) (Figure 4). This is expected since the degree of crystallinity of PVA ($\overline{M_n}$=35,420) membranes is slightly lower than PVA ($\overline{M_n}$=17,600) membranes. This is because the polymer with larger molecular weight has greater entanglements making it more difficult for the chains to align and form crystals. Therefore, the polymer chains in PVA ($\overline{M_n}$=35,420) membranes had a less restrained network for solute diffusion resulting in a higher flux.

It is interesting to note that the stirring speed did not seem to affect the solute diffusion across PVA membranes (Figure 5). A higher solute diffusion was expected with higher stirring speed if there were some FITC-dextran bound on the surface of the membranes due to decrease of the concentration polarization layer. Therefore, this result could possibly indicate that there is no FITC-dextran bound on the membranes. In contrast,

the PeS membranes exhibited a change in flux across the membrane with change in stirring speed, which is indicative of fouling. It was also found that the thinner the PVA membrane, the faster the solute diffusion (Figure 6). However, the membrane thickness could not be less than 100 μm, or the membrane would undergo rupture upon prolonged use.

As can be seen in Figure 7, the solute diffusion rates across PVA/Pluronic® membranes were significantly increased. Standard error is used instead of standard deviation only for the PVA/Pluronic® membranes due to the large variation that resulted from the relatively non-uniform blends formed between PVA and Pluronic® solutions. It is suggested that the hydrophilic parts of the Pluronic® swell extensively and enhance solute diffusive flux since they are not crystalline. These interactions reduce fouling and are expected to enhance solute diffusion. It also can be seen in Table 3 that the L-tryptophan diffusion coefficient across PVA/Pluronic® membranes was higher than across the heat-treated PVA membranes without Pluronic®. The other probable reason for this increase is dissolution and leaching out of Pluronic® from these membranes. This might also explain the large variation in solute diffusion rates due to the changes in the membrane performance over time. Since Pluronic® F68 has a slightly higher PEO content than Pluronic® F127, it was expected that solute diffusion would be faster across PVA membranes with Pluronic® F68. However, the difference was not significant as the solute flux through both membranes followed the same trend (Figure 7), and the diffusion coefficients of L-tryptophan for both membranes did not differ significantly (Table 3).

Figure 8 shows the amount of myoglobin retained in the donor cell and the amount that has diffused to the receptor cell. The amount of myoglobin in the donor cell declined by 6.9% after 4 hours of separation using PVA membranes and by 26% using PeS membranes. However, in the same time period, the amount of myoglobin diffusing across the membranes to the receptor cell is negligible. The amounts of myoglobin and L-tryptophan that diffused across the membranes are shown in Figure 9. It can be seen that the amount of L-tryptophan transported across the PVA and PeS membranes were approximately the same. Table 4 shows the fraction of the initial amount of myoglobin in the donor and receptor cells at different times and the unaccounted fraction of myoglobin. The unaccounted myoglobin is assumed to be on the membrane. As seen from the table, the fraction of myoglobin retained on the membrane (fouling) is much higher for PeS than for the PVA membranes. Another study was carried out by immersing 1 cm^2 of PVA and PeS membranes in 0.1mg/mL myoglobin solution for 18 hours. The ratios of concentrations of myoglobin in the membranes to that in the solution after 18 hours were found to be 3.60 for PVA membranes and 27.4 for PeS membranes. This study differs from the partition coefficient measurement in that the solutions did not reach equilibrium as in the partition coefficient study, and supports the conclusion that the PVA membranes display significantly lowered fouling compared to PeS membranes.

4. Conclusions

Ultrafiltration PVA membranes were successfully fabricated to separate macromolecules from small molecular weight solutes. The membrane strength was increased significantly by heat-treating the membranes at 100°C for 1 hour. The degree of crystallinity

of PVA ($\overline{M_n}$=35,420) was found to be lower than PVA ($\overline{M_n}$=17,600). The compressive moduli of heat-treated PVA membranes were comparable to those of the commercial PeS membranes even after the membranes were swollen in water for 5 hours. The tensile modulus of PVA membranes was lower than PeS membranes. It was found that the selectivity of crystallized PVA membranes was relatively high. The diffusion of L-tryptophan using heat-treated membranes was slower compared to untreated membranes. However, the membrane strength was increased significantly. The diffusion coefficient of L-tryptophan across untreated PVA membranes was also higher than across heat-treated PVA membranes. The L-tryptophan diffusion was faster for PVA ($\overline{M_n}$=35,420) membranes than PVA ($\overline{M_n}$=17,600). A higher stirring speed did not seem to effect the diffusion of L-tryptophan. As expected, thinner membranes gave faster L-tryptophan diffusion, however, the thickness could not be less than 100 μm. The PVA/Pluronic® membranes exhibited a faster solute diffusion rate but very little mechanical integrity compared to the heat-treated PVA membranes and the possibility of Pluronic® leaching out over time. The selectivity studies of myoglobin/L-tryptophan using heat-treated PVA and PeS membranes showed that membrane fouling was minimized with PVA membranes, thereby providing a better alternative to membranes that are currently being used.

Nomenclature

A membrane area exposed to diffusion [cm^2]

c_e solute concentration at equilibrium [mg/mL]

c_t solute concentration in the receptor cell at time t [mg/mL]

c_m solute concentration inside the membrane [mg/mL]

c_o initial solute concentration in the donor cell [mg/mL]

c_s solute concentration at equilibrium [mg/mL]

D_m diffusion coefficient [cm^2/s]

K_d partition coefficient

l membrane thickness in the swollen state[mm]

P permeability coefficient [cm/s]

V volume of half diffusion cell [cm^3]

V_m volume of solute in the membrane [cm^3]

V_s volume of solute in the solution [cm^3]

Acknowledgments. This project is funded by a grant from the Center for Advanced Technology Development. The authors would like to thank duPont for their generous gifts of PVA. The authors would also like to thank Dr. Steve Martin and Jeremy Schrooten for their help with the DMA experiments.

References

[1] M. Mulder, Nature of membranes, in J.A. Howell, V. Sanchez, R.W. Field (Ed.) Membranes in Bioprocessing Theory and Applications, Blackie Academic and Professional, London, 1993, pp. 13-32.

[2] R.H. Li and T.A. Barbari, Size-selective bioseparations using interfacially-crosslinked poly(vinyl alcohol) gel membranes, AIChE Symp. Ser., 88 no. 290 (1992) 65.

[3] W.R. Gombotz, W. Guanghui, T.A. Horbett, and A.S. Hoffman, Protein adsorption to poly(ethylene oxide) surfaces, J. Biomed. Mater. Res., 25 (1991) 1547.

[4] M. Amiji and K. Park, Prevention of protein adsorption and platelet adhesion on

surfaces by PEO/PPO/PEO triblock copolymers, Biomaterials, 13 no.10 (1992) 682.

[5] C.J. van Delden, J.M. Bezemer, G.H.M. Engbers, and J. Feijen, Poly(ethylene oxide)-modified carboxylated polystyrene lattices--immobilization chemistry and protein adsorption, J. Biomater. Sci. Polymer Edn., 8 no.4 (1996) 251.

[6] L.F. Gudeman and N.A. Peppas, Preparation and characterization of pH-sensitive, interpenetrating networks of poly(vinyl alcohol) and poly(acrylic acid), J. Appl. Polym. Sci. 55 (1995) 919.

[7] L.F. Gudeman and N.A. Peppas, pH sensitive membranes from poly(vinyl alcohol)/poly(acrylic acid) interpenetrating networks, J. Membr. Sci. 107 (1995) 239.

[8] M. Ebra-Lima and D.R. Paul, Hydraulic permeation of liquids through swollen polymeric networks. I. Poly(vinyl alcohol) – water, J. Appl. Polym. Sci., 19 (1975) 1381.

[9] K. Burczack, T. Fujisato, M. Hatada, and Y. Ikada, Protein permeation through poly(vinyl alcohol) hydrogel membranes, Biomaterials, 15 no.3 (1994) 231.

[10] M.G. Katz and T. Wydeven, Selective permeability of PVA membranes. I. Radiation - crosslinked membranes, J. Appl. Polym. Sci., 26 (1981) 2935.

[11] A.S. Hickey, and N.A. Peppas, Mesh size and diffusive characteristics of semicrystalline poly(vinyl alcohol) membranes prepared by freezing/thawing techniques, J. Membr. Sci. 107 (1995) 229.

[12] M.G. Katz and T. Wydeven, Selective permeability of PVA membranes. II. Heat-treated membranes, J. Appl. Polym. Sci., 27 (1982) 79.

[13] M.O. Ngui and S.K. Mallapragada, Understanding isothermal semicrystalline

polymer drying: Mathematical models and experimental characterization, J. Polym. Sci. Polym. Phys. 36 (1998) 2771.

[14] S. K. Mallapragada and S.F. Chin, Multilayered Semicrystalline Polymeric Controlled Release Systems, in I. McCulloch and S.W. Shalaby (Ed.) Tailored Polymeric Materials for Controlled Delivery Systems, ACS Symposium Series, 1998, pp. 176-185.

Table 1. Crystallinity of PVA membranes

Molecular Weight	**Uncrystallized/ Crystallized Membranes**	**Degree of Crystallinity**
$\overline{M_n}$=17,600	Uncrystallized	44 ± 0.7%
	Crystallized	51 ± 1%
$\overline{M_n}$=35,420	Uncrystallized	42 ± 0.9%
	Crystallized	49 ± 0.7%

Table 2. The compressive and tensile modulus of PeS and heat-treated PVA membranes

	PeS	PVA (swollen for 1 hour)	PVA (swollen for 5 hours)	PVA + 1%Pluronic F127 (swollen for 1 hour)
Compressive Modulus (MPa)	25 ± 7.0	39 ± 7.2	33 ± 5.8	11 ± 1.8
Tensile Modulus (MPa)	3600 ± 570	290 ± 57	180 ± 89	8.7 ± 0.92

Table 3. Diffusion coefficients

	L- tryptophan partition coefficients	L- tryptophan diffusion coefficients x 10^9 (cm^2/s)
Uncrystallized PVA	1512	10.5 ± 1.59
Crystallized PVA	1643	6.73 ± 0.557
PVA + Pluronic® F127	1363	9.63 ± 1.18
PVA + Pluronic® F68	1300	8.19 ± 0.586

Table 4. Distribution of myoglobin during selectivity studies of PVA and PeS membranes

Time (hrs)	PVA membranes			PeS membranes		
	Fraction of myoglobin in the donor cell	Fraction of myoglobin in the receptor cell	Fraction of myoglobin unaccounted	Fraction of myoglobin in the donor cell	Fraction of myoglobin in the receptor cell	Fraction of myoglobin unaccounted
0	1	0	0	1	0	0
1	0.958	0.002	0.040	0.888	0.001	0.111
2	0.961	0.002	0.037	0.826	0.002	0.173
3	0.946	0.004	0.50	0.772	0.002	0.226
4	0.925	0.006	0.069	0.739	0.003	0.258

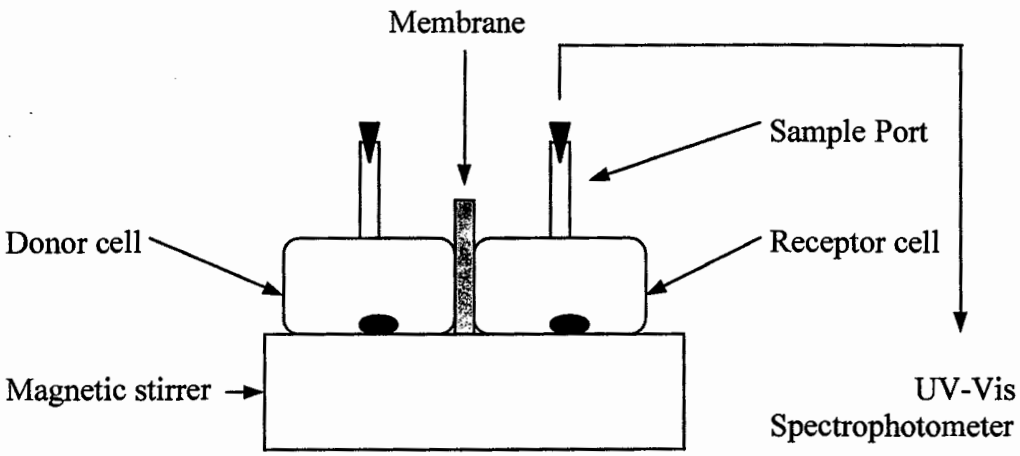

Figure 1. Diffusion cell apparatus

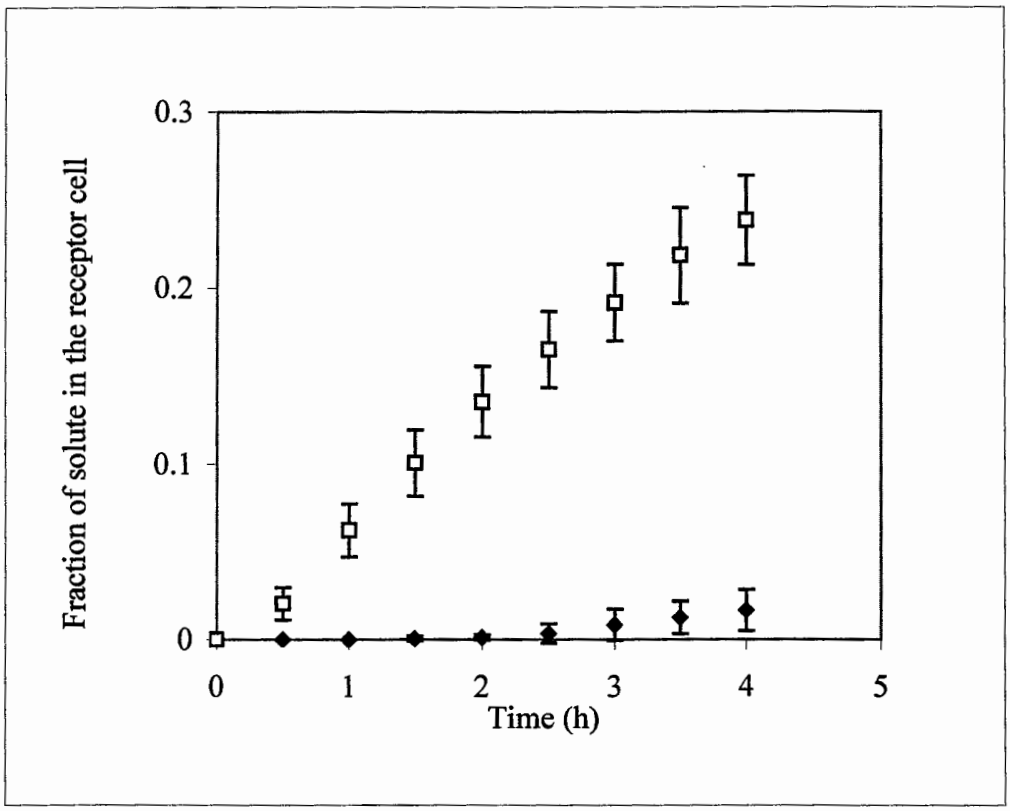

Figure 2. Separation of FITC-dextran from L-tryptophan using uncrystallized PVA membranes
◆FITC dextran; □L-tryptophan

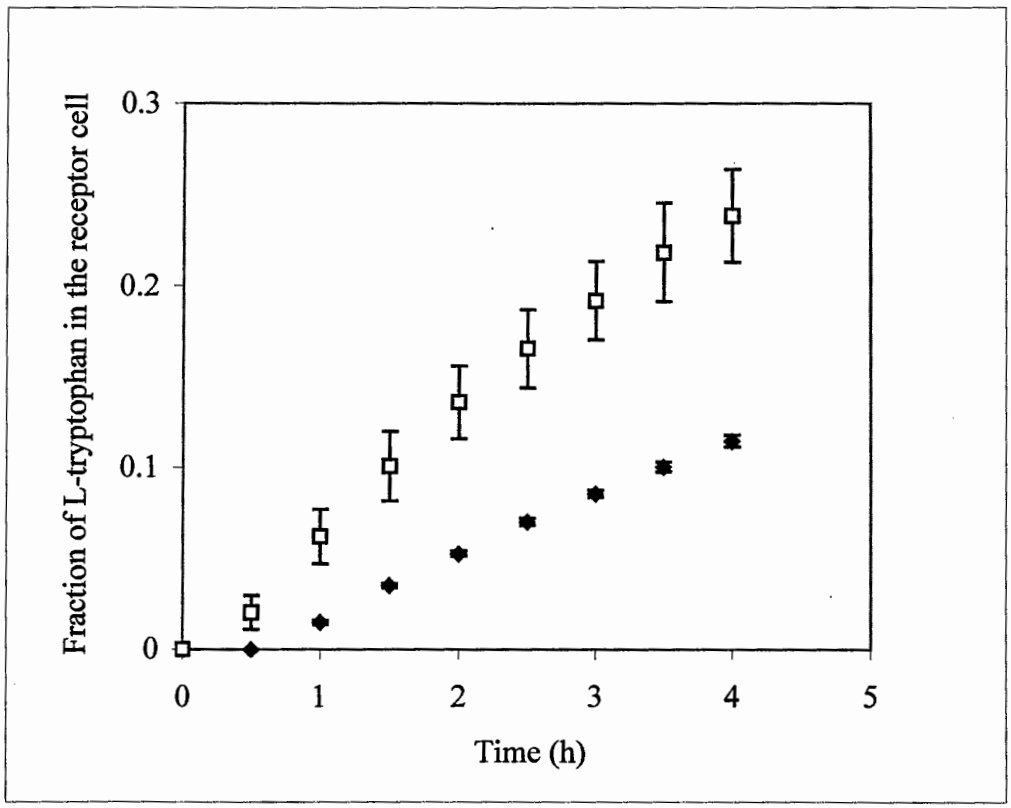

Figure 3. The effect of crystallinity on the separation of FITC-dextran and L-tryptophan using PVA membranes
◆Heat-treated membranes; □Untreated membranes

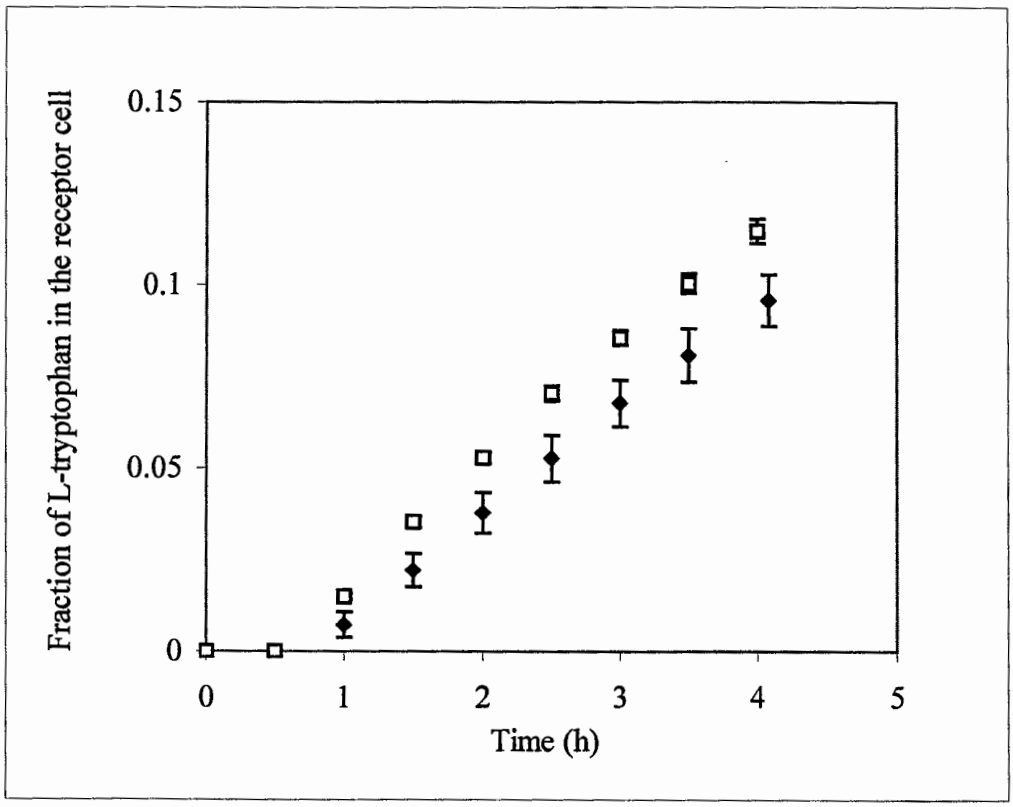

Figure 4. The effect of average molecular weight of PVA on the separation of FITC-dextran and L-tryptophan
◆$\overline{M_n}$=17,600; □$\overline{M_n}$=35,420

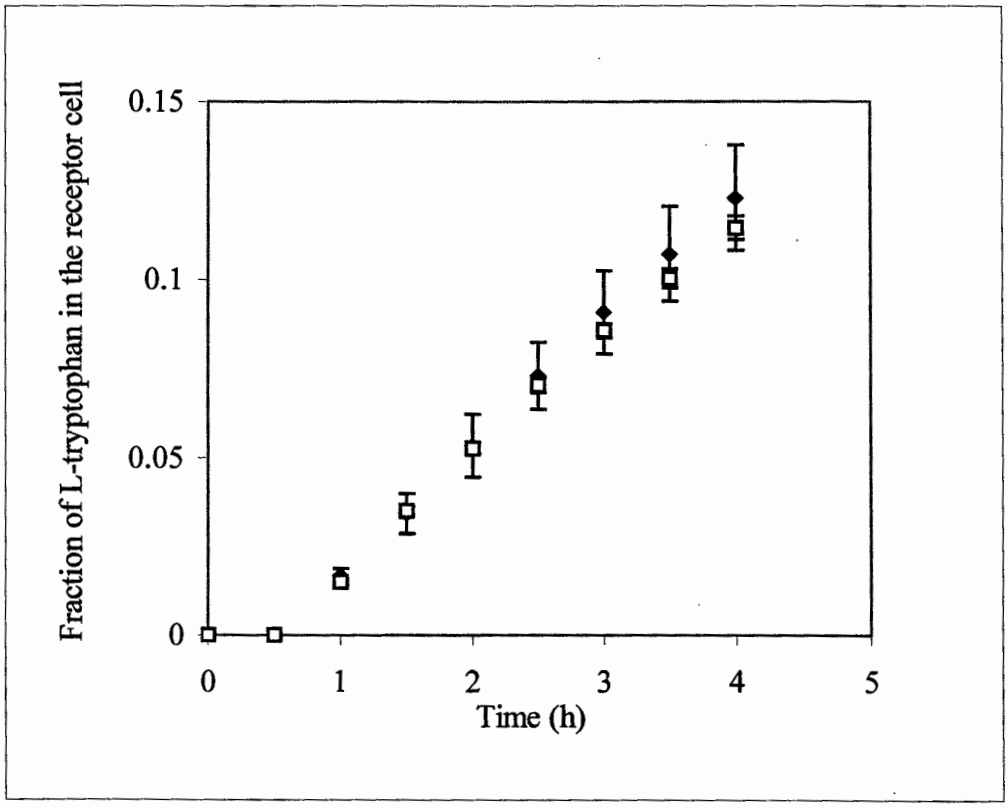

Figure 5. The effect of stirring speed on the separation of FITC-dextran and L-tryptophan
◆500rpm; □250rpm

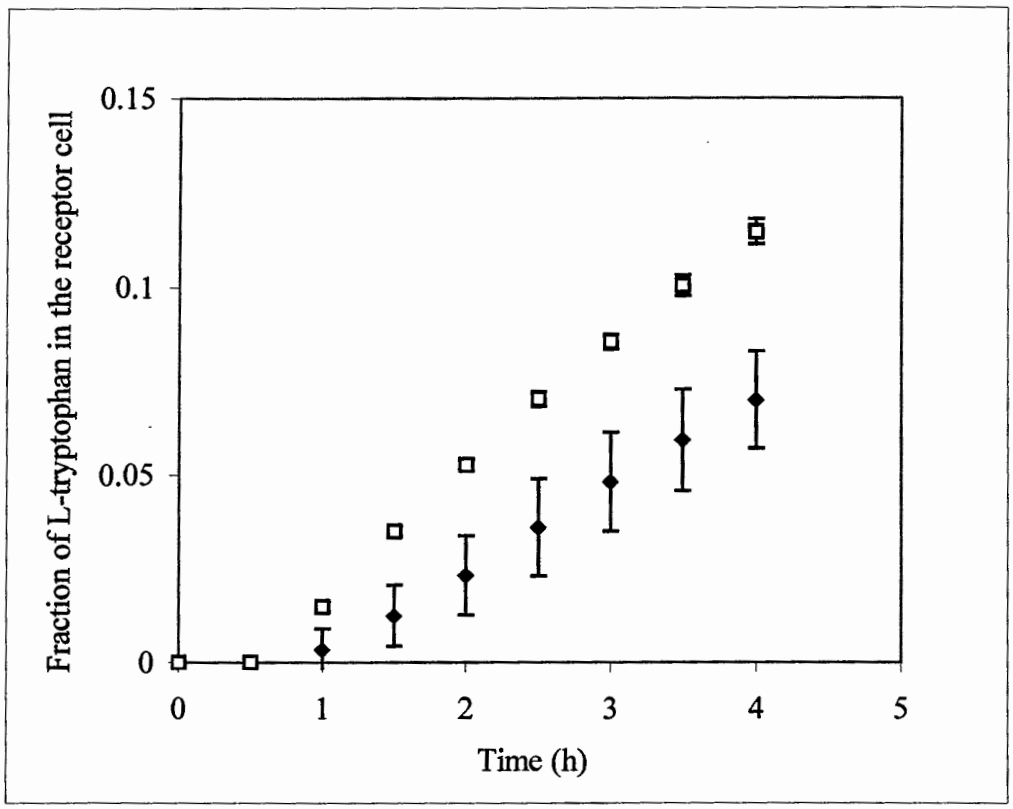

Figure 6. The effect of membrane thickness on the separation of FITC-dextran and L-tryptophan
◆0.20mm; □0.15mm

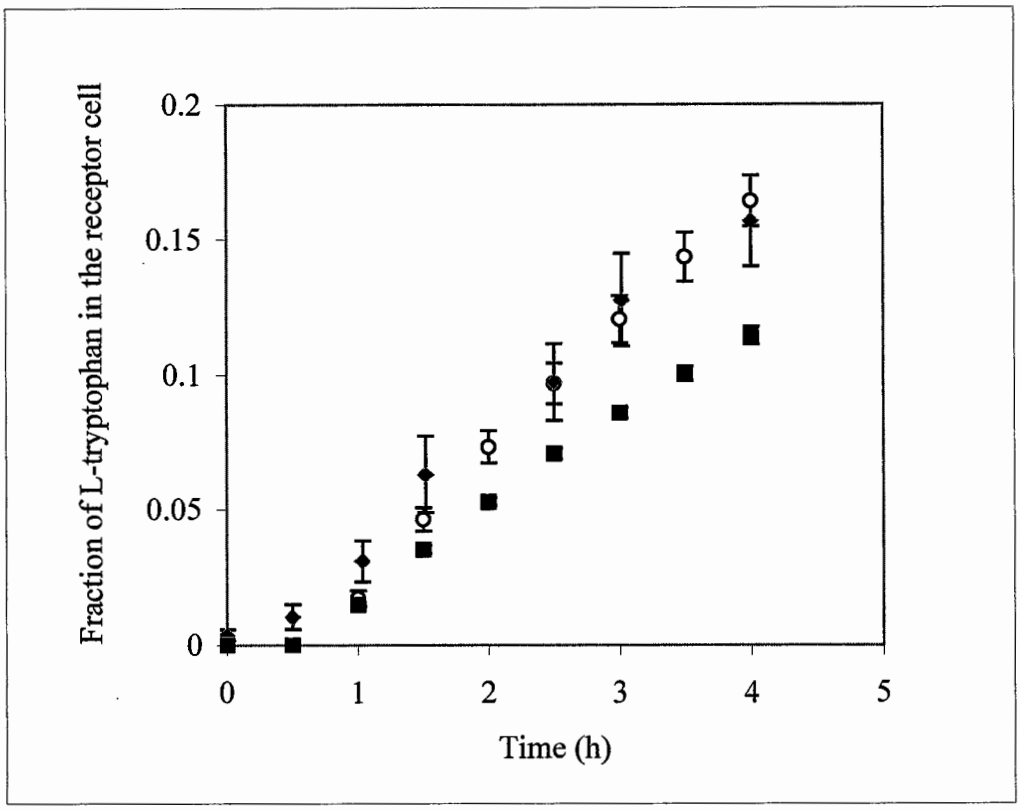

Figure 7. The effect of addition of Pluronic® on the separation of FITC-dextran and L- tryptophan
○with Pluronic® F68; ◆with Pluronic® F127; ■without Pluronic®

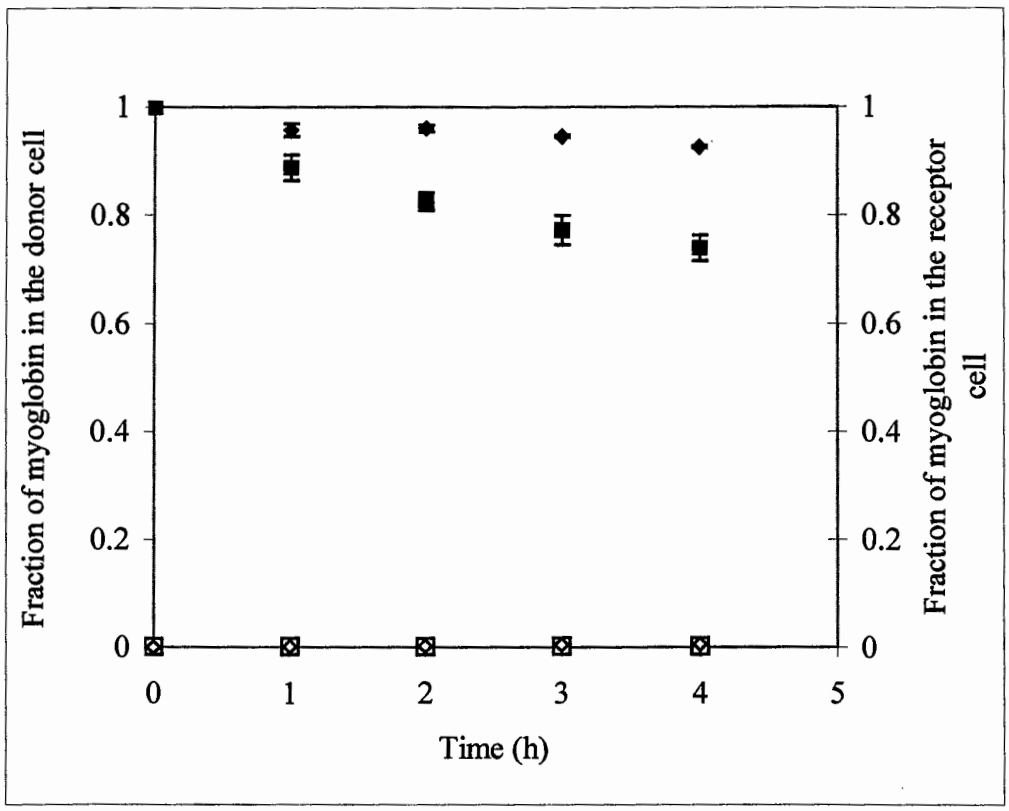

Figure 8. Weight fraction of myoglobin retained in the donor cell and diffused to the receptor cell
◆PVA membranes in donor cell; ■PeS membranes in donor cell; ◇PVA membranes in receptor cell; □PeS membranes in receptor cell

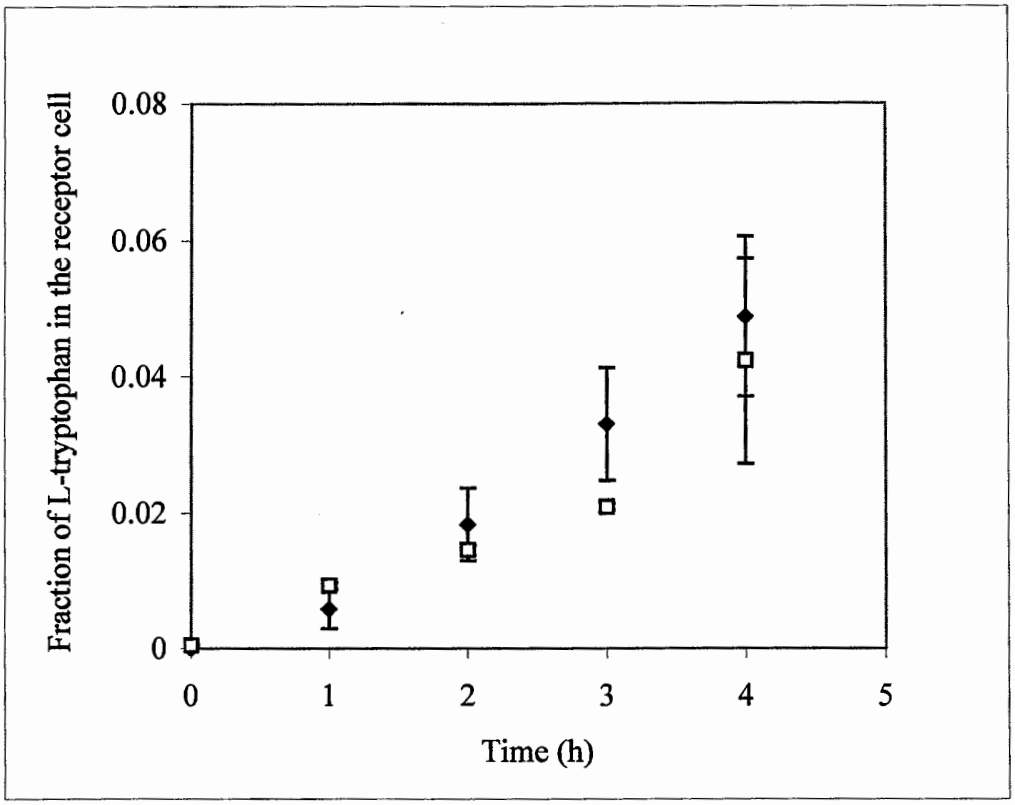

Figure 9. Weight fraction of L-tryptophan diffused to the receptor cell
◆PVA membranes; □PeS membranes

CHAPTER 3

PERMEABILITY STUDIES AND MAPPING OF PROTEIN FOULING ON HEAT-TREATED POLY(VINYL ALCOHOL), POLYETHERSULFONE, AND REGENERATED CELLULOSE MEMBRANES USING DIFFUSE REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPY

A paper submitted to Biotechnology Progress

Aurelia Amanda and Surya K. Mallapragada[1]

Abstract

Poly(vinyl alcohol) (PVA) membranes for ultrafiltration were fabricated by heat-treatment to separate macromolecules from microsolutes. PVA is a hydrophilic polymer with good mechanical properties because of its semicrystalline structure. The membranes were heat-treated at 100°C for 1 hour to increase their crystallinity, and thereby their mechanical strength. The mechanical strength of the membranes was evaluated using dynamic mechanical analysis by measuring their compressive and tensile moduli. Membrane permeabilities and protein fouling of heat-treated PVA membranes were compared to the commercial polyethersulfone (PeS) and regenerated cellulose membranes. Myoglobin from horse skeletal muscle was used as a model protein, and L-tryptophan was used as a model microsolute. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was used to identify protein fouling on the surfaces of these membranes. It was found from the permeability and DRIFTS studies that PVA membranes were more resistant to fouling than regenerated cellulose and PeS membranes.

---

[1] Corresponding author

Keywords: poly(vinyl alcohol), polyethersulfone, regenerated cellulose, hydrophilic membranes, fouling, diffuse reflectance infrared Fourier transform spectroscopy

1. Introduction

Fouling has been known to be a major drawback in membrane separations. Foulants deposited or adsorbed on the surface and inside the membrane cause the permeate flux to decrease, change the selectivity of the membranes, and cause a reduction in the output. Unlike concentration polarization, fouling is irreversible, and it is very difficult to acquire 100% flux recovery while still maintaining the desired membrane selectivity. Besides the shortcomings caused by the output cutback, membrane cleaning is required to remove the foulants and recover the permeate flux, and the efficiency of the membrane cleaning procedure will determine the membrane lifetime as well as the economics of the process. The cost of membrane cleaning and replacement of ultrafiltration membranes used to concentrate whey was approximately 47% of total cost *(1)*.

In the bioseparations industry where the feedstock mainly consists of proteins, one approach to reduce fouling is by adjusting the pH of the feed solution so that the proteins become more soluble. However, because there are usually many different proteins with different degree of solubilities present, this method becomes less practical *(2)*. Another approach to reduce fouling is to develop a 'superior' membrane that will minimize the interactions between solutes and membrane. The development of fouling-resistant membranes and a better understanding of fouling are known to be the main focus on research in ultrafiltration *(1)*, and these areas have attracted many researchers over the years.

Many membranes used in industry are made from hydrophobic polymers, or hydrophobic membranes that are rendered hydrophilic *(3)*. Polysulfone membranes are widely used because of their versatility: they have excellent temperature and pH stabilities, good selectivity and mechanical strength, and are capable to withstand rigorous cleaning. However, their hydrophobic nature makes them susceptible to fouling *(4)*. The hydrophobic parts of proteins can adsorb on these membranes very easily and cause severe fouling. Thus, there have been many attempts to render hydrophobic membranes or surfaces more hydrophilic by modifying the surface chemistry of these membranes, thus combining the excellent mechanical, chemical, and thermal stability of hydrophobic membranes with the fouling resistance of hydrophilic polymers *(5-10)*. The modified membranes were found to be more resistant to protein adsorption and cell adhesion. However, it is difficult to produce membranes with good stability using this method, and even though protein adsorption decreased by 40% on α,ω-diamino poly(ethylene oxide) (PEO) modified carboxylated polystyrene beads, they were not 'protein resistant' *(10)*.

Hydrophilic membranes have much better resistance to fouling, and protein adsorption on the hydrophilic membranes is easier to clean than on the hydrophobic ones *(4)*. However, commonly used hydrophilic membranes such as cellulose acetate have poor chemical and thermal stabilities, and they readily hydrolyze at high pH environment which make them very difficult to clean *(3)*. Poly(vinyl alcohol) (PVA) is another type of hydrophilic polymer that can minimize cell adhesion and protein adsorption. The minimized protein adsorption and biocompatibility of PVA are the main attraction for the popular use of PVA as biomaterials. PVA has been studied extensively as biomaterials for artificial kidney and pancreas *(11-13)*, glucose sensor *(14)*, immunoisolation membrane *(15)*, artificial

articular cartilage *(16)*, contact lenses *(17)*, and drug delivery devices *(18-21)*. There have also been studies on PVA membranes used in reverse osmosis *(22)* and bioseparations *(23)*. PVA is also non-toxic and easy to fabricate. It has excellent chemical and thermal stability, and its semicrystalline structure contributes to its good mechanical strength. Methods have been developed to increase its mechanical strength including chemical crosslinking *(23-27)*, irradiation *(25, 28-29)*, freezing and thawing technique *(30)*, and heat-treatment or annealing *(22, 31)*. Peppas and co-workers developed 'smart' membranes made of copolymers of PVA and poly(acrylic acid) (PAA) using a chemical crosslinking technique in which the mesh size of these membranes could be controlled by adjusting the pH of the feed solution and the ionic content of PAA *(27-28)*. Benign techniques become required in biomedical and food applications because of unreacted toxic crosslinking agents that can be harmful when they leach out. Heat-treatment was of particular interest because the technique is simple and membrane selectivities can be easily controlled by varying the conditions of annealing *(32)*. Heat-treated PVA membranes also allow faster microsolute transport than PVA membranes prepared by freezing/thawing technique *(30)*. Heat-treated PVA membranes with good stability can be obtained by heating the membranes less than 130°C for no longer than 2 hours *(31)*.

In this paper, the extent of protein fouling on fabricated heat-treated PVA membranes was compared to commercial polyethersulfone (PeS) and regenerated cellulose membranes by studying membrane permeabilities using diffusion cells and protein fouling using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). The membrane mechanical properties were also evaluated by measuring their tensile and compressive moduli.

2. Experimental

2.1 Membrane Preparation

The ultrafiltration (UF) membranes were prepared by dissolving PVA (Elvanol®, E.I. duPont de Nemours and Co., Wilmington, DE; $\overline{M_n}$=35,420, degree of hydrolysis >99%) in deionized water (10%w/v) at 90°C for 6 hours. The solution was cast in petri dishes and dried slowly at approximately 12°C. The membranes were heat-treated at 100°C for 1 hour to increase their mechanical strength. The membrane thickness was measured using calipers, and an average thickness was obtained for each membrane. The average dry thickness of PVA membranes used for the permeability and fouling studies was 248 ± 30 μm.

2.2 Membrane Characterization

Mechanical strength of regenerated cellulose membranes was evaluated by measuring their tensile and compressive moduli using a dynamic mechanical analyzer (DMA 7, Perkin-Elmer, Norwalk, CT). A comparison of the tensile and compressive moduli of PVA and PeS membranes was presented in a previous paper *(32)*. For the tensile moduli measurements, the membranes were cut into dumb-bell shapes, submerged in deionized water for 1 hour, clamped onto the sample holders of an extension film probe, and stretched at a constant rate of 100 mN/min from 100 to 1000mN. The compressive moduli were measured by increasing the static force at a constant rate of 100mN/min from 100 to 1000mN by placing an approximately 0.25 cm^2 sample on a parallel-plate probe. The tensile and compressive moduli were obtained from the slopes of static stress versus strain plots.

2.3 Permeability Studies

The permeability studies were carried out using side-by-side diffusion cells. The diffusion cells were custom made by the glass blowing shop at Iowa State University. The effective membrane area exposed for separation was 4.9 cm^2, and the volume of half diffusion cell was 18.5 mL. Two different apparatus set-ups were used: non-flow and flow under applied pressure (Figure 1). No pump was used for the non-flow system, and the effect of applied pressure on membrane fouling was studied. The pump used was a medium variable-flow peristaltic pump (Fisher Scientific, Pittsburgh, PA), and the pump flow rate was set at 9.0 ± 0.5 mL/min. The tubing used to connect the pump and the donor cell was made of silicone (Nalgene, Rochester, NY).

The model macromolecule used was myoglobin from horse skeletal muscle (MW~18,000; Sigma Chemical Company, St. Louis, MO), and the model small molecular weight solute used was L-tryptophan (MW=204.2; Sigma Chemical Company, St. Louis, MO). The maximum absorbances of myoglobin and L-tryptophan are 409 and 281nm, respectively. The receptor cell was initially filled with deionized water, whereas the donor cell was initially filled with an equal ratio by weight mixture of myoglobin and L-tryptophan (0.1mg/mL) solution in deionized water. The permeability and fouling of PVA membranes were compared to PeS and regenerated cellulose membranes (MWCO=10,000; Millipore Corporation, Bedford, MA). The amount of myoglobin depletion in the donor cell was used to determine the amount of protein deposited on these membranes. Since only a small amount of myoglobin passed through the membranes during 48 hours of permeability studies, the decrease of myoglobin absorbance in the donor cell was mainly caused by denaturation of myoglobin with time and adsorption of myoglobin on the tubing system and

on the membrane. Loss of myoglobin stability with time was studied by measuring the decrease of myoglobin absorbance over 48 hours period, which was the length of the permeability studies runs. The loss of myoglobin in the tubing was measured by replacing the membrane with a glass plate. The permeability studies were repeated thrice, and the error bars indicate standard error.

2.4 Diffuse Reflectance Infrared Fourier Transform Spectroscopy

Myoglobin fouling on PeS, regenerated cellulose, and PVA membranes was evaluated qualitatively using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS; Bio-Rad, FTS 40, Cambridge, MA). Prior to mapping membrane fouling using DRIFTS, permeability studies were carried out for PVA, PeS, and regenerated cellulose membranes. The permeability studies were varied from 5 minutes to 48 hours for the non-flow system. For the pressure-flow system, the permeability studies were only carried out for PeS and PVA membranes for 48 hours. After the permeability studies were completed, the membranes were rinsed carefully with deionized water to remove any solutions left on the membranes, and spectroscopic measurements on myoglobin deposited on the surface of the membranes were performed immediately. The membranes exposed to separation were cut into small pieces, and a piece was mounted on the holder inside the spectroscope. The permeability studies were repeated thrice for reproducibility. A spectrum of membrane fouled with myoglobin was obtained for every permeability study run, and an average spectrum was taken from the three permeability study run spectra. Spectra of pure myoglobin, unsoiled PeS, unsoiled regenerated cellulose, and unsoiled PVA swollen in water

for 48 hours membranes were also taken. The difference between spectra of unsoiled and soiled membranes was used to determine the extent of fouling on these membranes.

3. Results and Discussion

The tensile and compressive moduli of PVA and PeS membranes were compared in the previous study *(32)*. The tensile and compressive moduli of regenerated cellulose membranes after immersion for 1 hour in deionized water were 2428 ± 879 and 1.78 ± 0.25 MPa, respectively. The tensile and compressive moduli of PVA membranes (without any backing supports) swollen for 1 hour found in the earlier study were 290 ± 57 and 39 ± 7.2 MPa, respectively *(32)*. It was shown that the tensile modulus of regenerated cellulose membranes was an order of magnitude higher than PVA membranes, and this high tensile modulus of regenerated cellulose membranes was largely caused by the backing supports on the regenerated cellulose membranes. However, it was found that the compressive modulus of regenerated cellulose membranes was significantly lower than for PVA membranes. It was observed that after immersion in deionized water, the regenerated cellulose membranes 'shriveled' once they dried. To assess whether the membrane's strength decreases upon its contact with water, tensile and compressive moduli of unwetted membranes were measured. The tensile and compressive moduli of unwetted regenerated cellulose membranes were found to be 3745 ± 496 and 6.64 ± 1.20 MPa, respectively, which were significantly higher than the tensile and compressive moduli of regenerated cellulose membranes immersed in deionized water for 1 hour. Therefore, the reinforced regenerated cellulose membranes with backing supports undergo a significant loss of mechanical properties when brought into contact with water.

Absorbance of myoglobin from control solutions was measured periodically over 7 days. It was found that the maximum absorbance of myoglobin at 409 nm decreased linearly (Figure 2), however, the absorbance of myoglobin at 281 nm, which coincides with the maximum absorbance of L-tryptophan, stayed approximately the same over that period of time. It was thought that the decrease in the absorbance was caused by protein denaturation resulting from a prolonged exposure of myoglobin at room temperature.

A control solution of equal amount of L-tryptophan and myoglobin was used to monitor the decrease of absorbance of myoglobin and L-tryptophan. It was found that myoglobin absorbance of the control solution decreased to approximately 91% of its original value over 48 hours (Figure 3). The amount of myoglobin retained in the donor cell was used to observe the extent of fouling on the membranes. Figure 3 also shows that for the non-flow system with the exception of regenerated cellulose membranes, there was not much myoglobin detected in the receptor cell at the end of 48 hours. Therefore, the decrease in the absorbance of myoglobin in the donor cell was likely caused by either denaturation of myoglobin or myoglobin adsorption on the membranes. The amount of myoglobin in the donor cell decreased considerably to 54% of its original value after 48 hours for PeS membranes, and it was suspected that the amount of unaccounted myoglobin was incorporated on the PeS membranes and caused severe fouling on the membranes. The decrease of myoglobin to 69% of its original value in the donor cell on regenerated cellulose membranes was not as severe as PeS membranes, although it was significantly lower than PVA membranes in which the myoglobin in the donor cell decreased to 85%. For PVA membranes, the decrease of myoglobin in the donor cell follows approximately the same trend as the decrease of myoglobin of the control solution due to denaturation.

High selectivities of separation of PVA membranes were still maintained even after 48 hours of use for the non-flow system (Figure 4). PeS membranes had slightly higher selectivity than PVA membranes because over time, PVA membranes keep swelling upon contact with water. It is also observed that regenerated cellulose membranes had very poor selectivity compared to PeS and PVA membranes. Because of severe fouling on PeS membranes, the flux of L-tryptophan was lower for these membranes than for regenerated cellulose and PVA membranes.

Because of poor selectivities of the regenerated cellulose membranes under non-flow conditions, their selectivities were not tested using the flow system. The decrease of myoglobin in the donor cell was higher for the pressurized system for both PeS and PVA membranes (Figure 5) than for the non-flow system, but the decrease was greater for PeS membranes than PVA membranes. Besides myoglobin fouling on the membranes, this greater decrease of myoglobin in the donor cell might also be contributed by myoglobin adsorption on the pump tubing. To observe myoglobin adsorption on the pump tubings, the membrane was replaced with a glass plate, and the decrease of myoglobin absorbance in the donor cell was measured. The amount of myoglobin retained in the donor cell using a glass plate was 39% of its initial value, which was lower than PVA membranes (59%). This indicates that myoglobin also attached on the glass plate and on the surrounding diffusion cell wall. For PeS membrane using a flow system, the decrease was 28% of its initial value which was significantly higher than the decrease for PVA membranes. Thus, the observed decrease of myoglobin in the donor cell using PVA membranes and a pressurized system is likely caused by adsorption of myoglobin on the pump tubings as well as on the glass wall of the diffusion cell.

To verify this, myoglobin fouling on the membranes used in the permeability studies was then evaluated using DRIFTS. For myoglobin deposited on poly(ethylene) sheets, it was found that the peak at 1656 cm^{-1} corresponds to amide I region, whereas the peak at 1541 cm^{-1} corresponds to amide II region *(33)*. Figures 6-8 show two major peaks for myoglobin from horse skeletal muscle which were found between 1800 and 1600 cm^{-1} and between 1600 and 1480 cm^{-1} as well as the spectra of unsoiled and soiled PeS, regenerated cellulose, and PVA membranes (from 48 hours selectivity studies using non-flow systems). Figure 6 shows that there is an increase in the peak intensity between 1800 and 1600$cm^{-1}$and between 1600 and 1480 cm^{-1} on soiled PeS membranes. It is also seen that there were increases in the peak intensities at 1653 (amide I region) and 1559 cm^{-1} (amide II region). The increase in the peak intensity was difficult to distinguish for both regenerated cellulose and PVA membranes, and to see this increase more clearly, spectrum of unsoiled membrane was subtracted from the soiled membrane for all types of membranes and for various fouling times.

For PeS membranes, the differences at the two major peaks found for myoglobin were significant (Figure 9) between soiled and unsoiled membranes. For the non-flow system with PeS membranes, fouling was apparent even when the membranes were used in the permeability studies for only 5 minutes. The difference peaks increased as the time of the permeability studies was increased from 4 to 48 hours. The differences were found to be approximately the same for the non-flow and pressurized systems for the 48 hours permeability studies runs (Figure 10). This shows that most of the membrane fouling occurs very fast, in the first hour of operation and increasing the pressure does not seem to change the final amount of protein attached to the surface of PeS membranes.

There were essentially no differences in the spectra of soiled and unsoiled PVA membranes for up to 48 hours of permeability studies using the non-flow system (Figure 11). For the pressurized system for PVA membranes, it can be seen that there was a slight increase between 1800 and 1600 cm^{-1} and between 1600 and 1480 cm^{-1} (Figure 12), although the difference was much smaller than the ones found for PeS membranes under the same conditions. Figure 13 shows that for the non-flow system for regenerated cellulose membranes, there was a slight increase between 1600 and 1480 cm^{-1} and between 1800 and 1600 cm^{-1} indicating slight fouling. This agrees with the results obtained from the permeability studies where regenerated cellulose membranes undergo greater fouling than PVA membranes but less than on PeS membranes. The results found from DRIFTS support the findings found earlier through the permeability studies that PVA membranes were significantly more resistant to fouling than PeS and regenerated cellulose membranes. Since the selectivity of PVA membranes is better than regenerated cellulose membranes, and their extent of fouling is less than PeS membranes, they seem to be attractive candidates for bioseparations.

Figure 14 shows that the L-tryptophan flux was lower for pressurized system than for the non-flow system found earlier for PVA membranes. This was expected because some myoglobin fouling was observed on PVA membranes using the pressure flow system which was also seen from the DRIFTS, and this fouling hindered the transfer of L-tryptophan across the membrane. This was not observed for PeS membranes in which the flux was the same for both non-flow and pressurized systems. As seen earlier in the spectroscopy studies, there was no significant difference on the extent of fouling between non-flow and pressurized systems for PeS membranes, and therefore, the flux of L-tryptophan for both systems were

approximately the same. These studies seem to support the validity of the permeability and DRIFTS studies in ascertaining the fouling of various ultrafiltration membranes.

4. Conclusions

The PVA membranes even without any backing supports were found to exhibit good mechanical properties comparable to PeS and regenerated cellulose membranes with backing supports. From the permeability studies using the non-flow system, it was found that the decrease of myoglobin in the donor cell was much faster for PeS and regenerated cellulose membranes than for PVA membranes which indicated that both PeS and regenerated cellulose membranes underwent more fouling than PVA membranes. The extent of fouling of regenerated cellulose membranes was lower than for PeS membranes. For the non-flow system, the depletion of myoglobin in the donor cell was mainly caused by membrane fouling and the denaturation of myoglobin over time. For the pressurized system, adsorption of myoglobin on the pump tubing as well as on the glass wall of diffusion cell contributed to the decrease of myoglobin in the donor cell and was taken into account. For the pressurized flow system, after correcting for the above effects, PVA membranes were found to exhibit little or no fouling while the PeS membranes exhibited significant fouling.

DRIFTS confirmed the results found in the permeability studies. Significant differences in spectra of unsoiled and soiled PeS membranes between 1800 and 1600 cm^{-1} and between 1600 and 1480 cm^{-1} indicated that myoglobin fouled these membranes in both non-flow as well as pressurized systems. No significant fouling was observed for regenerated cellulose and PVA membranes using non-flow system, but there were small changes found with the pressurized systems on PVA membranes. These studies indicate the

PVA membranes exhibit good selectivities, mechanical properties, and are resistant to fouling and can be viable replacements for PeS and regenerated cellulose membranes.

Acknowledgments

This project was funded by a grant from Center for Advanced Technology and Development. The authors would like to thank Dr. Steve Martin and Jeremy Schrooten for the use of dynamic mechanical analyzer and diffuse reflectance infrared Fourier transform spectroscopy.

Literature Cited

(1) Eykamp, W. Ultrafiltration. In *Membrane Separation Systems – Recent Development and Future Directions*; Baker, R. W., Cussler, E. L., Eykamp, W., Koros, W. J., Riley, R. L., and H. Strathmann, Eds.; Noyes Data Corporation: New Jersey, 1991, pp. 360-395.

(2) Matson, S. L. Membrane Bioseparations. In *Membrane Science and Technology Series 2 – Membrane Separations Technology Principles and Applications*; Noble, R. D. and Stern, S. A. Eds.; Elsevier: Amsterdam, 1995, pp. 353-413.

(3) Mulder, M. Nature of Membranes. In *Membranes in Bioprocessing Theory and Applications*; Howell, J.A., Sanchez, V., Field R.W., Eds.; Blackie Academic and Professional: London, 1993, pp. 13-32.

(4) Howell, J. A. and Nystrom, M. Fouling Phenomena. In *Membranes in Bioprocessing Theory and Applications*; Howell, J.A., Sanchez, V., Field, and R.W., Eds.; Blackie Academic and Professional: London, 1993, pp. 203-241.

(5) Lee, J. H., Kopecek, J., and Andrade, J. D. Protein-Resistant Surfaces Prepared by PEO-Containing Block Copolymer Surfactants. *J. Biomed. Mater. Res.*, **1989**, *23*, 351-368.

(6) Gombotz, W.R., Guanghui, W., Horbett, T.A. and Hoffman, A.S. Protein Adsorption to Poly(ethylene oxide) Surfaces. *J. Biomed. Mater. Res.*, **1991**, *25*, 1547-1562.

(7) Amiji, M. and Park, K. Prevention of Protein Adsorption and Platelet Adhesion on Surfaces by PEO/PPO/PEO Triblock Copolymers. *Biomaterials*, **1992**, *13*, 682-692.

(8) Bergstrom, K., Osterberg, E., Holmberg, K., Hoffman, A. S., Schuman, T. P., Kozlowski, A., and Harris, J. M. Effects of Branching and Molecular Weight of Surface-Bound Poly(ethylene oxide) on Protein Rejection. *J. Biomater. Sci. Polym. Edn.*, **1994**, *6*, 123-132.

(9) Ueda, T., Ishihara, K., and Nakabayashi, N. Adsorption-Desorption of Proteins on Phospholipid Polymer Surfaces Evaluated by Dynamic Contact Angle Measurement. *J. Biomed. Mater. Res.*, **1995**, *29*, 381-387.

(10) van Delden, C.J., Bezemer, J.M., Engbers, G.H.M., and Feijen, J. Poly(ethylene oxide)-Modified Carboxylated Polystyrene Lattices--Immobilization Chemistry and Protein Adsorption. *J. Biomater. Sci. Polymer Edn.*, **1996**, *8*, 251-268.

(11) Burczack, K., Gamian, E., and Kochman, A. Long-Term *in vivo* Performance and Biocompatibility of Poly(vinyl alcohol) Hydrogel Microcapsules for Hybrid Type Artificial Pancreas. *Biomaterials*, **1996**, *17*, 2351-2356.

(12) Shih, C. Y. and Lai, J. Y. Poly(vinyl alcohol) Plasma Deposited Nylon 4 Membrane for Hemodialysis, *J. Biomed. Mater. Res.*, **1993**, *27*, 983-989.

(13) Young, T. H., Yao, N. K., Chang, R. F. and Chen, L. W. Evaluation of Asymmetric Poly(vinyl alcohol) Membranes for Use in Artificial Islets. *Biomaterials*, **1996**, *17*, 2139-2145.

(14) Liu, Y. C., Chen, X., Qian, J. H., Liu, H. Y., Shao, Z. Z., Deng, J. Q., and Yu, T. Y. Immobilization of Glucose Oxidase with the Blend of Regenerated Silk Fibroin and Poly(vinyl alcohol) and its Application to a 1, 1'-Dimethylferrocene-Mediating Glucose Sensor. *Appl. Biochem. and Biotechnol.*, **1997**, *62*, 105-117.

(15) Baker, R., Fournier, R. L., Sarver, J. G., Long, J. L., Goldblatt, P. J., Horner, J. M., and Selman, S. H. Evaluation of an Immunoisolation Membrane Formed by Incorporating a Poly(vinyl alcohol) Hydrogel within Microporous Filter Support. *Cell Transplant.*, **1997**, *6*, 585-595.

(16) Noguchi, T., Yamamuro, T., Oka, M., Kumar, P., Kotoura, Y., Hyon, S. H., and Ikada, Y. Poly(vinyl alcohol) Hydrogel as an Artificial Articular Cartilage: Evaluation of Biocompatibility. *J. Appl. Biomater.*, **1991**, *2*, 101-107.

(17) Hyon, S. H., Cha, W. I., Ikada, Y., Kita, M., Ogura, Y., and Honda, Y. Poly(vinyl alcohol) Hydrogels as Soft Contact Lens Material. *J. Biomater. Sci. Polymer Edn.*, **1994**, *5*, 397-406.

(18) Kim ,C. J., and Lee, P. I. Composite Poly(vinyl alcohol) Beads for Controlled Drug Delivery. *Pharm. Res.*, **1992**, *9*, 10-16.

(19) Wan, L. S. C. and Lim, L. Y. Drug Release from Heat-Treated Poly(vinyl alcohol) Films. *Drug Develop. Ind. Pharm.*, **1992**, *18*, 1895-1906.

(20) Mallapragada, S. K., Peppas, N. A., and Colombo, P. Crystal Dissolution-Controlled Release Systems. II. Metronidazole Release from Semicrystalline Poly(vinyl alcohol) Systems. *J. Biomed. Mater. Res.*, **1997**, *36 no. 1*, 125-130.

(21) Peppas, N. A. and Wright, S. L. Drug Diffusion and Binding in Ionizable Interpenetrating Networks from Poly(vinyl alcohol) and Poly(acrylic acid). *Eur. J. Pharm. Biopharm.*, **1998**, *46*, 15-29.

(22) Katz, M.G. and Wydeven, T. Selective permeability of PVA membranes. II. Heat-Treated Membranes. *J. Appl. Polym. Sci.*, **1982**, *27*, 79-87.

(23) Li, R.H. and Barbari, T.A. Size-Selective Bioseparations using Interfacially-Crosslinked Poly(vinyl alcohol) Gel Membranes. *AIChE Symp. Ser.*, **1992**, *88*, 65-71.

(24) Ebra-Lima, M. and Paul, D.R. Hydraulic Permeation of Liquids through Swollen Polymeric Networks. I. Poly(vinyl alcohol) – Water. *J. Appl. Polym. Sci.*, **1975**, *19*, 1381-1386.

(25) Burczack, K., Fujisato, T., Hatada, M., and Ikada Y. Protein Permeation through Poly(vinyl alcohol) Hydrogel Membranes. *Biomaterials*, **1994**, *15*, 231-238.

(26) Gudeman, L.F. and Peppas, N.A. Preparation and Characterization of pH-Sensitive, Interpenetrating Networks of Poly(vinyl alcohol) and Poly(acrylic acid). *J. Appl. Polym. Sci.* **1995**, *55*, 919-928.

(27) Gudeman, L.F. and Peppas, N.A. pH Sensitive Membranes from Poly(vinyl alcohol)/Poly(acrylic acid) Interpenetrating Networks. *J. Membr. Sci.* **1995**, *107*, 239-248.

(28) Peppas, N. A. and Merrill, E. W. Crosslinked Poly(vinyl alcohol) Hydrogels as Swollen Elastic Networks. *J. Appl. Polym. Sci.*, **1977**, *21*, 1763-1770.

(29) Katz, M.G. and Wydeven, T. Selective Permeability of PVA Membranes. I. Radiation - Crosslinked Membranes. *J. Appl. Polym. Sci.*, **1981**, *26*, 2935-2946.

(30) Hickey, A.S. and Peppas, N.A. Mesh Size and Diffusive Characteristics of Semicrystalline Poly(vinyl alcohol) Membranes Prepared by Freezing/Thawing Techniques. *J. Membr. Sci.* **1995**, *107*, 229-237.

(31) Mallapragada, S. K. and Chin, S.F. Multilayered Semicrystalline Polymeric Controlled Release Systems. In *Tailored Polymeric Materials for Controlled Delivery Systems*; McCulloch, I. and Shalaby, S.W., Eds.; ACS Symposium Series, 1998, pp. 176-184.

(32) Amanda, A. Kulprathipanja, A., Toennesen, M, and Mallapragada, S. K., Semicrystalline Poly(vinyl alcohol) Ultrafiltration Membranes for Bioseparations. *J. Membr. Sci.*, **2000**, *176*, 87-95.

(33) Sarver, R. W., Jr. and Krueger, W. C. Infrared Investigation on the Conformations of Proteins Deposited on Polyethylene Films. *Anal. Biochem.*, **1993**, *212*, 519-525.

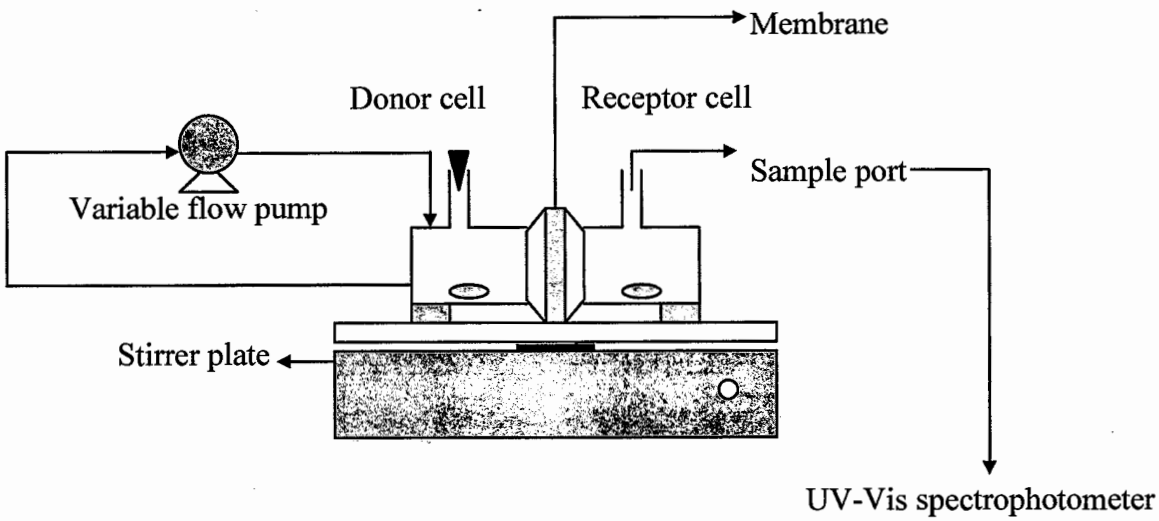

Figure 1. Permeability study apparatus for pressure-flow system

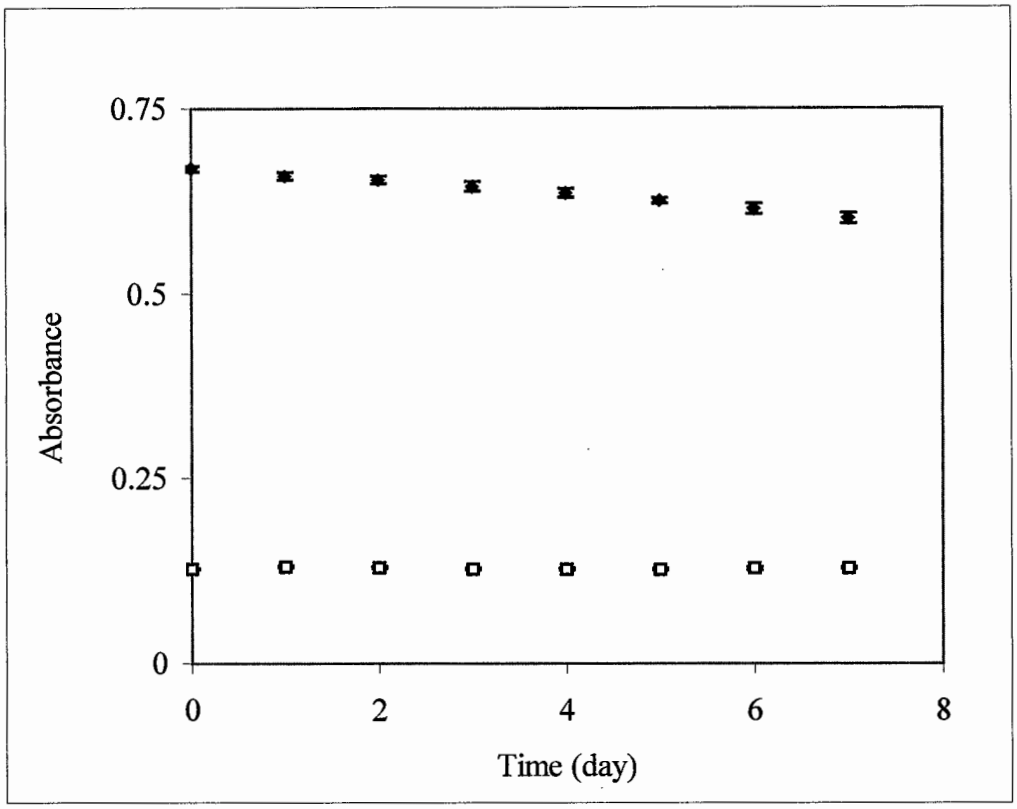

Figure 2. Stability of myoglobin from horse skeletal muscle
◆ 409nm □ 281nm

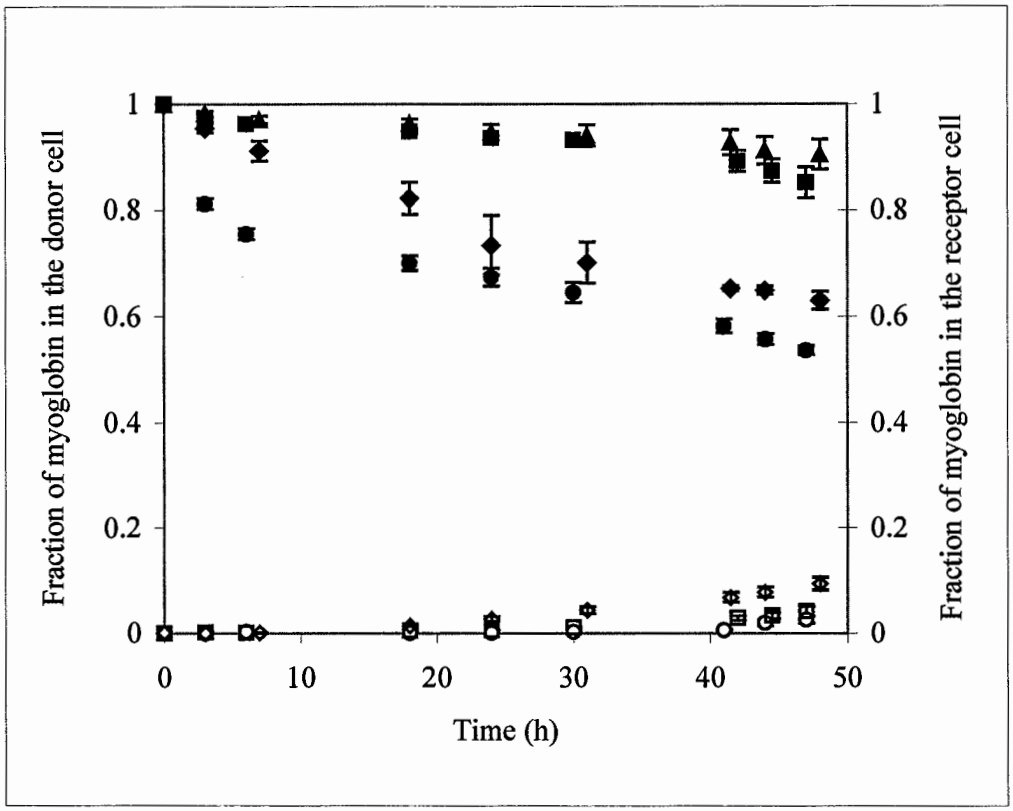

Figure 3. The extent of myoglobin fouling on PeS, regenerated cellulose, and PVA membranes for non-flow systems
●PeS (donor cell) ○PeS (receptor cell) ◆regenerated cellulose (donor cell)
◇regenerated cellulose (receptor cell) ■ PVA (donor cell) □PVA (receptor cell)
▲control solution – 0.1mg/mL each of myoglobin and L-tryptophan

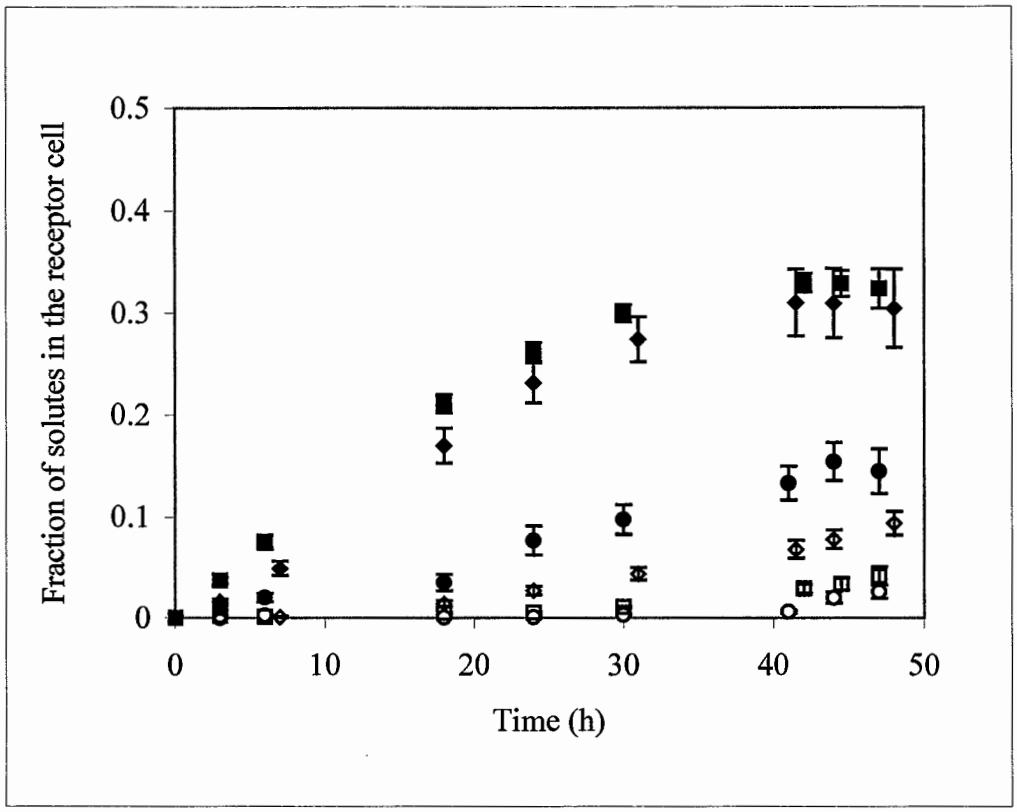

Figure 4. Comparison of permeabilities of PeS, regenerated cellulose, and PVA membranes for non-flow system
●PeS (L-tryptophan) ○PeS (myoglobin) ◆regenerated cellulose (L-tryptophan) ◇regenerated cellulose (myoglobin) ■PVA(L-tryptophan) □PVA (myoglobin)

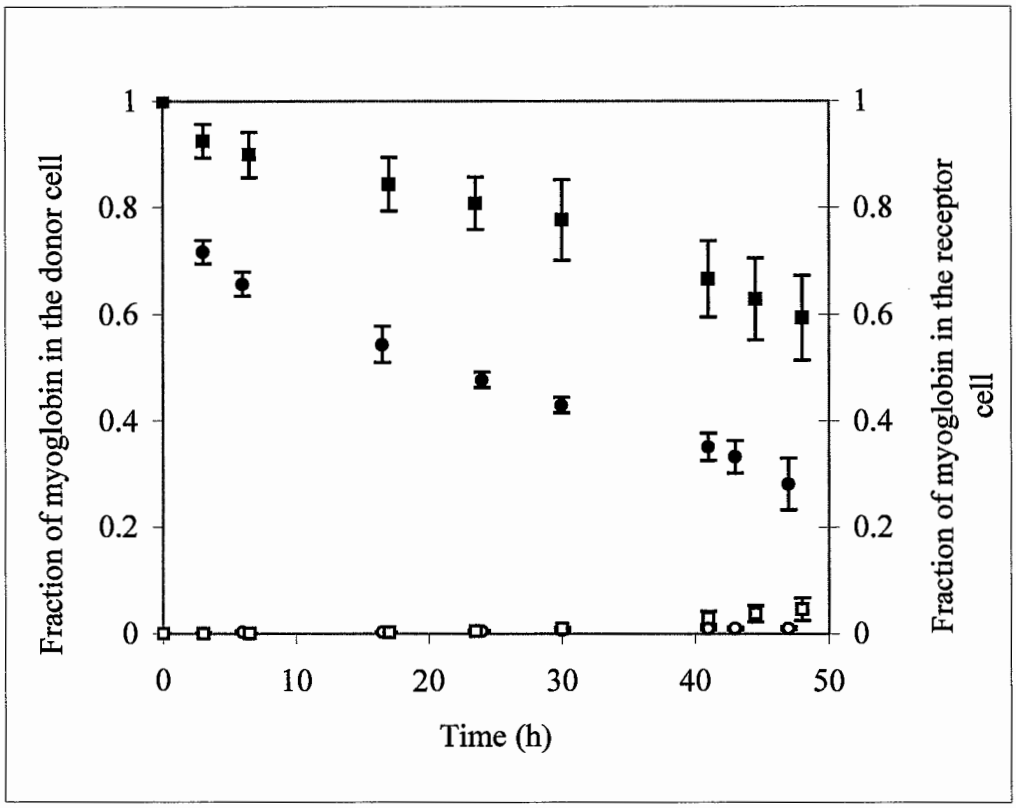

Figure 5. The extent of myoglobin fouling on PeS and PVA membranes for pressurized systems
● PeS (donor cell) ○PeS (receptor cell) ■PVA (donor cell)
□PVA (receptor cell)

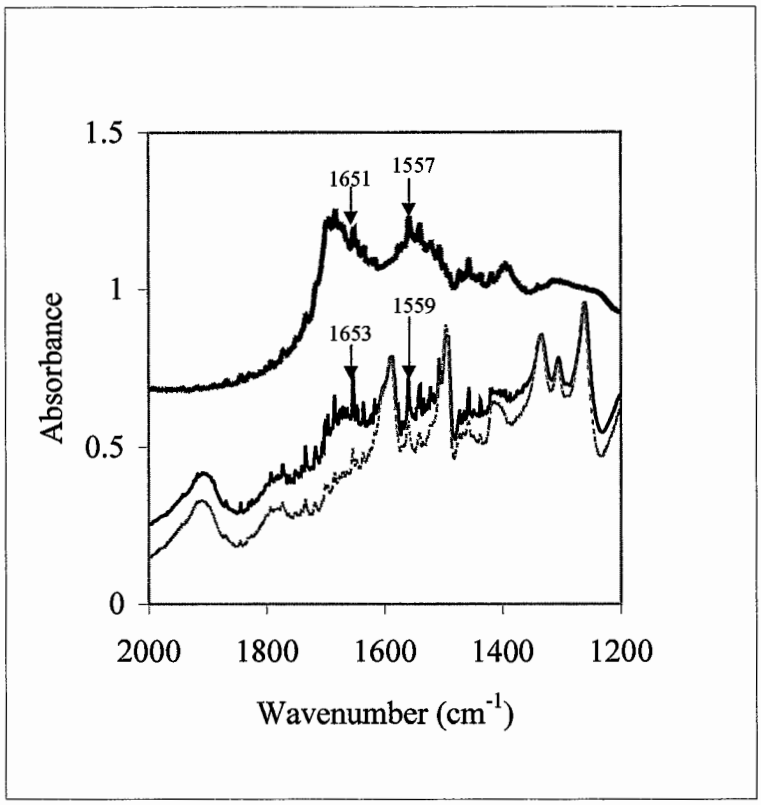

Figure 6. DRIFT spectra of PeS soiled with myoglobin for 48 hours for non-flow system
——pure myoglobin ····· unsoiled PeS membrane —— soiled PeS membrane

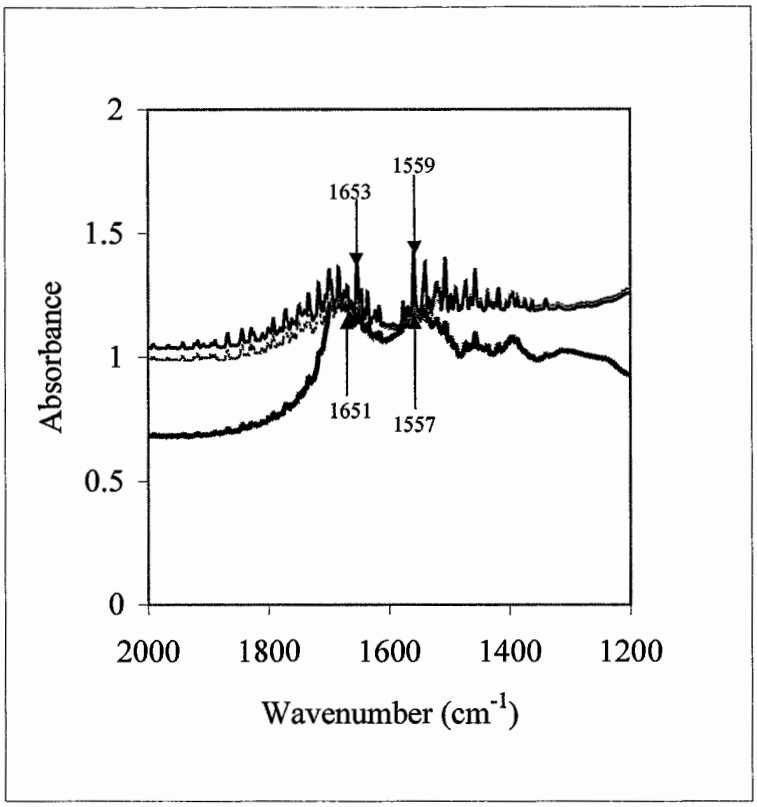

Figure 7. DRIFT spectra of regenerated cellulose soiled with myoglobin for 48 hours for non-flow system
—— pure myoglobin ····· unsoiled regenerated cellulose membrane
—— soiled regenerated cellulose membrane

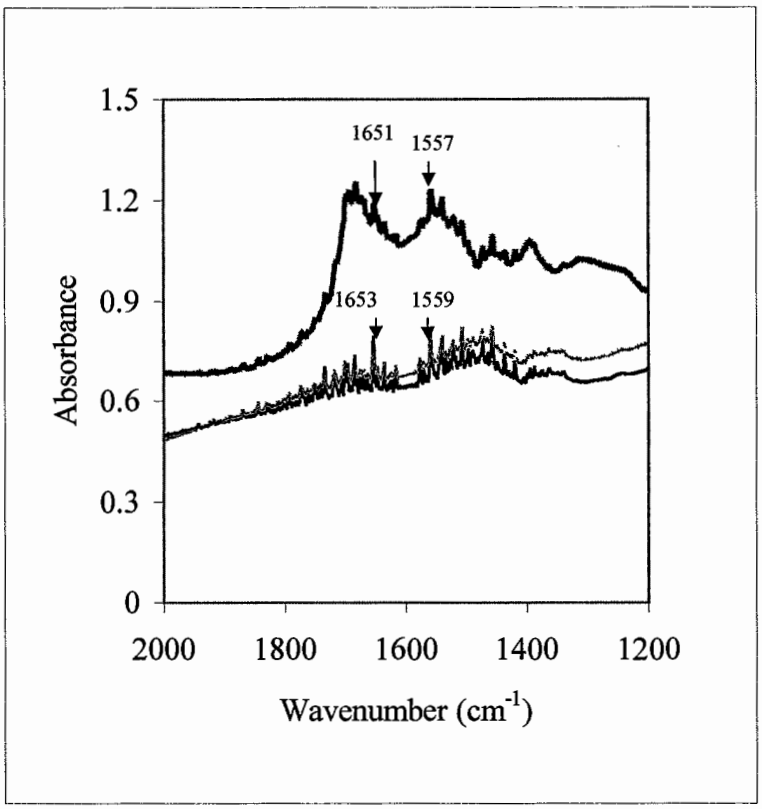

Figure 8. DRIFT spectra of PVA soiled with myoglobin for 48 hours for non-flow system
—— pure myoglobin ····· unsoiled PVA membrane ——soiled PVA membrane

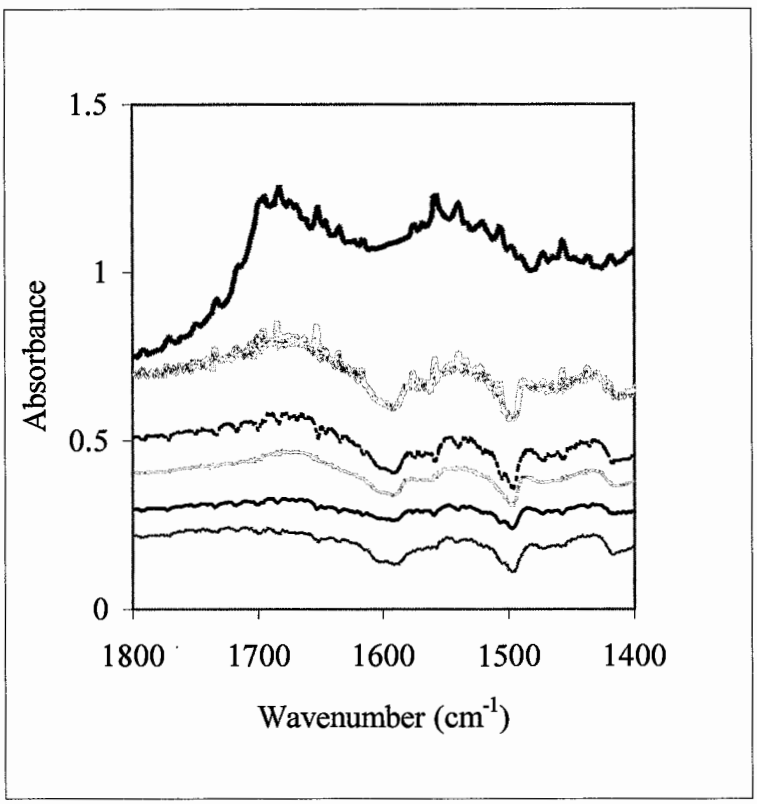

Figure 9. DRIFTS - differences of the spectra of unsoiled PeS membranes and PeS membranes soiled with myoglobin for different times using a non-flow system
myoglobin 5min 10min 1h 4h 48h

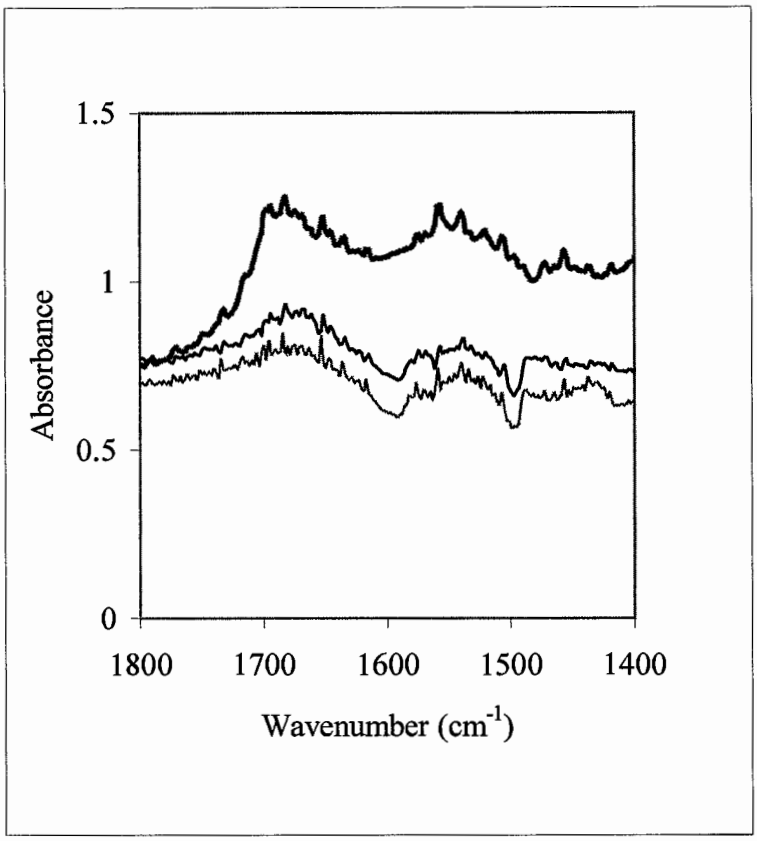

Figure 10. DRIFTS - differences of the spectra of unsoiled PeS membranes and PeS membranes soiled with myoglobin for 48 hours (non-flow vs. pressurized system)
—— myoglobin —— non-flow —— pressurized

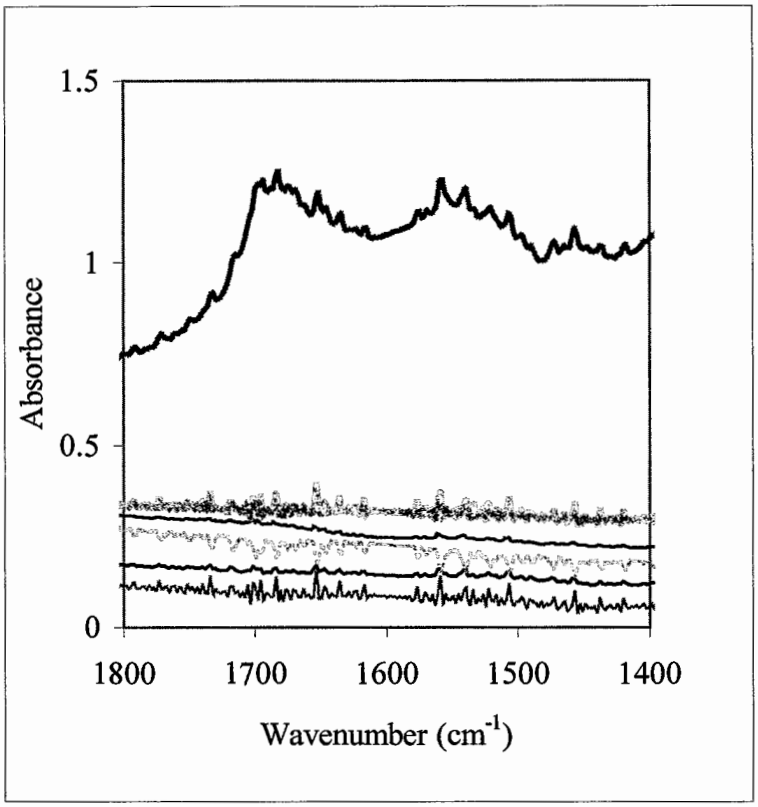

Figure 11. DRIFTS - differences of the spectra of unsoiled PVA membranes and PVA membranes soiled with myoglobin for different times using a non-flow system
myoglobin 5min 10min 1h 4h 48h

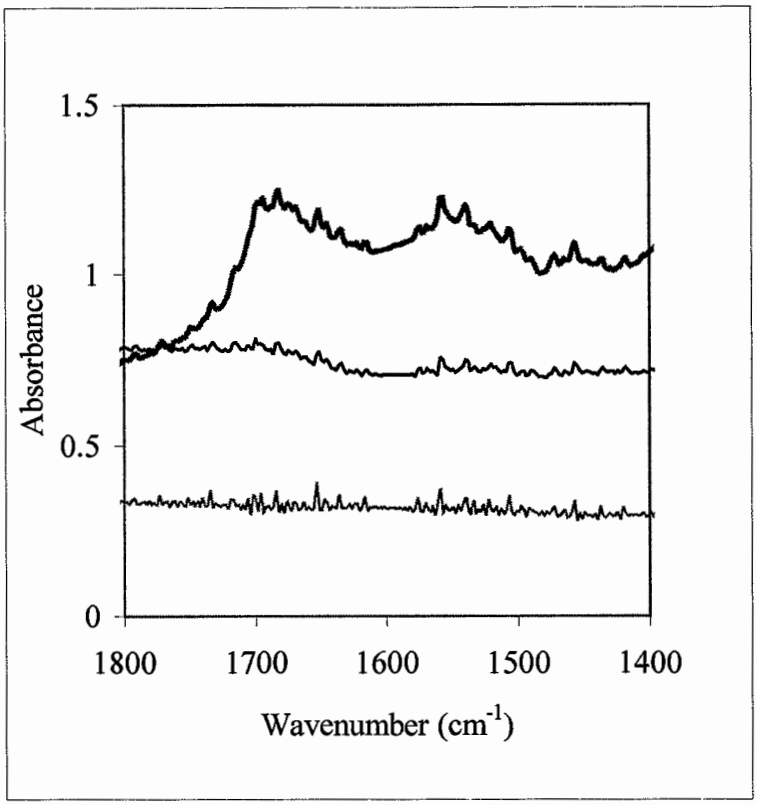

Figure 12. DRIFTS - differences on the spectra of unsoiled PVA membranes and PVA membranes soiled with myoglobin for 48 hours (non-flow vs. pressurized system)
myoglobin non-flow pressurized

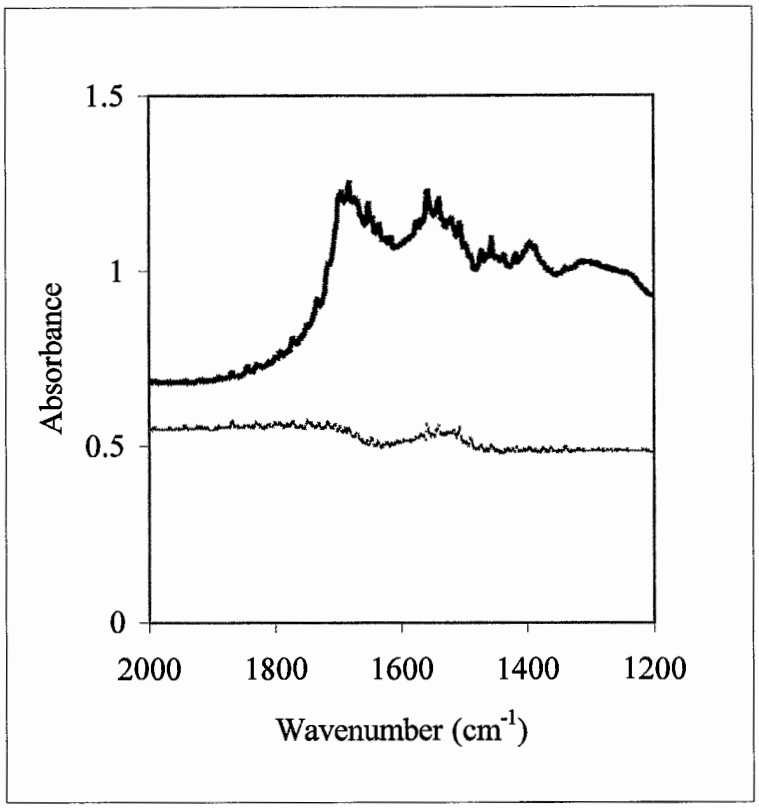

Figure 13. DRIFTS - difference spectra of unsoiled regenerated cellulose membranes and regenerated cellulose membranes soiled with myoglobin for 48 hours using a non-flow system

——— myoglobin ····· difference

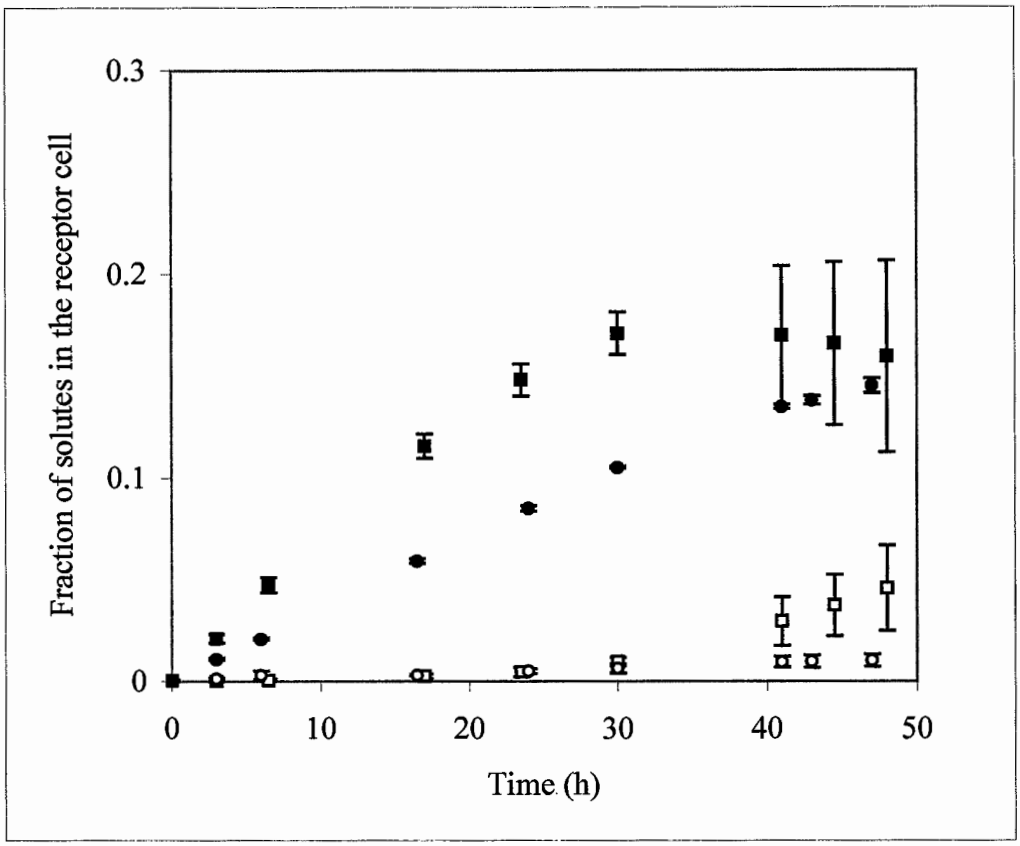

Figure 14. Permeabilities of PeS and PVA membranes for pressurized systems
●PeS (L-tryptophan) ○PeS (myoglobin) ■PVA(L-tryptophan)
□PVA (myoglobin)

CHAPTER 4

FABRICATION AND PERMEABILITY STUDIES OF ASYMMETRIC HEAT-TREATED POLY(VINYL ALCOHOL) MEMBRANES

Introduction

Asymmetric membranes are widely used in the membrane industry because they have high-flux, excellent selectivities, and good mechanical strength. An asymmetric membrane consists of a thin dense layer that contributes to its high selectivity and a porous support that provide mechanical strength for the membrane. The thin layer along with the porous support allows the solutes to diffuse faster across the membranes. Loeb and Sourirajan (1) synthesized asymmetric membranes made of cellulose acetate by phase inversion method, which had become the breakthrough in membrane technology. Asymmetric membranes are commonly produced in the industry using phase inversion method. One of the most popular phase inversion techniques is immersion precipitation (2). In this technique, the polymer is dissolved in a solvent, and the polymer solution is cast to form a thin membrane (3). Then, the membrane is placed in a non-solvent for the polymer, and this non-solvent has to be miscible with the solvent. Because of the instabilities at the interface, the polymer solution forms two phases and separates into thin dense layer and microporous support (Figure 1).

Li and Barbari (5) fabricated asymmetric PVA membranes by impregnating thin layer of PVA hydrogels within the pores of cellulose ester microfiltration membranes. The membrane surfaces were then interfacially crosslinked using toluene diisocyanate to form asymmetric membranes. The membranes produced had high flux, and the microporous supports imparted better mechanical strength. In a more recent study by Dai and Barbari (6), symmetric PVA membranes prepared by chemically crosslinking technique were compared

to asymmetric PVA membranes. The already crosslinked symmetric PVA membranes were interfacially modified with glutaraldehyde as crosslinking agent producing membranes with gradual increases in mesh size. The asymmetric membranes were shown to have high flux of microsolutes and better selectivities than the symmetric membranes, which had higher crosslinking densities. In another study by Young and co-workers (7), asymmetric membranes for artificial pancreas were fabricated by adding poly(ethylene glycol) into PVA solution to create pores on the surface layer. The solution was cast and immersed in coagulation bath containing Na_2SO_4, KOH, and water.

Korsmeyer and co-workers (8) studied drug release from porous hydrophilic films made of two different degrees of hydrolysis of PVA. PVA is commonly synthesized from poly(vinyl acetate) by substitution of the vinyl acetate groups with hydroxyl groups via hydrolysis (9). The residual vinyl acetate groups indicate the extent of hydrolysis of PVA: the higher the amount of residual vinyl acetate groups, the lower the degree of hydrolysis is, and these vinyl acetate groups weaken the strong hydrogen bonds form among the hydroxyl groups (10). The degree of hydrolysis of PVA determines the solubility of PVA in water, thus PVA with higher degree of hydrolysis is more difficult to dissolve in water. PVA with degree of hydrolysis of 98% or higher dissolves in water at a temperature above its glass transition temperature ($T_g = 85^oC$), but PVA with lower degree of hydrolysis (87 - 89% and approximately 80%) dissolves at room temperature (11).

In this work, the solubilities of PVA of different degree of hydrolysis were utilized to make asymmetric PVA membranes. Crystals PVA of low degree of hydrolysis (88%) were spread on top of cast PVA solution of high degree of hydrolysis (>99%). Dissolution of crystals PVA of low degree of hydrolysis in water takes place at room temperature leaving

micropores on the surface of PVA membranes of high degree of hydrolysis. The microporous layer is expected to increase the flux of low molecular weight solutes whereas the denser high hydrolysis PVA layer is supposed to maintain the high selectivities of regular heat-treated PVA membranes. By using this technique, the use of crosslinking agents which can be toxic when they leach out can be avoided (12). The membranes were heat-treated at 100°C for 1 hour to increase their mechanical strength. The mechanical strength, solute flux and selectivities of regular and asymmetric heat-treated membranes were compared to assess whether the asymmetric membranes could give comparable mechanical integrity, good selectivities, and higher permeabilities.

Experimental

Membrane Preparation

PVA with two different degree of hydrolysis were used (Elvanol®, E.I. duPont de Nemours and Co., Wilmington, DE; $\overline{M_n}$ = 35,420; degree of hydrolysis >99% and $\overline{M_n}$=23,000, degree of hydrolysis = 88%). A 10% (w/v) solution of PVA with degree of hydrolysis higher than 99% was prepared by dissolving PVA crystals in deionized water at 90°C for 6 hours. The solution was cooled off to room temperature and 25 mL of the solutions were cast into petri dishes with inside diameter of 9.7 cm. The cast solutions were kept in the refrigerator for 24 hours to form gels. The coarse crystals of PVA with degree of hydrolysis of 88% were milled using a coffee grinder and strained with sievers. A 0.5 gr of the crystals of US mesh size number 35 (sieve opening of 500 μm) were spread on top of the cast gel of high degree of hydrolysis via a strainer to create a uniform distribution of the crystals. Then, the cast membranes were dried slowly at around 12°C for 8 days. Once the

membranes had dried, they were peeled off the petri dishes and immersed in deionized water for 1 hour to dissolve the PVA with lower degree of hydrolysis. The dissolved PVA left pores on the surface of the membranes to form asymmetric membranes.

Membrane Characterization

The compressive and tensile moduli of heat-treated asymmetric PVA membranes were measured using a dynamic mechanical analyzer (DMA7, Perkin Elmer, Norwalk, CT). The compressive modulus was measured by placing a piece of asymmetric heat-treated PVA membrane that was swollen in deionized water for 1 hour between two-0.5 mm in diameter parallel plates. Then, the membrane was compressed with increasing static force from 100 to 1000 mN at a constant rate of 100 mN/min. For the tensile modulus measurement, the membranes were cut into dumb-bell shape, and a piece of the membrane was clamped onto extension film probe, and this time the membrane was stretched from 100 to 1000 mN at a constant rate of 100 mN/min. Since the membranes were in a swollen state when used in selectivity study experiments, the membranes were immersed in deionized water for 1 hour before the compressive and tensile moduli measurements.

Permeability Studies

The membrane was placed between two side-by-side diffusion cells with the dense layer facing the donor cell. By facing the dense layer to the donor cell, fouling of the membrane due to accumulation of macromolecules inside the pores can be avoided. A fiberglass screen (4.3 cm in diameter, Phifer Wire Products, Inc., Tuscaloosa, AL) acting as mechanical support for the membrane was placed behind the membrane. The diffusion cells

were custom made by the glass blowing shop at Iowa State University, with an separation area of 4.9 cm^2 and half-cell volume of 18.5 mL. Myoglobin from horse skeletal muscle (MW~18,000; Sigma Chemical Company, St. Louis, MO) was used as the model macromolecule, and L-tryptophan (MW=204.2; Sigma Chemical Company, St. Louis, MO) was used as the model microsolute. The maximum absorbances of myoglobin and L-tryptophan were 409 nm and 281 nm respectively. Initially, the receptor cell was filled with deionized water, and the donor cell was filled with 0.1 mg/mL each of myoglobin and L-tryptophan. The permeability study runs were carried out at room temperature. Samples were taken from both donor and receptor cells for 48 hours, and the absorbances of myoglobin and L-tryptophan were measured using UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD).

Results and Discussions

The mechanical strength of asymmetric heat-treated PVA membranes decreased considerably. The compressive and tensile moduli of asymmetric heat-treated PVA membranes swollen in deionized water for 1 hour were 2.6 ± 0.93 and 53.3 ± 23.0 MPa, respectively, which were significantly lower than regular heat-treated PVA membranes swollen in deionized water for 1 hour (see Chapter 2). Despite the low compressive and tensile moduli, the asymmetric membranes were still intact even after 48 hours of use in the selectivity study experiments.

Figure 2 shows that the amount of myoglobin retained in the donor cell using asymmetric PVA membranes was approximately the same as the regular heat-treated PVA membranes (see Chapter 3). As shown in Chapter 3, the decrease of myoglobin in the donor

cell was mainly caused by denaturation of myoglobin over time. Myoglobin denatured possibly because the operation temperature (room temperature) during permeability studies was much higher than myoglobin storage temperature. The selectivities of asymmetric PVA membranes were found to be approximately the same as regular heat-treated PVA membranes (Figure 3). But the flux of L-tryptophan across asymmetric PVA membranes was not higher than across regular heat-treated PVA membranes as expected earlier. The L-tryptophan flux across asymmetric PVA membranes was slightly higher during the first 10 hours of the experiment, and it started to follow the same trend as the flux of L-tryptophan across regular PVA membranes.

Conclusions

It was found that the asymmetric heat-treated PVA membranes formed by dissolution of low degree of hydrolysis crystals did not exhibit higher flux of L-tryptophan as expected. The asymmetric PVA membranes had approximately the same selectivities as regular PVA membranes, but the mechanical strength of the assymetric PVA membranes were significantly lower than regular PVA membranes as shown by the lower compressive and tensile moduli measurements.

Acknowledgments

This work was funded by the Center for Advanced Technology and Development. We would like to thank Dr. Steve Martin and Jeremy Schrooten for the use of dynamic mechanical analyzer.

Literature Cited

(1) Loeb, S.; Sourirajan, S. *Adv. Chem. Ser.* **1992**, *38*, 117.

(2) Mulder, M. Nature of Membranes. In *Membranes in Bioprocessing Theory and Applications*; Howell, J.A., Sanchez, V., Field R.W., Eds.; Blackie Academic and Professional: London, **1993**, pp. 13-32.

(3) Bell, G.; Cousins, R. B. Membrane Separations Processes. In *Engineering Processes for Bioseparations*; Weatherley, L. R., Ed.; Butterworth-Heinemann Ltd.: Oxford, **1994**, pp. 135-165.

(4) Mulder, M. *Basic Principles of Membrane Technology*; Kluwer Academic Publishers: **1991**, The Netherlands, pp. 281-311.

(5) Li, R. H.; Barbari, T. A. *J. Membr. Sci.*, **1996**, *111*, 115-122.

(6) Dai, W. S.; Barbari, T. A. *J. Membr. Sci.*, **1999**, *156*, 67-79.

(7) Hodge, R. M.; Edward, G. H.; Simon, G. P. *Polymer*, **1996**, *37*, 1371-1376.

(8) Young, T. H.; Yao, N. K.; Chang, R. F.; Chen, L. W. *Biomaterials*, **1996**, *17*, 2139-2145.

(9) Korsmeyer, R. W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N. A. *Int. J. Pharm.* **1983**, *15*, 25-35.

(10) Toyoshima, K. General Properties of Polyvinyl Alcohol in Relation to its Applications. In *Polyvinyl Alcohol*; Finch, C. A., Ed.; John Wiley & Sons: Great Britain, **1973**, pp.17-65.

(11) Toyoshima, K. Properties of Polyvinyl Alcohol Films. In *Polyvinyl Alcohol*; Finch, C. A., Ed.; John Wiley & Sons: Great Britain, **1973**, pp.339-389.

(12) Mallapragada, S. K.; Peppas, N. A.; Colombo, P. *J. Biomed. Mater. Res.*, **1997**, *36*, 125-130.

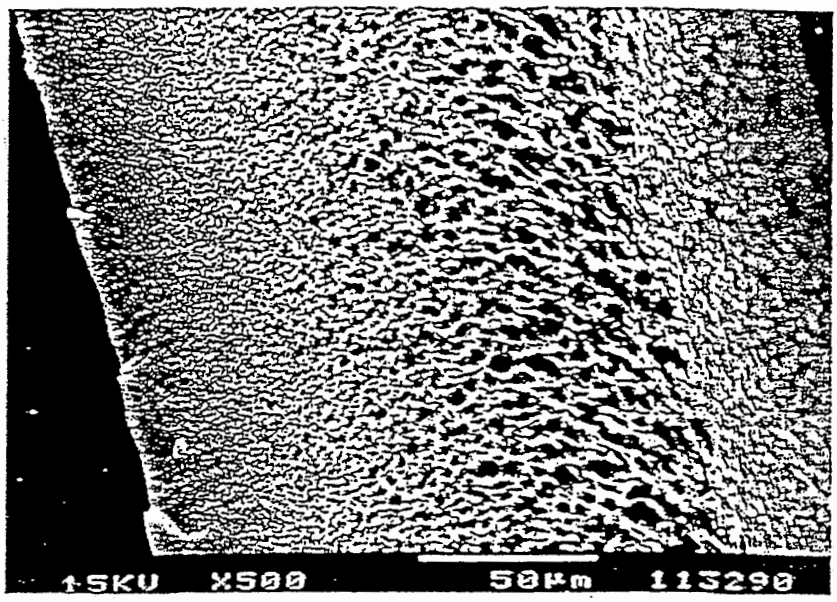

Figure 1. Asymmetric polysulphone membranes cross-section diagram (4; with kind permission from Kluwer Academic Publishers)

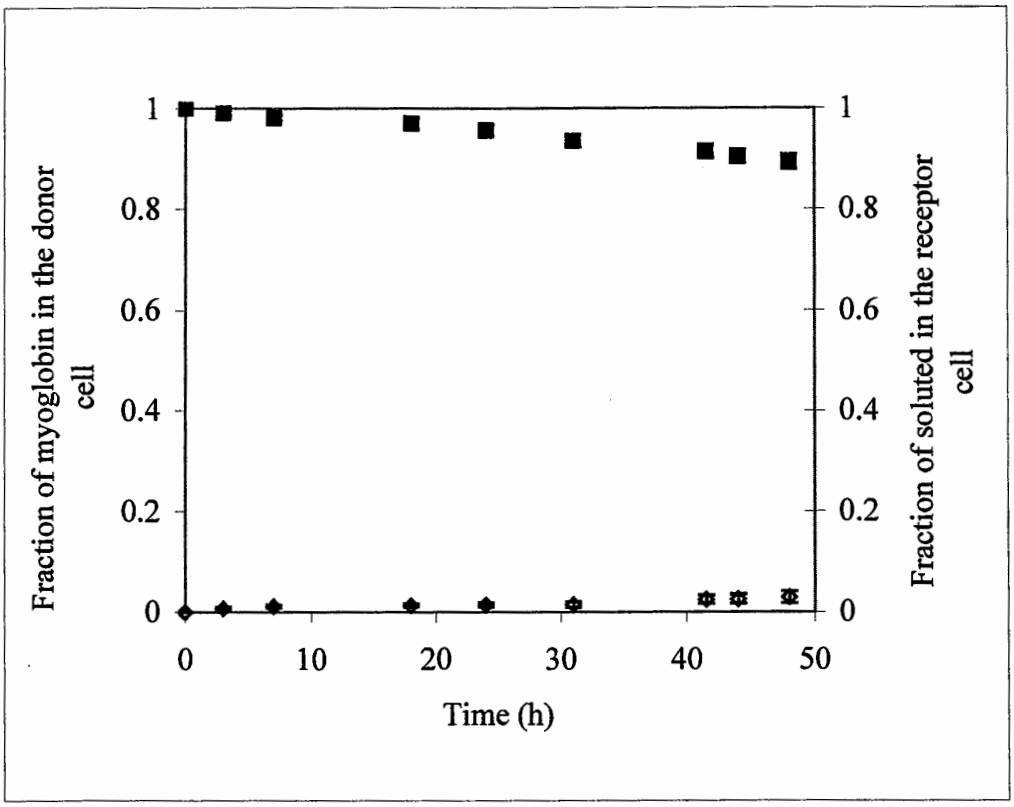

Figure 2. Extent of fouling on asymmetric heat-treated PVA membranes
■donor cell; ◇receptor cell

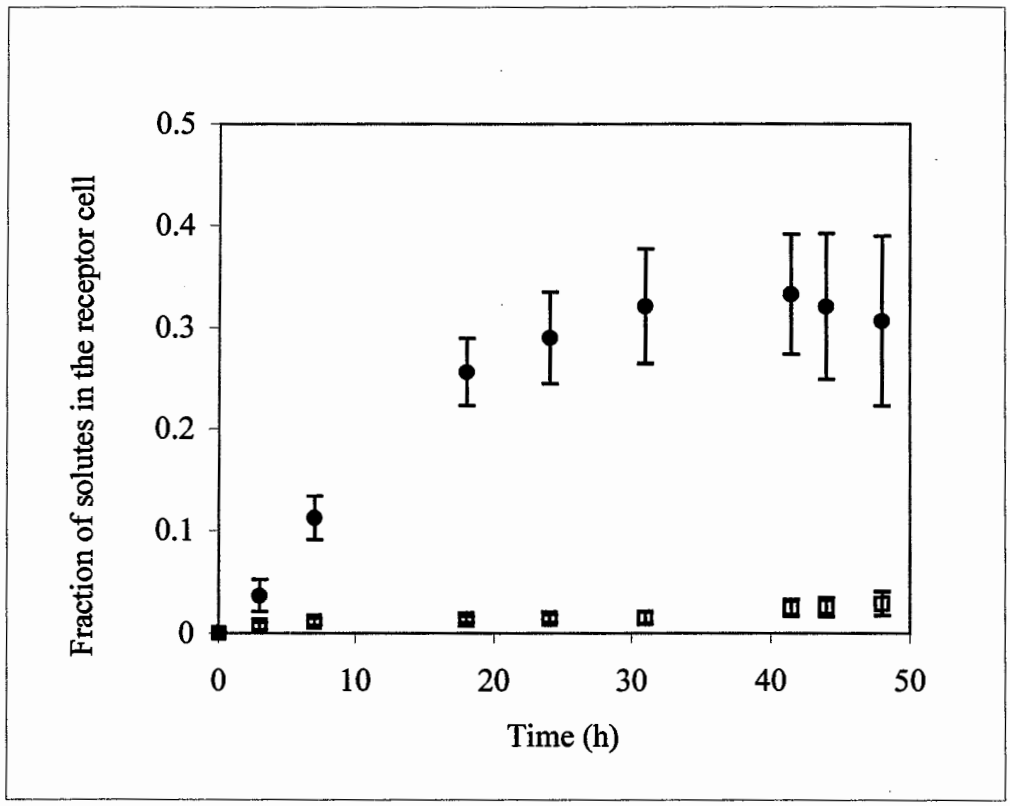

Figure 3. Permeabilities of asymmetric heat-treated PVA membranes
□myoglobin; ●L-tryptophan

CHAPTER 5

GENERAL CONCLUSIONS

Research Overview

Heat-treated PVA ultrafiltration membranes showing good mechanical integrity, good selectivities for bioseparations, and high fouling resistance were successfully fabricated and tested. The properties of the membranes tested showed comparable mechanical integrity and selectivities of separations and better fouling resistance than commercial PeS and regenerated cellulose membranes. The degree of crystallinity of PVA membranes after heat-treatment at 100°C for 1 hour did not seem to increase considerably from untreated PVA membranes. However, the strength of these heat-treated PVA membranes increased significantly as apparent from their mechanical properties and integrity during permeability studies. The heat-treated membranes were still intact after 48 hours of use, whereas the untreated PVA membranes were vulnerable to rupture after even less than 4 hours of use.

The compressive moduli of heat-treated PVA membranes swollen in deionized water for 1 hour was approximately the same as PeS membranes, but was higher than for regenerated cellulose membranes. Swelling of heat-treated PVA membranes in deionized water for 5 hours did not seem to decrease their compressive moduli. The tensile modulus of regular heat-treated PVA membranes without backing supports after swelling in deionized water for 1 hour was significantly lower than both PeS and regenerated cellulose membranes, and increasing the swelling time to 5 hours decreased their tensile modulus even more. It should be noted, however, that these commercial PeS and regenerated cellulose membranes tested had backing supports.

Separations of proteins and macromolecules from small molecular weight compounds were tested. Model macromolecules used were FITC-dextran and myoglobin, and model small molecular weight solute used was L-tryptophan. Heat-treated PVA membranes had higher selectivities but lower solute fluxes compared to untreated PVA membranes. It was found that solute flux increased with average molecular weight of PVA. For heat-treated PVA membranes, the stirring speed did not seem to affect solute diffusion across the membranes indicating that there was no concentration polarization near the membrane surface. As expected, thinner membranes gave higher solute fluxes although the membrane thickness could not be decreased below 100 μm to prevent membrane rupture. Despite the increase in solute diffusion across PVA/Pluronic® membranes, their mechanical properties were considerably inferior as shown by the low compressive and tensile moduli. Moreover, there were indications of Pluronic® leaching out from the membranes with time.

Solute fluxes across PeS and regenerated cellulose membranes were lower than across PVA membranes due to fouling caused by myoglobin. The extent of fouling was evaluated by measuring the decrease of myoglobin in the donor cell, and it was shown that the depletion of myoglobin in the donor cell was significantly higher using PeS and regenerated cellulose membranes than by using heat-treated PVA membranes. In fact, the decrease of myoglobin in the donor cell observed when using PVA membranes was mainly caused by myoglobin denaturation. It was also shown that PeS membranes exhibited higher extent of fouling than regenerated cellulose. The higher extent of fouling on PeS membranes was also confirmed by qualitatively mapping myoglobin fouling on PeS, regenerated cellulose, and PVA membranes using DRIFTS. The selectivities of separation of PeS and PVA membranes were found to be approximately the same, but the selectivities of

regenerated cellulose membranes were much lower than both PeS and PVA membranes. Permeability studies using pressurized system were carried out by connecting the donor cell to a variable pump to study the extent of fouling under flow conditions, and the studies showed that fouling was more severe on the membranes using the pressurized system than for the non-flow system.

The compressive and tensile moduli of asymmetric heat-treated PVA membranes swollen in deionized water for 1 hour were significantly lower than regular heat-treated PVA membranes under the same conditions. The selectivities of asymmetric heat-treated PVA membranes were approximately the same as the regular heat-treated PVA membranes, but the solute flux across the asymmetric membranes was not higher than across regular membranes as expected.

In conclusion, heat-treated PVA membranes were successfully fabricated to separate macromolecules from small molecular weight solutes. The heat-treated PVA membranes were shown to have good mechanical strength and selectivity and significantly higher fouling resistance than commercial PeS and regenerated cellulose membranes, which make them excellent candidates for use in the bioseparations industry.

Future Directions

Many laboratory studies are still required before the actual application of PVA membranes in larger scale bioseparations processes can be fully employed. Some important factors that have not been covered in this work include the measurement of water flux and salt rejection, which are common practices in the industry. Stirred cells can be used to

measure the water flux and salt rejections at the laboratory scale, and membrane performance can be assessed by evaluating the recovery of water flux and rejection coefficients.

The feed solutions used in this work only consist of one type of macromolecule and one type of small molecular weight solute. Feed solutions containing different proteins and microsolutes should be used to simulate the actual constituents of fermentation broths, and eventually feed solutions from fermentation broths should be employed to examine the performance of heat-treated PVA membranes under the actual conditions of use.

Most of the commercial membranes have backing supports to improve their mechanical integrity. Incorporating PVA membranes on top of porous supports via spin coating will permit the formation of thinner membranes with higher solute fluxes and better mechanical strength. The porous supports have to be able to withstand high temperature so that the supports can be heat-treated along with the thin layer of PVA coatings. The selectivities and solute fluxes of the composite membranes can be evaluated using both diffusion cells and stirred cells.

The asymmetric heat-treated PVA membranes prepared in this work did not give improved solute flux as expected. Addition of more water soluble polymers such as poly(ethylene glycol) is expected to increase the porosity on the surface of PVA membranes. It was also found that asymmetric PVA membranes did not have high mechanical strength, thus the incorporation of asymmetric PVA membranes on porous supports to impart better mechanical strength can also be evaluated.

Finally, microfiltration heat-treated PVA membranes can be prepared by laser etching. The microfiltration membranes will be able to retain cells and solid particulates but allow macromolecules, such as proteins and small molecular weight solutes to pass. The

proteins and small molecular weight solutes are passed through ultrafiltration membranes where they are further fractionated, and thus a series of microfiltration and ultrafiltration processes can be carried out simultaneously.

ACKNOWLEDGMENTS

I would like to give very special thanks to my advisor, Dr. Surya Mallapragada for her guidance and advice throughout my research and studies at Iowa State University. She has been very supportive in giving me directions and opportunities that are very useful toward my career goals. I feel very fortunate to have her as my advisor and mentor.

I personally thank Dr. Charles Glatz and Dr. Anthony Pometto for serving on my committee.

I also want to thank Dr. Steve Martin for letting me use the equipment in his laboratory and also his graduate student, Jeremy Schrooten for training and helping me with the dynamic mechanical analysis and diffuse reflectance infrared fourier transform spectroscopy measurements.

I greatly appreciate the help and ideas of undergraduate students and high school students who worked with me on this project: Ames, Saw-Choon, Merethe, Hanny, Chai-Kim, Sarah, Katherine, and Dana.

Special thanks also go to my family and friends who have been very supportive throughout my studies here at Iowa State University and to Dr. Mallapragada's group members: Anil, Meo-Ngui, Cheryl, Brian, Brandon, Greg, and Dave for their ideas, help, and friendship.

This project was funded by a grant from Center for Advanced Technology and Development.