ERASMUS MUNDUS MASTER IN
MEMBRANE ENGINEERING
FOR A SUSTAINABLE WORLD
EM3E-4SW

With the support of the
Erasmus+ Programme
of the European Union

MODIFICATION OF MEMBRANES SURFACES FOR BLOOD PROTEIN SEPARATION

Dissertation for obtaining the master's degree in Membrane Engineering.

Erasmus Mundus Master in Membrane Engineering for a Sustainable World

Sharath JAYAKUMAR

July 2021

University of Zaragoza, Spain

Adviser:	Christel CAUSSERAND	Professor, Universite Paul Sabatier Toulouse III, France
Co-Advisers:	Patrice BACCHIN	Professor, Universite Paul Sabatier Toulouse III, France
	Charaf MERZOUGUI	Ph.D, Universite Paul Sabatier Toulouse III, France
	Pierre ROBLIN	Dr., LGC, France
	Pierre AIMAR	Dr., CNRS, LGC, Universite Paul Sabatier Toulouse III, France
Overseer:	Reyes MALLADA	Professor, Universidad de Zaragoza, Spain

LABORATOIRE DE GÉNIE CHIMIQUE
TOULOUSE • UMR 5503

UNIVERSITÉ TOULOUSE III PAUL SABATIER
Université de Toulouse

Universidad Zaragoza
1542

www.em3e-4sw.eu
em3e-4sw-project@umontpellier.fr

The Erasmus Mundus Master in Membrane Engineering for a Sustainable Word (EM3E-4SW) is an education programme financed by the European Commission - Education, Audiovisual and Culture Executive Agency (EACEA), under Project Number-574441-EPP-1-2016-1-FR-EPPKA1-JMD-MOB. It is also supported by the European Membrane Society (EMS), the European Membrane House (EMH), and a large international network of industrial companies, research centers and universities.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to all my advisers for their guidance and for their knowledge that they have shared during the internship at Laboratory of Chemical Engineering (LGC) Toulouse. I thank all the LGC staff for their technical support given to me for performing experiments during my internship period. I consider it as a great privilege in being a part of the LGC.

I would like to thank all my professors and faculty members of the EM3E-4SW program and also my friends, classmates and my family for their constant encouragement and support that they have given me for successfully completing my master thesis.

CONTENTS

List of Tables and Figures

Tables

Figures

RESUMEN

La idea principal detrás de este proyecto es separar el factor de crecimiento derivado de plaquetas (PDGF) de las plaquetas, lo que tiene un papel prometedor que desempeñar para aplicaciones médicas como el tratamiento de úlceras diabéticas y quimioterapia. Las presentes técnicas utilizadas para separar PDGF consumen una gran cantidad de energía y el rendimiento también es bajo. Por tanto, un método alternativo para separar el PDGF de las plaquetas es mediante la separación por membrana. El objetivo principal de este proyecto es modificar la superficie de la membrana con un copolímero de bloque que puede capturar plaquetas de forma selectiva evitando interacciones no deseadas con otros componentes sanguíneos. El presente estudio se centrará en este primer paso de recubrimiento de la membrana y la evaluación de la adsorción no deseada de proteínas sanguíneas. Se variaron en varias condiciones de optimización, como el tiempo de recubrimiento, la concentración de copolímero, la longitud de la cadena y el proceso operativo, y el recubrimiento se analizó mediante mapeo FTIR. El tiempo de recubrimiento de 2 horas y la concentración de copolímero de 5 mg / mL después del proceso de inmersión, lavado, secado (IWD) fueron las condiciones óptimas. Se realizó una filtración sobre las membranas revestidas y se encontró que la permeabilidad disminuía con el aumento de tamaño del copolímero. Luego se realizó la filtración con solución de proteína y se calculó la concentración de proteína en el retenido y el permeado seguido del% de retención. El mapeo FTIR se realizó en la membrana de filtración para comprobar si se había eliminado el revestimiento de la superficie de la membrana. A partir del mapeo quedó claro que el revestimiento estaba presente en la superficie de la membrana incluso después de la filtración. Para estudios futuros, se recomienda utilizar otras proteínas sanguíneas como globulina y fibrinógeno en la adhesión con el copolímero. Se pueden utilizar otras herramientas de la membrana recubierta como SEM y AFM para analizar la morfología y la rugosidad de la superficie de la membrana recubierta, respectivamente.

ABSTRACT

The main idea behind this project is to separate platelet derived growth factor (PDGF) from platelets which has a promising role to play for medical applications such as treating of diabetic ulcers and chemotherapy. The present techniques used to separate PDGF consumes large amount of energy, and the yield is also poor. Hence an alternate method of separating PDGF from platelets is by membrane separation. The main objective of this project is to modify the membrane surface with a block copolymer that can selectively capture platelets by avoiding unwanted interactions with other blood components. The present study will focus on this first step of coating the membrane and evaluation of unwanted adsorption of blood proteins. Various optimization conditions like coating time, concentration of copolymer, chain length and operating process were varied, and the coating was analyzed using FTIR mapping. Coating time of 2 hours and the copolymer concentration of 5 mg/mL following the immersion, washing, drying (IWD) process were the optimum conditions. Filtration was done over the coated membranes and the permeability was found to decrease with increase in size of the copolymer. Then filtration was done with protein solution and the concentration of protein in retentate and permeate was calculated followed by retention %. FTIR mapping was done on filtration membrane to check if there was removal of coating from the membrane surface. From the mapping it was clear that the coating was present on the membrane surface even after filtration. For future studies, it is recommended to use other blood proteins like globulin and fibrinogen on the adhesion with the copolymer. Other characterization tools like SEM and AFM can be used to analyze the morphology and surface roughness of the coated membrane, respectively.

1. INTRODUCTION

Blood is a body fluid that contains cells which are suspended in plasma. It is the most easily accessible tissue in the body. The human blood comprises of 45% erythrocytes (red blood), leukocytes (white blood cells), thrombocytes (platelets) and 55% plasma. The most important function of the blood is to transport oxygen and nutrients to different parts of the body and remove waste such as carbon dioxide.[1] Erythrocytes contain hemoglobin and the main function is to transport oxygen throughout the body while the leukocytes are a part of the body's immune system. It attacks and destroys old cells and act against infectious agents and foreign substances. The Platelets help in blood clotting and in the wound healing process.[2] Platelets contain growth factors such as platelet derived growth factors (PDGF) which are separated from platelets upon activation. and have numerous medical applications. PDGF responds to injury and helps in the wound healing process. Transfusion of PDGF is highly necessary for patients undergoing treatment for diabetes, chemotherapy. Blood cells possess many scientific and medical applications because they serve to treat various problems in human body like hypertension, diabetes, chemotherapy, wound healing.[3, 4]

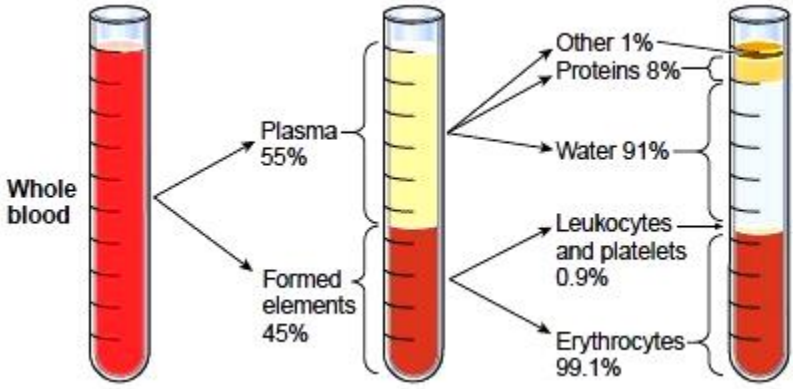

Figure 1.1 Constituents of blood [5]

In order to use the blood cells for above mentioned applications, we need to separate the blood cells from plasma. The separation of plasma can be done by several techniques. One such technique is centrifugation which makes use of centrifugal force for separating plasma. The drawback of centrifugation technique is that it consumes large amount of time and requires more

labor. Adding to it, if centrifugation is not done in proper conditions, it can lead to hemolysis of red blood cells (RBC) resulting in contamination of plasma.[6] Another method by which blood cells can be separated is flow Cytometry. The method was effective, but it consumed a lot of time and required expensive instrumentation and skilled personnel which made them less desirable.[7] Other technique is Filtration that involves a membrane. The pore size of the membrane is between 0.2 and 0.8 μm. Hence the blood cells are removed as they do not pass the membrane while the plasma passes through the membrane.[8] The advantage of using filtration is that it requires low fabrication cost and helps in easy integration.[9] Hemodialysis is a method of purifying the blood by using membranes. The dialysate side will have lower toxin concentration and the other side will have higher toxin concentration. The concentration gradient plays an important role in the dialysis process of blood purification.[10] Hence membrane based separation has proven to be the most preferred technique for separating blood components. The process can be improved by modifying the surface of the membrane by introducing functional groups that can interact or prevent interaction with the targeted molecule.

The objective of this project is to modify the surface of the membrane with a block copolymer that will help in selectively capturing platelets and leaving out other blood components. The copolymer is conjugated with a blood protein that will help in capturing platelets and followed by its activation and separating Platelet Derived Growth Factor (PDGF). Optimization of the copolymer coating on the membrane surface will be carried out using various block copolymers such as polystyrene-block-polyacrylic acid (PS-b-PAA), polystyrene-block-polyethylene oxide (PS-b-PEO) and investigate its interaction with blood proteins. Hence this report aims to propose an optimum coating conditions on the hydrophobic polyvinylidene fluoride (PVDF) membrane by characterizing the membrane using Attenuated Total Reflectance (ATR) - Fourier Transform Infrared (FTIR) spectroscopy and contact angle meter. The effect of coating on the membrane permeability was analyzed by performing filtration and static adhesion of blood protein on the membrane was investigated.

2. THEORETICAL BACKGROUND

2.1 Separation of Platelets using membranes.

Platelet separation and transfusion has important applications in the field of health care and medicine. They are a part of the physiological processes such as wound healing, chemotherapy, recovery of bones and soft tissues.[11, 12] Platelet Derived Growth Factor (PDGF) is a major mitogen for fibroblasts, smooth muscle cells and other type of cells. PDGF can be obtained from platelets which act as the major storage site.[13] PDGF plays a very important role in the wound healing process, embryonal development and regulate the interstitial fluid pressure.[14]

There are several ways by which PDGF can be extracted and purified from platelets. One such method of extracting PDGF is centrifugation which makes use of gravitational force. It results in settling of different fractions of blood components. The top layer will be plasma, the in-between layer will be leukocytes and platelets and the bottom layer will be erythrocytes. The process was found to be fast but consumed large amount of energy. In addition, the separation would be ineffective without the use of special equipment as the separation is based on size and density difference.[15] Hence there is a need for an alternate process to separate platelets from other blood components. This is possible with a help of modified porous membranes that can selectively capture platelets.

The most commonly used membranes for separation of blood components are hydrophobic polymer membranes such as polyvinylidene fluoride (PVDF) membranes as it possesses high mechanical strength, thermal stability and chemical resistence when compared to other polymers.[16] Since the process involves biological compounds such as blood proteins, there is a problem of membrane fouling caused by the hydrophobic nature of the PVDF membranes.[17]

The platelets cannot directly interact with the polymeric functional groups. Hence a material needs to be introduced that will have selective interaction with the platelets. One such material is the blood protein which is present within the human body. The interaction between polymeric surface and blood protein are widely discussed as it helps in platelet adhesion and it has applications in the area of health care engineering.[18] The proteins help in faster adhesion of platelets at a site of

an injury and leads to formation of blood clot and prevents the loss of blood. The human blood proteins like Albumin, Globulin and Fibrinogen are the most common blood proteins that play a major role in adhesion and activation of blood components. Among them, fibrinogen is said to be an adhesive protein that will regulate or modulate the adhesive response of platelets.[19] Hence by developing a membrane modified with protein on the surface will help in adhesion of platelets on its surface and further activate and release PDGF.

2.2 Modification of membrane surface

The membrane surface can be modified by coating, blending, composite materials, chemical reaction, grafting and a combination of various techniques. The most important criteria in surface modification are to check the flux after modification, simplicity of the process, reproducibility, environmental aspects, and cost effectiveness. In Blending method, two or more polymers are subjected to physical mixing to obtain the required properties. The problem with this technique is the mechanical strength of the coating which was found to be low. Hence it was less preferred method for membrane modification. In Chemical method, the membrane material is treated with modifying agents to introduce various functional groups on the membrane surface. The main drawback of chemical treatment is that the modifying agent was found to block the pores of the membrane. This resulted in increased reduction of the total flux of the membrane compared to that of the flux before coating.[20] Grafting is a technique where the monomers are covalently bonded onto the membranes. The stability of the membrane was found to be higher due to the strong interactions. In grafting, the monomer solution was made to come in contact with the membrane and polymer grows when additional energy is supplied to initiate the polymerization reaction. The drawback of this process is that it requires time consuming multiple steps, decrease in permeability during filtration and additional energy need to be supplied that increases the cost.[21]

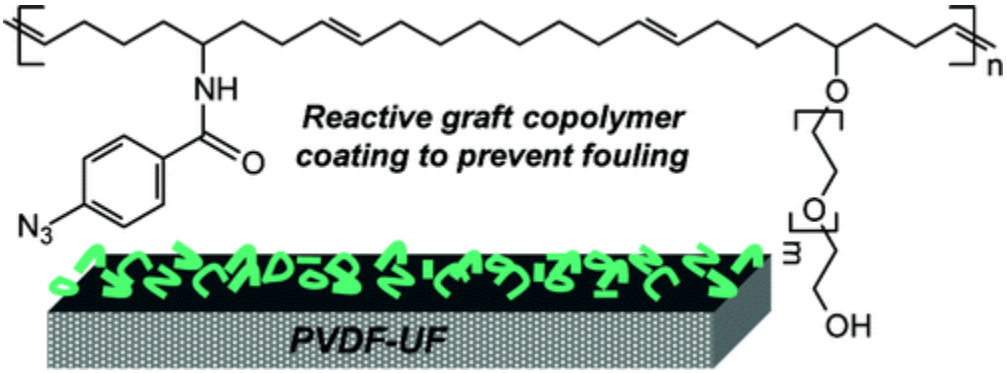

Figure 2.1: Grafting Poly(cyclooctene) with PEO on PVDF membrane for ultrafiltration[22]

On the other hand, Coating is a technique where a thin layer of the coating material is non covalently adhered to the membrane surface. Coating can be done through immersion, dip coating, spray coating and spin coating. This technique showed increased flux after coating and reduced fouling.[23] The stability of the modified surface depends on the type of interaction between the copolymer and the membrane surface. If the interaction is noncovalent, the stability can be low leading to detachment of the copolymer. Hence the interactions would play a major role in obtaining a proper coating of the copolymer.

2.3 Polymers used in membrane modification.

Various polymers like polyamide, polyurethane, polysaccharides are being used for blood purification and other medical applications. The polymer material when comes in contact with a blood protein for platelet adhesion and its activation, there is an important criteria to be considered which is biofouling. Hence the primary target of the polymer material is to construct a non-fouling surface. Polyethylene glycol (PEG) is one such material that has been identified as a non-fouling and non-thrombogenic material.[24, 25] The characteristic property of the PEG chain is that it can bind water molecules by the formation of hydrogen bonds and it forms a barrier in addition to the steric repulsion around the PEG chains,[26] and this helps the PEG modified surface to resist unwanted biomacromolecules like bacteria.[27] In order to ensure the stability of the membrane, the PEG chain is attached to a hydrophobic group forming a block copolymer and thereby increase membrane stability. One such copolymer is polystyrene (PS) and polyethylene glycol methacrylate (PEGMA) block copolymer coated over PVDF membrane. It was found that the PS-PEGMA coated membrane was highly hemocompatible. The hemocompatibility tests revealed that there

was no cell adhesion including platelets, erythrocytes, and leukocytes making the membrane possible for blood filtration. The resistence of fouling depends on the amount of copolymer blocks that maintains the hydrophobic-hydrophilic ratio that was coated on the membrane surface.[28, 29]

Certain polymers like poly-N-isopropylacrylamide (PNIPAM) can respond strongly to large physical conformational changes. PNIPAM is a thermo-responsive polymer that undergoes a sharp hydrophilic-hydrophobic transition in aqueous media at 32°C which is a low critical solution temperature (LCST).[30] When the PNIPAM chain is attached to another polymer to form a block copolymer, they have a wide range of biomedical applications due to their thermo-sensitive properties. They can adsorb blood proteins on the membrane surface for adhesion of platelets, and are also used as environmental friendly anti-microbial coatings.[31]

Few studies have suggested that polymers like Polyacrylic acid (PAA) helps in better adsorption of protein on its surface. Certain properties like pH need to be adjusted accordingly so that protein binds on the copolymer surface. A study showed that the polymer of low adsorption pH 3 had better adsorption capacity than the polymer of pH 5. This is because the low adsorption pH had the ability to generate high density -COOH groups that function on ion exchange sites and immobilizes the metal-ion complex that selectively bind the tagged protein.[32] Another study was done on Polyether Sulphone (PES) membranes that was blended with Polyacrylonitrile/acrylic acid (PAN/AA) copolymer. Here the copolymer coated membrane was grafted with bovine serum albumin (BSA) on its surface. This was made possible due to the interaction of amino group of the BSA and the carboxyl group of the polymer. It was noticed that after modification, the protein adsorption and platelet adhesion was low, and the contact angle decreased, and the water flux increased. These results suggested that PES modified membranes had improved the hydrophilicity and showed better blood compatibility.[33]

2.4 Properties affecting membrane fouling.

The mechanism of fouling occurring on the membrane can be due to the interaction forces between the colloidal particle and the membrane surface. The colloidal particles in protein and blood cells are usually charged and the aggregation or deposition can be prevented by the repulsive forces. A surface can be charged in water by two mechanisms, first is by the ionization or dissociation of surface groups that leads to the charging of the surface and the second mechanism is by the

adsorption of ions from solutions onto a previously uncharged surface. This leads to the formation of an electrical double layer with a charged surface and counterions in the aqueous solution.[34] Another force that could probably affect fouling are the hydrophobic forces. A hydrophobic surface has no polar or ionic group, hence water molecules cannot bind to the surface. Since the hydrogen bond of water molecule cannot come in contact with a hydrophobic surface, the water molecules are expelled into the bulk and the total free energy of the system is reduced. Hence the hydrophobic surface will form a barrier that will prevent the growth of clusters.[35]

One of the important surface characteristic that influences fouling is the surface roughness. Studies have suggested that hydrophobic membranes are more prone to fouling. It can be because of the low shear rate of the rough structure and also due to the acid base interactions of the polymeric membranes. The surface roughness of the membrane will provide greater surface area for the foulants to attach to the surface of the membrane ultimately leading to faster fouling rates.[36] Another parameter that influences the fouling in membranes is the surface charge. Most of the foulants carry a charge and the electrostatic charge of the membrane need to be considered to reduce fouling. When the surface and the foulant carry the same charge, electrostatic repulsion between the foulant and the membrane will prevent the colloidal and macromolecular deposition over the membrane thereby reducing fouling.[37] Hence the main goal in the membrane surface modification is to obtain a surface with minimum undesired interactions between the particles such that it prevents or reduces fouling, and these goals can be achieved by controlled modification techniques.

2.5 Surface Characterization of Membranes

2.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) Spectroscopy is a specialized tool to characterize the surface of the membranes to analyze the chemical groups that have been used to modify the membrane surface. The peaks that are obtained from the spectrum gives information about the stretching or deformation of the chemical bonds that corresponds to various functional groups of the modified samples.[38] The sample preparation was simple and very thin samples can also be analyzed with the help of this tool. However, the samples that are used for FTIR must be completely dried before the experiment is performed.

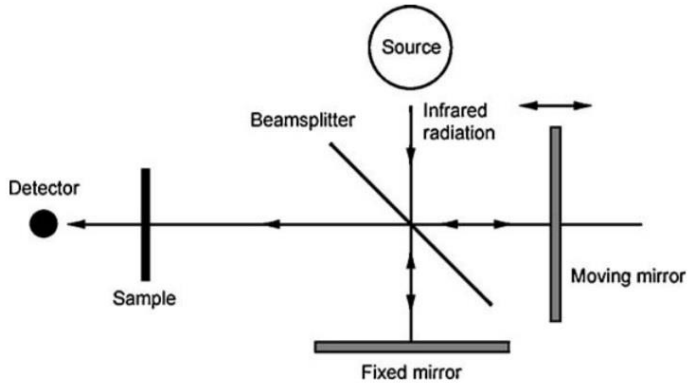

Figure 2. 2: Working of FTIR [39]

Attenuated Total Reflectance Spectroscopy (ATR) is used to analyze samples without complex sample preparation. In ATR sampling, an infrared beam is directed into a crystal of high refractive index. The infrared beam reflects from the internal surface of the crystal and creates a wave that is projected towards the sample which is in contact with the crystal. Some energy from the wave is adsorbed by the sample and the rest is transferred to the detector.[40] The detection and quantification of certain functional groups can be done by combining FTIR with ATR and it was found to be a high quality analysis tool for determining the chemical groups of complex samples. The mapping done on the membrane samples with a step size of 100x100 µm would provide information about area where there is more intensity of the coating on the sample.

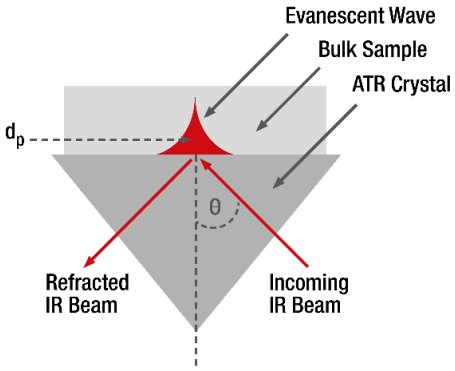

Figure 2. 3: Working of ATR [41]

Another extension of FTIR technique is FTIR microspectrometry. Here the infrared interferometer is coupled with a microscope that has specialized detectors to scan the surface of the sample. This method also provides information about the extent of fouling on the sample surface and detect various foulants such as proteins, polysaccharides, and various other inorganic species. This tool has found applications in biomedical, biomaterial and tissue studies.[42]

2.5.2 Contact Angle Meter

The other important characterization tool for membranes is Contact angle meter. The contact angle measurement will provide information about the hydrophobicity and hydrophilicity of the membrane surface. The hydrophilicity/hydrophobicity characteristics of the membrane surface will help in analyzing the surface wettability of liquids and govern the membrane performance in various applications.[43] The degree of wetting on a membrane surface can be determined by the properties of the material that is coated on the membrane surface that governs the interaction with water molecules and influenced by liquid pH, temperature, charge density and interfacial interaction energy.[44] The contact angle of a water droplet depends on the relative magnitude of the interfacial tension at three interfaces involved and this can be explained with the help of Young Equation:

$$\cos\theta_C = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}} \qquad \text{Equation 1}$$

Where γ_{SG}, γ_{SL}, γ_{LG} are the interfacial tensions for the solid-gas, solid-liquid, and liquid-gas boundaries, respectively.

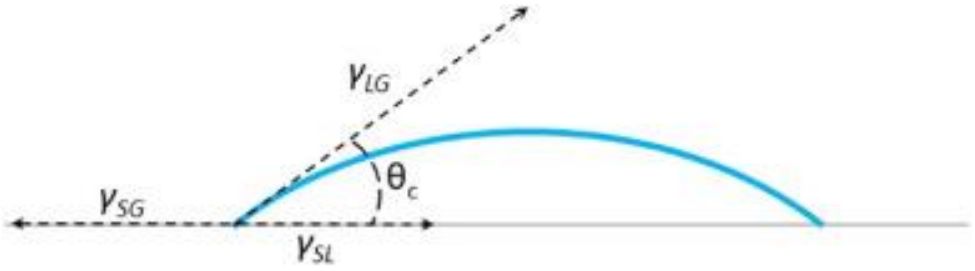

Figure 2. 4: Illustration of contact angle over a flat surface[45]

A study was done on investigating the wettability of hydrophobic membranes using contact angle analysis for pure water and aqueous solutions of alcohols that will complement the liquid entry

pressure tests. It was noted that when water was used to measure the contact angle of the membrane, the value of contact angle obtained was independent of the pore size of the membrane. It was also noted that roughness of the membrane surface increased the contact angle.[46]

2.6 Aim of the present project

In this project, we are mainly focused on modifying the hydrophobic PVDF membrane with a block copolymer. The block copolymers will possess functional groups that has minimum adhesion of unwanted blood components and only selectively capture platelets. The copolymers used in our study are Polystyrene-polyacrylic acid (PS-b-PAA) and Polystyrene-polyethylene oxide (PS-b-PEO). The copolymers are coated over the membrane surface and various optimization conditions are checked to analyze the effect of coating on the membrane surface. Then static adsorption of Human serum albumin (HSA), a protein which is present in the blood was analyzed. Filtration was done with modified membrane to determine the permeability and followed by filtration with HSA solution and the concentration of permeate and retentate was analyzed and the retention of HSA was calculated.

3. EXPERIMENTAL SECTION

3.1 Materials

The membranes used in our experiments were commercial polyvinylidene fluoride (PVDF) hydrophobic microporous membranes (VVHP, Millipore Co.) with an average pore size of 0.1 μm and thickness of 125 μm. A membrane area of 0.5cm^2 was used for performing experiments on checking various optimization conditions. The copolymers were purchased from Sigma Aldrich. The solvents that were used in our experiments to solubilize the copolymer samples were Tetrahydrofuran (THF) and Absolute Ethanol which were purchased from Acros Organics and VWR Chemicals Avantor, respectively. Phosphate Buffer Saline (PBS) solution 10X was obtained from Fischer Bioreagents and was diluted to PBS 1X using ultrapure water. Ultrapure water was purified using ELGA PURELAB classic water purification system with a final minimum resistivity of 18 MΩcm. The blood protein used in our experiments was Human Serum Albumin (HSA) which was purchased from Sigma Aldrich.

3.2 Methods

3.2.1 Coating of Membranes

The modification of the membrane surface was carried out by coating the hydrophobic membrane with a copolymer. The procedure was done by immersion technique where the membrane was immersed in a copolymer solution. The copolymer solution was initially prepared by dissolving the required amount of copolymer in a solvent containing 50% (v/v) THF/Ethanol. The copolymer was completely dissolved in the solvent with the help of a Ultrasonicator.

After the preparation of the copolymer solution, the hydrophobic PVDF membrane was cut for the required size and immersed in the copolymer solution of 1mL taken in Eppendorf tubes. A membrane sample with surface area of 0.5 cm^2 was immersed in 1 mL of solvent without copolymer solution and was used as a reference for comparative analysis of the results. The drying time of the coated membranes was maintained for 2 hours at 40°C and membrane washing was done by rinsing the coated membrane in 1 mL of PBS solution. The primary reason for washing the membrane is to remove the non-adsorbed copolymer from its surface. The optimization conditions of the copolymer coating were done by changing a parameter such as the concentration of the copolymer solution, coating time, change in process and change in chain length of the copolymer.

The concentration of the copolymer solution was varied by 1, 5, 10 mg/mL and a coating time of 2 hours was maintained. For the change in process conditions, the process was carried out either by immersion, washing, drying (IWD) or immersion, drying (ID) process to test the effect of washing on the copolymer coating. In the initial experiments, the coating time for the copolymer was varied by 2, 4, 6 hours. It was found that 2 hours of coating time was sufficient to coat the membrane and hence a coating time of 2 hours was fixed constant for further experiments. The other optimization condition was to check the copolymer samples of different chain length. Various copolymers such as PS-b-PAA, PS-b-PEO with different length of the hydrophilic and hydrophobic group of the block copolymer were tested at 5 mg/mL concentration and a coating time of 2 hours. This experiment will provide information about the effect of chain length on the stability of coating over the membrane surface.

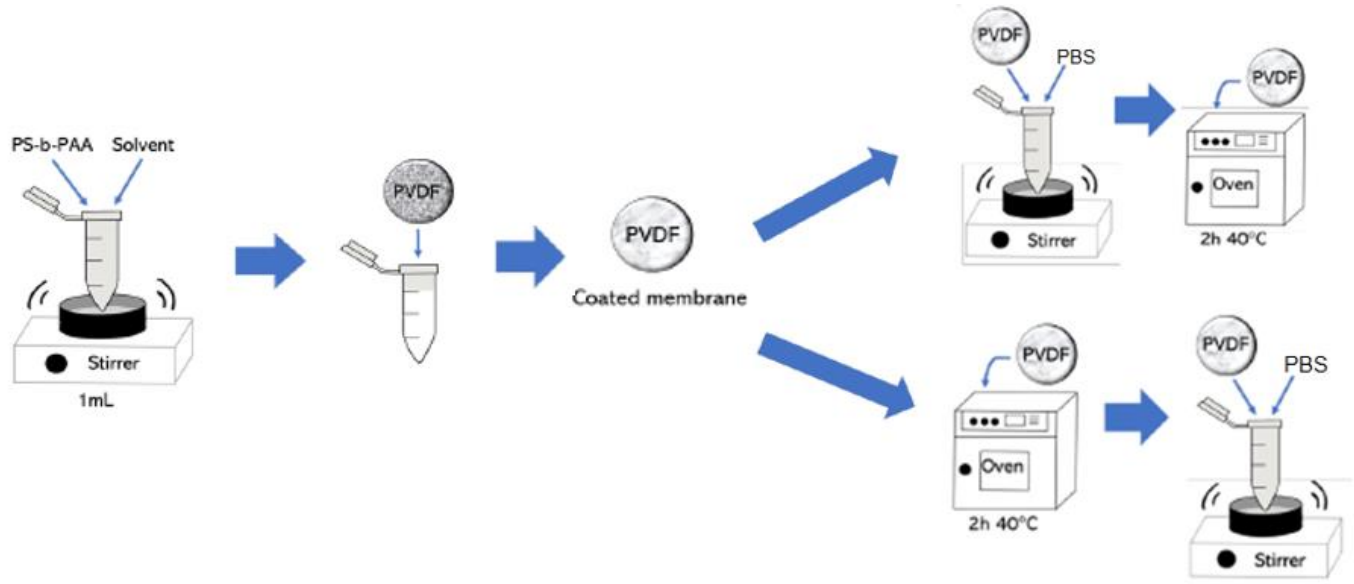

Figure 3. 1: Representation of coating procedure for the PVDF membrane

3.2.2 Static Adsorption of protein

In order to perform the experiments for static protein adsorption on the membrane surface, the protein solution was initially prepared by dissolving 1 mg of the desired protein with 1 mL of PBS solution and it was refrigerated overnight. The membrane was modified based on the optimization conditions as mentioned previously. After modification, the membrane was immersed in 1 mL of PBS solution overnight. This helps in hydrating the copolymer coated over the membrane surface. Afterwards, the PBS solution was replaced with 1 mL of 1 mg/mL protein solution that was prepared earlier. The membrane was immersed in protein solution for 2 hours at the room temperature and then removed from the solution and subsequently washed with PBS solution. By washing the membrane, the non-adsorbed protein was removed from the surface of the membrane and then the membrane was dried in the oven for 2 hours at 35°C.

3.2.3 Characterization

Attenuated Total Reflectance (ATR) spectroscopy (Nicolet 6700, Thermo Scientific) with diamond crystal at 45° incident angle, 16 scans with 4 cm^{-1} spectral resolution was used to obtain the spectra and verify the presence of coating and protein on the membrane surface. Initially the sample holder was cleaned with ethanol and background spectrum was collected to ensure there

was no disturbance in the spectra due to the presence of impurities. After obtaining the background spectrum, the membrane was placed over the sample holder and was tightened to the point where it was in contact with the diamond crystal. Then the sample spectrum was collected. The spectrum was obtained for a wavenumber range of 4000 to 400 cm^{-1}.

ATR-FTIR (iN10 Infrared Microscope, Thermo Scientific) with germanium crystal, 25° incident angle, 8cm^{-1} spectral resolution and 16 scans for each point was used for surface chemical mapping to determine the coverage of the coating and the protein adsorption. 40x40 points were measured and the size of scan was maintained at 100x100 μm. The tool had to be filled with liquid nitrogen to maintain the low temperature for the working of the detector. The ATR tip was cleaned with ethanol to ensure there was no impurities. The sample was prepared by fixing the membrane to the glass slide with an adhesive tape and ensured that the surface was flat to obtain proper results. The glass slide was fixed to the sample holder. The focus was adjusted such that the light falls on the center point of the membrane. The sample was set at the required position and the ATR tip was fixed to the microscope to perform mapping. In order to analyze the spectrum obtained from mapping, peaks that confirm the presence of coating and proteins were identified. Peaks with the highest absorbance and that corresponds to the material of interest was chosen for chemical mapping. After mapping, baseline correction was done to obtain an ideal baseline. The chemical maps generated are color coded from blue to red based on the intensity of the peak.

Contact Angle meter was used to determine the hydrophobicity of the membrane before and after coating. Initialization of the device was carried out by checking the base level of the contact angle meter to ensure that the sample holder was flat. The method used to determine the contact angle of the sample was by deposit drop method. The microscope attached to the device was tilted downwards between 0° and 1° at a position between 5 and 6 mm. The syringe used for measuring the contact angle was SY20 (28944) that was specifically used for deposit drop method. The syringe was cleaned with pure water twice to ensure removal of impurities from the syringe. Then the syringe was pressed gently to remove air gap. The substance that was used to measure contact angle was water and the phase environment was set to air. The sample was prepared in such a way that the membrane was fixed tightly to the glass plate with an adhesive tape and ensured that the membrane was flat to prevent errors during measurement of contact angle. Then the glass plate

was placed on the sample holder. The light was kept at high intensity to obtain a clear image of the measurement. The magnification of the microscope was set to 2.5X to obtain a precise image of the measurement. The adjustment knobs were used to position the sample at the center of the screen and the baseline was corrected. Now the measurement was taken by bringing the needle closer to the sample and running the procedure. Once the water droplet falls on the sample, the syringe is moved away, and the measurement was saved. In such a way, five measurements were taken for each sample The same procedure was followed for different samples.

3.2.4 Filtration Experiments

The main reason for performing filtration is to determine the permeability of the coated membrane and compare the results with pristine membrane followed by filtration using protein solution to determine the concentration of retentate and permeate followed by calculation of retention. Before the mentioned experiments are performed, the membrane needs to be compacted. The primary reason to perform membrane compaction is to maintain steady transport characteristics while applying pressure over the membrane. Since the membrane used in our experiments are hydrophobic PVDF membranes, they need to be wetted before performing the experiments. This can be done by passing 1 mL of ethanol into the cell fitted with membrane and removing it after one minute. In order to perform compaction, dead end filtration was conducted with the membrane using an Amicon cell (Series 8010, Merck Millipore) and ultrapure water. The pressure was set at 0.2 bars and the flux was measured and then the pressure was increased by 0.2 bar until 1 bar. At 1 bar, the flux was noted until it reaches steady state. Corresponding to the total compaction of membrane structure, the pressure was decreased in the same order and the permeability was determined from the curve for Flux vs Pressure.

After determining the permeability of the pristine membrane, the membrane was coated with copolymer at 5 mg/mL concentration for 2 hours and the filtration was performed using ultrapure water by varying the pressure for 0.2 bar to 1 bar. The weight of the permeate was collected for every 5 minutes until 20 minutes for each pressure. The flux J was calculated using the membrane area of 3.8 cm^2. Flux vs Pressure curve was plotted. Using Darcy's Law and the slope of the curve, the permeability was determined.

$$J = \frac{L_p}{\mu} \Delta P \qquad \text{Equation 3.1}$$

Where J is the water flux (m^3/m^2s), L_p is the permeability (m), μ is the viscosity of the fluid (Pa s) and ΔP is the transmembrane pressure (Pa).

After determining the permeability of the coated membrane, the protein (HSA) filtration was done to determine the concentration of permeate and retentate and calculate the retention. This experiment was carried out by filling the Amicon cell with 10 mL of protein solution of 1 g/L and pressure was set at 1 bar and the weight of the permeate was noted down for every 1 minute. The permeate and retentate were collected and the absorbance of the sample was noted at 280 nm by using UV-vis spectrophotometer. The absorbance value was noted down and the concentration of the permeate and retentate were calculated from the slope value of the calibration curve with absorbance of HSA at known concentrations. Then the retention was calculated using the following equation,

$$Retention\ (R) = 1 - \frac{C_P}{C_R} \qquad \text{Equation 3.2}$$

where C_P and C_R are the concentration of permeate and retentate, respectively.

4. RESULTS AND DISCUSSIONS

This section will include results of the experiments that were performed as part of the optimization of the copolymer coating that was done over the PVDF membrane. It was followed by the static adsorption of protein over the membrane surface that evaluates the capturing of platelets. The hydrophobicity of the membranes was determined using contact angle meter. The results of the filtration experiments over the modified membranes were explained and various parameters like membrane flux, permeability of the coated membrane, evaluation of the concentration of protein in the permeate and retentate were discussed followed by calculation of retention %.

4.1 Optimization of the coating process

The results obtained from optimization conditions include change in coating time, change in process conditions, change in concentration, and change in copolymer chain length which are discussed below. Before starting the optimization of the coating over the membrane surface, the ATR spectra of pristine PVDF membrane and the copolymer samples were compared to differentiate the peaks of the membrane and the copolymer as shown in figure 4.1 below. The peaks corresponding to various functional groups of the copolymer is summarized in table 4.1.

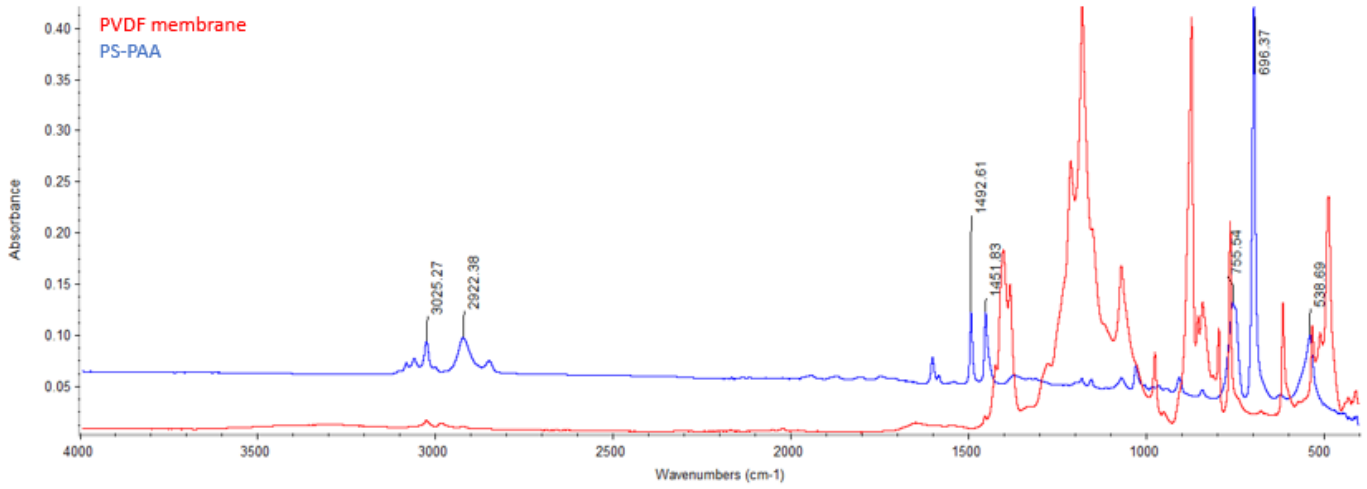

Figure 4.1 ATR spectrum of pristine membrane and copolymer sample in powder form

Table 4.1: Absorption of functional groups in PS-PAA copolymer

Wavenumber (cm^{-1})	**Functional groups**
3105-3000	=C-H stretching in PS
3000-2850	-C-H stretching in polymer backbone
1600,1500,1450	C=C stretching of aromatic in PS
700	-C-H deformation of monosubstituted aromatic in PS

The hydrophilic part of the copolymer PAA should have absorption at 1770 cm^{-1} for -C=O stretching. However, the peaks were not clearly visible at all times. This can be due to the small length of PAA chain when compared to the PS chain. Hence the absorption peak at 700 cm^{-1} was

chosen to analyze the presence of copolymer over the membrane surface as it had strong absorbance and it did not overlap with the peaks of the membrane material (PVDF).

4.1.1 Variation of coating time

The membrane samples were prepared by varying the time of immersion in the copolymer solution. The membranes were immersed in a copolymer solution of $PS_{30}PAA_8$ for 2, 4, 6 hours and the samples were prepared for mapping as mentioned previously. By increasing the coating time, the amount of copolymer coated over the membrane surface was found to increase. This is because the copolymers were given enough time to self-assemble over the membrane surface. From figure 4.2, we were able to see that with 2 hours of coating time, the copolymer was coated over the membrane surface. Similar results were observed with $PS_{30}PAA_5$ coated membrane. Hence for further experiments, the coating time of 2 hours was kept constant for performing the experiments.

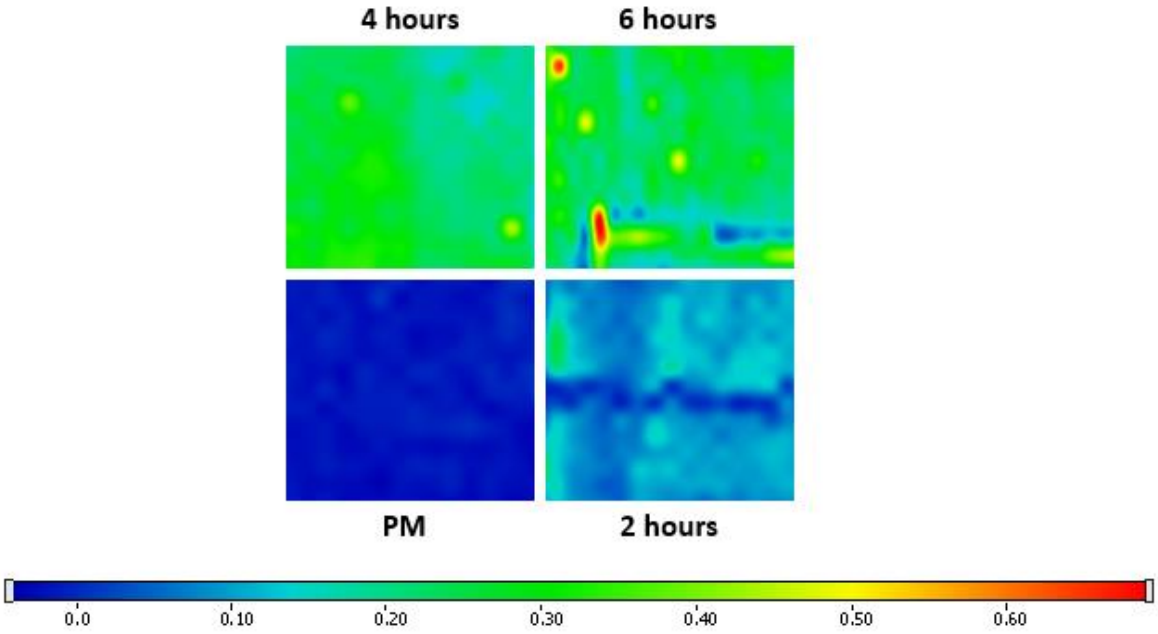

Figure 4.2: FTIR mapping of $PS_{30}PAA_8$ coated membrane at 700 cm^{-1} with change in coating time

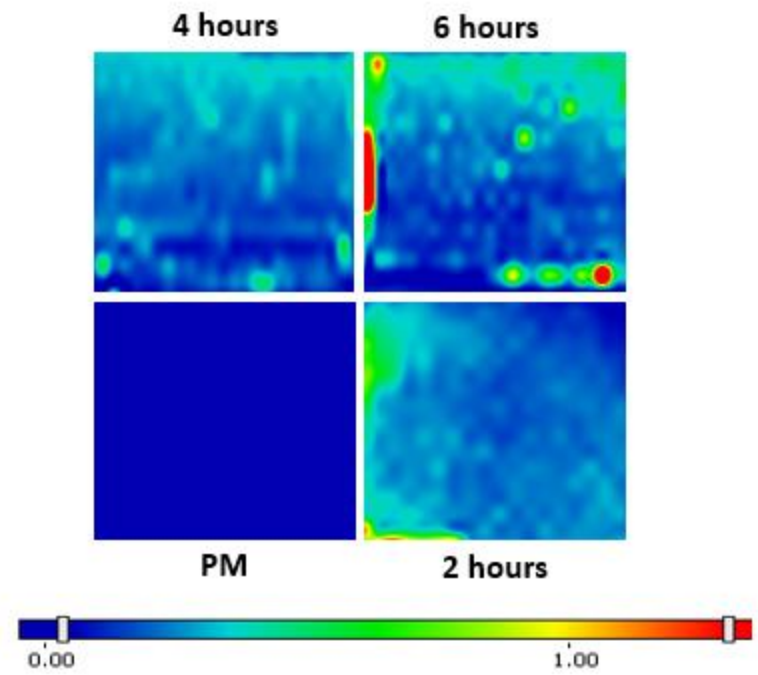

Figure 4.3: FTIR mapping of $PS_{30}PAA_5$ coated membrane at 700 cm^{-1} with change in coating time

4.1.2 Variation of operating process

The main reason for checking the various operating process is to analyze the effect of washing on the copolymer coated over the membrane surface. Three operating process were tested in the following order as Immersion, Washing, Drawing (IWD); Immersion, Drying, washing (IDW); Immersion, Drying (ID). From the figure 4.4, it is clear that the copolymer has adhered to the membrane surface and the intensity of coating on the membrane with ID process was higher comparing to the IDW and IWD process. The ID process ensures optimum coating of the copolymer over the membrane surface. But there are chances for the copolymer to block the pores of the membrane which might be a drawback during filtration. The IDW process consumes more time for optimization as it involves 6 hours for preparing the sample for mapping. In IWD process, we were able to obtain considerable amount of coating on the membrane surface and by washing and then drying, the non-adsorbed copolymer was removed from the membrane surface. Hence IWD process was chosen as the appropriate operating process in coating the copolymer over the membrane surface.

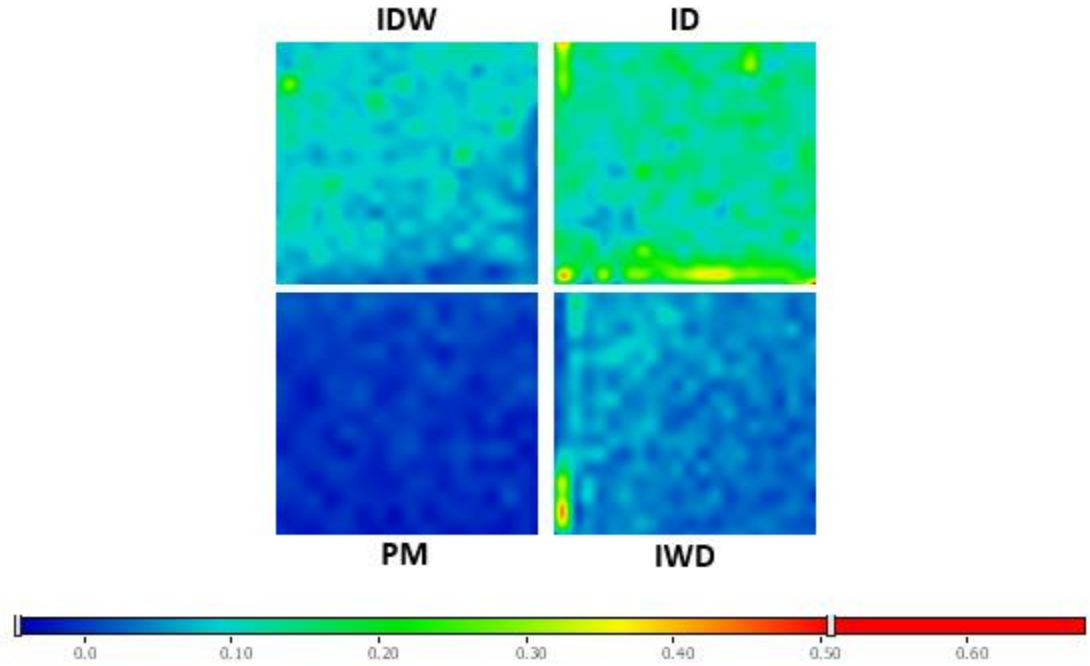

Figure 4.4: FTIR mapping for $PS_{30}PAA_8$ coated membrane at 700 cm^{-1} with different operating process

4.1.3 Variation of copolymer concentration

The concentration of the copolymers coated over the membrane surface was varied for 1, 5, 10 mg/mL. The main reason for changing the concentration of the copolymer is to obtain a homogenous coating over the membrane surface. From figure 4.5, it is clear that with the increase in concentration of $PS_{100}PAA_{107}$, the intensity of copolymer coating over the membrane surface also increases. For 1 mg/mL, the amount of coating on the membrane surface was found to be very low and for 10 mg/mL, the amount of coating on the membrane surface was in excess. Hence the intermediate concentration of 5 mg/mL was chosen as the ideal concentration of the copolymer coated over the membrane surface and the same concentration was maintained in membrane coating for filtration experiments. Contact angle was determined from the membrane with the help of a contact angle meter, and we were able to notice that for higher concentration of 10 mg/mL, the contact angle decreased slightly comparing to other concentration because the absorbance of

hydrophilic group PAA was higher at high concentration. Similar results of FTIR mapping were obtained from membrane coated with $PS_{28}PEO_{13}$ as shown in figure 4.6.

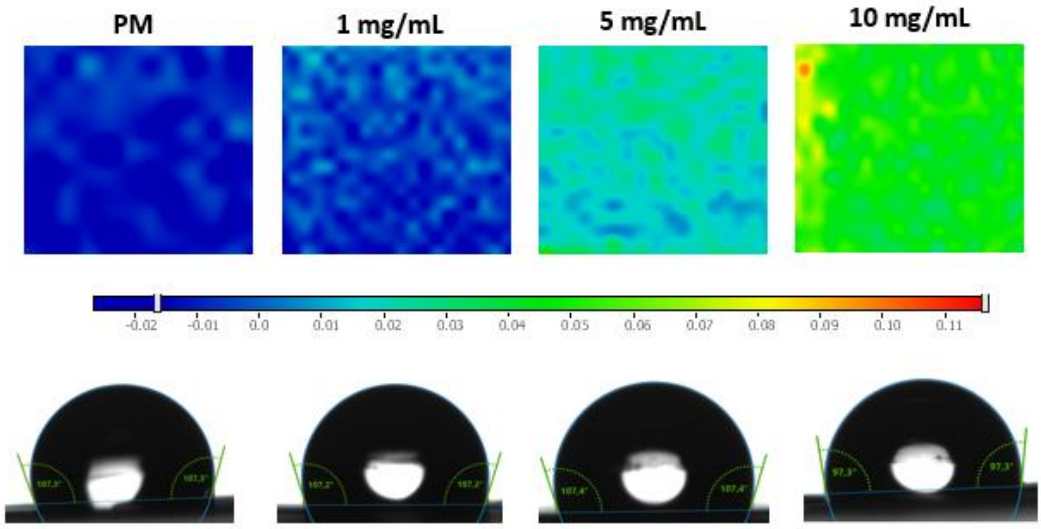

Figure 4.5: FTIR mapping for $PS_{100}PAA_{107}$ coated membrane at 700 cm^{-1} for change in concentration with contact angle measurements.

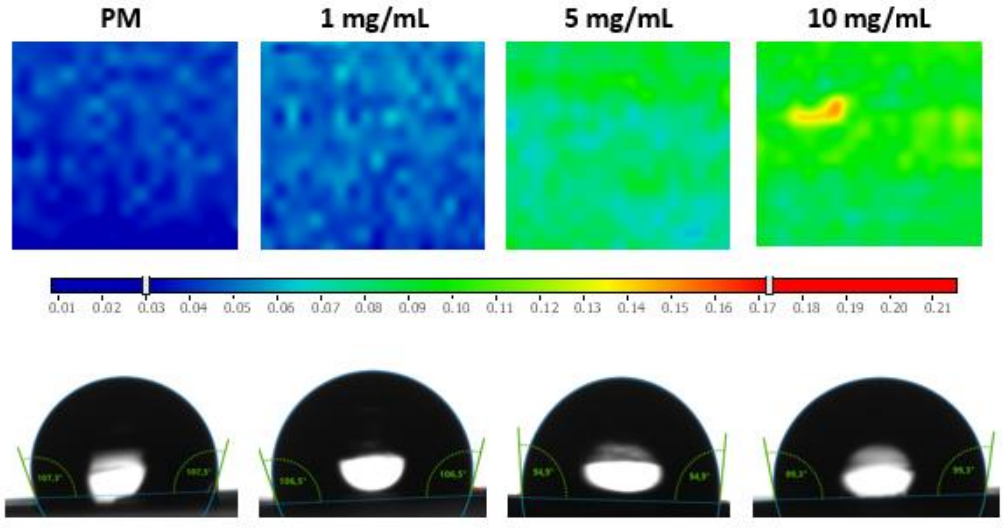

Figure 4.6: FTIR mapping of $PS_{28}PEO_{13}$ coated membrane at 700 cm^{-1} for change in concentration with contact angle measurements.

Table 4.2: Contact angle measurement for modified membrane at different concentration

Sample	1 mg/mL	5 mg/mL	10 mg/mL
$PS_{30}PAA_5$	110°	105°	104°
$PS_{26}PAA_{76}$	104°	91°	92°
$PS_{100}PAA_{107}$	107°	107°	97°
$PS_{28}PEO_{30}$	106°	94°	99°
$PS_{12}PEO_{30}$	91°	89°	99°

4.1.4 Variation of copolymer chain length

Membranes coated with copolymers with different chain length were chosen to analyze the effect of coating with varying chain length of hydrophobic and hydrophilic part of the copolymer. From figure 4.7, we can see that $PS_{30}PAA_2$ coated membrane had higher coating intensity at 700 cm^{-1} compared to other modified membranes, because the size of the hydrophobic part (PS) was far bigger than the hydrophilic part (PAA). Hence the FTIR tool could easily detect the PS part of the copolymer whereas in other modified membranes, the hydrophilic part PAA was longer, and it reduced the intensity of the PS part. Similarly for PS-PEO modified membranes, the $PS_{28}PEO_{13}$ modified membrane had higher intensity of coating over the membrane surface compared to other copolymer coated membranes because the length of the PS chain was higher than the PEO chain. Whereas in other copolymer coated membranes, the hydrophobic part was visible, but the intensity was lower due to the smaller chain length. Hence, we cannot conclude from the varying chain length the optimum coating conditions of the copolymer over the membrane because the hydrophilic part of the copolymer alters the analysis of FTIR mapping. Hence the intensity of hydrophobic part observed on the FTIR mapping would vary depending on the chain length of the hydrophilic part of the copolymer.

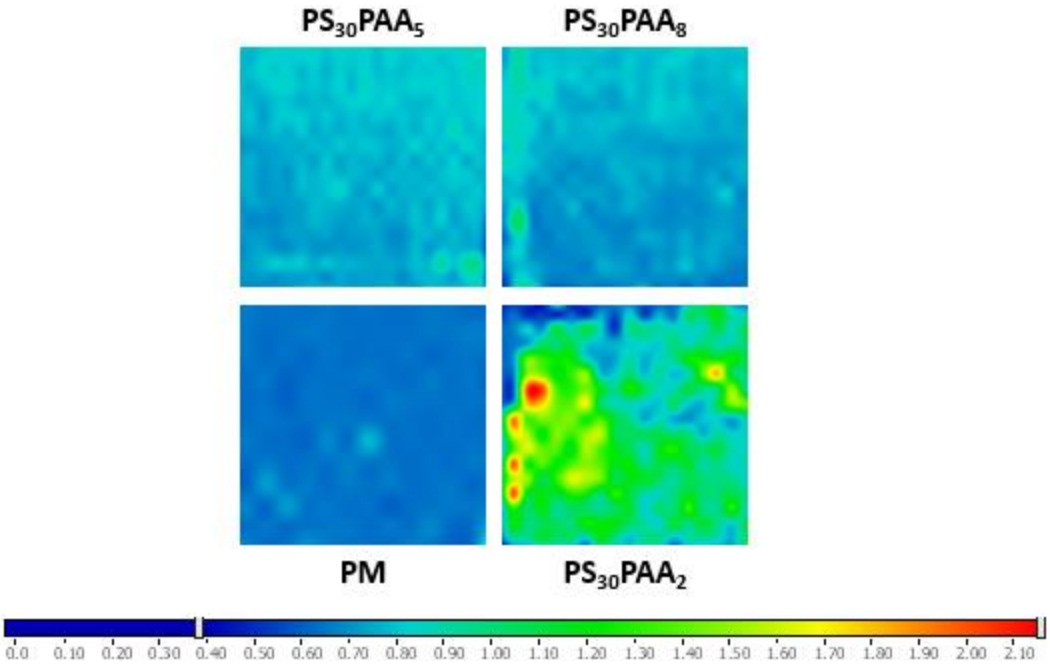

Figure 4.7: FTIR mapping of PS-PAA modified membranes at 700 cm^{-1} for change in copolymer chain length.

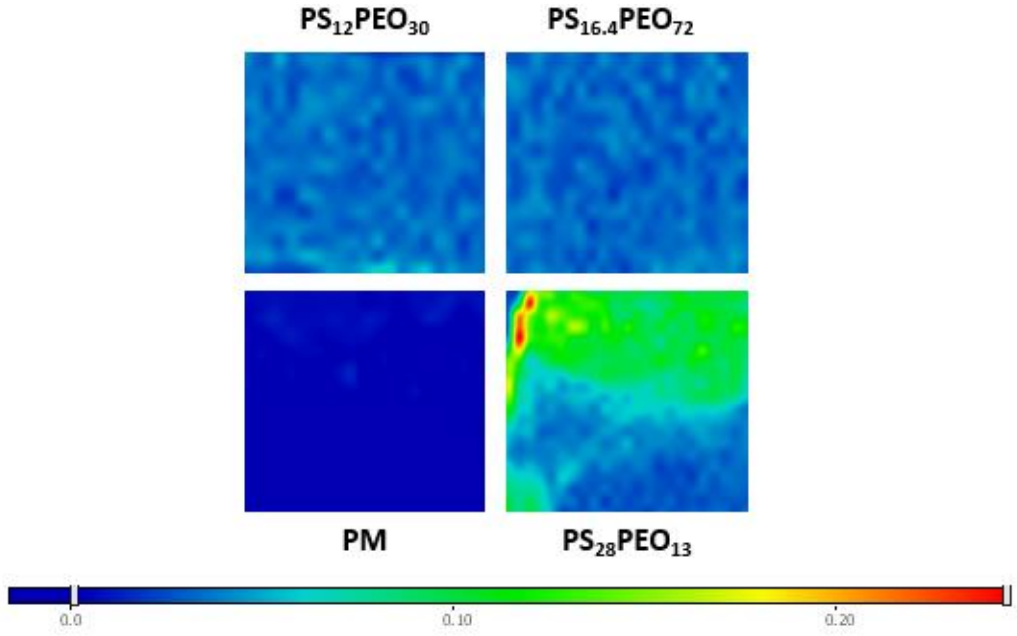

Figure 4.8: FTIR mapping of PS-PEO modified membrane at 700cm^{-1} for change in copolymer chain length.

4.1.5 Static Adsorption of protein

Adsorption of blood protein like human serum albumin (HSA) was studied with pristine membrane and copolymer coated membrane. In order to identify the peaks of the functional groups corresponding to the proteins, the ATR spectra of the pure protein samples were analyzed. The proteins consist of different amino acids which are connected by peptide bonds. All amino acids have -NH and C=O groups suggesting that these could be the most abundant functional groups in protein. Hence the most notable peaks that were obtained in the ATR spectrum would correspond to these functional groups.

Table 4.3: Absorption of functional groups in proteins

Wavenumber (cm^{-1})	**Functional groups**
3500-3070	Amide N-H stretching vibration
1680-1630	Amide C=O stretching vibration
1570-1515	Amide N-H deformation and C-N stretching vibrations

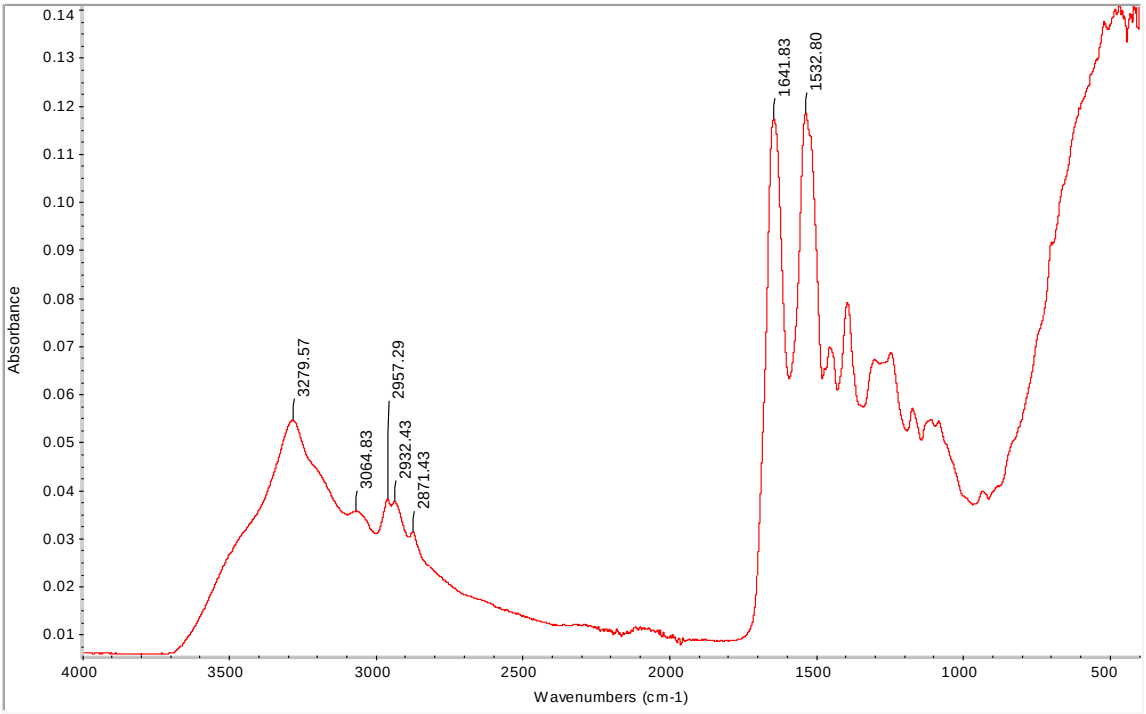

Figure 4.9: ATR spectrum of pure human serum albumin (HSA) in powder form

Adsorption of HSA over the modified membrane surface was analyzed with the help of ATR-FTIR. The membrane was modified with $PS_{30}PAA_8$ copolymer with varying concentration of 1, 5, 10 mg/mL. Then the membrane was immersed in HSA for 2 hours and dried. The concentration of HSA was kept constant at 1 mg/mL for all samples. As shown in figure 4.10, it was observed that with the increase in concentration of the copolymer coated over the membrane surface, the amount of HSA adsorbed over the surface of the membrane also increased. This is because the hydrophilic part of the copolymer PAA has a strong interaction with HSA. This helped in better adsorption of HSA over the membrane surface.

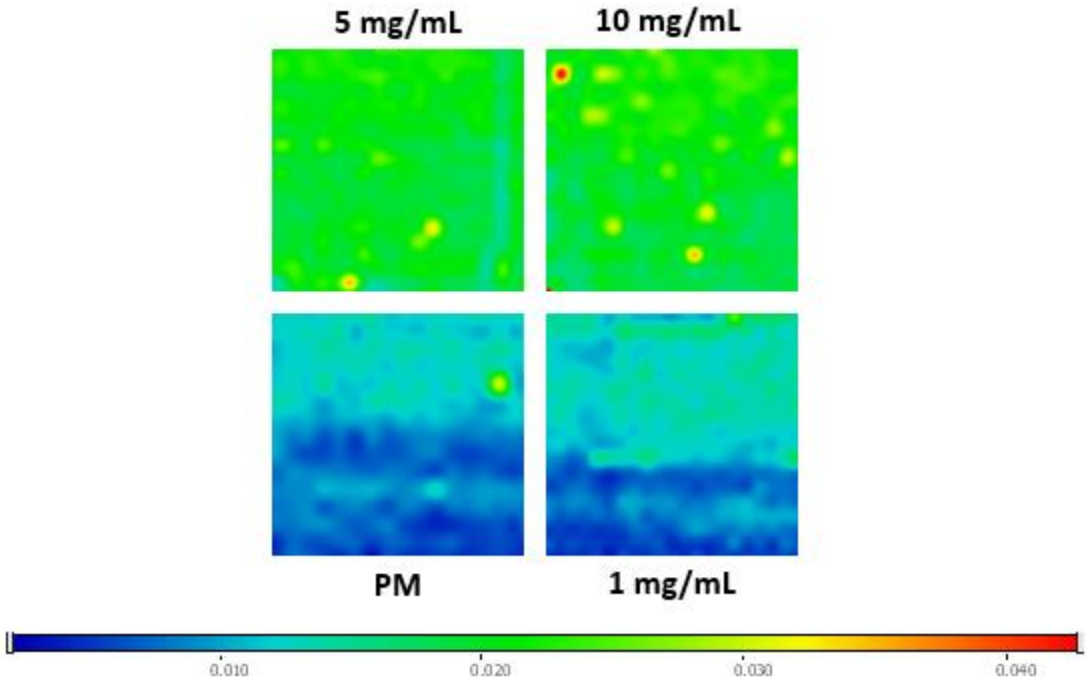

Figure 4.10: FTIR mapping of $PS_{30}PAA_8$ coated membrane for different copolymer concentration at 1650 cm^{-1} for HSA adsorption (1 g/L)

4.2 Filtration Experiments

The membranes used for filtration need to be compacted before determining the permeability of the pristine and the coated membrane. The main reason behind membrane compaction is to avoid decline in transport properties during permeability measurements. Hence the membrane compaction is done until the flux is stabilized as shown in figure 4.11. After membrane compaction, the permeability of the pristine membrane is determined by decreasing the pressure from 1 bar until 0.2 bar. Then the membrane was coated with copolymer by the method as mentioned previously and the flux was determined, and the permeability were calculated. Filtration was done with $PS_{30}PAA_5$, $PS_{26}PAA_{76}$, $PS_{100}PAA_{107}$ coated membranes at 5 mg/mL and the permeability of the membranes were calculated. The decline in permeability was then determined as mentioned in the table 4.4 below.

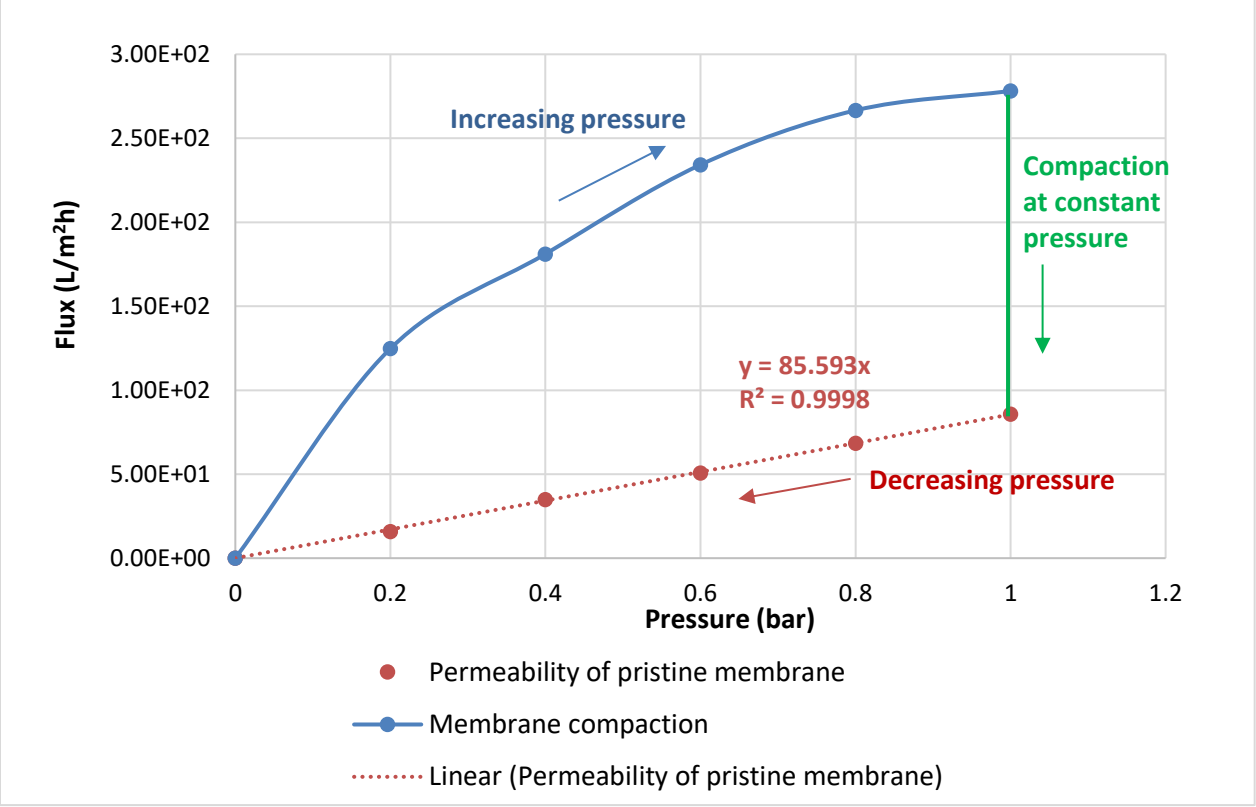

Figure 4.11: Compaction of pristine membrane.

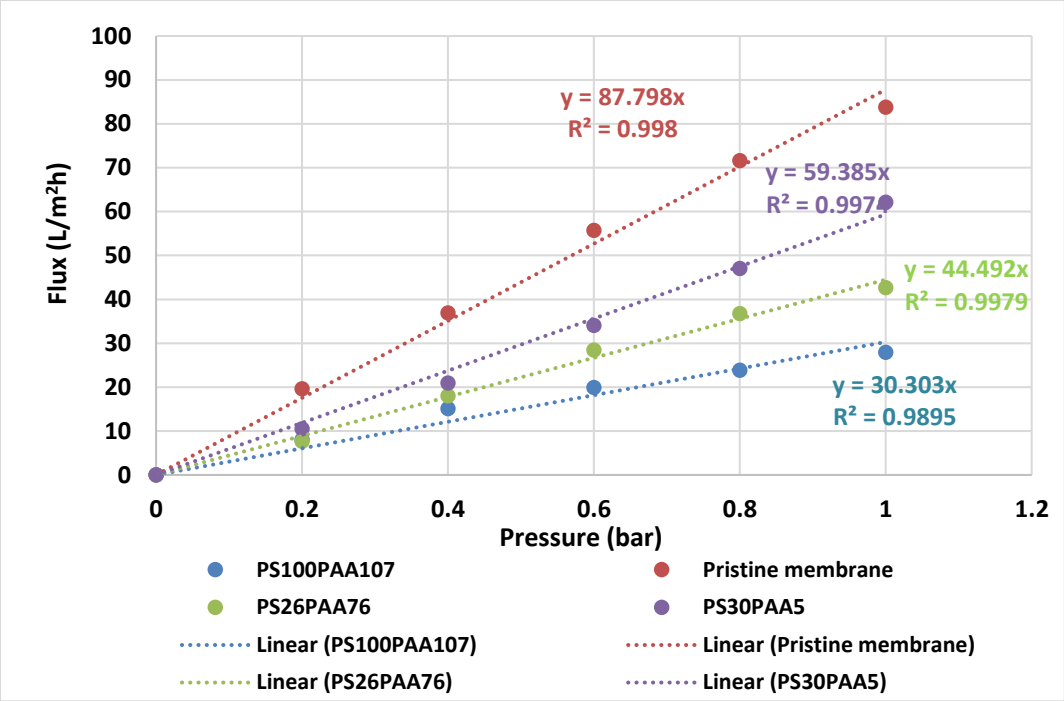

Figure 4.12: Flux evolution of pristine and copolymer coated membranes with pressure.

Table 4.4: Calculation of permeability of pure water filtration with percentage decline

Sample	Permeability (L/m^{2}hbar)	Permeability decline% after filtration
Pristine membrane	87	—
$PS_{30}PAA_5$ coated membrane	59	32
$PS_{26}PAA_{76}$ coated membrane	44	49
$PS_{100}PAA_{107}$ coated membrane	30	65

From the table above, we can see that the permeability decreases with the increase in size of the copolymer coated over the membrane surface. This is because the larger chain length of the copolymer coated over the membrane surface blocks the passage of water through the membrane by creating additional resistence to the system thereby reducing its permeability. Then the filtration

was performed by passing 10mL of protein solution of HSA (1 mg/L) as feed at a pressure of 1 bar. In order to calculate the concentration of HSA in permeate and retentate, the absorbance calibration was done for known concentration of HSA solution at 280 nm wavelength as shown in the figure 4.13 below.

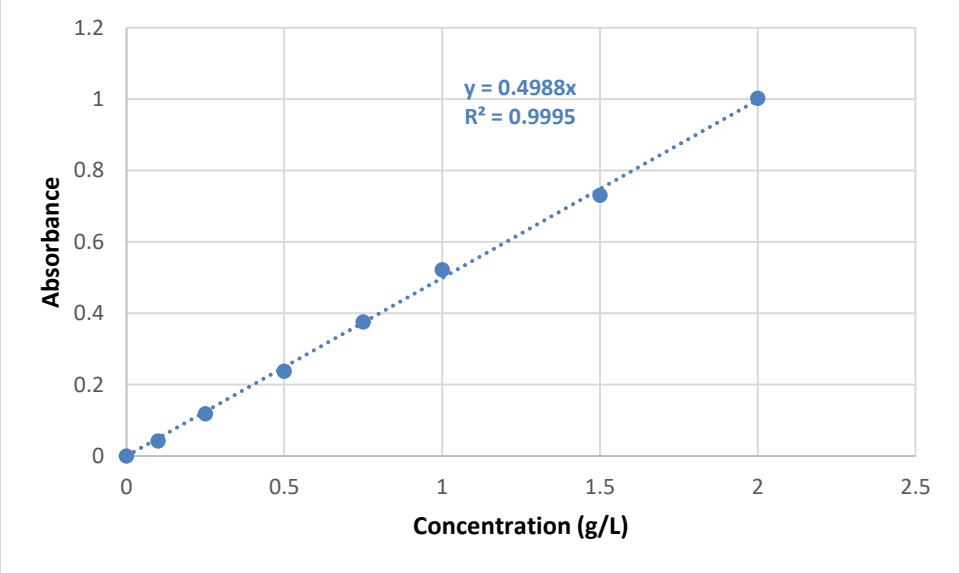

Figure 4.13: Absorbance calibration of HSA at 280 nm wavelength for different concentration

After calibration, the concentration of the permeate and retentate was calculated from the absorbance value obtained using UV-vis spectrophotometer. Then the retention coefficient was calculated.

Table 4.5: Calculation of concentration of permeate and retentate followed by retention %

Sample	Concentration of permeate (g/L)	Concentration of retentate (g/L)	Retention %
Pristine membrane	0.97	1.08	10.1
$PS_{30}PAA_5$ coated membrane	0.82	1.22	32.8
$PS_{26}PAA_{76}$ coated membrane	0.09	3.53	97.4

From the table 4.5 above, we can see that copolymer coated membrane with longer chain length has more retention of HSA when compared to the other membranes. This is because, the

hydrophilic part of the copolymer PAA can adhere proteins to its surface which in turn helps in capturing platelets to its surface whereas in pristine membrane, the retention of protein is low because the capability of pristine PVDF membrane to retain the protein to its surface is much lower.

In order to confirm non removal of copolymer from the membrane surface after filtration, ATR-FTIR mapping was done over the filtration membrane to analyze the presence of copolymer. The intensity of coating on the membrane surface can be analyzed and compared with the pristine membrane. From the mapping it is clear that copolymer coating was present even after the filtration was performed as shown in figure 4.14. The peaks obtained from the ATR spectrum of the coated membrane at 700 cm^{-1} and 1700 cm^{-1} confirms the presence of copolymer as shown in figure 4.16. Similarly, the presence of HSA after filtration was analyzed by performing FTIR mapping on the same $PS_{30}PAA_5$ coated membrane and it was compared with a pristine membrane as shown in figure 4.15. The intensity of the HSA coating was higher for the copolymer modified membrane than the pristine membrane. The peaks obtained from the ATR spectrum at 1650 cm^{-1} and 3300 cm^{-1} confirms the presence of HSA on the membrane surface as observed in figure 4.16.

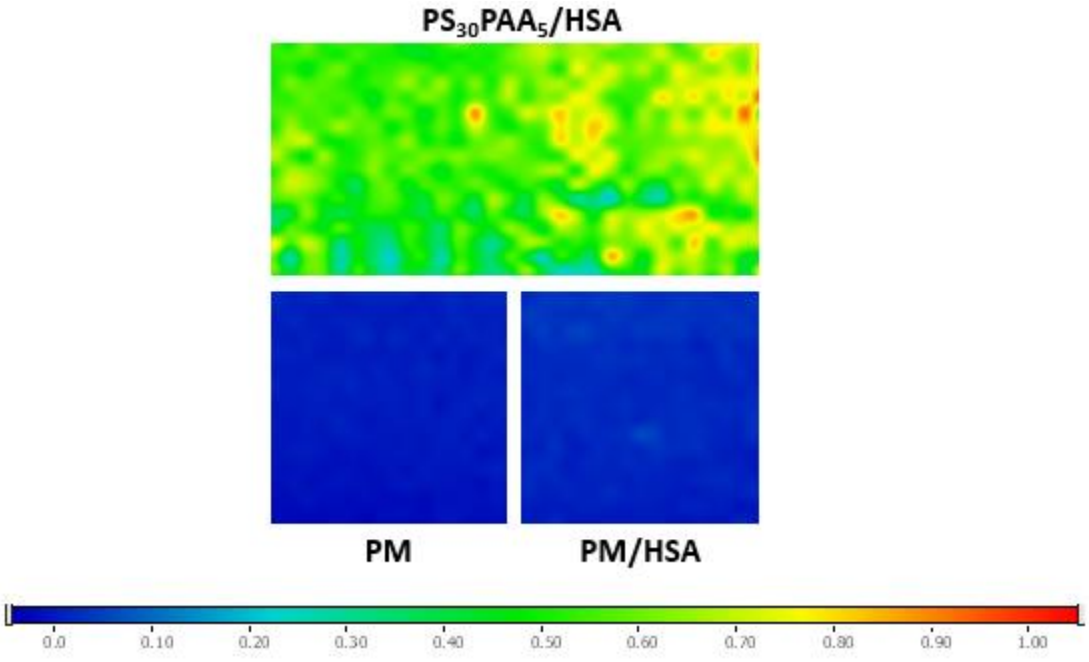

Figure 4.14: FTIR mapping of pristine and $PS_{30}PAA_5$ coated membrane to detect PS part of copolymer at 700 cm^{-1} after HSA filtration.

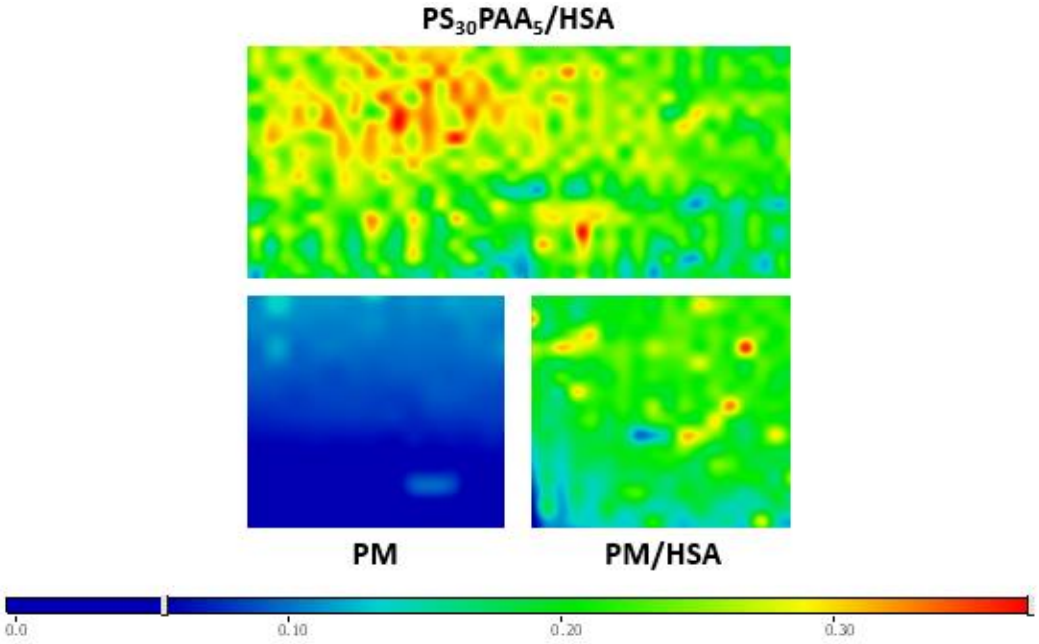

Figure 4.15: FTIR mapping of pristine and $PS_{30}PAA_5$ coated membrane to detect HSA at 1650 cm^{-1} after HSA filtration.

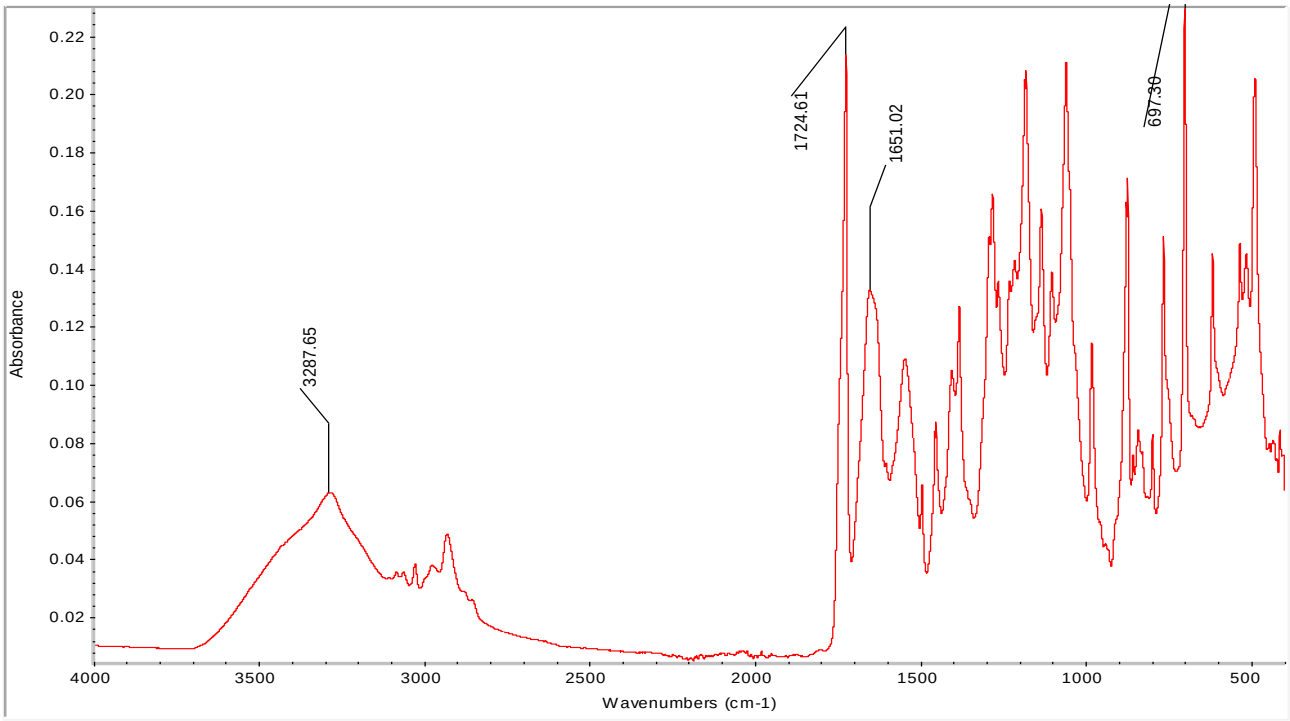

Figure 4.16: ATR spectra of $PS_{30}PAA_5$ coated membrane after HSA filtration

Contact angle measurements were done for membranes modified with copolymer after HSA filtration and the values of the contact angle was compared with that of the pristine membrane as shown in figure 4.17. It was found that the values were lower for the modified filtration membranes after filtration with HSA solution. HSA exhibits hydrophilic character, and it contributes to the low value of contact angle. From the table 4.6, it is clear that the chain length of the hydrophobic and hydrophilic group of the copolymer would play a major role in measuring the contact angle of the modified membrane.

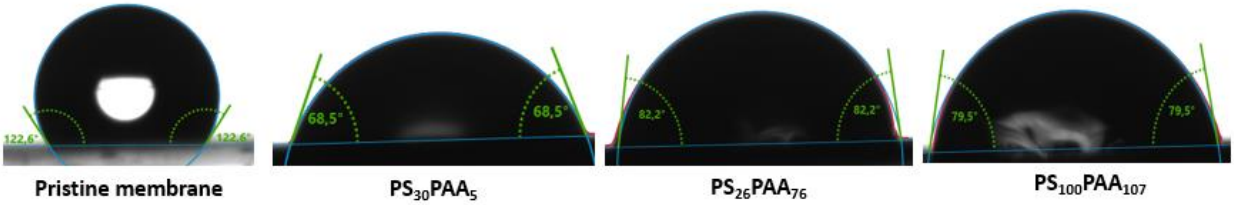

Figure 4.17: Contact angle measurements for modified membranes after HSA filtration.

Table 4.6: Contact angle measurements for modified membranes after HSA filtration

Sample	Contact angle (degree)
Pristine membrane	122° ± 4°
$PS_{30}PAA_5$	65° ± 2°
$PS_{26}PAA_{76}$	81° ± 8°
$PS_{100}PAA_{107}$	79° ± 3°

5. CONCLUSION AND FUTURE TRENDS

Based on the results obtained from our experiments, we were able to optimize the coating of copolymer over the membrane surface and investigate the interaction between the copolymer coating and the human serum albumin (HSA) protein.

During the optimization process of the coating on the membrane surface, the immersion time for membranes was maintained for 2 hours. The process conditions followed for the experiments were Immersion, Washing, Drawing (IWD) and we were able to confirm that there was no removal of copolymer from the membrane surface during the washing process. For all filtration experiments, the concentration of copolymer coated over the membrane surface was maintained at 5 mg/mL as it was the most optimum concentration for coating. Permeability of pure water over the coated membrane was determined and there was a decline in the value of permeability with the increase in size of the copolymer coated over the membrane. Filtration was done with HSA solution and the concentration of HSA in permeate and retentate was calculated followed by calculation of retention. It was found that membrane modified with longer PAA chain had higher retention of HSA at 97.4 %. To analyze the presence of coating after filtration with HSA solution, FTIR mapping was done, and we were able to confirm the presence of copolymer coating over the membrane surface.

For future studies, it is recommended to study the effect of other blood proteins like globulin and fibrinogen on the adhesion with the copolymer. Optimization can be done by modifying membranes at different pH and analyzing the effect of pH on the copolymer coating over the membrane surface. It is highly recommended to use other characterization tools as it provides some additional information about the coating and the modified membrane surface. Scanning Electron Microscopy will provide information about the structural changes on the membrane surface after copolymer coating. Atomic Force Microscopy can be used to determine the surface roughness of the membrane modified with the copolymer.

APPENDIX

A.1 Supporting data for Results and Discussions.

A.1.1 Optimization of copolymer coating

The ATR spectrum was obtained for copolymer samples in powder form so that it would be easier to differentiate the peaks of the membrane and the copolymer coating during optimization. The ATR spectrum of all the copolymers used for analysis have been shown in the figure below.

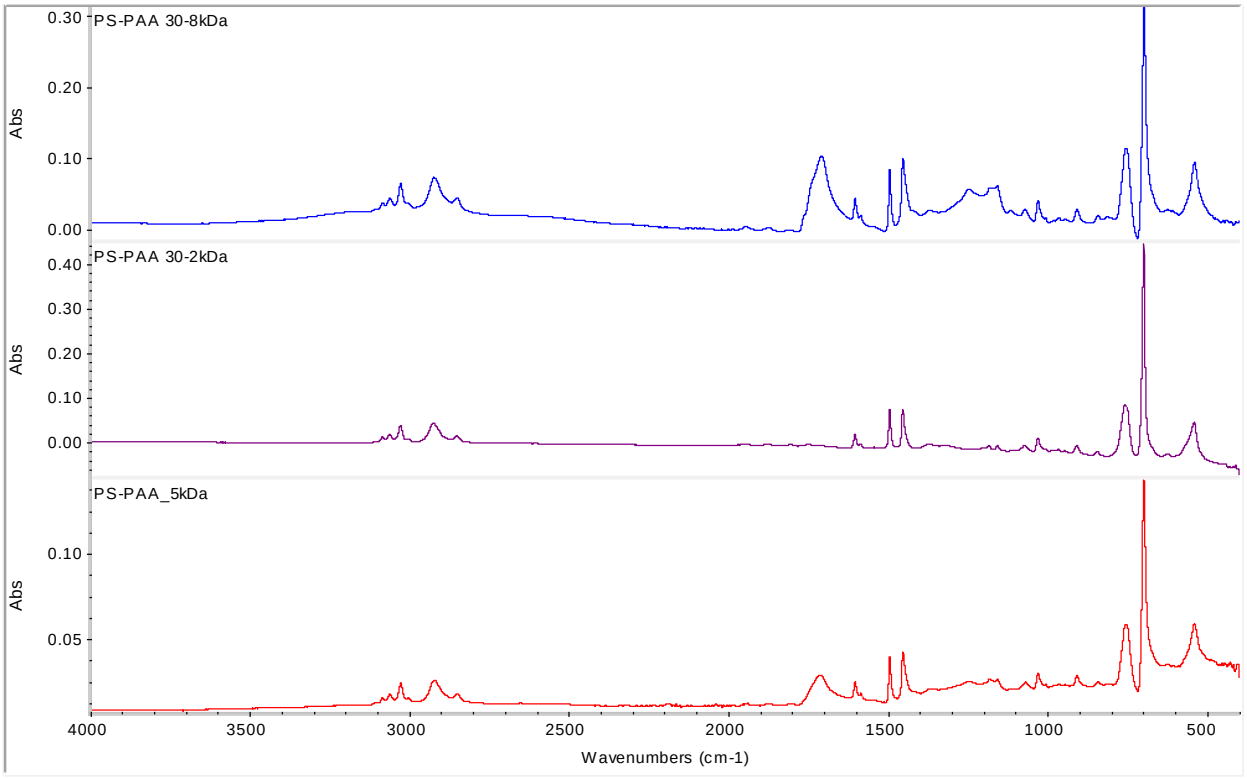

Figure A.1: ATR spectra of $PS_{30}PAA_2$, $PS_{30}PAA_5$, $PS_{30}PAA_8$ copolymer in powder form

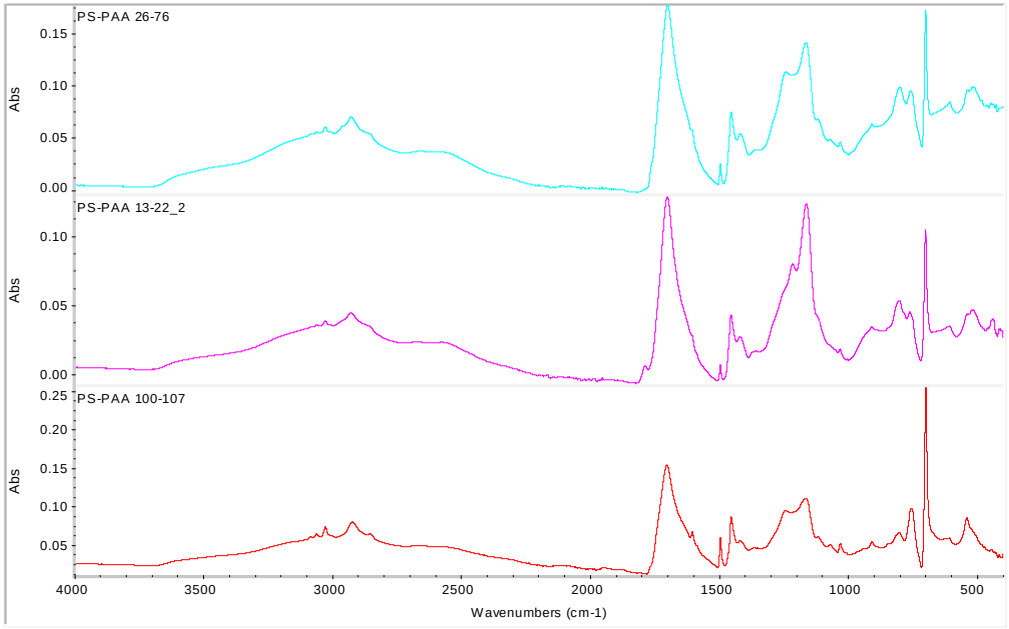

Figure A.2: ATR spectra of $PS_{13}PAA_{22}$, $PS_{26}PAA_{76}$, $PS_{100}PAA_{107}$ copolymers in powder form

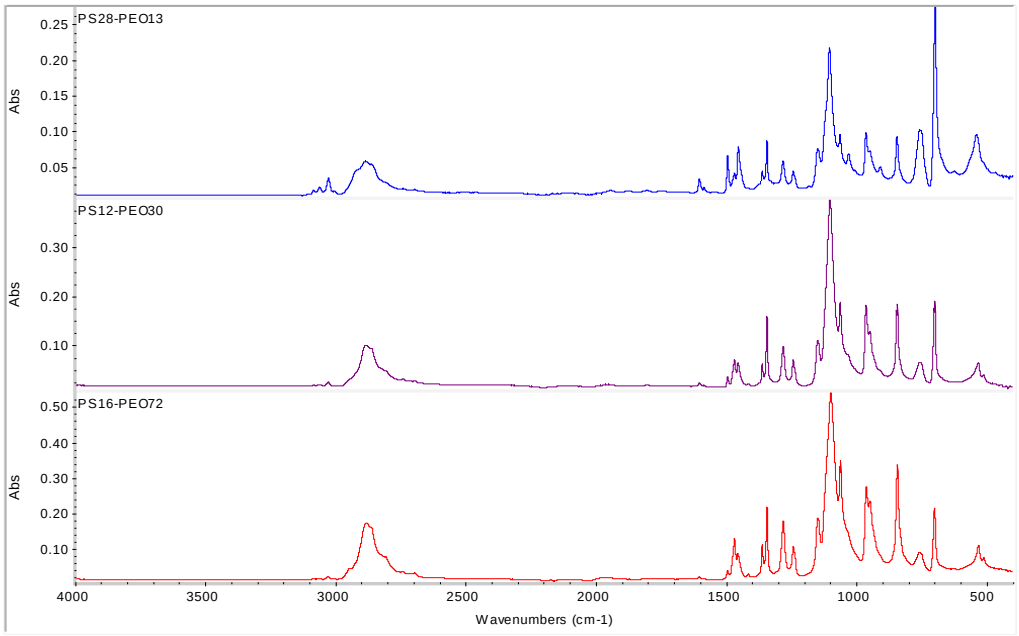

Figure A.3: ATR Spectra of $PS_{12}PEO_{30}$, $PS_{28}PEO_{13}$, $PS_{16.4}PEO_{72}$ copolymers in powder form

Similarly, ATR spectra of the proteins were analyzed in powdered form to differentiate the peaks of the proteins and the membrane during the static adsorption of protein over the membrane surface. The ATR spectra of the proteins are shown in the figure below.

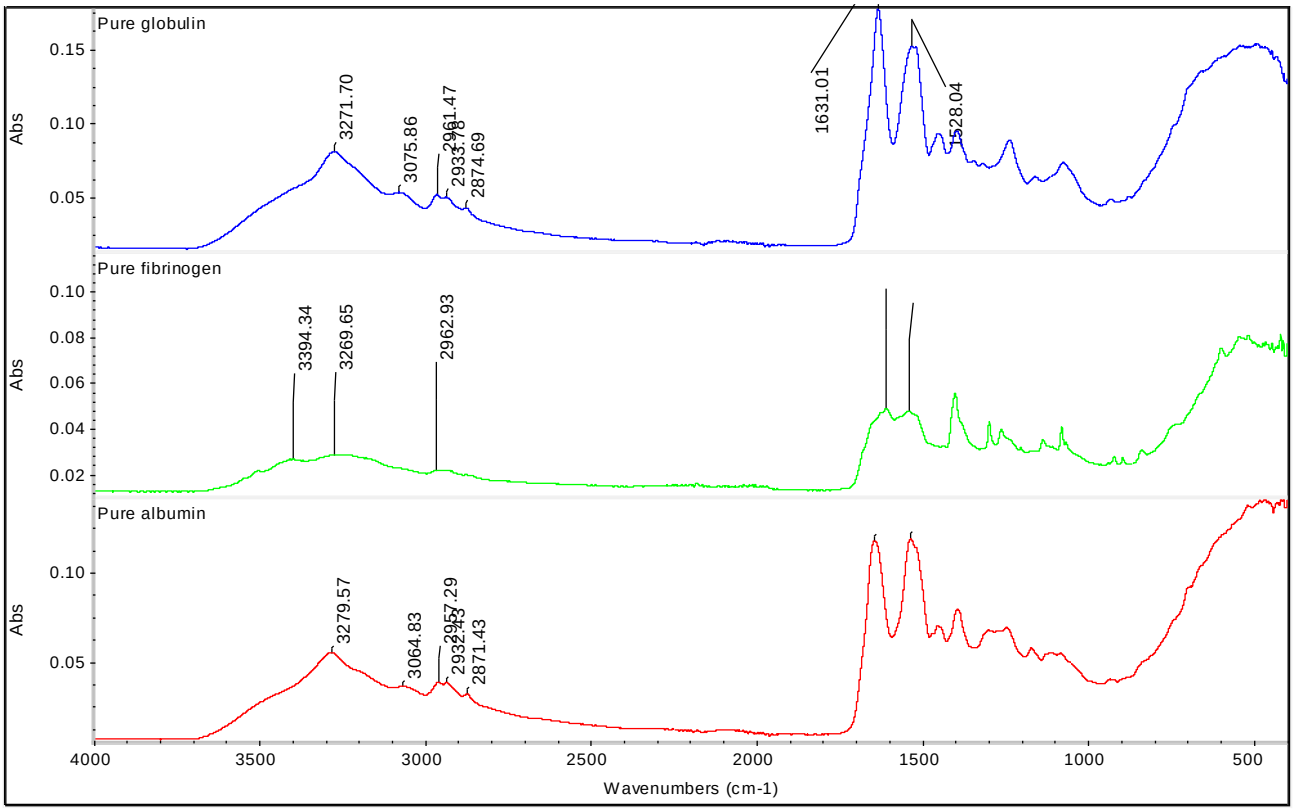

Figure A. 4: ATR Spectra of the proteins such as Albumin, globulin, and fibrinogen in powdered form

A.1.2 Permeability Measurements

The flow rate of the pristine membrane and the copolymer modified membranes were calculated by measuring the weight of the water collected for every five minutes at different pressures. We see that the flow rate decreases with the increase in size of the copolymer coated over the membrane surface.

Table A. 1: Measurement of flow rate at different pressure

	Pristine membrane		$PS_{30}PAA_5$ coated membrane		$PS_{26}PAA_{76}$ coated membrane		$PS_{100}PAA_{107}$ coated membrane	
Pressure (bar)	Weight (g)	Q (g/min)	Weight (g)	Q (g/min)	Weight (g)	Q (g/min)	Weight (g)	Q (g/min)
0.2	0.66	0.132	0.37	0.074	0.27	0.054	0.24	0.048
	0.59	0.118	0.34	0.068	0.2	0.04	0.28	0.056
	0.6	0.12	0.3	0.06	0.24	0.048	0.26	0.052
	0.63	0.126	0.32	0.064	0.22	0.044	0.24	0.048
0.4	1.22	0.244	0.71	0.142	0.59	0.118	0.48	0.096
	1.18	0.236	0.65	0.13	0.55	0.11	0.48	0.096
	1.14	0.228	0.66	0.132	0.51	0.102	0.47	0.094
	1.13	0.226	0.63	0.126	0.55	0.11	0.48	0.096
0.6	1.81	0.362	1.12	0.224	0.86	0.172	0.64	0.128
	1.78	0.356	1.07	0.214	0.83	0.166	0.61	0.122
	1.74	0.348	1.08	0.216	0.82	0.164	0.63	0.126
	1.72	0.344	1.04	0.208	0.8	0.16	0.64	0.128
0.8	2.31	0.462	1.51	0.302	1.13	0.226	0.76	0.152
	2.29	0.458	1.49	0.298	1.1	0.22	0.77	0.154
	2.32	0.464	1.48	0.296	1.07	0.214	0.72	0.144
	2.24	0.448	1.47	0.294	1.07	0.214	0.77	0.154
1	2.76	0.552	2	0.4	1.27	0.254	0.9	0.18
	2.64	0.528	2	0.4	1.28	0.256	0.9	0.18
	2.62	0.524	1.96	0.392	1.27	0.254	0.89	0.178
	2.58	0.516	1.9	0.38	1.26	0.252	0.85	0.17

REFERENCES

1. Tripathi, S.; Varun Kumar, Y. V. B.; Prabhakar, A.; Joshi, S. S.; Agrawal, A., Passive blood plasma separation at the microscale: a review of design principles and microdevices. *Journal of Micromechanics and Microengineering* **2015,** *25* (8), 083001.

2. Basu, D.; Kulkarni, R., Overview of blood components and their preparation. *Indian journal of anaesthesia* **2014,** *58*, 529-37.

3. Bukowska, D. M.; Derzsi, L.; Tamborski, S.; Szkulmowski, M.; Garstecki, P.; Wojtkowski, M., Assessment of the flow velocity of blood cells in a microfluidic device using joint spectral and time domain optical coherence tomography. *Opt. Express* **2013,** *21* (20), 24025-24038.

4. Vadhan-Raj, S., Management of Chemotherapy-Induced Thrombocytopenia: Current Status of Thrombopoietic Agents. *Seminars in Hematology* **2009,** *46*, S26-S32.

5. Laxmi, V.; Tripathi, S.; Joshi, S. S.; Agrawal, A., Microfluidic Techniques for Platelet Separation and Enrichment. *Journal of the Indian Institute of Science* **2018,** *98* (2), 185-200.

6. Lippi, G.; Avanzini, P.; Cervellin, G., Prevention of hemolysis in blood samples collected from intravenous catheters. *Clinical Biochemistry* **2013,** *46* (7), 561-564.

7. Givan, A. L., *Flow cytometry: first principles*. John Wiley & Sons: 2013.

8. Hirano, R.; Namazuda, K.; Suemitsu, J.; Harashima, T.; Hirata, N., Plasma separation using a membrane. *Transfusion and Apheresis Science* **2017,** *56* (5), 649-653.

9. Thorslund, S.; Klett, O.; Nikolajeff, F.; Markides, K.; Bergquist, J., A hybrid poly(dimethylsiloxane) microsystem for on-chip whole blood filtration optimized for steroid screening. *Biomedical Microdevices* **2006,** *8* (1), 73-79.

10. Sakai, K., Dialysis membranes for blood purification. *Frontiers of Medical and Biological Engineering* **2000,** *10* (2), 117-129.

11. Anitua, E.; Andia, I.; Ardanza, B.; Nurden, P.; Nurden, A., Autologous platelets as a source of proteins for healing and tissue regeneration. *Thrombosis and haemostasis* **2004,** *91 1*, 4-15.

12. Eppley, B. L.; Woodell, J. E.; Higgins, J., Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plastic and reconstructive surgery* **2004,** *114* (6), 1502-1508.

13. Heldin, C.-H.; Westermark, B., Mechanism of Action and In Vivo Role of Platelet-Derived Growth Factor. *Physiological Reviews* **1999,** *79* (4), 1283-1316.

14. Alvarez, R. H.; Kantarjian, H. M.; Cortes, J. E., Biology of Platelet-Derived Growth Factor and Its Involvement in Disease. *Mayo Clinic Proceedings* **2006,** *81* (9), 1241-1257.

15. Hardwick, J., Blood processing. *ISBT Science Series* **2008,** *3* (2), 148-176.

16. Liu, F.; Hashim, N. A.; Liu, Y.; Abed, M. M.; Li, K., Progress in the production and modification of PVDF membranes. *Journal of membrane science* **2011,** *375* (1-2), 1-27.

17. Yu, L.-Y.; Xu, Z.-L.; Shen, H.-M.; Yang, H., Preparation and characterization of PVDF–SiO2 composite hollow fiber UF membrane by sol–gel method. *Journal of Membrane Science* **2009,** *337* (1), 257-265.

18. Brash, J. L.; Lyman, D. J., Adsorption of plasma proteins in solution to uncharged, hydrophobic polymer surfaces. *Journal of Biomedical Materials Research* **1969,** *3* (1), 175-189.

19. Tanaka, M.; Motomura, T.; Kawada, M.; Anzai, T.; Yuu, K.; Shiroya, T.; Shimura, K.; Onishi, M.; Akira, M., Blood compatible aspects of poly(2-methoxyethylacrylate) (PMEA)—relationship between protein adsorption and platelet adhesion on PMEA surface. *Biomaterials* **2000,** *21* (14), 1471-1481.

20. Nabe, A.; Staude, E.; Belfort, G., Surface modification of polysulfone ultrafiltration membranes and fouling by BSA solutions. *Journal of Membrane Science* **1997,** *133* (1), 57-72.

21. Nady, N.; Franssen, M. C. R.; Zuilhof, H.; Eldin, M. S. M.; Boom, R.; Schroën, K., Modification methods for poly(arylsulfone) membranes: A mini-review focusing on surface modification. *Desalination* **2011,** *275* (1), 1-9.

22. Revanur, R.; McCloskey, B.; Breitenkamp, K.; Freeman, B. D.; Emrick, T., Reactive Amphiphilic Graft Copolymer Coatings Applied to Poly(vinylidene fluoride) Ultrafiltration Membranes. *Macromolecules* **2007,** *40* (10), 3624-3630.

23. Kim, K.; Fane, A.; Fell, C., The performance of ultrafiltration membranes pretreated by polymers. *Desalination* **1988,** *70* (1-3), 229-249.

24. Harris, J. M., *Poly (ethylene glycol) chemistry: biotechnical and biomedical applications*. Springer Science & Business Media: 1992.

25. Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A., "Non-fouling" oligo (ethylene glycol)-functionalized polymer brushes synthesized by surface-initiated atom transfer radical polymerization. *Advanced Materials* **2004,** *16* (4), 338-341.

26. Gombotz, W. R.; Guanghui, W.; Horbett, T. A.; Hoffman, A. S., Protein adsorption to poly (ethylene oxide) surfaces. *Journal of biomedical materials research* **1991,** *25* (12), 1547-1562.

27. McPherson, T. B.; Shim, H. S.; Park, K., Grafting of PEO to glass, nitinol, and pyrolytic carbon surfaces by γ irradiation. *Journal of biomedical materials research* **1997,** *38* (4), 289-302.

28. Venault, A.; Wu, J.-R.; Chang, Y.; Aimar, P., Fabricating hemocompatible bi-continuous PEGylated PVDF membranes via vapor-induced phase inversion. *Journal of Membrane Science* **2014,** *470*, 18-29.

29. Venault, A.; Ballad, M. R. B.; Liu, Y.-H.; Aimar, P.; Chang, Y., Hemocompatibility of PVDF/PS-b-PEGMA membranes prepared by LIPS process. *Journal of Membrane Science* **2015,** *477*, 101-114.

30. Chang, Y.; Yandi, W.; Chen, W.-Y.; Shih, Y.-J.; Yang, C.-C.; Chang, Y.; Ling, Q.-D.; Higuchi, A., Tunable bioadhesive copolymer hydrogels of thermoresponsive poly (N-isopropyl acrylamide) containing zwitterionic polysulfobetaine. *Biomacromolecules* **2010,** *11* (4), 1101-1110.

31. Jhong, J.-F.; Venault, A.; Hou, C.-C.; Chen, S.-H.; Wei, T.-C.; Zheng, J.; Huang, J.; Chang, Y., Surface zwitterionization of expanded poly (tetrafluoroethylene) membranes via atmospheric plasma-induced polymerization for enhanced skin wound healing. *ACS applied materials & interfaces* **2013,** *5* (14), 6732-6742.

32. Bhattacharjee, S.; Dong, J.; Ma, Y.; Hovde, S.; Geiger, J. H.; Baker, G. L.; Bruening, M. L., Formation of High-Capacity Protein-Adsorbing Membranes through Simple Adsorption of Poly(acrylic acid)-Containing Films at Low pH. *Langmuir* **2012,** *28* (17), 6885-6892.

33. Fang, B.; Ling, Q.; Zhao, W.; Ma, Y.; Bai, P.; Wei, Q.; Li, H.; Zhao, C., Modification of polyethersulfone membrane by grafting bovine serum albumin on the surface of

polyethersulfone/poly(acrylonitrile-co-acrylic acid) blended membrane. *Journal of Membrane Science* **2009,** *329* (1), 46-55.

34. Israelachvili, J., Intermolecular and surface forces. Academic Press, London. *Intermolecular and surface forces. 2nd ed. Academic Press, London.* **1992**, -.

35. Israelachvili, J.; Pashley, R., Measurement of the hydrophobic interaction between two hydrophobic surfaces in aqueous electrolyte solutions. *Journal of colloid and interface science* **1984,** *98* (2), 500-514.

36. Vrijenhoek, E. M.; Hong, S.; Elimelech, M., Influence of membrane surface properties on initial rate of colloidal fouling of reverse osmosis and nanofiltration membranes. *Journal of membrane science* **2001,** *188* (1), 115-128.

37. Al-Amoudi, A.; Lovitt, R. W., Fouling strategies and the cleaning system of NF membranes and factors affecting cleaning efficiency. *Journal of Membrane Science* **2007,** *303* (1-2), 4-28.

38. Belfer, S.; Fainchtain, R.; Purinson, Y.; Kedem, O., Surface characterization by FTIR-ATR spectroscopy of polyethersulfone membranes-unmodified, modified and protein fouled. *Journal of Membrane Science* **2000,** *172* (1-2), 113-124.

39. Leng, Y., *Materials characterization: introduction to microscopic and spectroscopic methods*. John Wiley & Sons: 2009.

40. Barbeş, L.; Rădulescu, C.; Stihi, C., ATR-FTIR spectrometry characterisation of polymeric materials. *Romanian Reports in Physics* **2014,** *66* (3), 765-777.

41. Griffiths, P. R.; De Haseth, J. A., *Fourier transform infrared spectrometry*. John Wiley & Sons: 2007; Vol. 171.

42. Benavente, L.; Coetsier, C.; Venault, A.; Chang, Y.; Causserand, C.; Bacchin, P.; Aimar, P., FTIR mapping as a simple and powerful approach to study membrane coating and fouling. *Journal of Membrane Science* **2016,** *520*, 477-489.

43. Al-Amoudi, A.; Williams, P.; Al-Hobaib, A. S.; Lovitt, R. W., Cleaning results of new and fouled nanofiltration membrane characterized by contact angle, updated DSPM, flux and salts rejection. *Applied Surface Science* **2008,** *254* (13), 3983-3992.

44. Rosa, M.; De Pinho, M., Membrane surface characterisation by contact angle measurements using the immersed method. *Journal of membrane science* **1997,** *131* (1-2), 167-180.

45. Johnson, D. J.; Oatley-Radcliffe, D. L.; Hilal, N., State of the art review on membrane surface characterisation: Visualisation, verification and quantification of membrane properties. *Desalination* **2018,** *434*, 12-36.

46. García-Payo, M. d. C.; Izquierdo-Gil, M. A.; Fernández-Pineda, C., Wetting study of hydrophobic membranes via liquid entry pressure measurements with aqueous alcohol solutions. *Journal of colloid and interface science* **2000,** *230* (2), 420-431.