A new technique to fabricate high-performance biologically inspired membranes for water treatment

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Abstract:

Aquaporin, a highly selective water channel protein, has received worldwide attention because of its potential to form biomimetic membranes with high flux and rejection for water reuse and desalination. In this study, purified aquaporins were incorporated into the active layer of the polybenzimidazole (PBI) nanofiltration membrane. Aquaporins were dispersed in gum arabic and embedded in amphiphilic polyvinyl alcohol with alkyl side chains (PVA-alkyl). PVA-alkyl embedded with treated aquaporins was then attached to flat sheet PBI membranes using carbodiimide chemistry. PVA-alkyl acted as support for aquaporins to prevent their chemical alteration and also gave the membranes mechanical strength. It was found that membranes modified with PVA-alkyl-AqpZ displayed lower flux declines and higher flux recoveries as compared to unmodified PBI membranes. Higher protein and salt rejections were also observed using PVA-alkyl-AqpZ modified membranes as compared to unmodified PBI membranes.

Keywords: Biomimetic, Aquaporin, Nanofiltration, Water purification

Abbreviations: PBI, Polybenzimidazole; PVA-alkyl, Polyvinyl alcohol with alkyl side chains; AqpZ, AquaporinZ; GA, Gum Arabic; PVA, Polyvinyl Alcohol; DMAc, N, N-Dimethylacetamide; LiCl, Lithium Chloride; DI water, Deionized water; CMBA, 4-chloromethyl benzoic acid; PVA-COOH, carboxy methyl polyvinyl alcohol; NaOH, Sodium Hydroxide; ClCH₂COONa, Sodium monochloroacetate; DMSO, Dimethyl Sulfoxide; CH₃(CH₂)₁₄CHO, Hexadecanal; HCl, Hydrochloric acid; LB, Luria Broth; SDS-PAGE, Sodium dodecyl sulfate- Polyacrylamide Gel electrophoresis; OG, n-octyl-D-glucopyranoside; EDCH, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride; NHS, N-hydroxysuccinimide; MES- 2-(N-morpholino) ethanesulfonic acid; NaCl, Sodium Chloride; FTIR, Fourier Transform Infrared spectroscopy; ATR, Attenuated Total Reflectance; BSA, Bovine Serum Albumin; UV-VIS, Ultraviolet- visible; ESEM, Environmental Scanning Electron Microscopy; CaCl₂, Calcium Chloride.

1. Introduction:

Water is an essential component in all separation processes involved in any application. Water is required in varying degrees of purity depending on the application for which it is used. Membrane separations play an important role in different industrial applications related to water, energy, pharmaceutical and life sciences. These membranes provide an alternative to conventional separation processes to obtain cost effective and high quality water [1-3]. However, there are some drawbacks involved with membrane separation processes; in particular, desalination membranes provide very low water flux values. In addition, limited lifetime of membranes, insufficient pollutant rejection, further treatment of concentrates, and chemical resistance of membranes are some of the problems faced while dealing with membrane separations [4-8]. Nanofiltration membranes can be used for brackish water desalination but membrane fouling, pretreatment, membrane cleaning, limited recoveries and feed water loss, and short lifetimes of membranes are some of the problems involved in it [1]. Therefore, even though membrane separations are now established processes for water treatment, there is still need to develop improved membranes that would avoid these limiting issues.

Aquaporin is a bidirectional water channel protein present in cell membranes, and it regulates the flow of water in and out of cells. Aquaporin has, therefore, a potential to improve the water flux through incorporation into synthetic polymeric membranes. Water passes through porous structures as a single unbroken column of molecules with hydrogen bonds between adjacent water molecules. These water molecules can carry protons as well through these structures in the form of H_3O^+ ions. However, this flow of protons along with water is prevented while passing through aquaporins. Aquaporins have pores which are constricted in the middle

and wider at the openings. This constriction leads to a high dielectric barrier for charged entities like protons or other ions, while allowing the passage of small, neutral solutes. The backbone amide and carbonyl groups in the Asparagine-Proline-Alanine motif that lines the pore walls in aquaporin make hydrogen bonds with the water oxygen atoms, causing reorientation of water molecules to become perpendicular to the pore axis. In this orientation the water hydrogen atoms can't make H-bonds with adjacent water molecules. All of the other groups present on the pore-walls are hydrophobic, leaving the water hydrogen atoms without hydrogen bonding partners. This raises the energy by nearly equal 3 kcal/mol [9-12] thus allowing water passage with a minimal energy barrier. The activation energy for the transport of water through Aqp is about 3 kcal which is close to hydrogen-bonding energy, suggesting breakage of hydrogen bonds in the water flow [13].

For this research project, AqpZ, a water channel protein found in *Escherichia coli* was used, since it is inexpensive and can be expressed in large quantities. AqpZ has been shown to be robust under different reducing conditions and at low temperatures. It can retain 100% activity for up to 6 months of storage at 4°C. [11, 14]. The overarching purpose is to improve water permeability while enhancing ion rejection, by incorporating AqpZ into the membrane. Successful formulation could then lead to the formation of biomimetic membranes with high selectivity and high water flux. In this study, aquaporins were treated with gum arabic (GA), a polysaccharide used previously to disperse carbon nanotubes to PVA successfully with the key purpose of preventing excessive functionalities that lead to disruption of the original structures.[15]. Gum arabic is a water soluble polysaccharide produced by Acacia Senegal trees. . In modern times, the most important applications of gum arabic have been as an emulsifier in the food and pharmaceutical industries and as an adhesive [16-18]. Gum arabic has been used in

the food industry as a stabilizer, thickener, emulsifier, anti-caking agent as well as color preservative [19]. It has also been used to slow down the deterioration of inks [20, 21], and it has been observed to be significantly enhance the stability of iron-gall inks [22]. Gum arabic has been used to increase the durability of the functional textiles and add value to them [23]. Gum arabic conjugated polysaccharides showed an unusually high tolerance to salts, thermal instability and lower stability in alkaline conditions [24]. This ability of GA to prevent functionality of materials is exploited here with the purpose to have it protect the aquaporins from being functionalized in the presence of PVA. GA acted as an intermediate layer to enhance the interfacial interaction between the selective layer and the substrate, thus making the assembly stronger. Aqp-GA was then dispersed in a polyvinyl alcohol matrix carrying alkyl side chains (PVA-alkyl). PVA-alkyl is amphiphilic in nature with high hydrophilicity of PVA and hydrophobicity of the long alkyl side chains. This polymer has good film forming properties and outstanding physical and chemical stability [25], and is proposed to be an excellent material to support aquaporins. Thus, by attaching PVA-alkyl matrix with Aqp-GA dispersed in it to the hydrophilized PBI membranes, the assembly was made mechanically stronger and was designed to withstand higher hydraulic water pressure gradients.

2. Research Objective:

The objective of the project is to make a new class of biomimetic nanofiltration membranes made of aquaporin dispersed in a membrane selective layer and capable of operation under high hydraulic pressure. The PVA-alkyl with embedded aquaporins will be used as the nanofiltration membrane active layer (Figure 1). Aquaporins are dispersed into PVA-alkyl layer,

but not necessarily aligned into the layer. Aquaporins are bidirectional in nature; hence, even if their orientation is different than aligned, it does not affect the transmembrane water transport.

Figure 1. Schematic of biomimetic membranes.

3. Methodology:

3.1 PBI membranes casting:

The dope polymer used to cast the backbone of the membranes was polybenzimidazole (PBI). PBI is stable polymer, which has robust mechanical strength with thermal stability for a wide range of high temperature applications and it also provides chemical stability over a wide pH range. PBI membranes are hydrophobic [26, 27], and are strong but brittle [28-30]. The structure of PBI molecule is shown in Figure 2. The imidazole ring of PBI contains two nitrogen atoms, one protonated to serve as a potential hydrogen bond donor and the other nitrogen has a lone pair, which can act as a proton acceptor.

Figure 2. PBI molecule structure.

The solvent used to make the dope solution was N, N-Dimethylacetamide. Commercially-available 26% w/w dope solution, containing 26% PBI polymer, 72% N, N-Dimethylacetamide (DMAc) and 2% Lithium chloride (LiCl), was used and obtained from PBI Performance Products, inc. (Charlotte, NC). LiCl served the function of a pore former, preventing PBI polymer from phasing out of the solution [29, 31] and imparting long shelf life to the solution. The dope solution was diluted to 21% PBI by adding solvent, and the solution was sealed with parafilm to prevent air bubbles from being trapped inside the solution and affecting its homogeneity. Because of very high viscosity of the solution, the solution was kept in the sonicator and degassed for 2 days in order to ensure homogeneous mixing of the solvent and the solute. After sonication, the solution was allowed to come to room temperature and then the solution was ready for membrane casting using the phase inversion process. Phase inversion is the phenomenon whereby the phases of a liquid-liquid dispersions interchange such that the dispersed phase spontaneously inverts to become the continuous phase [30, 32]. The non-solvent phase that was used in this process was water. The PBI chemistry used here was identical to that of previous studies which made nanofiltration membranes with a pore size of 0.61 nm, so the unmodified PBI membranes of this study should also be suitable for nanofiltration[28].

A casting knife, or doctor's blade (Paul N Gardner Co, U.S. Pat 4869200, Pompano Beach, FL.) was used to make flat sheet membranes. A clean glass mirror was used as a surface, which provides optimum hydrophobicity to the membranes and helps for detachment of polymer

films during phase inversion [32]. The solution was placed in an even line on the surface and the casting knife was used to push the solution across the glass surface to make a thin film. The thickness of the membranes was kept between 150 μm and 200 μm . A water coagulation bath was used to induce phase inversion with subsequent pore formation within the membranes. Once the phase inversion had taken place, the membrane came out of the surface of the water. The membrane was thoroughly washed with water and kept in a 50/50 glycerol-DI water solution. Glycerol was added to DI water to ensure that the membranes would stay wet during storage because when they are dried, they can become brittle and susceptible to breakage [28]. The membranes were kept in the solution at least one day before they were analyzed.

3.2 Surface activation of membranes:

The polybenzimidazole membrane surface needs to be activated for further modifications with PVA-alkyl and additions of Aqp-GA to the PVA-alkyl matrix. Activation was achieved by reaction of a highly reactive chlorine atom, from 4-chloromethyl benzoic acid (CMBA) purchased from Sigma-Aldrich (USA), with the secondary amine group in the imidazole ring of the repeat unit in PBI backbone (Figure 2). CMBA adds a carboxylic group to the surface, which serves two purposes: 1) to impart negative charge on the membrane surface, and 2) to act as a platform for subsequent functionalization of the membrane. It is important to note that there are two secondary amine sites in PBI molecule, so after the reaction, carboxylic groups are added on both sites on the molecule. For simplicity, the reaction at only one site is shown (Figure 3).

Figure 3. CMBA modification of membrane.

Modification was performed according to previous studies [28]. For the reaction, 1 wt% solution of sodium persulphate in water was prepared for use as a free radical initiator for the reaction. 200 ml DI water were taken in a 500mL beaker with a stir bar. 2.02g sodium persulphate was added to the water, and the solution was stirred on hot plate at 40°C. Two membranes were added to the solution, which were kept fully submerged and were not stuck under the stir bar. In a second beaker, 0.5 wt% solution of CMBA in acetone was prepared. 0.788 g CMBA was added to 200 mL acetone and was stirred until dissolved. Then acetone/CMBA solution was slowly added to the beaker on a hot plate while stirring, and then covered. This was done in order to prevent the precipitation of CMBA as it is insoluble in water. The final solution was a 50/50 mixture. The temperature of the solution was kept at 40°C and it was stirred for 24 hours to keep all the reactants in solution and prevent the evaporation of CMBA. Once the reaction was finished, the membranes were washed with copious amounts of DI water to remove excess sodium persulphate and immediately placed in glycerol/water bath.

3.3 Preparation of PVA-alkyl:

PVA-alkyl is polyvinyl alcohol carrying long alkyl side chains. It is amphiphilic as it has both hydrophilic (PVA) and hydrophobic (alkyl chains) elements present. This polymer has good film forming properties and outstanding physical and chemical stability. Using PVA as skin layer provides the resulting membranes with high water permeation rate, good antifouling nature, and excellent integrity in acidic and alkaline and remarkable resistance to abrasion [25, 33]. PVA-alkyl was prepared in two steps [34, 35], the preparation of (1) carboxy-methyl PVA followed by (2) PVA-alkyl.

3.3.1) Preparation of carboxy-methyl PVA (PVA-COOH):

The preparation of carboxy-methyl PVA (PVA-COOH) followed literature methods [36, 37], summarized here. Initially, 50 mL water were taken in a 100 mL beaker, 1 g PVA (Acros Organics, USA) was added to it, and the solution was kept at 70°C. The mixture was stirred for 1 hour continuously to prevent PVA from sticking to the bottom of the beaker until it completely dissolved. The solution was transferred to a 500 mL beaker, and 50 g of sodium monochloroacetate (Fisher Scientific, Pittsburgh PA) were added. The solution was then covered with aluminum foil and was incubated at 4 °C for 24 hours. After that, 42 mL water were taken in a 100 mL beaker, 42 g sodium hydroxide (Fisher Scientific, Pittsburgh PA) were added, and the mixture was stirred until the sodium hydroxide dissolved.

NaOH/water solution was added to the incubated solution of PVA and sodium monochloroacetate in water, and stirred at room temperature for 24 hours. Then, it was neutralized using a 6 M solution of hydrochloric acid (Fisher Scientific, Pittsburgh PA) in water was prepared in a separate beaker with pH being continuously monitored. This neutralized

solution was dialyzed against deionized water. The molecular weight cut-off for the dialysis was chosen as high as possible, which was 12-14 kDa in order to maximize dialysis rate. The dialysis tubing (Fisher Scientific, Pittsburgh PA) used was soaked in water for 3 hours in order to open it and fill it with the solution. It was then sealed with dialysis locking membrane clamps. A 2000 mL beaker with a stir bar in it was filled with DI water to be used as a dialysate. It was kept stirring and the water was changed after every 4-5 hours to make sure that the driving force for the dialysis is high. The procedure was continued for 3 days, and the remaining solution in the tube was taken out and stored in another beaker. Then, the stored solution after dialysis was deionized using ion-exchange resins. DOWEX 1X8 (Acros Organics, USA) was used for negatively charged ions and DOWEX 50WX8 (Acros Organics, USA) was used for positively charged ions. The output solution after the ion exchange was lyophilized using freeze dryer. For that, the solution was kept in centrifuge tubes and was allowed to freeze dry for 3 days. PVA-COOH was obtained as a white solid after freeze-drying. The weight of the product was 0.45 g and the yield was 39%. The overall reaction is shown in Figure 4.

Figure 4. PVA-COOH synthesis.

3.3.2) Preparation of PVA-alkyl:

The second step of making PVA-alkyl was synthesis of hexadecanal. Chemicals used for the preparation were celite, pyridinium chlorochromate and 1-hexadecanol. Dichloromethane was used as the solvent. 11.2 mL dichloromethane were taken in a 50 mL beaker while stirring, and 0.95 g celite, 0.95 g pyridinium chlorochromate and 0.5 g 1-hexadecanol were added to the beaker under a fume hood. The solution was sealed with aluminum foil and stirred for 6 days at room temperature. After that, the reaction mixture was diluted by adding 40 mL diethyl ether. Florisil columns were used to remove excess celite, pyridinium chlorochromate and 1-hexadecanol. Then the mixture was evaporated, and hexadecanal was obtained as a white solid.

PVA-COOH obtained after first reaction was dissolved in DMSO. Hexadecanal and 200 μ L of 12 M hydrochloric acid were added to the solution, which was maintained at 70 °C and stirred for 25 hours. The reaction mixture was extracted with diethyl ether. The mixture was then neutralized with 1M sodium hydroxide. The neutralized solution was dialyzed against DI water using dialysis tubing of 12-14kDa following the same procedure as described previously. The solution was then desalted with ion exchange resins previously mentioned and lyophilized using a freeze dryer for 3 days. PVA-alkyl was obtained as a white solid with yield of 49% at a final weight of the product was 0.22 g. The overall reaction for the second step of the process is shown in Figure 5.

Figure 5. PVA-alkyl synthesis.

3.4 AqpZ expression and purification:

Aquaporin Z (AqpZ) is a bacterial aquaporin from *Escherichia coli*, which shows high degree of homology to its mammalian counterparts and can be expressed in a bacterial host. Furthermore, it is highly stable and resists denaturing due to heat, detergent, voltage, and pH changes [11, 14]. This makes the protein suitable for commercial use.

The pET-NH6 plasmid (a generous gift from Dr. Michael Weiner, University of Virginia) was transformed into an *Escherichia coli* C43(DE3) cell line for protein expression. Single colonies bearing the plasmid were used to inoculate four liters of LB media containing 100 µg/mL ampicillin at 37 °C with shaking at 250 rpm. After reaching an OD600 of 1.0, AqpZ protein expression was induced by addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) with further incubation for 3 hours at 30 °C and shaking at 250 rpm. Cells were harvested and stored at -80 °C until use.

The protein was purified by using a modified version of a previously published protocol [38]. Five grams of cells paste were resuspended in 50 ml cold buffer (20 mM Tris-HCl pH 7.4, 500 mM NaCl and 1 mM Pefabloc). The suspension was lysed by ultrasonication using 30 sec pulse on and 2 min pulse off cycle for a total exposure time of 8 min. The lysate was centrifuged at 12,500 rpm for 30 minutes to pellet cell debris. Membrane fractions were collected by high speed at 40,000 rpm for 1 hr. The total membrane pellet was resuspended in cold buffer (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 1 mM Pefabloc and 10% glycerol) and 270 mM of n-octyl-D-glucopyranoside (OG) was added with incubation at room temperature on a rocking platform.

The solubilized membrane was loaded onto a 5 mL TALON superflow metal affinity (Co²⁺-IMAC) equilibrated with Buffer A (20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 10%

glycerol and 40 mM OG) using an ÄKTA chromatography system. The column was washed with 10% buffer B (20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 10% glycerol, 40 mM OG and 500 mM imidazole) for 10 column volumes. The protein was eluted with a linear gradient from 50 mM to 500 mM imidazole and the peak fractions were analyzed by SDS-PAGE (NuPAGE Novex 4-12% Bis-Tris gels). The calculated monomer mass of hexahistidine-tagged AqpZ is 27 kDa, but on this gel the AqpZ protein run at a lower electrophoretic mass of approximately 22 kDa, as shown in Figure 6. The AqpZ containing fractions were pooled and stored at -80 °C until ready for incorporation into the membranes.

Figure 6. SDS- PAGE showing purification of AqpZ with the protein band migrating at 22 kDa.

3.5 Treatment of AqpZ with gum arabic:

A 15wt. % solution of gum arabic (Fisher Scientific, USA) in water was prepared. AqpZ was added to this solution and sonicated under mild conditions for 4 hours in order to disperse it

in the gum arabic solution. The concentration of AqpZ was 1ml/3ml of GA. Gum arabic has been used previously to disperse carbon nanotubes to PVA without either shortening the nanotubes or disrupting their structures. After the addition of GA to carbon nanotubes solution, the suspension was found to be stable over few months and was not affected by centrifugation. Hence, it was proposed to protect the structure of aquaporins. The weight ratio of AqpZ to gum arabic was kept at 1:4. The mixture was kept stirring at room temperature for 72 hours and then heated slowly to remove water [15, 39]. Gum arabic-treated AqpZ were then added to de-ionized water and sonicated for 30 minutes. The solution with gum arabic-treated AqpZ was physically dispersed into the PVA-alkyl solution. The ratio of AqpZ-GA to PVA-alkyl solution was 1:1 by volume. Aquaporin is bidirectional water channel protein; hence the orientation of the protein isn't expected to change the water flux through the protein [40].

3.6 Surface modification of PBI membrane using Aqp-PVA-alkyl:

Aqp-PVA-alkyl was attached to the membrane using carbodiimide chemistry [28], for the reaction between a carboxylic group and the hydroxide group present in PVA-alkyl molecule. For the reaction, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDCH) and N-hydroxysuccinimide (NHS), both purchased from Sigma-Aldrich were used. The reactions were performed in 2-(N-morpholino) ethanesulfonic acid (MES) buffer (Sigma-Aldrich, USA).

400 mL water were taken in a 500 mL beaker and 7.85 g MES buffer was added with stirring, Followed by the addition of 11.75g NaCl with stirring. The solution was titrated to pH 6 using NaOH, 0.23 g NHS and 0.153 g EDCH were added to the solution and stirred well. CMBA modified PBI membranes were added to the solution, stirred for 15 minutes, and then the

reaction mixture was titrated to pH 7 using NaOH. PVA-alkyl was added to the solution and stirred for 24 hours, and then the membranes were removed and rinsed well with DI water and stored in a beaker filled with DI water. The membranes were stored for 24 hours before using it for analysis. The overall reaction is shown in Figure 7.

Figure 7(a)

Figure 7(b)

Figure 7(a) and (b). Carbodiimide membrane activation chemistry.

3.7 Membrane characterization:

3.7.1) Fourier Transform Infrared Spectroscopy (FTIR):

A vibrational spectrum is a characteristic of every molecule and is considered a unique property of that molecule [41]. FTIR was used in ATR mode to determine the chemistry of the proposed reactions and modifications on the membrane surface. Digilab UMA 600 FT-IR microscope was used for all the analysis of membrane samples performed in this study.

3.7.2) Contact angle measurements:

Contact angle is defined as the measure of wettability of a surface. Cam-Plus Micro contact angle meter (Tantec Inc., Schaumburg, IL) was used for the contact angle measurement of all the membrane samples. A small drop of water was placed on the membrane surface and resultant angle of the droplet to the surface was measured as shown in Figure 8. The higher the hydrophobicity of the membrane, the higher the contact angle.

Figure 8. Contact angle measurement [42].

3.7.3) Flux analysis:

Dead end filtration was used to monitor the flux decline of both PBI and PVA-alkyl-AqpZ membranes. Filtration experiments were performed using Amicon filtration cell (Amicon Stirred Cell 8010 – 10 ml). Using a constant membrane surface area of 4.1 cm^2 , the time to

collect a 2-ml permeate sample was measured for each feed and flux was calculated. A constant pressure of 70 psi (4.83 bar) for laboratory purposes and continuous stirring was applied in all tests. Flux values were calculated as L/m²-hr and plotted against the total time of filtration. Membrane samples were cut into circular pieces of area 4.1 cm² and supported by a WhatmanTM filter paper (110 mmø). Each membrane was precompacted with DI water for 7-8 hours until a stable flux was reached. Precompaction was followed by filtration of protein solutions of 10 ppm each of bovine serum albumin (BSA) and lipase protein in water. The same filtration cell was used for protein filtration under the same conditions of pressure and stirring. Protein rejections were calculated using UV-VIS spectrometer (Varian Inc. Cary 50, Agilent Technologies, Santa Clara, CA) according to following equation (1):

$$R = \left(1 - \frac{C_p}{C_f}\right) \times 100\%$$

where C_p and C_f are solute concentrations in permeate and feed solutions respectively.

Salt rejection was tested using five solutions of different concentrations of sodium chloride and calcium chloride in DI water: 3.4, 10, 20, 35 and 100 mM solutions. Solutions were run through the 10 ml dead-end cell under the same conditions as used for the pure water flux testing. Continuous stirring was applied inside the cell to prevent salt build-up on the membrane surface. Two mL permeate samples were collected for each feed. Salt rejections were calculated using conductivity meter. The apparent solute rejection R (%) was calculated using equation (1).

After each feed water filtration, the membrane was backwashed for 1 hour with DI water and filter paper support was changed. The flux recovery of the membrane was measured after backwash.

3.7.4) Morphological characterization:

Environmental scanning electron microscopy (ESEM) was used to verify the asymmetric morphology of the membranes and monitor the surface of both unmodified and PVA-alkyl-AqpZ modified membranes before and after filtration. An FEI Quanta 3D FEG Dual Beam Electron Microscope (FEI, USA) was used to test the samples. By freezing small samples of the membranes in liquid nitrogen and cracking them, smooth cross-sectional areas could be observed. In order to get an image of cross-section of the membranes, the frozen and cracked samples were attached vertically to a carbon tape while the samples were attached horizontally to the carbon tape to get an image of membrane surface. The surfaces of the samples were dusted with a thin layer of palladium-gold using a Cressington 108 auto sputtering device and then observed under scanning electron microscope.

4. Results and Discussion:

4.1 Chemical analysis:

Figure 9 shows the FTIR analysis of PBI, CMBA and PVA-alkyl-AqpZ membranes, and Table 1 identifies all peaks. FTIR showed peaks at $\sim 1050\text{ cm}^{-1}$ for C-O stretch, $\sim 1200\text{ cm}^{-1}$ for alkyl substituted ether, which is observed after PVA-alkyl is attached to CMBA-modified PBI molecule using carbodiimide chemistry (Figure 10). A peak at 1660 cm^{-1} corresponds to C=O stretch that is present in the final molecule due to addition of CMBA. FTIR spectrum of PBI molecule also shows a peak at 1650 cm^{-1} , which identifies the presence of secondary amine group present in PBI. Peak 4 corresponds to O-H stretch, which is present in CMBA-modified PBI molecule as well as PVA-alkyl-AqpZ-modified molecule (Figure 10). Peak 5 in the analysis

is due to long alkyl chains that are present in the PVA-alkyl-AqpZ-modified molecule [41]. A very broad peak around 3400 cm^{-1} is observed in all the membrane samples, which is associated with N-H stretching [43]. Bonds corresponding to different functional groups are indicated in Figure 10.

Figure 9. FTIR analysis of unmodified, CMBA modified and PVA-alkyl modified membranes.

Table 1: Functional groups and corresponding wave numbers in IR spectra.

Band number	Functional group	Wave number (cm^{-1})
1	C-O	1057
2	C-O (ether)	~ 1200
3	C=O	1670-1820
4	OH	~ 2670

5	C-H	~ 2850
6	>N-H	1550-1650

Figure 10. AqpZ-PVA-alkyl modified PBI molecule.

4.2 Hydrophobicity:

Contact angle was used as a measure of hydrophobicity, and results are shown in Figure 11. CMBA modified membranes were found to be more hydrophilic than PBI membranes [28, 30]. This was most likely due to addition of a $-\text{COOH}$ group in the modified molecule and its increased ability to form hydrogen bonds because of the presence of oxygen with a lone pair. However, the change is not statistically significant because of the overlap of standard deviations. After the addition of PVA-alkyl to the membranes, the contact angle decreased further showing a significant increase in the hydrophilicity of the membrane. This was most likely due to high

hydrophilicity of PVA [25]. After AqpZ was added to the membrane, there was no significant difference between its contact angle and that of the PVA-alkyl membranes, which is likely because the aquaporins that were added to PVA-alkyl matrix were embedded inside the matrix and not on the surface of the membrane.

Figure 11. Hydrophobicity via contact angle.

4.3 Flux analysis:

4.3.1) Flux profile:

Before comparing observations between PBI and PVA-alkyl-AqpZ (or AqpZ-modified) membranes, it is important to separate the effects of the addition of PVA-alkyl only and PVA-alkyl-AqpZ to the membrane in order to separate the effect of the presence of the amphiphilic/polar PVA-alkyl inclusions from that of the presence of AqpZ. Experiments were conducted in parallel comparing PBI, PVA-alkyl and PVA-alkyl-AqpZ membranes using BSA and lipase solution filtration. The flux analyses are shown in Figures 12, 13 and 14. For PBI membranes, the pure water flux was 7.90 L/m²-h (LMH), the initial flux for BSA filtration was 5.77 LMH, the recovery after BSA filtration (i.e. after DI backwash) was 5.47 LMH (or a 69%

flux recovery, as compared to the initial pure water flux), and the final flux after lipase filtration was 3.82 LMH. BSA and lipase rejections were approximately 86% and 84%, respectively. After the addition of PVA-alkyl (no aquaporins), the pure water flux was lower at 6.0 LMH, which is likely due to the extra resistance added by the addition of PVA-alkyl to the surface of the membrane. The initial flux for BSA filtration was 4.36 LMH, the recovery after BSA filtration was 4.80 LMH (or an 81% flux recovery), and the final flux after lipase filtration was 4.13 LMH. BSA and lipase rejections were approximately 90% and 87%, respectively. While flux values for the PVA-alkyl membranes were not as high as for the PBI membranes, it is clear that the flux decline during filtration was improved and the final flux after the 36-hour filtration period was higher. This was associated with the increase in hydrophilicity of PVA-alkyl (Figure 11). Lastly, after the addition of PVA-alkyl with embedded aquaporins (PVA-alkyl-AqpZ), the initial pure water flux was lower at 5.32 LMH, which again is likely due to the extra resistance. The initial flux for BSA filtration was 5.0 LMH, the recovery after BSA filtration was 5.0 LMH (or a complete recovery of the filtration flux, or 96% flux recovery as compared to the initial flux), and the final flux after lipase filtration was 4.43 LMH. BSA and lipase rejections were approximately 91% and 87%, respectively. Protein rejection and flux recovery data is shown in Table 2. Therefore, it is concluded that the addition of aquaporins to the membrane led to a more consistent flux value and a nearly complete recovery of flux back to its initial pure water flux after cleaning.

Figure 12. Flux analysis of unmodified PBI membrane.

Figure 13. Flux analysis of PVA-alkyl modified PBI membrane.

Figure 14. Flux analysis of AqpZ-PVA-alkyl modified PBI membranes.

Table 2: Protein rejection and flux recovery comparisons.

	Unmodified PBI membranes	PVA-alkyl modified PBI membranes	AqpZ-PVA-alkyl modified PBI membranes
BSA Rejection	86% $\pm$ 1%	90% $\pm$ 1%	91% $\pm$ 1%
Lipase Rejection	84% $\pm$ 1%	87% $\pm$ 1%	87% $\pm$ 1%
Flux Recovery	69%	81%	96%

More in-depth flux comparisons were then conducted between PBI and PVA-alkyl-AqpZ membranes for a period of 140 hours with protein solutions and salt solutions. These flux analyses are shown in Figures 15 and 16, respectively. For unmodified PBI membranes, initial flux values observed were higher than for the PVA-alkyl-AqpZ membranes due to extra increased thickness, and hence resistance, associated with the addition of PVA-alkyl to the

membrane matrix. However, the flux decline observed was greater in unmodified membrane than modified membrane.

Figure 15. Flux analysis of unmodified PBI membranes.

Figure 16. Flux analysis of AqpZ modified PBI membranes.

For PBI membranes, initial pure water flux was 8.77 LMH, initial flux for BSA filtration was 7.89 LMH and that for lipase filtration was 6.79 LMH. The flux recovery obtained after BSA filtration was 7.07 LMH (or an 80% flux recovery as compared to the initial pure water flux). Final flux after lipase filtration was 6.37 LMH and flux recovery after DI backwash was 6.41 LMH (a 73% of initial water flux). Rejections for protein filtrations are shown in Table 3.

BSA and lipase rejections for unmodified PBI membranes were 84% and 83%, respectively, and these were not significantly different from our initial PBI studies. For PVA-alkyl-AqpZ membranes, initial pure water flux was 6.74 LMH, which again was likely lower due to the additional resistance to flow from the addition of PVA-alkyl to the surface of PBI membranes. Initial flux for BSA solution for these membranes was 6.32 LMH and for lipase solution was 6.30 LMH. The flux recovery after BSA filtration was 6.68 LMH (or a 99% as compared to initial pure water flux). The final flux after lipase filtration was 5.96 LMH, and flux recovery after that was 6.24 LMH (a 92% of initial water flux). Rejection values for both BSA and lipase solutions increased for PVA-alkyl-AqpZ membranes. For BSA solution, an 88% and for lipase, an 86% rejection was observed. This might be due to addition of AqpZ in the PVA-alkyl matrix since aquaporins block the flow of protein molecules through the aquaporin structure. Any protein going through the membrane would be moving through the membrane regular pores.

Table 3: Rejection and flux recoveries for protein filtrations

	PBI membranes	AqpZ-PVA-alkyl membranes
BSA rejection	84% $\pm$ 1%	88% $\pm$ 1%
Lipase rejection	83% $\pm$ 1%	86% $\pm$ 1%
Flux recovery	61.11%	87.98%

Filtrations with protein solutions were followed by filtration with solutions of NaCl and CaCl₂ in water. Five different concentrations of salt solutions were chosen for filtration studies of both unmodified and modified membranes. For unmodified PBI membranes, initial flux for 3.4 mM NaCl feed solution was 6.14 LMH and final flux was 6.10 LMH, with a flux recovery of 6.58 LMH (75%). For PVA-alkyl-AqpZ membranes, the initial flux for the same NaCl solution

was 6.22 LMH, the final flux was 6.03 LMH and flux recovery was 6.38 LMH (94.6%). A 10 mM NaCl solution was used as feed after DI water backwash. For PBI membranes, the initial flux was 6.16 LMH, the final flux was 6.02 LMH and flux recovery was 6.44 LMH (73.43%), while for PVA-alkyl-AqpZ membranes, the initial flux was 6.30 LMH, the final flux was 6.29 LMH and flux recovery was 6.87 LMH (100%). When the NaCl solution concentration was increased to 20 mM, the unmodified PBI membranes showed an initial flux 5.80 LMH, final flux was 5.81 LMH and flux recovery was 6.15 LMH (70%). For PVA-alkyl-AqpZ membranes, initial flux was 6.80 LMH, final flux was 6.69 LMH and flux recovery obtained was 6.81 LMH (100%). Upon another increase in the NaCl feed solution concentration to 35 mM, the initial flux for the PBI membranes was 5.30 LMH, the final flux was 5.41, and flux recovery was 5.41 LMH (61.7%). Again, for PVA-alkyl-AqpZ membranes, flux values remained not significantly different from previous solutions with an initial flux of 6.73 LMH, final flux of 6.47 LMH and flux recovery of 6.33 LMH (93%). For 100 mM NaCl feed solution, PBI membranes showed an initial flux of 4.98 LMH, a final flux of 5.10 LMH and a flux recovery of 5.60 LMH (63.8% flux). For this this concentration of NaCl, the initial flux of PVA-alkyl-AqpZ membranes decreased slightly 6.10 LMH, a final flux was 5.90 LMH and flux recovery of 5.95 LMH (88.3%).

While flux declines during filtration were lower and flux recoveries higher with the PVA-alkyl-AqpZ membranes, flux values after the final recovery were approximately the same as PBI membranes; however, the greatest advantage of the PVA-alkyl-AqpZ membranes seems to be with respect to sodium chloride rejection, as shown in Figure 17. AqpZ-PVA-alkyl membranes showed higher rejections for the solutions as compared to PBI membranes. Unmodified PBI membranes showed 19% rejection during filtration of the 3.4 mM NaCl solution, and as the

NaCl concentration went up to 100 mM, the rejection decreased to 5.3%. On the other hand, PVA-alkyl-AqpZ membranes showed a much better rejection of 73.5% for 3.4 mM feed solution of NaCl and 36% for 100 mM NaCl.

Figure 17. Sodium chloride and calcium chloride filtration rejection.

After NaCl filtration and the final flux recovery, CaCl₂ solutions of five different concentrations were filtered through the membranes (flux declines and recoveries are shown in Figures 12 and 13). For a 3.4 mM CaCl₂ feed solution, PBI membrane had an initial flux of 4.95 LMH, a final flux of 5.10 LMH and a flux recovery of 6.33 LMH (72.2%). PVA-alkyl-AqpZ membrane displayed an initial flux of 5.94 LMH, a final flux of 5.85 LMH and flux recovery of 6.58 LMH (97.6%) for the same solution. This was followed by filtration of 10 mM CaCl₂ solution. For PBI membranes, the initial flux was 4.69 LMH, final flux of 4.65 LMH and flux recovery of 5.97 LMH (68%), while for PVA-alkyl-AqpZ membranes, these were 6.40 LMH, 6.20 LMH and 6.03 LMH (89.5%), respectively. After that, the concentration of the feed solution was increased again to 20 mM CaCl₂. PBI membranes gave initial flux of 4.92 LMH, final flux

of 4.56 LMH and flux recovery of 6.02 LMH (68.6%), while PVA-alkyl-AqpZ membranes displayed 6.01, 5.80 LMH and 5.56 LMH (82.5%), respectively. For a feed solution of 35 mM CaCl_2 solution, the initial flux for PBI membrane was 5.39 LMH, final flux was 4.71 LMH and flux recovery was 5.76 LMH (65.7%). For the same solution, PVA-alkyl-AqpZ membranes had an initial flux of 5.47 LMH, final flux of 5.31 LMH and flux recovery of 4.77 LMH (70.8%). When feed concentration was increased to 100 mM of CaCl_2 , the initial flux for PBI membranes was 4.63 LMH, the final flux of 4.36 LMH and flux recovery of 5.36 LMH (61.1%), while PVA-alkyl-AqpZ membranes had an initial flux of 4.42 LMH, final flux of 4.28 LMH and a flux recovery of 5.93 LMH (88%). Figure 17 also shows CaCl_2 salt rejections. For CaCl_2 salt solutions of low concentration (3.4 mM), the maximum salt rejection observed for PBI membranes was 24.3% while for PVA-alkyl-AqpZ membranes was 76.2%. As the concentration of CaCl_2 feed solution was increased to 100 mM, the rejection for PBI membranes dropped to 8%, while that for PVA-alkyl-AqpZ membranes was 39.6%. Therefore, as with NaCl filtrations, the final rejection of salts by aquaporin membranes remained significantly higher than for PBI membranes. It is important to note here that salt rejections observed for both the salt solutions for AqpZ-PVA-alkyl modified PBI membranes were not 100%. This might be because of the feed solution going around aquaporins that are embedded in the PVA-alkyl matrix.

4.4 Morphological analysis:

Figures 18(a), (b) and (c) show the SEM images of cross sectional areas of clean unmodified PBI, PBI membranes with only PVA-alkyl added and PVA-alkyl-AqpZ modified PBI membranes, respectively, at a magnification of 50 μm before filtration. The cross sectional area of PVA-alkyl modified PBI membrane showed the addition of an extra layer added to the surface of PBI membrane due to the attachment of PVA-alkyl. The thickness of the PVA-alkyl

layer was approximately 10 μm , and it can be seen in Figure 18(b). Similarly, in case of PVA-alkyl-AqpZ modified PBI membranes (Figure 18(c)), a PVA-alkyl-AqpZ layer of thickness of approximately 10 μm was added to the PBI membrane. It is important to note that the layer of PVA-alkyl was added to both sides of the membrane. Unmodified PBI membranes showed more porous structures as compared to modified PBI membranes. Since the modification of the PBI membranes was not controlled to only surfaces, some of the modification might have occurred inside the pores of the PBI membranes resulting in the less porous structure of modified membranes observed. This is in agreement with the lower pure water flux values observed for the PVA-alkyl-AqpZ membranes, shown in Figures 15 and 16 (during precompaction).

Figures 18(a), 18(b) and 18(c) SEM images of cross sectional areas of unmodified PBI, PVA-alkyl and PVA-alkyl-AqpZ modified PBI membranes.

Figures 19(a) and (b) show SEM images of the clean surfaces of both unmodified PBI and PVA-alkyl-AqpZ modified PBI membranes, respectively, at a magnification of 10 μm . The surface of unmodified PBI membranes displayed a smoother texture as compared to that of PVA-alkyl-AqpZ modified PBI membranes. This was likely due to the addition of the extra layer of PVA-alkyl containing AqpZ. On the other hand, Figures 20(a) and (b) show SEM images of

surfaces of both unmodified PBI and PVA-alkyl-AqpZ modified PBI membranes, respectively, at a magnification of 10 μm after filtration. The surface of unmodified PBI membranes showed a rougher texture as compared to PVA-alkyl-AqpZ modified membranes. The reason is hypothesized to be due to the accumulation of foulants on the surface of unmodified PBI membranes. PVA-alkyl-AqpZ modified membranes showed better antifouling characteristics than unmodified PBI membranes. This might be due to addition of aquaporins in PVA-alkyl layer added on the surface of PBI membranes. The addition of aquaporins likely helped during backwash by providing more water channels for water to pass through the membrane resulting in higher removal of foulants. This agrees with flux recoveries shown in Figures 15 and 16 as well as Table 3.

19(a)

19(b)

Figure 19(a) and (b) SEM image of surface area of unmodified PBI membrane and PVA-alkyl-AqpZ modified PBI membrane respectively

Figure 20(a) and 20(b) SEM image of surface area of unmodified PBI membrane and PVA-alkyl-AqpZ modified PBI membrane respectively after filtration

5. Discussion:

While the initial water flux values of PVA-alkyl-AqpZ membranes were slightly lower than unmodified PBI membrane, the flux decline was less when using PVA-alkyl-AqpZ membranes, and the final flux after 140 hours of flux experiments was higher for PVA-alkyl-AqpZ membrane as compared to PBI membrane. Therefore, it is believed that the presence of aquaporins helped to increase and maintain the water flux through the membranes, and the addition also led to better flux recoveries. Aquaporins are bidirectional, hence during backwash, aquaporins might have also helped in the increased water flux through the membrane and more removal of reversible fouling resulting in better flux recovery of the membrane. The embedment of AqpZ in the modified membranes is hypothesized to have facilitated water transport through the membrane and brought about an increase in the water flux, which agrees with previous studies [44, 45]. Furthermore, the increase in the hydrophilicity of the membrane after modification (Figure 11) could also be associated with the increase in the water being transported

across the membrane [46]. Aquaporins selectively filter water molecules through their porous structures while preventing the passage of ions and other solutes (Figure 1). Thus they form channels for water molecules to pass through them and in turn increase the water flux and the flux recovery of the PVA-alkyl-AqpZ membrane.

It is hypothesized that the presence of aquaporins in the PVA-alkyl-AqpZ membranes acted as water channels and prevented the flow of ions and salt solutions that flow along with water molecules. This helped in the increase in water flux and rejection of salt solutions of different concentrations. Previously, Chung et al. [45] prepared Aqp-modified cellulose acetate membranes functionalized with methacrylate end groups. Aqp was introduced into the membranes using vesicle rupture of triblock (ABA) copolymer vesicles and UV polymerization. Maximum salt rejection obtained with NaCl salt solution of concentration 3.4 mM was 32%. Fane et al. [47] prepared a thin film composite aquaporin-based biomimetic membrane by interfacial polymerization method. A microporous polysulfone substrate was soaked with an m-phenylene-diamine (MPD) aqueous solution which contained AQP-based proteoliposomes. The substrate was then exposed to trimesoyl chloride (TMC) to form a three-dimensionally crosslinked polyamide layer with the proteoliposomes embedded. Maximum salt rejection obtained for NaCl salt solution of concentration 10 mM was $96.3 \pm 1.2\%$ at 5 bar. Also, lipid bilayer was attached to commercial NF-270 membrane under conditions suitable for AqpZ survival and AqpZ was incorporated into the lipid bilayer subsequently [48]. Maximum salt rejection obtained for NaCl salt solution of concentration 1 mM was 20% at 1 bar. In this study, PBI membranes modified with PVA-alkyl-AqpZ showed 73.5% rejection for 3.4 mM NaCl feed solution, whereas for 10 mM NaCl feed solution, the salt rejection was 68% using nanofiltration

membranes. Salt rejections observed in this study were not 100% likely because some of the feed solution might have leaked around the aquaporins embedded in the PVA-alkyl matrix.

6. Conclusions:

Aquaporins have received worldwide attention due to their potential to significantly improve water flux across synthetic membranes. Aquaporins provide more water channels while rejecting everything else that is present in the feed including ions and dissolved salts. However, under high pressure, aquaporins can get chemically altered and not function properly, resulting in the failure of the assembly of synthetic membrane and the protein. In this study, aquaporins were protected with gum arabic and dispersed into a PVA-alkyl layer, which was crosslinked to a synthetic PBI membrane backbone using carbodiimide chemistry to minimize the disruptive effects of high pressure. Membranes modified with aquaporins showed higher water fluxes, better flux recoveries and greater ion selectivities as compared to unmodified synthetic membranes. The biomimetic membranes presented here showed promising performance with respect to lower fouling and ion rejection.

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