

**Title.** High-performance biologically inspired membranes for water treatment

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### **Problem and Research Objectives, Methodology, and Principal Findings and Significance**

This project relies on the idea of combining the ultra-efficient functioning of biological molecules with the productivity of synthetic membranes. Aquaporin is a bidirectional water channel protein present in the cells, which regulates the flow of water in and out of the cells. It allows water molecules to pass through in single file while the transport of ions, salts is prevented by electrostatic tuning mechanism of channel interior. Larger molecules are rejected due to size exclusion. Aquaporin is well-known for its potential to form biomimetic membranes.

#### *Problem –*

Challenges involved in the incorporation of the Aquaporin proteins in the membranes limit their applicability. One challenge is to attach aquaporins to membranes without chemically altering or damaging the aquaporin during chemical binding. The second challenge is to design and prepare an assembly that will allow biomimetic membranes with aquaporins to sustain high hydraulic water pressure gradients without losing their integrity and performance.

#### *Research objective –*

The objective of the project is to make a new class of biomimetic nanofiltration membranes by modifying their surface and making them chemically and mechanically stable. We propose that aquaporins can be treated with polysaccharides to protect them, and then can be embedded in amphiphilic PVA-alkyl matrix. The PVA-alkyl with embedded aquaporins will be used as the nanofiltration membrane active layer.

#### *Methodology –*

##### 1. PBI membranes casting -

The dope polymer that was used to cast 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 range of pH. PBI membranes are hydrophobic. They are strong but brittle. The structure of PBI molecule is shown in Figure 1. Within the imidazole group of PBI, the heterocycle has two nitrogen atoms. One is attached to the hydrogen atom as a site to form hydrogen bond while other nitrogen has a lone pair, which can act as a proton acceptor.

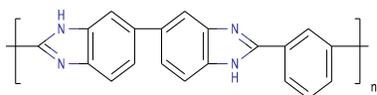


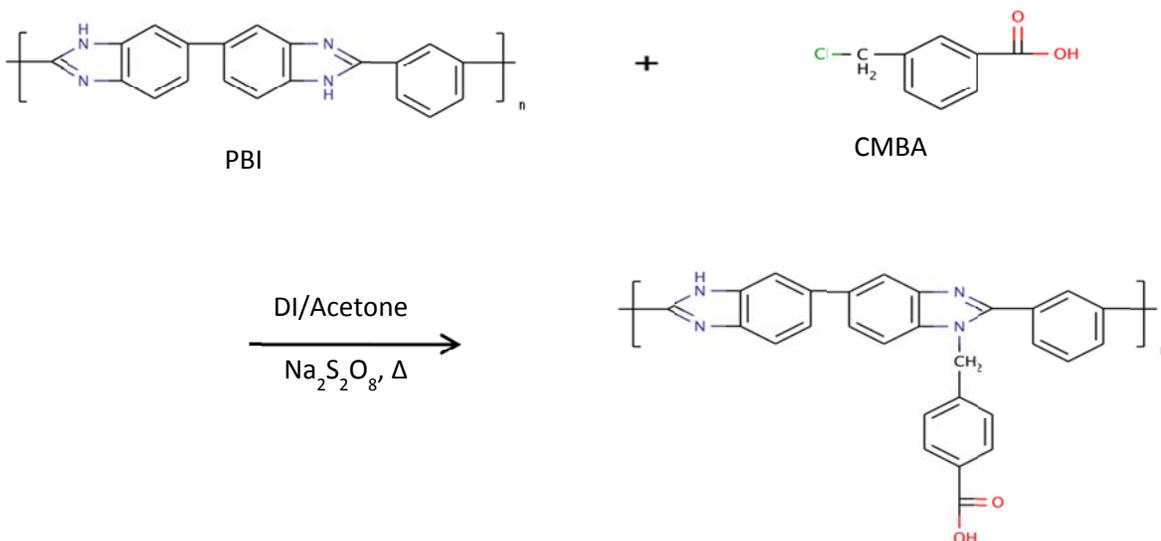
Figure 1. PBI molecule structure

The solvent used to make the dope solution was N, N-Dimethylacetamide. Commercially 26% w/w dope solution is available which contains 26% PBI polymer, 72% N, N- Dimethylacetamide (DMAc) and 2% Lithium chloride (LiCl). LiCl served the function of preventing PBI polymer from phasing out of the solution. Hence, it imparts long shelf life to the solution. The dope solution was diluted to 21% PBI by adding solvent to it, and the solution was sealed with parafilm in order to prevent the air bubbles from being trapped inside the solution affecting its homogeneity. Because of very high viscosity of the solution, the solution needed to be kept in the sonicator and degassed for 2 days in order to ensure complete mixing of the solvent and the solute and make the solution homogeneous. After sonication, the solution was allowed to come to room temperature and then the solution was ready to make the membranes. The membranes were cast using 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. The non-solvent phase that is used in this process is water.

A casting knife or doctor's blade was used to make flat sheet membranes. A clean glass mirror was used as a hydrophobic surface to make sure that the solution would not become stuck to the membrane. 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 $\mu$ m and 200 $\mu$ m. A water coagulation bath was used to induce phase inversion subsequent pore formation within the membranes. Once the phase inversion had taken place, the membrane came out of the surface of the membrane. The membrane was thoroughly washed with water and kept in a 50/50 glycerol-DI water solution. Glycerol was added to DI water due to possibility of water evaporation. If the membranes get dry, they become brittle and susceptible to breakage. The membranes were kept in the solution at least one day before they were analyzed.

## 2. Surface activation of the membranes –

The PBI membranes prepared are hydrophobic. Hence, for subsequent modifications, the surface of the membranes needed to be activated. It was achieved by way of reaction of highly reactive chlorine atom of 4-chloromethyl benzoic acid (CMBA) with the secondary amine group in the imidazole ring of repeat unit in PBI backbone. CMBA added a carboxylic group to the surface. This served two purposes: 1) It imparted negative charge on the membrane surface 2) It acted as a platform for subsequent functionalization of the membrane. There are two secondary amine sites in PBI molecule. Hence, after the reaction, carboxylic group was added on both sites on the molecule. For simplicity, reaction at only one site is shown in the diagram below. Overall reaction is as follows:



For the reaction, 1 wt% solution of sodium persulphate in water was prepared. Sodium persulphate was used as a free radical initiator for the reaction. 200 ml DI water was 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. The temperature was set at 40°C. 2 membranes were added to the solution and it was made sure that the membranes are 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 all CMBA was dissolved. Then acetone/ CMBA solution was slowly added to the beaker on hot plate and stirring was done at a slow rate. This was done in order to prevent the precipitation of CMBA as it is insoluble in water. After complete addition of the solution, it was covered with parafilm. The final solution had 50/50 mixture of both solutions. The temperature of the solution was kept at 40°C and it was stirred for 24 hours. The temperature was chosen to keep all the reactants in solution and prevent the evaporation of CMBA. Once the reaction was done, the membranes were washed with copious amounts of DI water to remove excess sodium persulphate and placed in glycerol/water bath as soon as possible.

### 3. Preparation of PVA–alkyl:

PVA-alkyl is polyvinyl alcohol carrying long alkyl side chains. It is amphiphilic as it has both hydrophilic and hydrophobic elements present in it. PVA has hydrophilic properties and long alkyl chains account for the hydrophobicity of the molecule. PVA-alkyl was prepared in the lab in two steps.

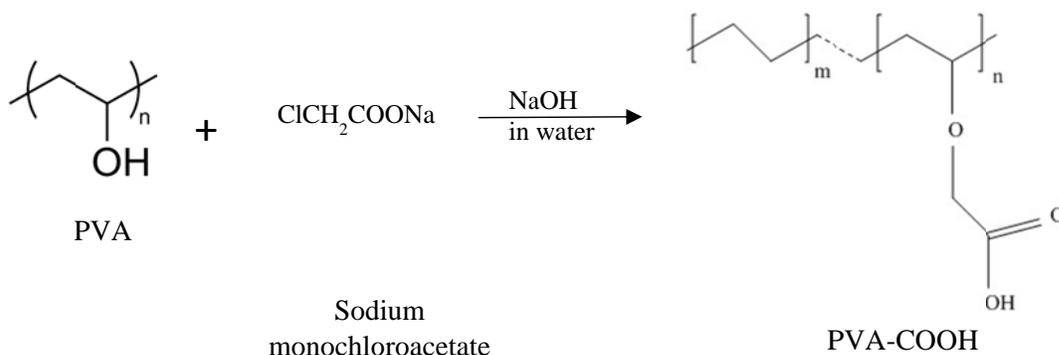
#### a) Preparation of carboxy-methyl PVA (PVA-COOH):

Initially, 50mL water was taken in a 100 mL beaker and 1g PVA was added to it and the beaker was kept on a hot plate. The temperature of the water was maintained at 70°C. The mixture was stirred for 1 hour continuously with stir rod to prevent PVA from sticking to the bottom of the beaker. The stirring was continued until PVA was completely dissolved in water. The solution was transferred to a 500mL beaker and 50g sodium monochloroacetate was added to it. The solution was then covered with aluminium foil and was incubated at 4°C for 24 hours.

After that, 42mL water was taken in a 100mL beaker and 42g sodium hydroxide was added to it. The mixture was stirred until sodium hydroxide was completely dissolved in it.

NaOH/water solution was added to the incubated solution and it was kept stirring at room temperature for 24 hours. Then it was neutralized using hydrochloric acid. For that, 6M solution of hydrochloric acid with water was prepared in separate beaker and benchtop pH meter was used to continuously monitor the pH of the final solution. This neutralized solution was dialyzed against deionized water. The molecular weight cut-off for the dialysis was 12-14 kDa. The dialysis tubing used was soaked in water for 3 hours in order to open it and fill it with the solution and was sealed with dialysis locking membrane clamps. A 2000mL beaker with a stir bar in it was filled with DI water. The tubing was attached to foam to keep it suspending in water. 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. Remaining solution in the tube was taken out and stored in another beaker.

Then, it was deionized using ion-exchange resins. DOWEX 1X8 was used for negatively charged ions and DOWEX 50WX8 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 as follows:



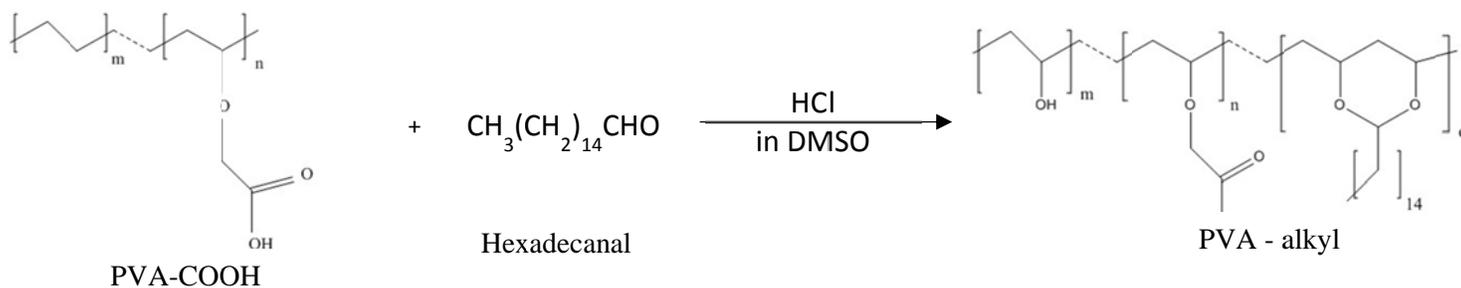
#### b) Preparation of PVA-alkyl:

First part 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. 11.2mL dichloromethane was taken in a 50mL beaker with stir bar in it. 0.95g celite, 0.95g pyridinium chlorochromate and 0.5 g 1-hexadecanol were added to the beaker. The addition was done under fume hood. The solution was sealed with aluminium foil and kept on stir plate. It was stirred for 6 days at room temperature. After that, the reaction mixture was diluted by adding 40mL diethyl ether to it. Florisil columns were used to remove excess celite, pyridinium chlorochromate and 1-hexadecanol. Then the mixture was evaporated. Hexadecanal was obtained as a white solid.

PVA-COOH obtained after first reaction was dissolved in DMSO. The mixture was prepared in 100mL beaker with stir bar. Hexadecanal and 200µm of 12M hydrochloric acid were added to the solution and it was kept on hot plate. The temperature was maintained at 70°C

and kept stirring for 25 hours. The reaction mixture was extracted with diethyl ether. The mixture was then neutralized with 1M sodium hydroxide.

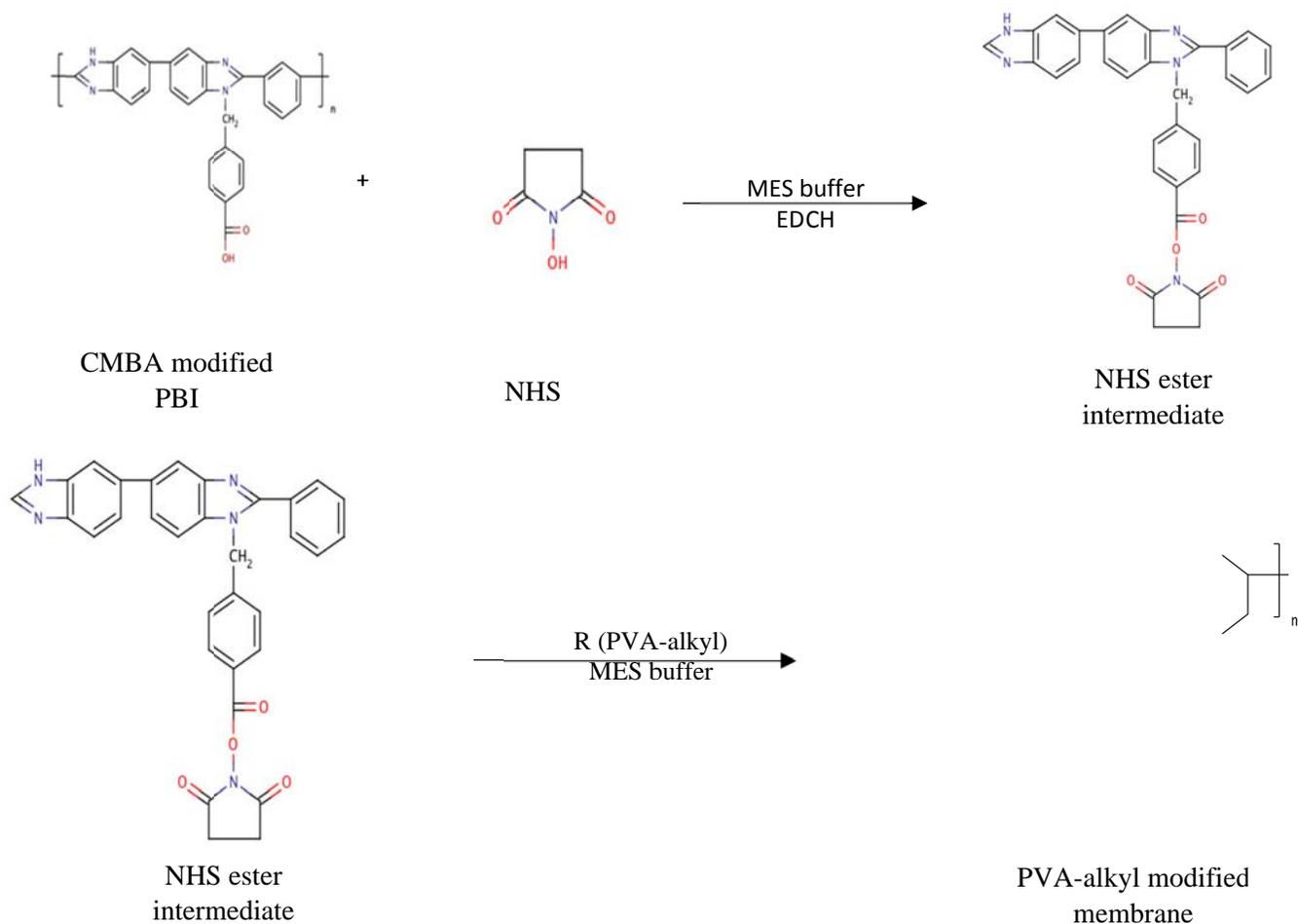
Neutralized solution was dialyzed against DI water using dialysis tubing of 12-14kDa. Same procedure as described above was used for dialysis. Then the solution was desalted with ion exchange resins mentioned above and lyophilized using a freeze dryer for 3 days. PVA-alkyl was obtained as a white solid with yield of 49%. Final weight of the product was 0.22g. The overall reaction for the second step of the process is as follows:



#### 4. Surface modification of PBI membrane using PVA-alkyl:

PVA-alkyl was attached to the membrane using carbodiimide chemistry. Carbodiimide chemistry is used for the reaction between carboxylic group and a nucleophile. The nucleophile that is used in this case is hydroxide group present in PVA-alkyl molecule. For the reaction, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDCH) and N-hydroxysuccinimide (NHS) were used. The reactions were performed in 2-(N-morpholino) ethanesulfonic acid (MES) buffer.

400mL water was taken in a 500mL beaker and 7.85g MES buffer was added to it. The solution was stirred until MES was completely dissolved in water. 11.75g NaCl was added to the solution and stirred until dissolved. The solution was titrated to pH 6 using NaOH. Benchtop pH meter was used to monitor pH with addition of NaOH. 0.23g NHS and 0.153g EDCH were added to the solution and stirred well. CMBA modified membrane was added to the solution and was stirred for 15 minutes. After 15 minutes, the reaction mixture was titrated to pH 7 using NaOH as soon as possible. PVA-alkyl was added to the solution and it was kept stirring for 24 hours. After 24 hours, membrane was taken out of the solution and rinsed well with DI water and stored in a beaker filled with DI water. The membrane was stored for 24 hours before using it for analysis. Overall reaction is as follows:



### *Preliminary Results –*

Virgin PBI and CMBA-modified membranes were tested using Fourier Transform Infrared Spectroscopy in attenuated total reflectance mode (FTIR-ATR). The difference in the FTIR curves of both the membranes can be seen in Figure 2. The appearance of peak 1 in the curve for the CMBA-modified membrane was associated with the presence of a C-O bond in the molecule. Peak 2 corresponded to carbonyl group, while peak 3 was associated with the presence of an O-H bond. Secondary amine groups, present in the PBI molecule, displayed peaks in the same region as carbonyl groups ( $1620\text{-}1640\text{ cm}^{-1}$ ).

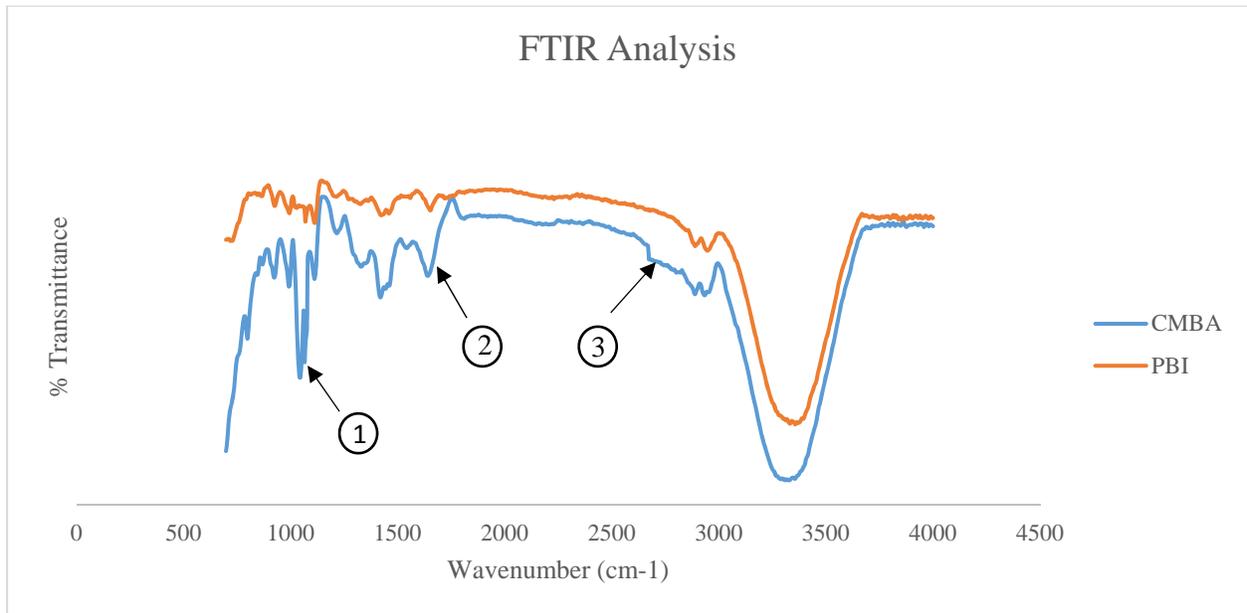


Figure 2 FTIR Spectra of virgin PBI and CMBA-modified membranes

Virgin PBI membranes were analyzed to determine the flux decline during operation. The decrease in the permeate flux over the time period of operation was measured using membranes prepared using 21% dope solution. A 10-mL Amicon dead-end filtration cell was used for this purpose. A constant pressure of 70 psi was maintained for all the tests and the membranes were supported with a filter paper. The time required to collect 2mL of permeate was measured, and the flux was calculated. The first step of filtration was precompaction, during which deionized water was filtered through the membrane, and the flux decline with time was measured. Filtration experiments were also performed using bovine albumin serum (BSA) and lipase protein solutions after precompaction. The concentration of feed for both solutions was kept 10 mg/L. After BSA filtration, the membrane was backwash for one hour. For this purpose, the membrane was flipped and then DI water was filtered through the membrane under the same operating conditions as above. Flux recovery of the membrane was measured after backwash. Flux decline is shown in Figure 3.

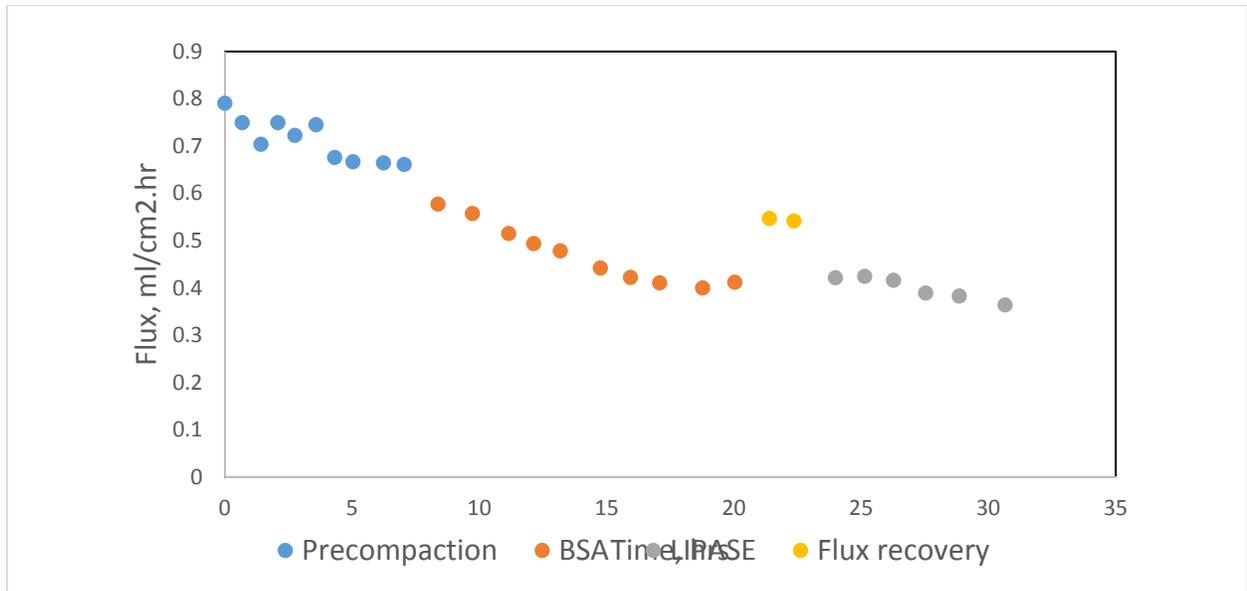


Figure 2 Flux analysis of PBI membrane

*Ongoing Studies –*

The following tasks are underway:

- Produce AqpZ, treat it with GA, and incorporate it in amphiphilic PVA-alkyl.
- Use chemical surface modifications to polymerize the AqpZ-GA in PVA-alkyl to the PBI membrane to form biomimetic membranes.
- Characterize the biomimetic membranes chemically and structurally.
- Characterize the biomimetic membranes with respect to flux and solute rejection.