

Highly Hydrophilic Ultrafiltration Membranes Synthesized From Acrylic Acid Grafted Polyethersulfone For Downstream Processing Of Therapeutic Insulin And Cobalamin

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Abstract

The significance of the present study is to purify small therapeutic biomolecules such as urea, cobalamin, and insulin of molecular weights below 10 kDa through surface grafted polyethersulfone (PES) ultrafiltration (UF) membranes. The membranes were synthesized by adding an additive of 6 kDa polyethylene glycol (PEG) and grafted with acrylic acid (AA) by varying the concentrations from 2 to 6 wt. % under UV-induced photocatalytic reaction. These membranes were characterized by various tools such as Scanning electron microscope ((SEM), Fourier transforms infrared spectra (FTIR), thermogravimetric analysis (TGA), and contact angle for membrane morphological, structural, thermal stability, and hydrophilicity, respectively. The degree of grafting and their MWCOs of the indigenous membranes were analyzed using various molecular weight solutions of PEG. After PEG doping, the PWF of the membrane was enhanced to $41.50 \text{ L m}^{-2} \text{ h}^{-1}$ for PES [6+] [0], and a similar trend was also observed for the PEG doped PES grafted membranes. From the experimental results, the synthesized membranes of additive loaded with 5 and 6 wt. % AA grafted PES reject 90 % of the insulin and cobalamin. The results are found to be in correlation with the MWCO values of these membranes ranging from 1 to 10 kDa. From the overall characterization and experimental observations, it can be confirmed that these indigenously synthesized flat sheet grafted membranes showed excellent permeabilities and higher % rejection towards the therapeutic biomolecules.

Introduction

Biomolecule purification is an important research frontier today, as the world market of proteins and their production is growing tremendously. For effective purification of the biomolecules, the most efficient separation techniques have to be focused. An ideal biomolecule purification strategy is achieved in few steps through easy and low-cost separation techniques, which have a high demand for mass production [1][2]. The membranes used in the separation process can conduct without chemical treatment at an ambient condition where the membrane act as a selective sieving barrier. Hence, membrane filtration is used to separate biological components such as amino and organic acids, salts, proteins, sugars, peptides, and vitamins, growing in the biotechnological industries [3]. Ultrafiltration (UF) is a separation process extensively used as a preferred technique and an alternative to size exclusion chromatography for protein concentration and buffer exchange [4]. UF membranes have a pore size in the range of 10–100 nm, especially for high retention of macromolecules and protein [5].

UF membranes can be prepared from various synthetic polymers, have high thermal stability, chemical resistivity, and restrict somewhat harsh cleaning chemicals [6]. Polyethersulfone (PES) is a widely used UF membrane material because of its high rigidity, creep resistance, moderate hydrophilicity, good thermal and dimensional stabilities [7]. The choice of the membrane is usually guided by its molecular weight cut-off (MWCO), which defines the capability of the membrane where it could reject 90 % of the equivalent molecular size of the protein. PES-UF membranes, either hollow fiber or flat sheet, were extensively studied to separate proteins [8–10].

Various researchers have been synthesizing the polymeric membranes by adding additives to enhance the process efficiency of UF membranes. Li et al., 2014 [11] prepared blend membranes of PPTA and poly(vinylidene fluoride) (PVDF) by in situ polycondensation using various additives such as PEG, inorganic salt of lithium chloride (LiCl), and Tween-80 surfactant were additives for the adequate performance of the membrane. Otitoju et al. [12] reviewed the blending of PES with different additives such as polyvinylpyrrolidone (PVP), polyethylene oxide (PEO), PEG, and inorganic nanoparticles to improve the hydrophilicity and performance in terms of flux, solute rejection, and reduction of fouling. As per Rata et al., [13] polysulfone (PSf) and cellulose acetate UF membranes (UF1-UF36) were prepared by phase inversion method using 1000, 2000, and 4000 Da PEG additives for separation of whey protein. Lin et al. [14] synthesized a novel UF membrane by blending brominated poly(phenylene oxide) (BPPO) with its additive of quaternary phosphonium derivative (TPPOQP-Br) through the phase inversion method for suitable rejection property with high water flux.

On the other hand, the surface modification of the membranes plays a vital role in the separation process, especially for developing the selective separation of the solute molecules through molecular sieving [13]. Akbari et al. [15] developed NF membranes from PSf UF membrane surface modification with sodium p-styrene sulfonate (NaSS) and [2-(acryloyloxy)ethyl]trimethyl ammonium chloride (AC) by sensitive UV-photo induced grafting technique. These membranes were successfully removed six different dyes according to their charge and produced reused water in the process house. Shi et al. [16] fabricated methacrylic acid (MAA) onto polyethersulfone (PES) using a chemical initiator of benzoyl peroxide (BPO) by facile graft polymerization. PES membranes were prepared using six different monomers such as [2 neutral (N-2-vinyl pyrrolidone (NVP), 2-hydroxyethyl methacrylate (HEMA)), 2 weak (carboxylic) acids (acrylic acid (AA), 2-acrylamidoglycolic acid (AAG)), and 2 strong (sulfonic) acids (3-sulfopropyl methacrylate (SPMA), 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS))] as grafting agents and Taniguchi and Belfort [17] compared their performance. Moreover, the AA grafted PES exhibited high protein fluxes and BSA rejection with good compatibility for harsh cleaning chemicals and durability compared to the unmodified membranes by Blanco et al. [17]. The ideal UF membrane shows a higher separation factor and permeability studied by Metha and Zydney [18] and Cramer et al. [19]; such membranes are currently unavailable in the market. Hence, this study aims to prepare new membranes of high selectivity and enhanced throughput for utilization in biotechnology.

The present study mainly focuses on the purification of semi-synthetic small therapeutic biomolecules of size below 10 kDa by UF membranes. There are several chromatographic methods in literature to purify such molecules. However, these methods have two limitations, i.e., expensive nature and difficulty in scale-up the process. Therefore, this paper addresses the preparation of PEG additive added and AA grafted synthetic PES-UF membranes to purify small biomolecules below 10kDa. These porous UF membranes were synthesized using PES polymer with desired modification by adding PEG 6 kDa additive and grafted with various concentrations of AA (2 to 6%) on the membrane surface. These indigenous membranes were characterized by FTIR, SEM, TGA, and contact angle before and after membrane modification. The degree of grafting and MWCOs of various concentrations of AA grafted PES membranes were determined in terms of pure water flux (PWF). These membranes were applied to the

separation of various biomolecules such as Urea, cobalamin, and Insulin, based on their size exclusion. The separation specificity and improved biocompatibility of these membranes are discussed in this manuscript.

Materials And Methods

2.1. Materials

The polymer PES was procured from Solvay, Vadodara, India, to synthesize flat sheet membranes. The solvent N-methyl-2-pyrrolidone (NMP), a crystalline compound of bismuth subnitrate (BiONO_3) and hydrochloric acid (HCl) with 35–38 % concentration, were purchased from Sd Fine Chemicals, Mumbai, India. The molecular weight cut-off of the synthesized membrane was determined using PEG with different molecular weights, i.e., 600, 1000, 2000, 4000, 6000, 10,000, and 20,000, along with AA were supplied from Sigma-Aldrich Chemical Private Limited, USA. Urea, a therapeutic biomolecule, was purchased from LOBA Chemie Pvt Ltd., Mumbai, India. Analytical grade purity of potassium iodide (KI) and sodium hydroxide (NaOH) purchased from Molychem, Mumbai, India provided. Human mixtard and Cobalamin insulin injections were purchased from NOVO Nordisk India Pvt Ltd., Bangalore, and Mankind Pharma Ltd., New Delhi, India. The deionized water with TDS < 2 ppm was used for sample preparation and experimental studies in the laboratory using the RO membrane cascade system.

2.2. Methods

2.2.1. Synthesis of flat sheet membranes

The UF flat sheet membranes were synthesized and analyzed for their pore size and MWCOs. The method used in this study for the fabrication of the membrane was by phase inversion. Initially, the honey-like viscous polymer solution was prepared by dissolving 17 % of PES in 83 wt % of the NMP solvent to synthesize the pristine membrane. On the other hand, 8 wt % 6,000 Da molecular weight of PEG solution was prepared using NMP solvent and stirred continuously for 30 mins at 900 rounds per minute (rpm) under ambient conditions until it dissolves completely. Then the desired amount of polymer was added into that PEG solution was provide in Table 1. The obtained solution was stirred for another 2 h at 45 ± 2 °C using a magnetic stirrer to get the homogeneous solution, further degassed to make it bubble-free. The obtained polymer solution was cast on polyester (PE) non-woven fabric support using a doctor's blade to the desired thickness. While casting the solution, the air gap between the blade and the support fabric was maintained at such a distance to get the resultant membrane of 60 μm polymeric coating, measured using a micron gauge. After casting the membrane on the support, it was transferred immediately into a non-solvent bath, where type-II water was used as non-solvent and kept for 30 min to obtain desired porous membrane.

Table 1
Various combinations of PES membranes and their % of AA grafting

Membrane	PES Wt %	NMP Wt %	PEG Wt %, (molecular weight)	AA Wt %
Pristine PES	17	83	-	-
PES[0][6]	17	83	-	6
PES[6+][0]	17	75	8 (6 kDa)	-
PES[6+][2]	17	75	8 (6 kDa)	2
PES[6+][3]	17	75	8 (6 kDa)	3
PES[6+][4]	17	75	8 (6 kDa)	4
PES[6+][5]	17	75	8 (6 kDa)	5
PES[6+][6]	17	75	8 (6 kDa)	6

2.2.2. Membrane modification by UV- induced graft polymerization

Initially, 6 wt % of AA, a weak acid considered a monomer, was selected to grafting the membrane. After the synthesis of membranes through phase inversion, washed thoroughly with type-II water to remove excess residual solvents from the matrix. These membranes were subjected to grafting by immersing them into an AA solution under 265 nm UV light at 50 cm distance for about 40 min as the monomer gets attached to the PES polymer and forms polyacrylic acid around the pores, which leads to a decrease in the membrane pore size. The reaction mechanism of UV-induced AA graft polymerization at the pores of the PES support was provided in Scheme 1. The prepared membranes were denoted in the order of PES and used additive PEG to prepare the membrane represented as the molecular weight (kDa), followed by the percentage of AA used for grafting. For example, the membrane synthesized by PES polymer solution with additive PEG 2 kDa and grafted with 6 % AA represented as PES [2+] [6]. The combinations of PES membrane with PEG additive and AA grafting polymers in terms of weight percentage used in this present study were provided in Table 1.

2.2.3. Preparation of Standard solution of insulin

The insulin stock solution of 1000 ppm was prepared by dissolving 1 gm in 1 L of 0.01 M HCl solution. It was further diluted into various concentrations ranging from 20 to 100 ppm with a step of 20 ppm. The known concentrations of standard solutions were kept aside for about 12 h to attain uniform concentrations throughout the solution [20][21]. The prepared solutions were employed to calibrate the plots by UV-VIS spectrophotometer at a maximum of 310 nm wavelength.

2.2.4. Preparation of standard solution of urea

A stock solution of 1000 ppm urea was prepared by adding 1 g of urea in 1 L Type-II water. Various concentrations of standard urea solutions, i.e., 20, 40, 60, 80, and 100 ppm, were prepared from the stock

and analyzed the absorbance values using UV-VIS spectrophotometer to generate the calibration plots [22].

2.2.5. Reagent preparation for urea analysis

The reagent solution of aldehyde-ethanol was prepared by mixing 4 g of *P*-dimethyl amino benzaldehyde (DMAB) with 200 ml of 95% Ethanol. The concentrated HCl solution of 40ml was added to the obtained aldehyde-ethanol solution, which further turns into a yellowish-green color. This reagent was used to detect urea in the solution through a UV-VIS spectrophotometer [23].

2.2.6. Sample preparation for urea analysis

Before analyzing the standard urea samples using UV-VIS spectrophotometer, 1 ml of the sample was added to the 10 ml of the reagent in a 25 ml volumetric flask, where deionized water was used further to make up the solution. A yellowish-green color solution of the obtained sample was subjected to UV-VIS spectrophotometer against a blank solution comprising 10 ml of reagent diluted with distilled water with 15 ml in 25 ml volumetric flask. The sample's absorbance was noted at a maximum wavelength of 420 nm [24].

2.2.7. Preparation of standard solution of cobalamin (vitamin B12)

Cobalamin standard solution of 500 ppm was diluted to obtain 20 ppm to 100ppm solutions using deionized water. These diluted solutions were used as standard solutions to prepare the standard calibration chart with UV-VIS Spectrophotometer against deionized water as blank. The maximum absorbance of the samples was recorded at a wavelength of 435 nm [25] [26].

2.2.8. Preparation of bismuth subnitrate reagent (dragendorff) for PEG analysis

Dragendorff reagent was prepared to determine the various PEG concentrations. Initially, 1.6 g of BiONO_3 dissolved in 20 ml of (35 to 38 %) HCl of 50 ml volumetric flask and make up the solution diluting with type II to obtain bismuth subnitrate solution. On the other hand, 20 g of KI powder dissolved in 50 ml distilled water, and the prepared KI solution was stored in a brown volumetric flask. Further, 5 ml of each solution was taken into the 100 ml of brown volumetric flask and diluted with type II water up to mark [27].

2.2.9. Preparation of various concentrations of PEG solution

Initially, various molecular weights of PEG standard solutions i.e., 600, 2000, 4000, 6000, 10,000, and 20,000 with different concentrations of 20, 40, 60, 80, and 100 ppm were prepared in 100 ml volumetric flasks. To determine the corresponding absorbance values of PEG, 1.0 ml of the prepared standard PEG was added to 8 ml of 0.01 M HCl and 1.0 ml of dragendroff reagent. After preparing the reaction mixture, the absorbance of each concentrated solution was determined using a UV-vis spectrophotometer at different wavelengths.

2.2.10. Experimental Set-up and procedure

The experimental laboratory setup for a UF system using flat-sheet membranes is shown in Fig. 1. The system consists of a 1 L capacity feed connected to a flat-sheet membrane module with a membrane

surface area of 90 cm² through 300 gallons per day (GPD) pump. The module another has an exit of permeate and reject streams, where the reject line is directly connected to the feed tank, and the permeate was collected into permeate tank. Pressure gauge and control valves were fixed at the reject line to measure the applied pressure on the membrane module, and the control valve was used to restrict the flow in the system.

Initially, the feed container was filled with 500ml of PEG solution of desired molecular weight and passed across the membrane under 30 psi feed pressure in a cross-flow manner. Before collecting the final permeate, the system permeates and rejects streams recycled for about 30 min to attain a steady state. The feed and permeate samples were then collected every 5 min time intervals to analyze the samples. The UV-VIS spectrophotometer was used to analyze the concentrations of the samples over the standard calibration curve. The same procedure was followed for all the membranes to find MWCO.

2.2.11. Membrane characterization studies

2.2.11.1. FTIR

The Fourier transform infrared spectroscopy (FTIR) was used to analyze the functional groups and grafting of AA on the surface of the PES membrane before and after modification by a Nicolet-740, Perkin –Elmer-283B FTIR spectrophotometer, Boston, MA, USA. The spectrum was measured in the wavelength ranging from 400–4000cm⁻¹ at 25°C.

2.2.11.2. SEM

The surface morphology of pristine, PEG loaded, and AA grafted PES membrane before and after surface modification was analyzed using scanning electron microscopy (SEM) instrument with model Quanta 200 SEM. Before subjecting the samples to SEM, it was fractured under liquid nitrogen followed by a thin layer of gold coating to produce conductive on the surface.

2.2.11.3. TGA analysis

Thermal stability of the unmodified and modified membranes was tested using an SDT Q 600 V20.9 Build 20 analyzer, Japan. The thermal stability of the samples was tested in the temperature range of 25–900°C with a heating rate of 10°C/min under continuous purge gas of pure N₂ flowing at 20 mL/min.

2.2.11.4. Contact angle (CA) measurement

The surface hydrophilicity of pristine, after PEG additive loading and various % of AA, grafted PES membranes were characterized at 25°C using a sessile drop method with a CA analyzer with model Dino-Lite Basic AM211 digital microscope, obtained from Taiwan. The readings were measured when the 3-μL droplet of water was placed on the dried membrane surface within 5 s after dropping to ensure the accuracy of the CA value.

2.2.12. Analysis with UV-VIS Spectrophotometer

Spectro photometry involves both emission and absorption processes in the analysis. The qualitative analysis of the UV-VIS absorption method was based on the fact that the wavelength of the absorbed light depends on the properties of absorbing atoms, ions, and molecules. The calibration curves for the various samples such as PEG of different MWCOs, Urea, vitamin B12, and insulin were analyzed against the respective blank solutions. Before calibration, the samples were scanned from wavelength 900 to 180 nm to identify the maximum absorbance at the wavelength [27].

2.2.13. Mathematical Equations

2.2.13.1 Degree of grafting

The degree of grafting is used calculated the amount of AA grafted on the membrane surface and is estimated as the following equation;

$$\text{Degree of grafting (\%)} = \frac{W_2 - W_1}{W_1} \times 100 \quad (1)$$

where W_1 and W_2 are the weight of the samples before and after grating the PES membrane, respectively.

2.2.13.2 Pure Water Flux (PWF)

The volumetric flow rate of the permeate is measured as a function of time during the UF process. The permeate flux is estimated by accounting for the volumetric flow rate per unit effective area of the membrane, as shown in Eq. (2).

$$\text{Pure water flux (J)} = \frac{Q}{A} \quad (2)$$

Where permeate volumetric flowrate Q and Membrane effective area A

2.2.13.3. Percentage Rejection

Percentage rejection is one of the essential parameters that define the potentiality of the membrane in particle retention. The percentage of rejection is calculated from Eq. (3).

$$\% \text{ Rejection} = \left[1 - \frac{C_p}{C_f} \right] \times 100 \quad (3)$$

Where C_p and C_f the solute concentrations of permeate and feed, respectively.

Results & Discussion

3.1. SEM studies

Morphological studies of the PES membrane before and after adding additive and surface grafting were shown in Fig. 2. The pristine PES membrane without grafting and without any additive resulted in uneven

distribution of pores throughout the surface (Fig. 2a). After adding the PEG 6000 additive to pristine PES, the pore density was increased from 0.86 (pristine) to $2.45 \mu\text{m}^{-2}$ on the surface with uniform distribution throughout the membrane can be seen from Fig. 2b. Various percentages of AA, i.e., 2, 4, and 6 % grafted on (8 % PEG 6000) additive added PES substrates, and surface pores were cured by crosslinking of AA monomer at the pores, which can be clearly seen from Figs. 2c, d, and e. The crosslinking mechanism at the pores was provided in Scheme 1 and further confirmed from FTIR in the following section. This observation increases membrane performance in the separation process by increasing the pore density on the surface PEG and shrinking these pores by AA grafting, which helps develop different MWCO membranes.

3.2. FTIR studies

The FTIR spectra of the pristine PES and modified with acrylic acid surface functional groups can be observed from Fig. 3 (a and b). From Fig. 3a, the pristine PES membrane exhibited the peaks at 1149, and 1320 cm^{-1} represented the symmetric and asymmetric stretching vibration of $\text{O}=\text{S}=\text{O}$ groups, respectively. The peaks at 1437, 1484, and 1576 cm^{-1} were attributed to the aromatic ring C-C stretching vibrations. The ether (C-O-C) group stretching and bending vibrations were found to be around 1009 cm^{-1} and 1239 cm^{-1} , respectively. These functional group characteristic peaks appearance in Fig. 3 confirmed the structure of the PES membrane. The PES membrane surface modification with AA through graft polymerization is confirmed by forming new peaks at 1736, and 2967 cm^{-1} represents the carbonyl (C=O) and hydroxy (-OH) groups stretching vibrations of grafted AA. The characteristic peaks in the range of 1100 to 1400 cm^{-1} shift with the lower intensity from pristine to grafted PES membrane, which means the possible bond formation between the sulfonated functional groups of PES with AA groups [28]. The IR spectrum represents the pristine PES membrane was photochemically modified and formed bonds with AA.

3.3. Contact angle

The contact angle of the pristine, PEG additive loaded and various concentrations of AA grafted PEG loaded PES membranes were measured and provided in Table 2. From Table 2, it can be observed that the PES with PEG loaded membrane (PES [6+][0]) shows a lower contact angle than pristine, which is due to the presence of encapsulated PEG in the membrane matrix. Similarly, the grafted PES in the absence of (PES [0][6]) the membrane contact angle was found to be 60.3° , which is due to the free carboxylic acid groups of AA on the membrane surface. The contact angle further decreased with increasing the degree of grafting, i.e., from 53.2 to 41.4° of PES [6+][2] to PES [6+][6], respectively. The decrease in contact angle indicates an increase in hydrophilicity, which helps to enhance the separation performance of the membrane [29].

Table 2
Contact angles of the pristine, PEG, and AA grafted PES membranes

S. No.	Membrane Name	Contact Angle in Degrees
1	Pristine PES	65.4
2	PES [0][6]	60.3
3	PES [6+][0]	55.7
4	PES [6+][2]	53.2
5	PES[6+][3]	50.5
6	PES[6+][4]	44.2
7	PES[6+][5]	42
8	PES[6+][6]	41.4

3.4. Thermo Gravimetric Analysis (TGA)

The TGA analysis of the various compositions of flat sheet PES membranes was illustrated in Fig. 4. The pristine PES and PEG-loaded PES[6+][0] membrane showed the single-stage weight loss from 400 to 420 °C, whereas the grafted membranes, i.e., PES [0][6], PES[6+][2] to PES[6+][6] exhibited additional weight loss from 450 to 550 °C. From Fig. 4., it can be clearly observed that as the degree of grafting increases the derivative, second weight loss increases, which is possibly due to the degradation of grafted polyacrylic acid (PAA) [29]. From TGA analysis, it can be concluded that the PAA deposition on the PES porous substrate increased with the grafting solution concentration and also supports the structural elucidation of FTIR.

3.5. Effect of AA concentration on the degree of grafting

The AA concentration in the grafting bath was varied from 2 to 6 % to understand the trade-off. All the membranes were grafted under the same operational condition, i.e., UV light working distance and grafting period. From Fig. 5., it is observed that the percentage of AA monomer increased from 2 to 6 %, resulting in the grafting percentage increased from 12.4 % to 17.8 %. The reason is possibly due to the availability of monomer units increased on the membrane surface, which is further confirmed from TGA analysis, where the additional weight loss of the grafted films, i.e., PES[6+][2] to PES[6+][6] is corresponding to the grafted PAA.

3.6. Calibration curve for PEG concentration determination

The calibration curve was generated using various prepared known concentrations of 20, 40, 60, 80, and 100 ppm of PEG 1000 Da solution; spectra were recorded in the range of 300 to 700 nm wavelength. The maximum wavelength was observed at 465 nm for PEG 1000 Da, and the absorbance values are linearly increasing with PEG concentration, shown in Fig. 6a. The first-order linear regression is fitted to recorded

absorbance values at the maximum wavelength of 465 nm to establish the co-relation between PEG concentration and its corresponding absorbance, as shown in Fig. 6b. Similarly, the calibration plots for 600, 2000, 4000, 6000, 10000, and 20000 Da PEG were recorded using the same steps.

3.7. Determination of MWCOs of PES membranes and pure water flux (PWF)

Molecular weight cut-off (MWCO) is a pivotal characterization technique in porous films used to determine the retention capabilities, which is > 90 % of solute molecules. The percentage rejection of different molecular weights of PEG solutions, i.e., 600, 1000, 2000, 4000, 6000, 10000, and 20000 Da, when passed through the indigenous UF membranes, the % rejection was determined and illustrated in Fig. 7. The rejection of each PEG solution grade gradually increases with the degree of grafting increased on the membrane surface, i.e., from pristine to PES [6+] [6]. The pristine and PES [6+] [0] membranes show the rejection % of 20 kDa less than 60%, which means the MWCO of these membranes is more than 20 kDa, whereas the PWF of the membrane was observed to be $25.03 \text{ L m}^{-2} \text{ h}^{-1}$ (Fig. 8). The PES [0] [6] membrane exhibited 89% rejection for 4 kDa PEG; the % rejection decreased for lower molecular weights. As per the experimental observations, the pristine and additive doped membranes without grafting (PES [6+] [0]) show a similar rejection pattern. However, after doping, the membrane PWF was enhanced from 25.03 to $41.50 \text{ L m}^{-2} \text{ h}^{-1}$ due to increased pore density on the membrane surface [30]. On the other hand, the MWCO of grafted pristine (PES [0] [6]) membrane decreased to 4 kDa due to the reduction in pore size after grafting on the surface, which leads to a drastic drop in PWF, i.e., $8.5 \text{ L m}^{-2} \text{ h}^{-1}$ which is clearly seen from Fig. 8. From these experimental and graphical observations, it can be concluded that the additive enhances PWF; in contrast, the grafting reduces the MWCO for effective separation of solute molecules. With this background, the membrane performance was increased by adding additive and increased grafting on the surface [31].

From Fig. 7, it is also observed that the % rejection of various AA grafted membranes, i.e., PES[6+][2] to PES[6+][6], shows different MWCOs. The PES[6+][2] membrane allows all the PEG solutions ranging from 0.6 kDa to 20 kDa, which indicates the membrane has MWCO more than 20 kDa. However, the % rejections for 10 and 20 kDa significantly improved with the 2% AA grafting solution compared to pristine and PES[6+][0] membranes; this is due to the pore grafting on the surface. In PES[6+][3], the membrane could pass the PEG solutions up to 10 kDa and retained the 20 kDa, partially indicating the membrane MWCO lying between 10 to 20 kDa. The 4% AA grafted membrane PES[6+][4] achieved 90 % rejection for 6 kDa PEG solutions; therefore, the MWCO of this membrane was above 5 kDa. Further, the resulting PES[6+][5] and PES[6+][6] membranes show about 87 and 90% rejections for 2kDa PEG solution, which confirms the MWCO can be around 2kDa, respectively. Hence, this infers that the MWCO is less than 2kDa for these membranes of PES[6+][5] and PES[6+][6] are nearly similar in pore size and possess comparable MWCO. On the other hand, from Fig. 8, it can be observed that the PWF gradually decreased from 38.5 to 25 for PES [6+] [2] to PES [6+] [6] membranes, respectively, which is due to the increasing degree of grafting causes reducing pore size on the surface of the membrane. Hence, the additive increases the

pore density and PWF, whereas the degree of grafting enhanced membrane performance for selective separation.

3.8. Application of PES[6+][2] to PES[6+][6] membranes for separation of therapeutic biomolecules

Various grafted membranes prepared in this study were tested for separation performance of small biomolecules of urea, cobalamin (vitamin B12), and insulin. Figure 9 represents the permeability data of urea, cobalamin, and insulin solutions of 50ppm concentration that was passed through PES[6+][2] to PES[6+][6] membranes. From this graphical observation, it can be noted that permeability was decreased from $30 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ to $25 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ for Urea from PES[6+][2] to PES[6+][6], respectively. Similarly, the permeability of cobalamin and insulin was decreased from 27 to $12 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ and from 22 to $4 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$, respectively (Fig. 9). The decreasing permeability trend is due to the size exclusion of biomolecules in urea < cobalamin < insulin.

From Fig. 10, it can be seen that the negligible declination in permeate concentration of urea was observed for PES[6+][2] to PES[6+][6] membranes at 30 psi pressure. The reason behind this is due to the smaller molecular size of the urea, i.e., < 0.5 kDa, allowed by these membranes. On the other hand, the permeability of cobalamin and insulin solutions showed a similar trend for the membranes of PES[6+][2] and PES[6+][3]. However, in the case of the membrane PES[6+][4], a drastic decline in insulin concentration and negligible change for cobalamin was observed at the permeate side. Moreover, for PES[6+][5] and PES[6+][6] membranes (Fig. 10), the insulin and cobalamin were getting rejected, and the concentration of this molecule in permeate is minimal. Therefore, the insulin and cobalamin permeability and permeate concentrations were decreased with increasing the degree of grafting on the membranes. As per the size exclusion principle, the low molecular weight biomolecule of urea permeates faster through the membrane than other therapeutic molecules. Therefore, the size exclusion of biomolecules is evaluated in terms of the membrane transport phenomenon provided in Fig. 11. From the graphical and transport properties of the grafted membrane, it can be concluded that these membranes could pass urea molecules easily and concentrate the remaining biomolecules in the reject stream. Hence, from the overall characterization and experimental observations, it can be confirmed that these indigenously synthesized flat sheet grafted membranes showed excellent permeability for all the biomolecules and applicable for the separation of therapeutic molecules with an MWCO range of 1 to 10 kDa.

Conclusions

In the present study, the PES -UF membranes were prepared by phase inversion method in 6 kDa PEG as a pore-forming agent to enhance the surface pore density. Surface modification was performed by AA grafting through UV-induced photo-catalytic reaction. The molecular weight cut-off of the indigenous membranes was controlled using various monomer grafting concentrations. The characterization studies were carried out with various SEM, FTIR, TGA, and contact angle tools, where SEM revealed the role of PEG additive in the dope solution and AA grafting on the membrane surface. Before and after grafting,

the functional groups and new bonds formed on the membrane surface were clearly evident from the FTIR studies. The thermal analysis of the grafted membranes exhibited an additional weight loss of PAA, supporting the structural analysis. The trade-offs between the grafting solution concentration with a degree of grafting and corresponding MWCs of the prepared membranes were established. From the experimental results, the PWF of the grafted membranes was significantly enhanced in the presence of the additive (PES [6+] [6]) than the pristine grafted membrane (PES [0] [6]). The urea permeability for all the membranes was found to be similar, whereas the retention of cobalamin and insulin was maximum in the case of PES[6+][6] and PES[6+][5]. All the essential peptides and proteins present in human blood above 2kDa will be retained. Only uremic toxins, which are less than 1 kDa, are removed with these membranes. Therefore, from the results, it can be concluded that PES[6+][6] and PES[6+][5] membranes are helpful to remove uremic toxins for renal patients instead of tedious dialysis procedure by surgically implanting this membrane cassette into the chronic renal patients so that the permeate is attached to the urinary bladder and reject back to the bloodstream, where the uremic toxins are removed continuously. The continuous removal of small uremic toxins will reduce these small toxins binding to significant proteins and peptides present in the blood.

Declarations

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Consent for Publication: All authors willing to publish this work.

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Authors Contributions:

Conceptualization: Gayatri Nimmagadda, Shiva Prasad Nandala; *Methodology:* Gayatri Nimmagadda, Shiva Prasad Nandala, Nagasandhya Bala; *Formal analysis and investigation:* Shiva Prasad Nandala, Nagasandhya Bala; *Writing - original draft preparation:* Shiva Prasad Nandala, Kalyani Swayampakula; *Writing - review and editing:* Gayatri Nimmagadda, Kalyani Swayampakula, Sridhar Sundargopal; *Funding acquisition:* Sridhar Sundargopal; *Resources:* Sridhar Sundargopal; *Supervision:* Sridhar Sundargopal.

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Figures

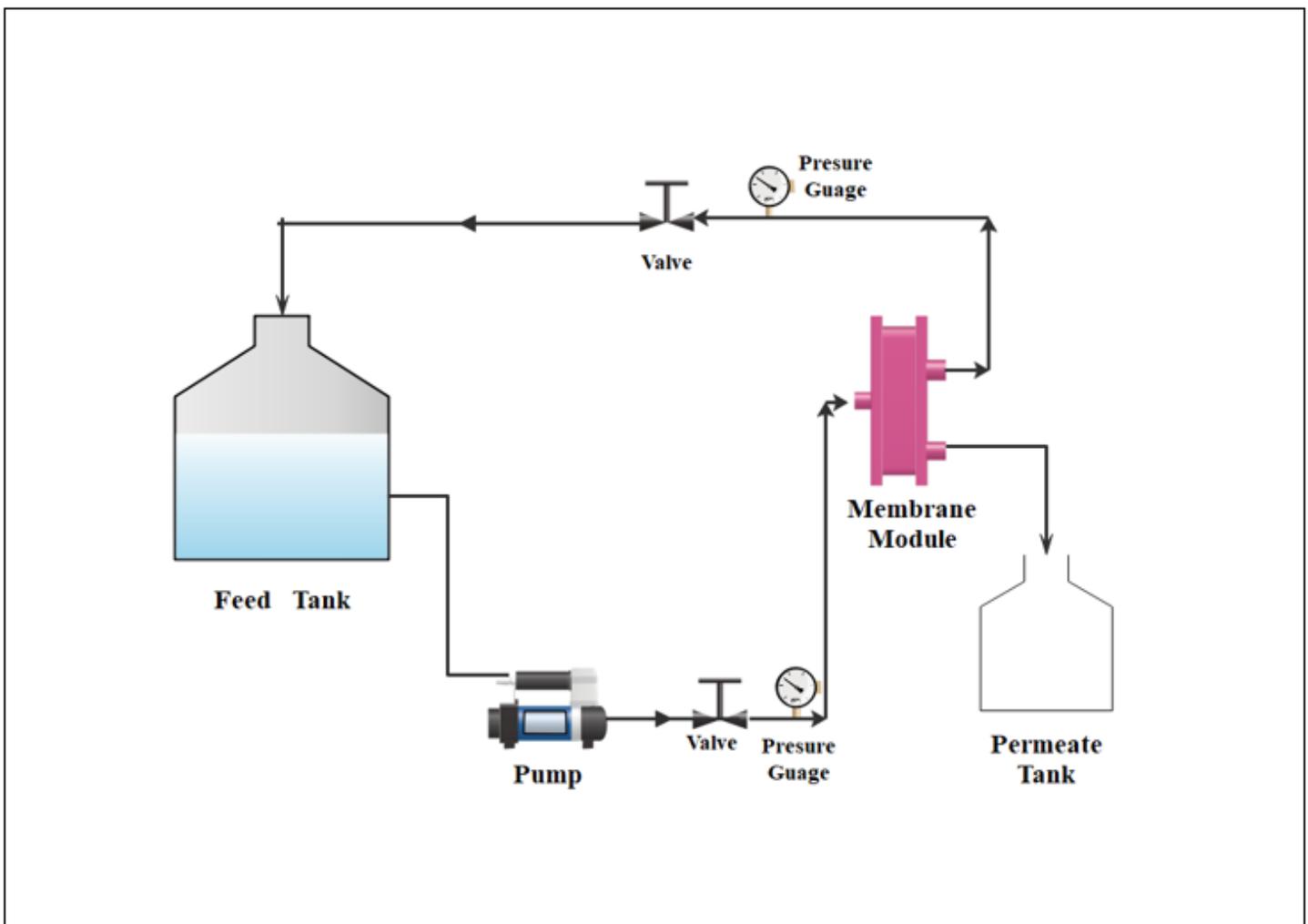


Figure 1

Process flow diagram of the laboratory experimental UF system

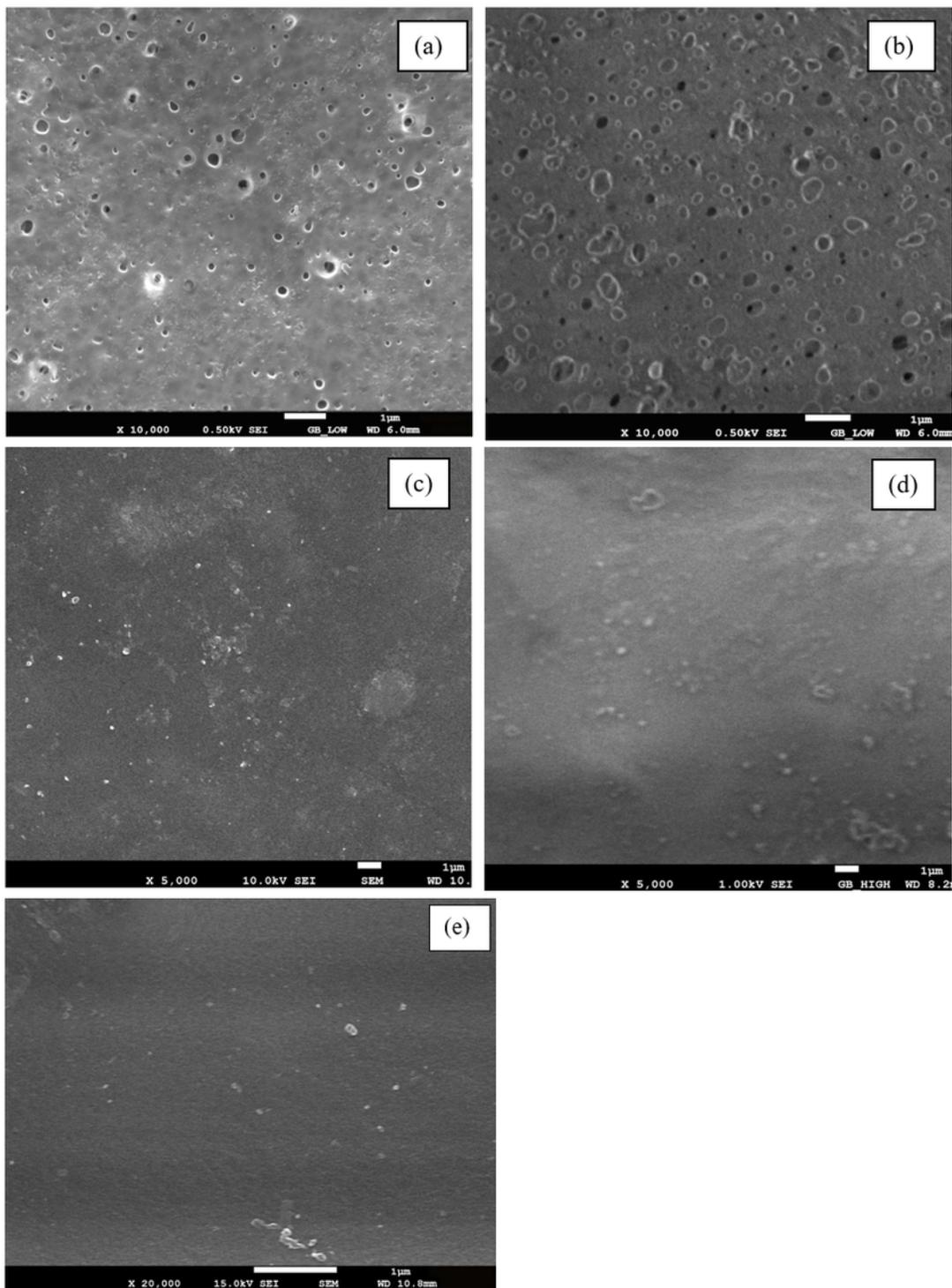


Figure 2

Surface morphologies of (a) pristine PES, (b) PES [6+] [0], (c) PES [6+] [2] (d) PES [6+] [4] and (e) PES [6+] [6] membranes

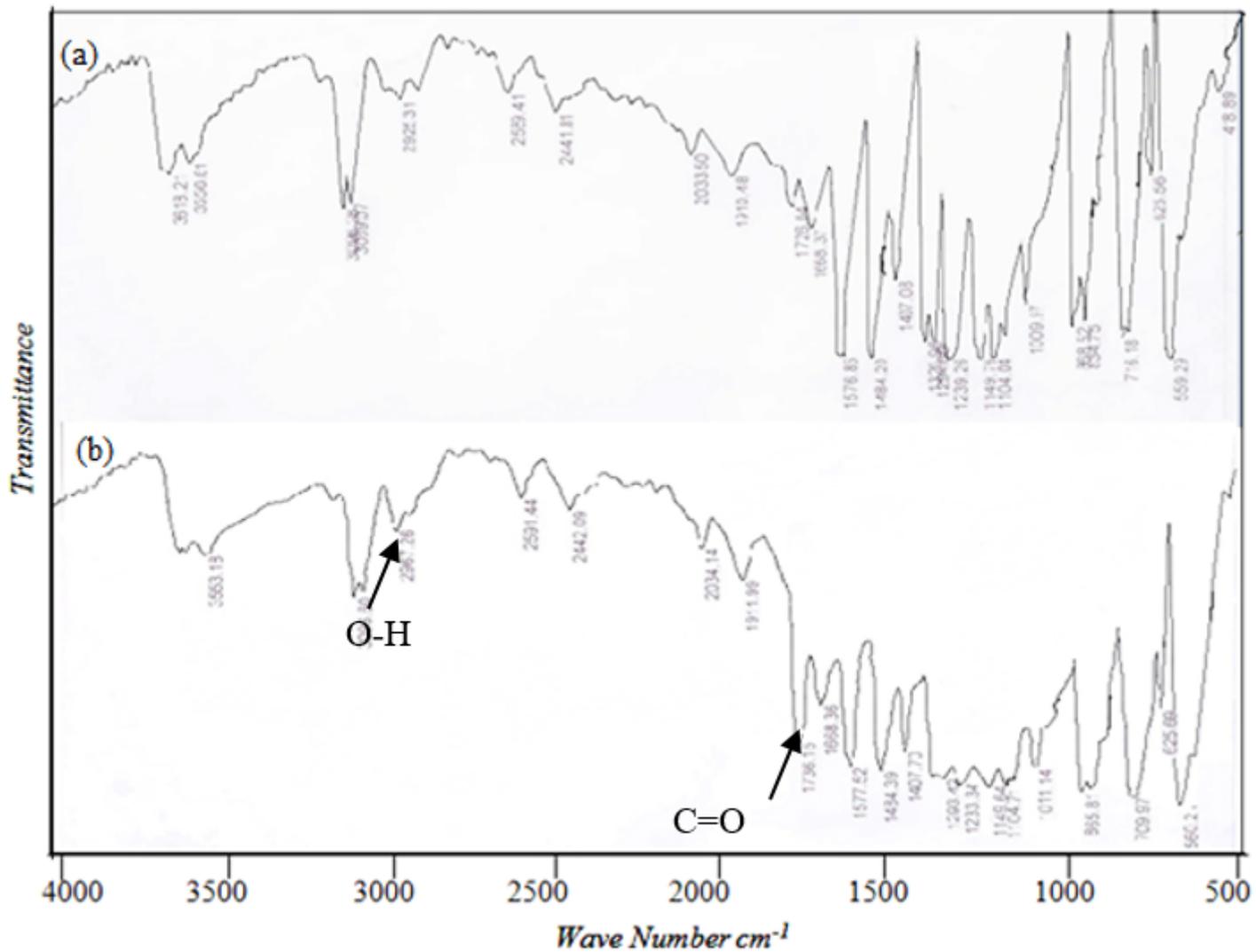


Figure 3

FTIR spectra of (a) pristine and (b) grafted PES

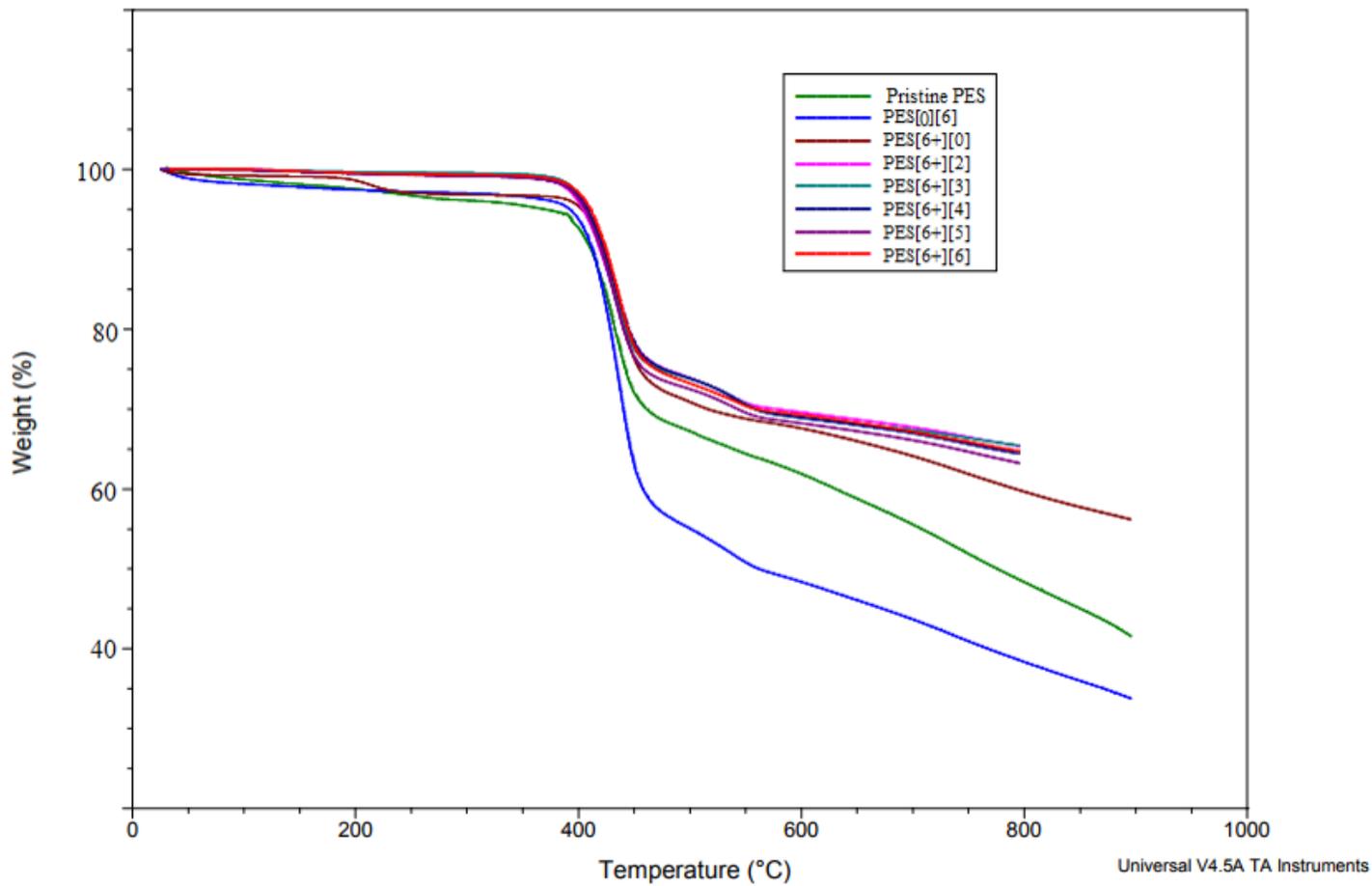


Figure 4

TGA of pristine, PES[6] [0], PES [0] [6] and PES [6+] [2] to PES [6+] [6] membranes

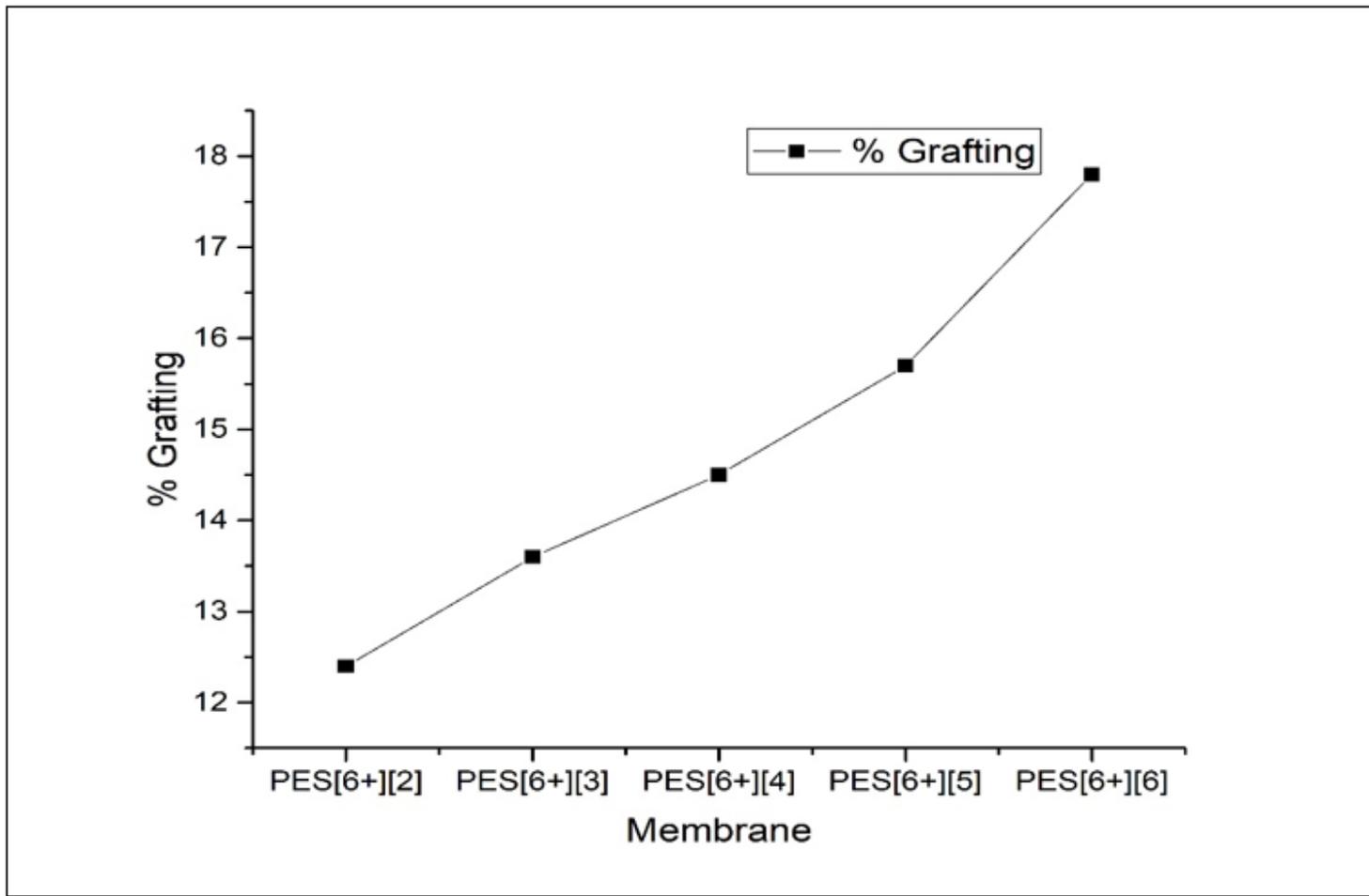


Figure 5

Effect of AA concentration on the degree of grafting

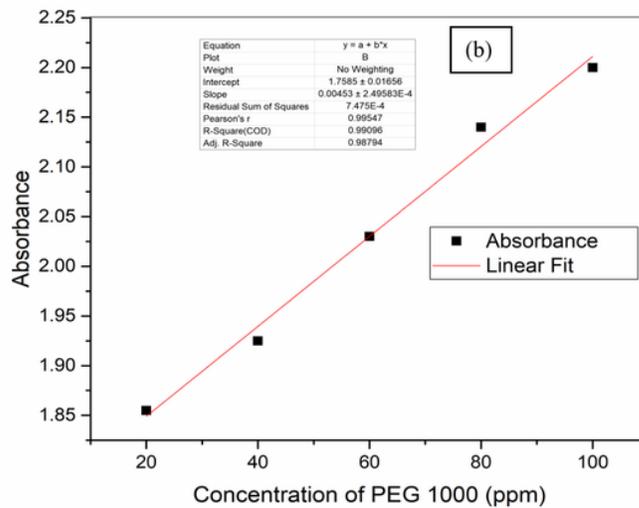
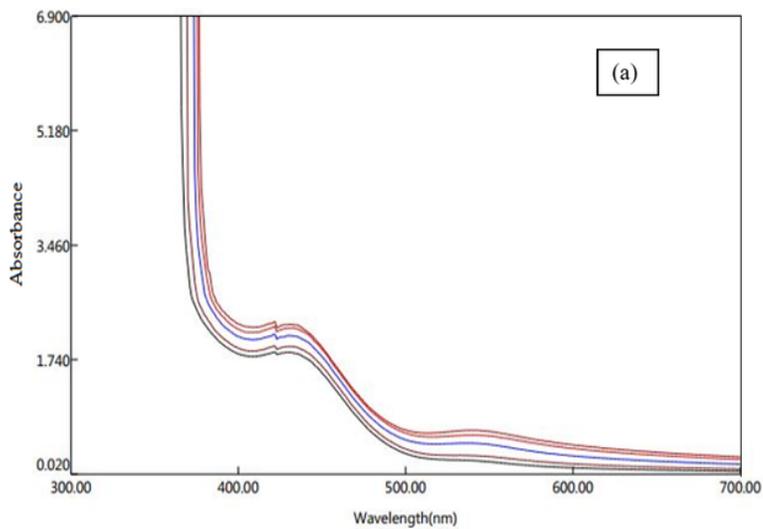


Figure 6

(a) Absorption spectra of various concentrations of PEG 1,000 kDa solution and (b) The calibration curve of PEG 1,000 kDa

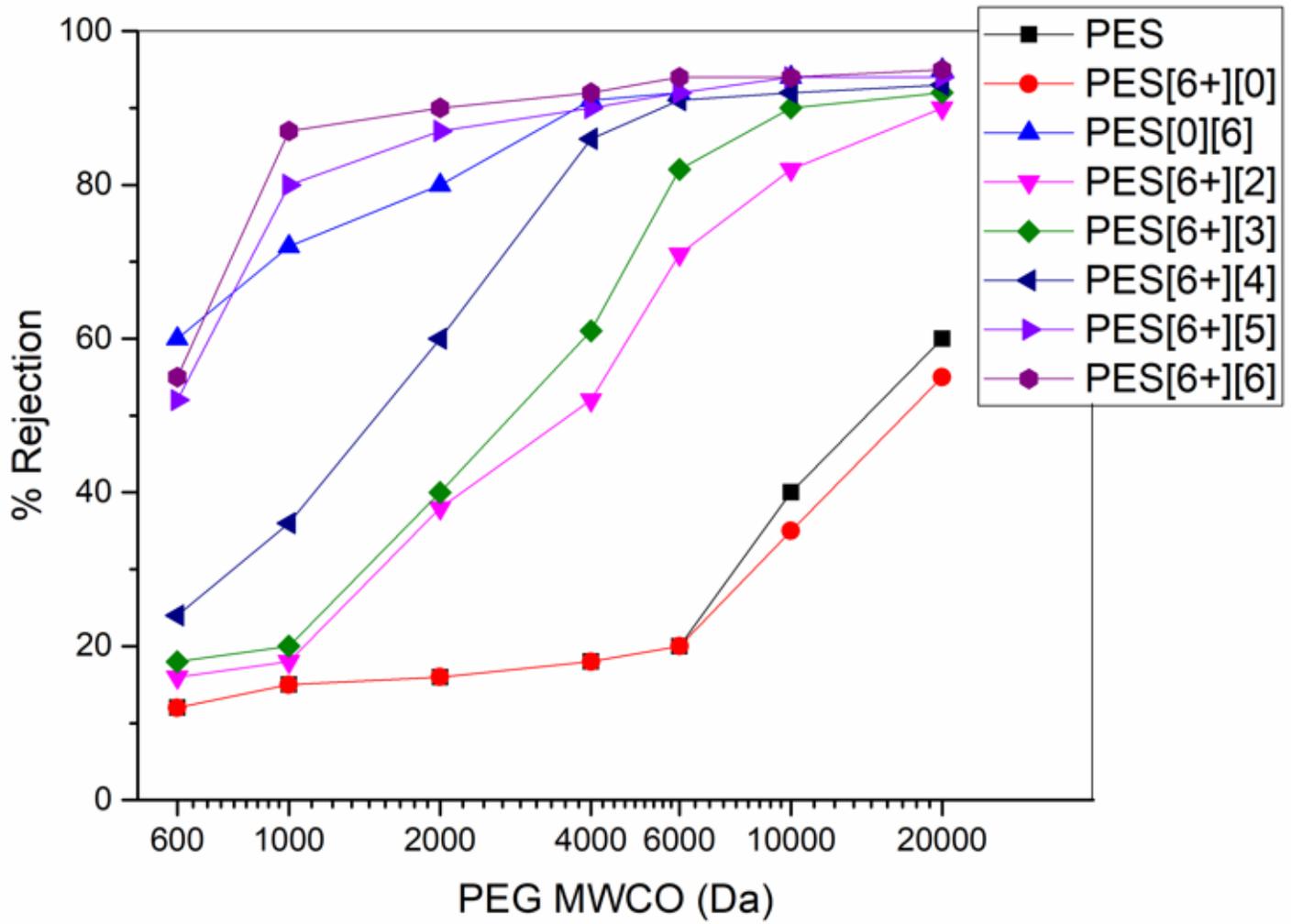


Figure 7

Effect of grafting on MWCOs of the membrane

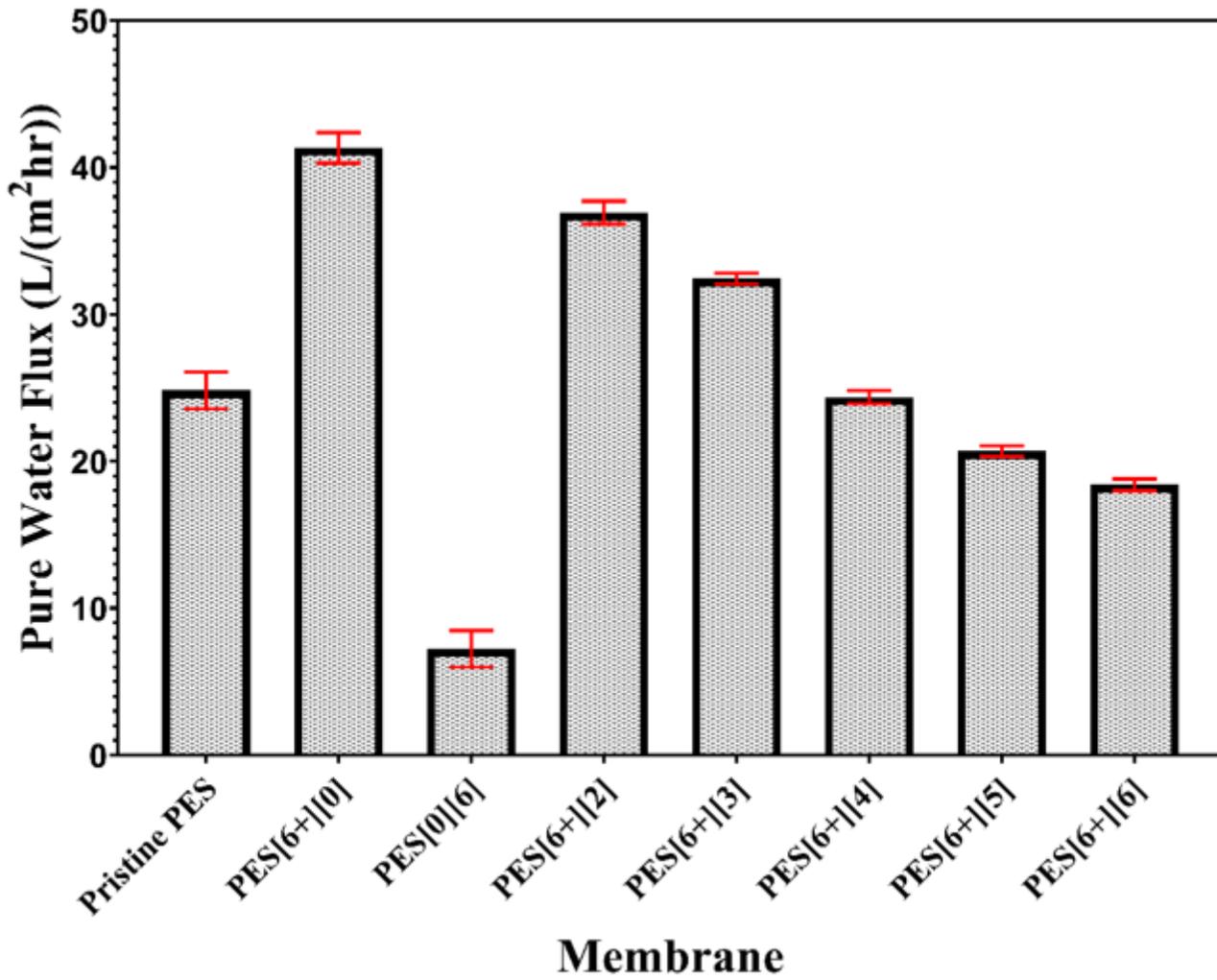


Figure 8

Effect of degree of grafting on PWF

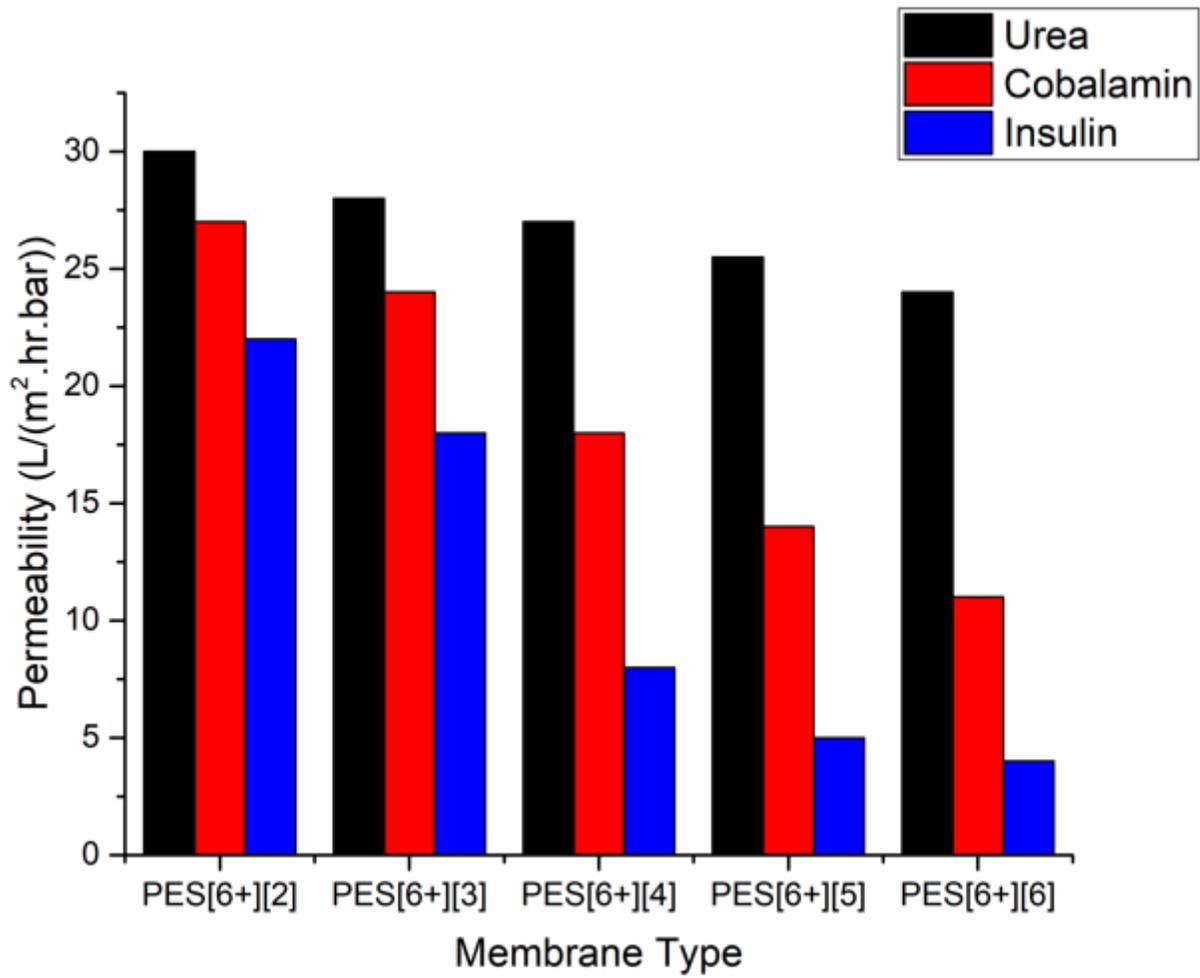


Figure 9

Permeability trend of urea, cobalamin, and insulin for PES[6+][2] to PES[6+][6] membranes

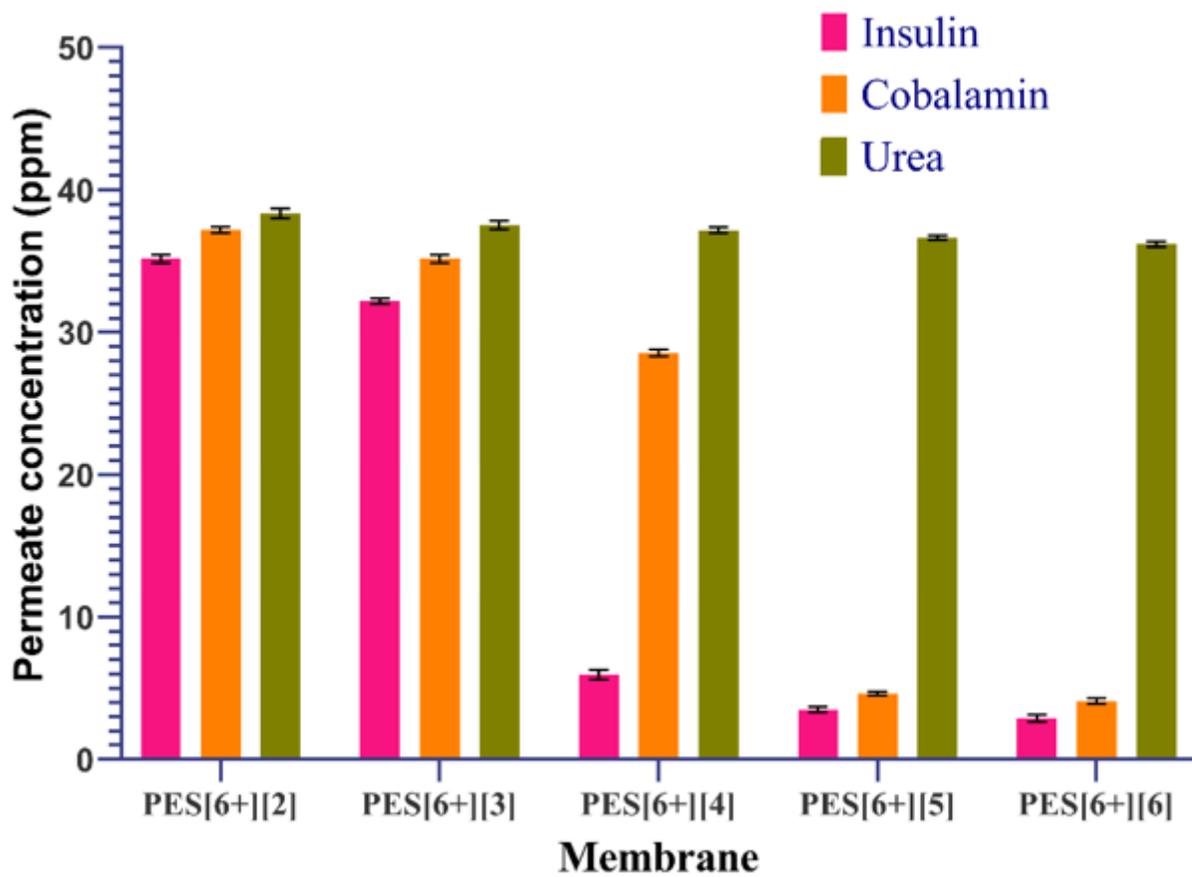


Figure 10

Effect membrane grafting on permeate concentration

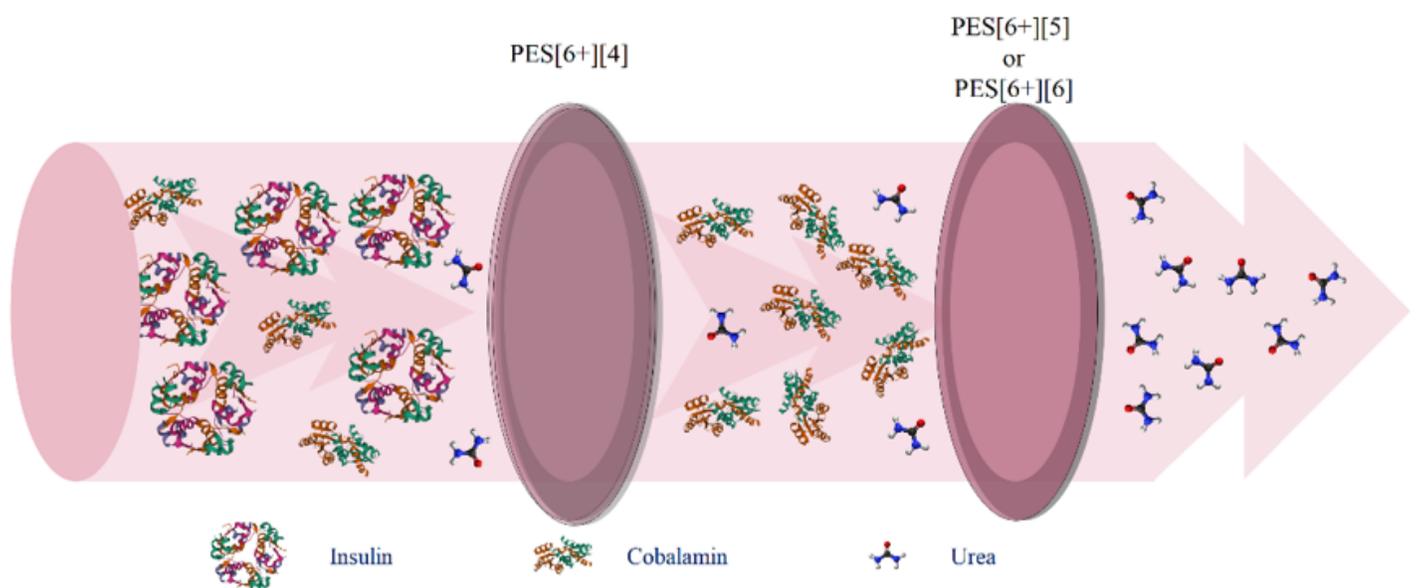


Figure 11

Schematic of transport phenomenon of biomolecules

Supplementary Files

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- [Scheme1.png](#)