Controlling the Rejection of Protein During Membrane Filtration by Adding Poly-Vinyl Pyrrolidone (PVP)

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Abstract

Mixed matrix membranes were synthesized by combining Polyvinylpyrrolidone (PVP) with Cellulose Acetate (CA). The preparation of the CA/PVP protein filtration membrane was carried out using the diffusion-induced phase separation approach. To investigate the impact of adding PVP to CA membranes, the morphology, water permeation efficiency, and bovine serum albumin (BSA) rejection were examined. Membrane samples were examined using contact angle, FTIR and SEM to determine their surface shape. The homogeneous and considerable mixing of PVP components into the pure CA matrix was confirmed by SEM. The percentage of protein rejection and water permeation were used to gauge the performance of blended membranes. The greater hydrophilicity of CA/PVP membranes was clearly demonstrated by the extraordinary reduction of contact angle from 83° to 69°. Over 90% of CA/PVP membranes were rejected. By adding 1% by weight PVP (M-1), the hydrophilicity, water permeability, and protein rejection percentage of the CA membrane were all enhanced.

Keywords: Cellulose Acetate, Dialysis Membranes, Poly Vinyl Pyrrolidone, BSA Rejection Diffusion Induced Phase Separation

Introduction

In protein separation and purification processes, enhanced efficiency in terms of protein separation is of chief significance. High performance protein fractionation and purification, especially in pharmaceutical applications, dairy, biotechnology and food is of noticeable commercial interest nowadays. The protein separation and purification are subjected to improve purity and stability of selective protein and to eliminate impurities (Zydney, 2009). Filtration through membranes is an extensively used technique in industrial protein filtration. A membrane is a selective barrier which allows selective molecules or ions to pass across via diffusion and sieving mechanism collectively. Separation via membrane requires low energy and reduced pressure and temperature conditions (Pinelo and Jonsson 2009). The protein-membrane interaction that takes place all through protein separation and purification procedures via membrane is of major importance. The electrostatic attraction, Van Der Waals forces, hydrogen bonding and hydrophobic interactions are involved for the adsorption and deposition of protein on membrane surface (Mendret, 2013). These interactions affect the resolution, rejection and stability of protein (Yeu, 2008). Thus, the performance of the membrane for protein filtration is based on its selectivity. In both industrial and environmental backgrounds, wastewater comprising bovine serum albumin (BSA) offers distinct potential and issues for resource retrieval and treatment. BSA is a frequently utilized protein in the field of biotechnology and pharmaceutical. However, the effluent from these applications may allow this crucial protein to enter wastewater systems, leading to a host of environmental issues. When BSA is present, wastewater's chemical and biological oxygen demands (known as COD and BOD) may increase. In aquatic environments, high BOD and COD levels can lead to oxygen depletion, which reduces the amount of dissolved oxygen available to aquatic species (Taha et al. 2024). Membranes for ultrafiltration (UF) are essential for biotechnological applications. They are frequently used in processes like enzyme extraction and proteinaceous solution concentration for protein filtration and separation. Polymers and ceramics are the main materials used in membrane production in modern technology. However, polymeric membranes function better than their ceramic counterparts because they are more easily shaped into different shapes and have an inherent flexibility (Prihandana et al. 2024).

Gao et al. used the co-deposition of diglycolamine and dopamine to create a ZrO_2 membrane surface that is resistant to proteins. They discovered that when the antifouling membrane was filtered into a bovine serum albumin (BSA) solution, its flow rose by 43%. Lee et al. grafted organosilanes with sulfonic acid groups to adjust the charge on surface of a ceramic membrane. Due to electrostatic repulsion, the resulting membranes showed an outstanding flux recovery ratio of about (80%) into humic acid (Yahan ye et al. 2023). In another study, the ZIF-8 and UiO-66 NH_2 NMOFs were synthesized using a microwave heating technique and added to PVDF and PVDF/chitosan nanofibrous membranes. The performance of generated membranes was studied for bovine serum albumin (BSA) protein and Cr(VI) ions separation (M Pishnamazi et al. 2020). Certain ionic and molecular species are prohibited from passing across a membrane by its selective barrier function. Membrane filtering offers better throughput for bulk processes than chromatography since it can be carried out at lower temperatures with less energy consumption, doesn't require additives or solvents, and is simple to scale up to high flow rates. Moreover, membrane processes are easily integrated into other separation or reaction processes and run continuously. Since membranes can overcome most of the drawbacks of traditional extraction techniques, their employment in the food and pharmaceutical industries is continually expanding (Alavi et al. 2023). As a result of membrane fouling, the extensive employment of polymeric membrane in the UF technique is limited. Enhancing the polymeric membrane's antifouling properties is therefore desperately needed. To stop pollutants from adhering to the UF membrane, a fouling-resistant membrane design is essential. Currently, grafting, physical blending, and immobilization techniques can all be used to improve a membrane's antifouling properties. Physically blending a membrane with hydrophilic additives is seen to be one of the most practical and effective techniques among them. (Purushothaman et al. 2023). Since the sizes of many proteins are comparable, it is expected that their retention

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will be similar as well. Making use of a UF membrane that allows solutes to pass through (open UF) is necessary for moderate retention in order to achieve separation, and using separation mechanisms other than size exclusion may increase the process's selectivity. The outline for quantitatively characterizing the transport of a protein across open UF membranes has not yet been well described, despite the fact that many studies discuss enhancing fractionation of protein with UF by changing the membrane or the system conformation. This is because of the complexity associated with the instantaneous action of numerous driving forces and the presence of some components in the system (Y Song et al. 2023).

The aim of the current study is to investigate the role of polyvinyl pyrrolidone (PVP) incorporation within cellulose acetate (CA) polymer for separation of protein present in the feed solution during membrane filtration. For this purpose, the effect of addition of PVP to CA was estimated in terms of Bovine serum albumin (BSA) rejection percentage. The leading hypothesis behind the study is that modification of CA with PVP can result in lowering of permeation and elevation of BSA rejection.

Experimental Procedure:

a) Material

To separate proteins, asymmetric polymeric membranes were synthsized. Sigma Aldrich provided the cellulose acteate (average molecular weight of 30,000 Dalton and 39.8% degree of acetylation), 99.7% pure acetic acid, and bovine serum (molecular weight of 66,000 Dalton). Fluka supplied PVP with an average molecular weight of thirty thousand.

b) Methodology/System Description

Several CA/PVP/Acetic acid mix casting solutions with a constant Cellulose Acetate weight percentage of 18.5 were made. The contents of the ready-made casting solutions are displayed in Table 1. The solutions were continuously stirred until all of the components—polymer, solvent, and additive—were completely mixed. To eradicate trapped air bubbles, the treated solution was degassed using an ultrasonic bath for two hours. After degassing, the casting solutions were stored in the dark to hold back the aging course. The casting solution was spread out on a plate made of glass using a doctor blade with a 200µm wet membrane thickness. Diffusion-induced phase separation occurred when the glass plate was immediately immersed in a distilled water bath (Sivakumar and Mohana Sundaram, 1998). Exchange of solvent (acetic acid) and non-solvent (water) took place during phase separation. After that, the synthesized flat sheet membrane was moved to a new trough with fresh water and cleaned to remove any leftover solvent. There were three to four washing cycles. Lastly, before the last experiment, the constructed membrane was stored in distilled water for a whole day. Membrane casting systematic procedure is shown in Figure 1.

Table I
Composition of synthesized membranes

Sample	CA wt. %	PVP wt. %	Solvent Wt. %
M-0	18.5	0	8I.5
M-1	18.5	1	80.5
M-2	18.5	3	78.5
M-3	18.5	5	76.5

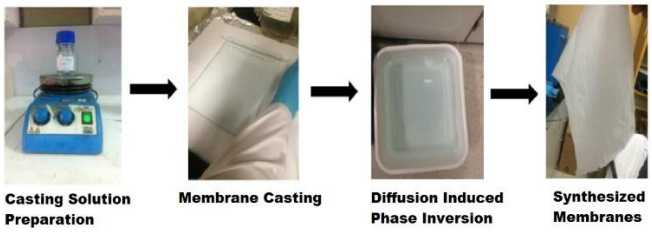

Figure 1. Systematic description of CA/PVP protein separation membranes

Analysis

a. Contact Angle Measurement

Contact angles measurements are used to determine a membrane's hydrophilicity. At a Tantec contact angle meter, the sessile drop method was used to determine contact angle (Kee and Idris, 2010). Using a micro syringe, a single drop of distilled water was applied to a flat, dried membrane surface. The contact angle was measured after ten seconds of testing in a saturated water vapor atmosphere. To obtain an average value, a minimum of eight readings of the contact angle were recorded, and each reading was taken at room temperature.

b. Pure Water Flux

The performance of the synthesized flat sheet hemodialysis membrane was estimated in terms of pure water permeation flux (PWF) in a dead end filtration cell (having 250 mL capacity) having an actual area of 12.57 cm^2 at 2.0–3.0 bar pressure with double distilled water. The setup is shown in Figure 2. The experimental procedures were repeated numerous times to evade data uncertainty. The PWF was measured using (1):

$$Flux\ (J) = {}^{Q}/_{\Delta t} \times A \qquad (1)$$

In (1), J represents the permeation flux (Lm-2h-1) of pure water, Q denotes the volume of infiltrated solution, A signifies the active site area of membrane and Δt denotes the permeation time (h) (Irfan and Idris, 2014).

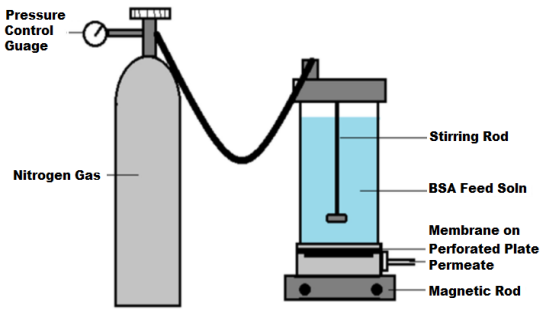

Figure 2. Dead-end filtration assembly for protein rejection measurement

c. Scanning Electron Microscopy (SEM)

To investigate the impact of adding PVP to the CA matrix, SEM analysis was conducted. Using the JSM 6409A SEM model from JEOL, Japan, the surface morphology, pore dispersion, and uniformity were examined. The samples

were placed onto brass plates with the help of double sided cellophane tapes in a lateral orientation after being fractured, sputter-coated with platinum in liquid nitrogen.

d. Fourier transform Infra-Red spectroscopy

FTIR Spectrum 100 PerkinElmer, MID-IR instrument was used to measure FTIR measurements for manufactured membranes. After being sliced into tiny pieces, the pristine and blend membranes were put in a pallet holder. After that, the holder was installed in an FTIR device (PerkinElmer). At room temperature, the FTIR was operated in the wave number range of 450–4000 cm^{-1} with a transmission mode resolution of 1 cm^{-1}.

e. Protein Rejection measurement

The protein rejection % of the synthesized membrane was analyzed using dead end filtration set up. The operation conditions were all the same as for water flux analysis. The feed solution was prepared with 1 mg/ mL BSA concentration. The protein rejection was calculated using equation (2) where Cp and Cr stand for permeate and retentate concentrations correspondingly (Qin and Oo, 2005).

$$Percentage\ Rejection = \left(1 - {}^{Cp}/_{Cr}\right) \times 100 \quad (2)$$

Results and Discussions

a. Impact of Adding PVP on Hydrophilicity of Cellulose Acetate Membranes

If membrane has a low contact angle, it is highly hydrophilic. Contact angle values for CA and PVP fabricated CA membranes are presented in Figure3. It is seen that increasing the concentration of PVP up to 5% decreased the particular contact angle. The evaluated results indicated that the integration of PVP increases the hydrophilicity of Cellulose Acetate membrane obviously.

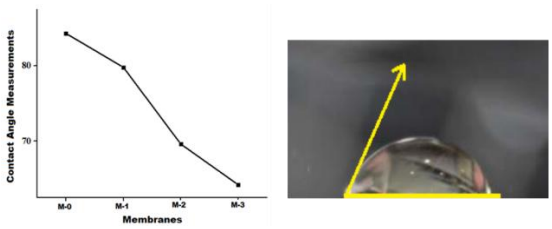

Figure 3. Contact angle of synthesized membranes

b. Impact of PVP Addition on Surface Configuration and Efficiency of Cellulose Acetate Membranes

The SEM micrographic view of the cross-sectional configurations of PVP and CA in each produced membrane is displayed in Figure 4. SEM micrographs show that adding PVP to the CA matrix creates membranes having asymmetry with a compact top surface layer and a sub-layer having porous structures throughout that resemble fingers. From these pictures, it can be observed that macro spaces and a porous structural morphology having good water flux developed when the bath temperature was kept constant at 25°C and the PVP concentration increased from 0 to 3 weight percent. However, increasing the concentration of PVP content above 4 weight percent caused huge visible pores to grow, rendering the membranes useless for dialysis, filtration, and purifying processes. The presence of the hydrophilic addition PVP was shown to cause instantaneous demixing in the coagulation bath and to create macro gaps in the membrane structure up until the concentration dropped to less than 3 weight percent (Castillo-Ortega and Najera-Luna, 2011).

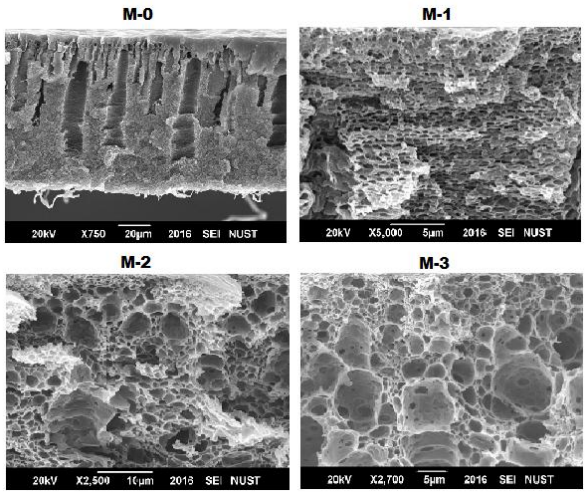

Figure 4. SEM Image of synthesized membranes

*__M0__ with 0wt% of PVP **M1** with 1wt% of PVP
M2 with 3wt% of PVP **M3** with 5wt% of PVP

c. FTIR Analysis of CA/PVP Blended Membranes

The FTIR spectra seen in Figure 5 underwent band and peak broadening and shifting as a result of the addition of PVP to the CA matrix. The carbonyl group (−C=O) stretching vibration observed at 1734cm^{-1} and the −CH group stretching vibration observed at 2960cm^{-1} were clearly visible in the FTIR spectra of the CA/PVP blended membranes. These are the characteristic peaks of CA. (Cai and Song, 2017). Additionally, M-1, M-2, and M-3 displayed the bending vibration of the −CH2− group at ~1498cm^{-1} and the stretching vibration of the carbonyl group of the (C=O-N) amide at ~1656cm^{-1}. At around 1295 cm, the C-N group also displayed tertiary stretching vibration. It is evident from the spectrum that the peaks get sharper as the weight percentage or concentration of PVP rises. Although the findings were not as significant as they were for the other two membranes, M-3 exhibits the same tendency as M-1 and M-2 (Gao and Sun, 2013).

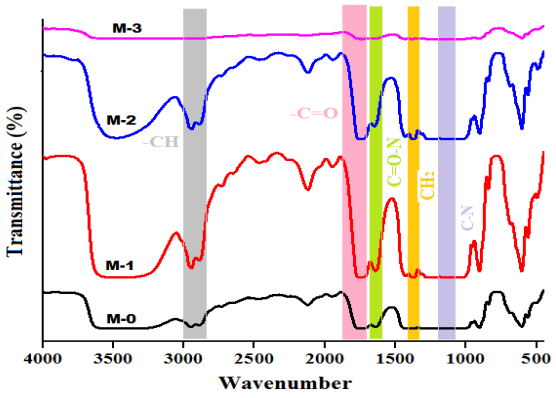

Figure 5: FTIR Image of synthesized membranes

d. Water Permeation of CA/PVP Modified Membrane

Membrane performance tests were based on pure water flux (PWP) and the extent of rejecting bovine serum albumin from feed solution. Membrane's pure water flux was found to be 13, 39, 41and 54 $Lm^{-2}h^{-1}$ for M-0, M-1, M-2 and M-3 respectively as shown in Figure 6.

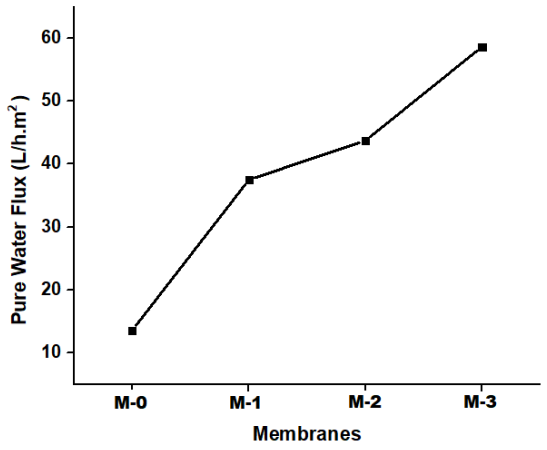

Figure 6. Pure water flux of synthesized membranes

e. BSA Rejection % of CA/PVP Blended Membranes

To obtain good protein filtration or rejection, albumin loss should be evaded during procedure. Figure 7 indicates the BSA rejection % and PWP of all pristine CA and CA/PVP blended membranes. M-1, M-2 and M-3 have BSA rejection above 90% i.e 96.7%, 97.8% and 99.2% respectively which is comparatively smart for commercial protein filtration. PWP of the synthesized membrane was found to be optimum in case of CA/PVP blends. Membrane M-1is having PWP of 39 $Lm^{-2}h^{-1}$ and BSA rejection % of 96.7% whereas M-2 is with PWP of 43 $Lm^{-2}h^{-1}$ and BSA rejection of 97.8%. The best performing among these four is M-3, with highest PWP and BSA rejection of 67.4 $Lm^{-2}h^{-1}$ and 99.3% respectively. Further analysis of the best membrane was made for porosity and was found that it is 69.64% porous, offering limited passage for protein flow across it. Hence picking M-3 as the applicable membrane with optimum porosity and highest protein rejection in protein filtration application.

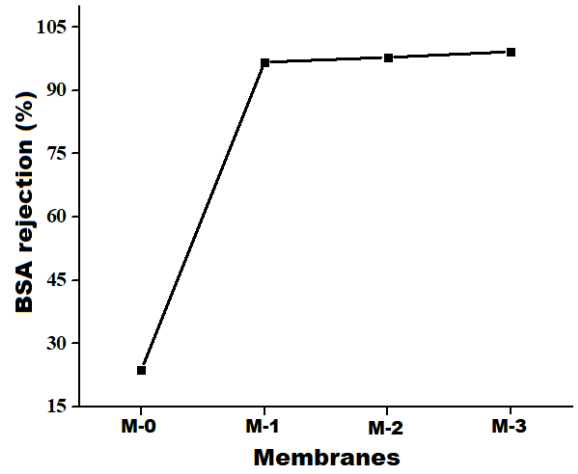

Figure 7. BSA rejection % of synthesized membranes

Conclusion

In this study, the phase inversion approach was used to synthesize CA flat sheet membranes with the addition of PVP. When examined in cross section, the CA/PVP membranes revealed a dense structure with a porous surface layer that contained macro voids. FTIR analysis demonstrated that PVP and CA effectively bonded. The hydrophilic character was investigated by contact angle measurements as well. Each of these characterizations demonstrated that adding PVP to the CA matrix enhanced hydrophilicity. The results of the performance tests indicated that the percentage of BSA rejection and PWP flux values of the CA/PVP blended membranes were more efficient. According to this study, PVP is a suitable addition to improve protein rejection. In summary, the protein filtration through membrane may reject 96.7 percent of BSA protein using the CA blend containing 5% by weight of PVP.

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