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Separation of proteins from aqueous solution using cellulose acetate/poly (vinyl chloride) blend ultrafiltration membrane

Lakshmi Krishnamoorthy · Pathan Mohammed Arif · Rasheeth Ahmedkhan

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Abstract Cellulose acetate (CA) membranes are widely used for ultrafiltration applications. CA and poly (vinyl chloride) (PVC) blend membranes are prepared by using polar solvent of N, N-dimethylformamide (DMF) by phase inversion method. Polyethylene glycol (PEG 600) is used as polymeric additive in the casting solution. The effect of polymer composition and additive concentration on surface morphology, porosity are studied. The morphology of prepared membranes is found to have asymmetric structure with dense skin layer and porous sub-layer. Applications of these membranes are carried out for rejection and permeation of macromolecular proteins such as trypsin, pepsin, egg albumin, bovine serum albumin. On increasing the concentration of PVC and PEG 600, protein rejection decreases whereas permeate flux increases.CA/PVC/PEG (70/30/10 wt%) blend membrane shows the highest permeation flux of 211.1 lm^{-2} h^{-1} for trypsin.

Introduction

Separation of proteins from food and bio-related industrial waste streams are gaining increased visibility due to environmental concern and to save precious materials. The presence of proteins in effluents generates biological oxygen demand (BOD) and chemical oxygen demand (COD) in surface water [1]. Efforts are taken to purify proteins for their wide application in scientific research [2]. Many researchers have attempted to separate proteins using various methods and they have concluded that ultrafiltration (UF) membrane is a suitable technique for the separation of macromolecular proteins because of non-destructive process and allows only limited denaturation of proteins to take place [3, 4].

Among different polymers cellulose acetate (CA) is often used for biological separation due to its biocompatibility, high flux, non-toxic [5, 6]. In CA membranes, fouling is greatly reduced because of its excellent hydrophilicity [7, 8]. CA membranes are well known for their use in ultra-filtration and desalination by reverse osmosis process [9]. Blending offers a novel material with suitable properties [10].The performance of membrane can be improved by blending it with appropriate polymers which results in the enhancement of permselectivity and permeability [11]. Poly (vinyl chloride) (PVC) has excellent physical, chemical, and mechanical properties. Polymers such as SAN, PVP, and PVB are blended with PVC to improve water flux [12–14].

The presence of additive into the casting solution is one of the major factors. Additive is used to obtain optimal membrane structure [15]. Number of researchers has reported their observation on the role of additive in membrane structure [16–19]. Arthanareeswaran et al. [20] have reported that the properties of CA membranes can be improved in the presence PEG 600 as an additive.

In this study, CA is blended with PVC in different composition using PEG 600 as pore former. The effect of polymer composition and additive concentration on surface morphology, porosity, rejection, and permeation flux of macromolecular proteins such as bovine serum albumin (BSA), egg albumin (EA), pepsin, and trypsin are discussed.

L. Krishnamoorthy (✉) · P. M. Arif
Post Graduate and Research Centre, Department of Chemistry, Maulana Azad College, Aurangabad, Maharastra 431001, India
e-mail: lakshmichemy@gmail.com

R. Ahmedkhan
Post Graduate and Research Department of Chemistry, The New College, Chennai 600 014, India

Materials and methods

Materials

Commercial grade CA was procured from Mysore Acetate and Chemical Co. Ltd., Mysore, India. PVC was obtained from Gharda chemicals Pvt. Ltd., India. Analytical grade N, N-dimethylformamide (DMF), sodium lauryl sulfate (SLS), polyethylene glycol (PEG) 600, oxalic acid, maleic acid, and acetone were procured from SRL Ltd., India. Anhydrous sodium monobasic phosphate and sodium dibasic phosphate heptahydrate were procured from CDH chemicals Ltd., India. These were used for the preparation of phosphate buffer solutions in protein analysis. Proteins such as BSA ($M_w = 69$ KDa), pepsin ($M_w = 35$ KDa), and trypsin ($M_w = 20$ KDa) were procured from SRL chemicals Ltd., India. EA ($M_w = 45$ KDa) was obtained from CSIR Bio-Chemical Centre, New Delhi, India. Deionised and distilled water were used in the preparation of protein feed solution and the gelation bath preparation.

Preparation of solution blending

Cellulose acetate and PVC (total polymer composition = 17.5 wt%) were blended by dissolving the two polymers in different compositions of 100/0, 90/10, 80/20, 70/30 wt% in the presence of additive PEG 600 (0, 5, 10 wt%) in a solvent, DMF (72.5–82.5 wt%) under constant stirring in a two neck round bottom flask for 4 h at 40–45 °C. The homogenous solution was kept as such in room temperature for 1 day in an air tight condition to get rid of air bubbles.

Preparation of blend membrane

In this study, the asymmetric membranes were prepared by phase inversion technique [21]. The polymer solution was poured on a smooth glass plate and spread by using metal rod. The membrane-casting chamber was maintained at a temperature of 24 ± 2 °C and a relative humidity of 50 ± 2%. The casting and gelation conditions were maintained constant throughout since morphology and performance of membrane would affect largely even for small changes in thermodynamic conditions.

Before casting, 2 L of gelation bath consisting of 2.5% (v/v) DMF solvent (to reduce liquid–liquid demixing and macrovoids) and 0.2 wt% surfactant SLS (to reduce surface tension at polymer-non-solvent interface) in distilled water (non-solvent) was prepared and kept at 20 ± 1 °C.

After casting, the solvent was allowed to evaporate for 30 s which resulted in the preparation of membrane. Then, the cast film along with the glass plate was gently immersed in the gelation bath for 1 h. The membrane formed was peeled off from the glass plate slowly. The peeled out membrane was removed from gelation bath and washed thoroughly with distilled water to remove solvent and surfactant from the membranes. Finally, the membranes were stored in distilled water containing 0.1% formalin solution to prevent from microbial growth.

Experimental setup

The UF experiments were carried out in a batch type, dead end cell (UF cell-S76-400-Model, Spectrum, USA) fitted with teflon-coated magnetic paddle as shown in Fig. 1. The effective membrane area available for ultrafiltration was 38.5 cm^2. The solution filled in the cell is stirred at 400 rpm using a magnetic stirrer. The permeating solution was collected from the bottom of the cell. This cell was connected to a compressor with a pressure control valve and gauge through a feed reservoir.

Membrane characterization

SEM analysis

The morphology of all the prepared blend membranes is studied by using Scanning Electron Microscope (SEM) (Leica Stereoscan 440, UK). The membranes are cut into various sizes and mopped with filter paper and are immersed in liquid nitrogen for 30 s and the frozen membranes are preserved in desiccator for SEM studies. The top surface and cross-sectional view of the membranes are observed by SEM under high vacuum at 20 kV.

Porosity

The surface porosity of the membrane is calculated by assuming that only skin layer of the membrane is effective in separation. Porosity is calculated as follows [22]:

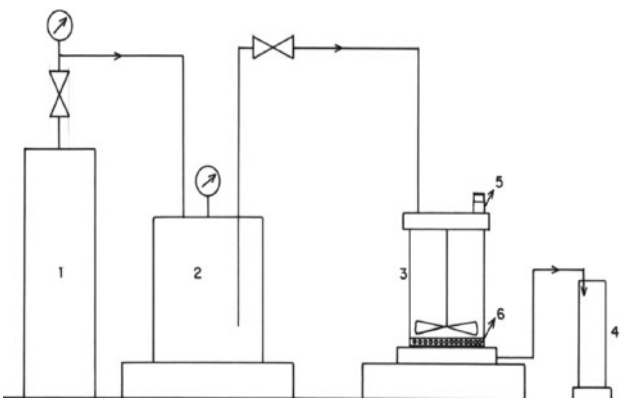

Fig. 1 Schematic diagram of ultrafiltration test kit cell. *1* Compressor, *2* feed tank, *3* UF cell, *4* permeate collector, *5* pressure relief valve, *6* membrane

$$P(\%) = \frac{(W_w - W_d)}{AT} \times 1000, \quad (1)$$

where P is porosity of membrane (%), W_w is wet sample weight (g), W_d is dry sample weight (g), A is area of the membrane (m^2), and T is thickness of the membrane (mm).

Application studies

In this study, an attempt is made to remove the valuable proteins using modified CA-based membranes prepared in the absence and presence of polymeric additive PEG 600.

Rejection of proteins

The protein (BSA, EA, pepsin, and trypsin) is dissolved in 0.1 wt% phosphate buffer (0.5 M, pH 7.2) solution and used as standard feed solution for the analysis of proteins. After the membrane is mounted in the UF test kit, the chamber is filled with the individual protein solution and pressurized under a nitrogen atmosphere at 345 kPa which is maintained constant throughout the run. Permeate is collected over measured time intervals in graduated tubes, and the tube contents are analyzed for protein content by ultraviolet spectrophotometer (Hitachi, model U-2000) at λ max = 280 nm. The percentage solute rejection (%SR) is calculated from the concentration of the feed (C_f) and permeate (C_p) using the following equation:

$$\%SR = \left[1 - \frac{C_p}{C_f}\right] \times 100. \quad (2)$$

Results and discussions

Ultrafiltration membranes based on CA and PVC are prepared with various compositions. The composition of PVC in the blend solution is varied from 0 to 30 wt% (total polymer composition 17.5 wt%) in the casting solution. By increasing PVC composition beyond 30 wt% in the casting solution results in phase separation due to incompatibility between CA and PVC polymers [23]. Hence, composition of PVC in the blend is restricted to 30 wt%. Further, maximum concentration of additive (PEG 600) is found to be 10 wt% in all the prepared blend membranes. Beyond this concentration, the blend solution exhibits incompatibility with the additive, as soon as solution blending is mechanically arrested. Hence, the maximum additive concentration in this system is restricted to 10 wt%.

Morphological studies

In order to understand the permeation results, top surface and cross-section morphologies of blend membranes characterized by SEM analysis are shown in Fig. 2a and b, respectively.

Effect of polymer composition

From Fig. 2a it can be observed that with pure CA membranes (0% additive), the pores are smaller and distributed evenly. It can be observed that the pores of the blend membranes are larger than that of pure CA membranes. As PVC composition is increased in the casting solution, pore size increases proportionately. It is noted that the pore size of CA/PVC (70/30 wt%) blend membrane is bigger than those of pure CA and other blend membranes.

Cross section of all blend membranes establishes asymmetric structure. When PVC added to CA, the asymmetric structure of the blend is changed significantly. At the base CA content, increasing PVC content gives substantial and systematic increase in the membrane permeability.

Effect of additive concentration

From Fig. 2a SEM observation illustrates when PEG 600 increases, pore size of the top surface becomes larger and the distance from the top surface to the starting point of macrovoid formation becomes larger. The decrease in protein rejection and increase in flux are due to increase in the pore size of top surface. The thermodynamically less stable membrane-forming system enhances the precipitation rate in the coagulation bath and makes porous membranes [24]. When the concentration of PEG 600 is increased in the casting solution, a thermodynamic enhancement of phase separation takes place by reducing the miscibility of solvent (DMF) and water resulting in the instantaneous demixing. This facilitates the formation of macrovoids in the membrane structure [25, 26].

From Fig. 2b, when the concentration of additive is increased from 0 to 10 wt%, the sub-layer seems to have finger like cavities as well as macrovoids. Large macrovoids would result in increase in the permeability. Due to hydrophilic nature of additive (PEG 600) instantaneous demixing takes place which results in the formation of finger like cavities in the sub-layer.

Porosity

Effect of polymer composition

Porosity is considered as an important characterization of membrane. The prepared membranes should posses more number of pores with smaller size. More number of pores increases the permeability and smaller size pores increases the selectivity. In order to have higher flux, the surface porosity should be in suitable range. Porosity of all the prepared blend membrane is shown in Table 1. For pure

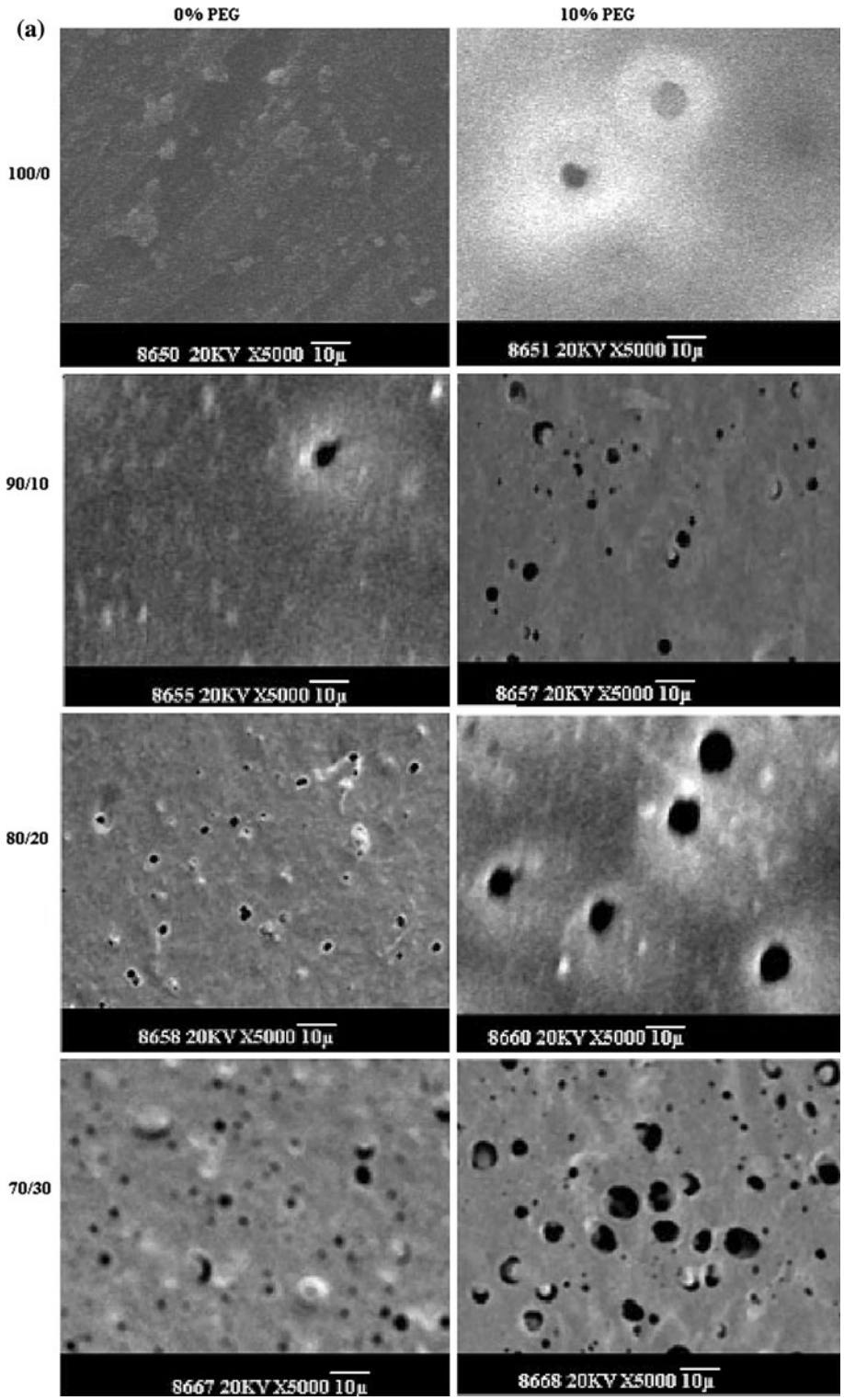

Fig. 2 **a** SEM top surface images on different polymer composition and additive concentration of CA/PVC blend membranes. **b** SEM cross-section images on different polymer composition and additive concentration of CA/PVC blend membranes

CA membrane in the absence of additive porosity is found to be 52.3%; with the addition of 10% PVC, the porosity of the membrane is increased to 55.7%, in principle, would lead to increase in the permeability; further porosity enhances to 60.8%, when there is an increase in the PVC composition to 30% in the blend solution.

Effect of additive concentration

The membranes prepared in the absence of additive shows low porosity when compared to the membranes prepared in the presence of additive. By increasing the concentration of pore former there is increase in porosity for all the blend

Fig. 2 continued

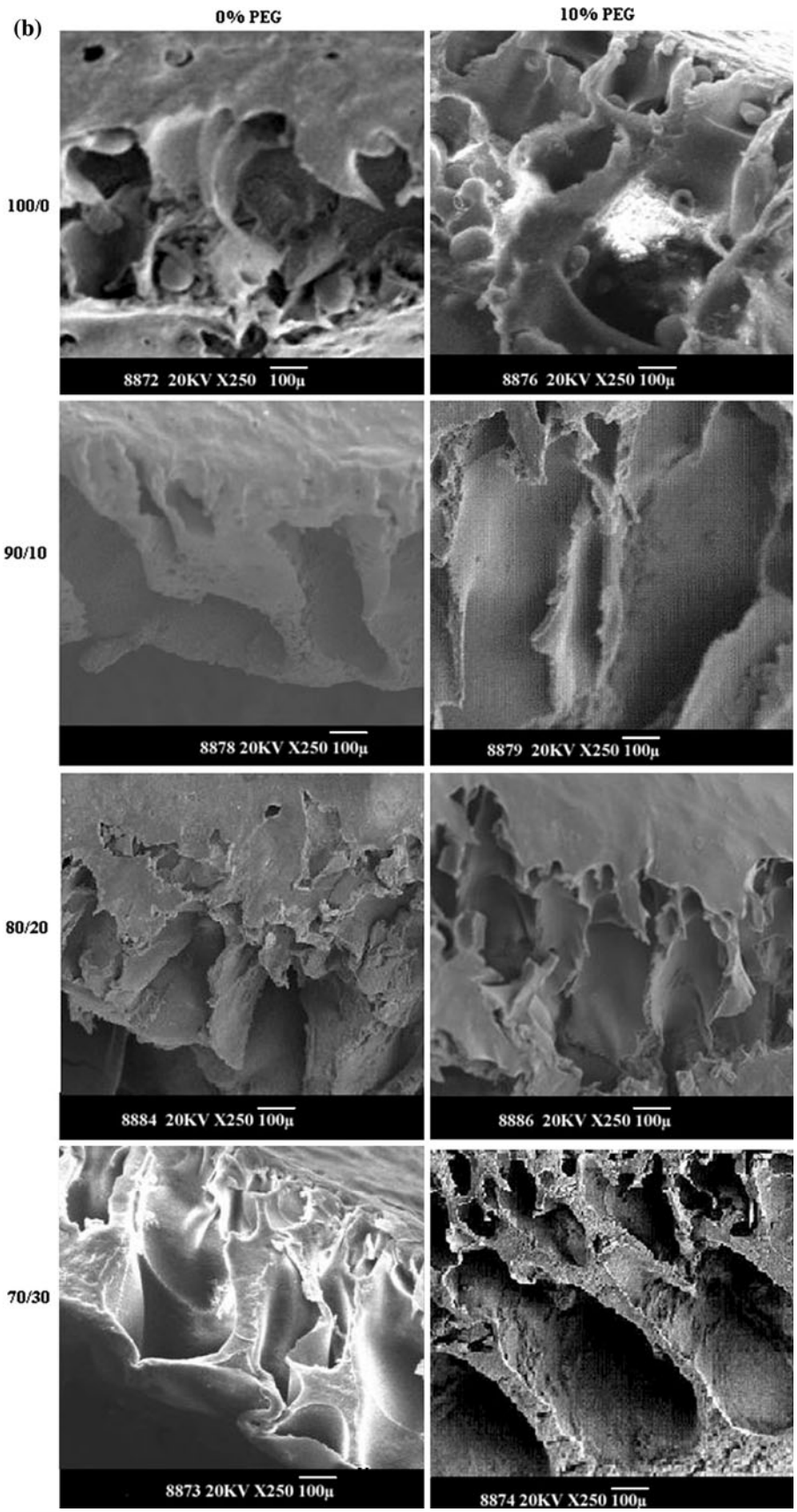

Table 1 Effect of polymer composition and additive concentration on porosity of CA and CA/PVC blend membranes

Blend composition (17.5 wt%)		Additive (wt%)	Solvent	Porosity (%)
CA	PVC		DMF (wt%)	
100	0	0	82.5	52.3
90	10	0	82.5	55.7
80	20	0	82.5	57.9
70	30	0	82.5	60.8
100	0	5	77.5	54.2
90	10	5	77.5	58.0
80	20	5	77.5	61.7
70	30	5	77.5	63.9
100	0	10	72.5	57.8
90	10	10	72.5	62.3
80	20	10	72.5	67.6
70	30	10	72.5	69.7

Table 2 Effect of polymer composition and additive concentration of proteins by CA and CA/PVC blend membrane

Polymer blend composition (17.5 wt%)		Additive (wt%)	Percentage rejection			
CA	PVC		BSA	EA	Pepsin	Trypsin
100	0	0	96	94	85	81
90	10	0	93	85	75	72
80	20	0	91	87	74	69
70	30	0	89	82	71	65
100	0	5	90	89	74	72
90	10	5	88	82	72	65
80	20	5	85	79	65	63
70	30	5	79	75	64	61
100	0	10	77	73	68	62
90	10	10	75	70	58	54
80	20	10	72	67	53	50
70	30	10	70	60	52	48

membranes. The porosity is found to be 69.7% for 70/30/10 wt% (CA/PVC/PEG 600) which is higher than the other blend membranes. Porosity increases when additive concentration increases is due to leaching of the additive from the surface of membrane during gelation. Similar results are reported by Arthanareeswaran et al. [27].

Application studies: separation of proteins

The rejection of macromolecular solutes such as BSA ($M_w = 69$ kDa), EA ($M_w = 45$ kDa), pepsin ($M_w = 35$ kDa), trypsin ($M_w = 20$ kDa) are attempted using pure CA, 90/10, 80/20, 70/30 wt% (CA/PVC) blend membranes with different concentrations of additive such as 0, 5, 10 wt%. The lowest molecular weight protein, trypsin, is taken first for the study to prevent fouling and cake formation by high molecular weight proteins. Therefore, separation of proteins is performed in the order of trypsin, pepsin, EA, and BSA.

Protein rejection studies

The pH of the individual feed solutions is kept constant at 7.2, since a change in pH may increase the fouling of the membranes [28]. Furthermore, intermolecular forces between protein molecules and membrane predominates and affects the efficiency of membranes if the pH of the solution changes.

Effect of polymer composition

The percentage rejection of proteins is shown in Table 2. The composition of the polymer plays an important role in the separation of proteins. Pure CA membrane shows the rejection of 96% for BSA and 81% for trypsin in the absence of additive. As PVC content is increased from 0 to 30 wt% in CA/PVC blend (absence of additive) the percentage of rejection decreases to 89%. This may probably due to the higher PVC content creates inhomogeneity between the polymer matrices, resulting in the formation of aggregate pores in the membranes. For 70/30 wt% blend composition, the percentage rejection values are 89, 82, 71, and 65 for BSA, EA, pepsin, trypsin, respectively. BSA shows higher rejection when compared to trypsin is due to solute size of the proteins. The rejection decreased in the order of

BSA > EA > pepsin > trypsin.

Effect of additive concentration

The effect of the PEG 600 concentration on the rejection behavior of pure CA and CA/PVC blend membranes is shown in Table 2. The concentrations of additive and percentage rejection are inversely proportional. For pure CA membrane with 5 wt% additive the BSA rejection is 90% and decreases to 77% with increase in the additive concentration of 10 wt%. In EA, for 80/20 wt% (CA/PVC) blend membrane when additive concentration increases from 5 to 10 wt%, rejection decreases from 79 to 67%. A similar trend is observed for other proteins also. This may be due to leaching out of the additive from the membranes during gelation, which creates pores proportionately in the membranes. The pure water flux studies carried out for these membranes exhibited similar trend. Similar results are observed by other researchers [29].

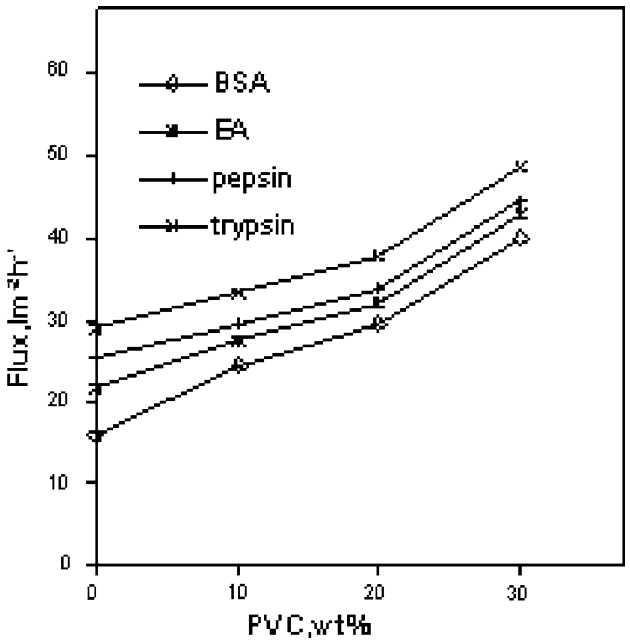

Fig. 3 Effects of composition of PVC on permeate flux of proteins for CA membranes

Protein permeate flux

Effect of polymer composition

Figure 3 illustrates the permeate flux of all the proteins for pure CA and CA/PVC blend membranes. 100% CA membrane shows the lowest permeate flux of 15.8 lm^{-2} h^{-1} for BSA in the absence of additive. The other proteins such as EA, pepsin, and trypsin show comparatively higher fluxes with pure CA membranes. In pepsin, when PVC content in the blend is increased from 0 to 30 wt%, the flux increases from 25.2 to 44.6 lm^{-2} h^{-1}. A similar trend is observed for all the other proteins. This is due to low molecular attractive forces between the blend components. As a result, there is an increase in the void volume of membranes, so the flux increases with increase in the PVC content.

Effect of additive concentration

The presence of additive in the casting solution has a significant role in morphology and in turn on the flux of the

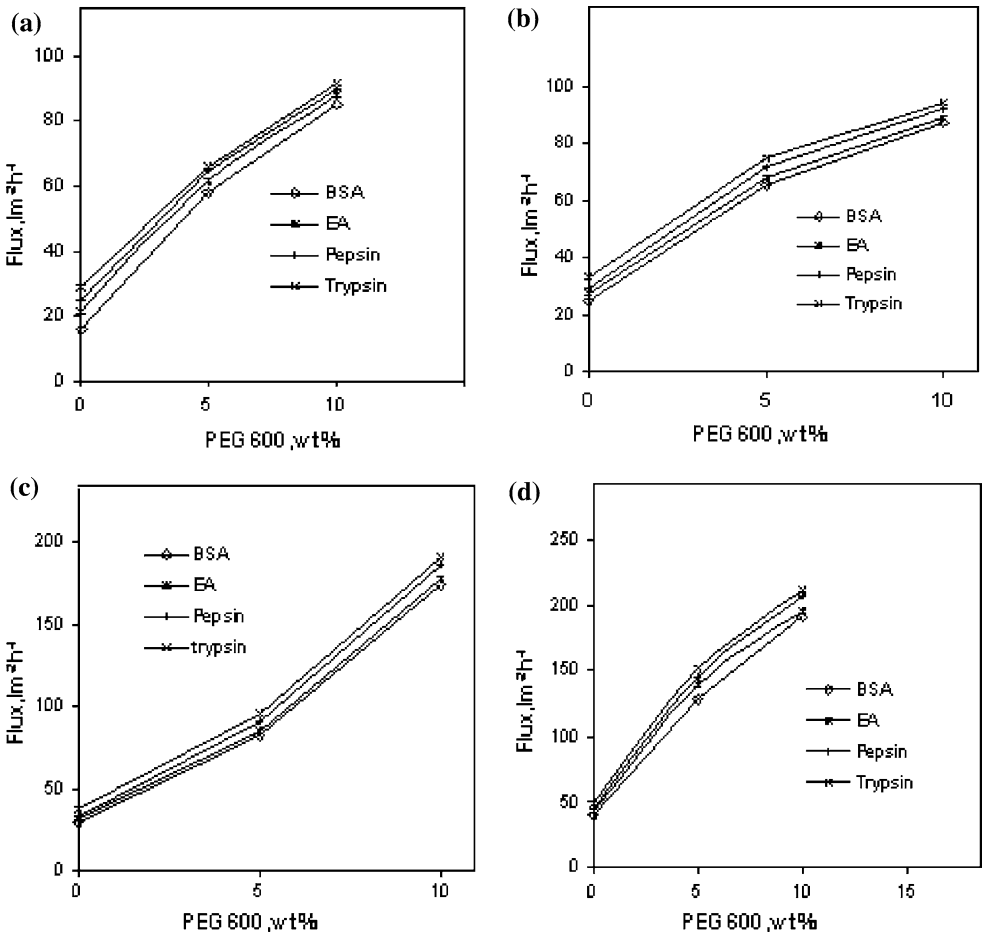

Fig. 4 Effect of PEG 600 concentration on the flux of proteins. **a** Pure CA membranes, **b** 90/10 wt% CA/PVC blend membranes, **c** 80/20 wt% CA/PVC blend membranes, and **d** 70/30 wt% CA/PVC blend membranes

resulting membranes. From Fig. 4a, pure CA membrane for BSA has a flux of 15.8 for 0% PEG 600 and 85.1 lm^{-2} h^{-1} for 10 wt% PEG 600. The other proteins also exhibited a similar trend. From Fig. 4d, for EA when additive is increased from 0 to 10 wt% protein permeate flux increases from 42.9 to 195.7 l m^{-2} h^{-1}. All the blend membranes exhibited similar behavior when additive concentration increases. Fouling reduces by increasing the concentration of additive. This may be due to the faster rate of leaching out of the additive during gelation which results in macrovoids. The order of protein flux is found to be

trypsin > pepsin > EA > BSA.

Among the protein separation, BSA is found to have the lowest flux since, the molecular weight and the flux are inversely proportional to one another.

Conclusions

Flat sheet asymmetric polymeric blend membranes based on CA and PVC are prepared using phase inversion technique. Phase separation of blends is seen beyond 30 wt% of PVC and 10 wt% of additive (PEG 600) in the casting solution. The addition of PEG 600 increases the exchange rate of additive and non-solvent, resulting in an enhancement of the macrovoid formation, which is confirmed by SEM analysis. When the composition of PVC and concentration of PEG 600 is increased in CA/PVC blend, there is increase in the porosity.

Protein separation has been studied using CA/PVC blend membrane in the presence and absence of additive. By increasing the concentration of PVC and PEG in the casting solution, there is an increase in the flux of proteins. The flux of protein is in the order of trypsin > pepsin > EA > BSA, which is directly proportional to the molecular weight of the proteins. The rejection of proteins in the presence of additive is lower and flux is higher for CA/PVC/PEG blend membranes when compared to CA/PVC and pure CA membranes.

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