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PREPARATION OF CELLULOSE ACETATE DIALYSIS MEMBRANE FOR SEPARATION OF BOVINE SERUM ALBUMIN

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Abstract. Flat sheet dialysis membranes were casted using phase inversion method, using cellulose acetate as the polymer and acetic acid as the solvent. Modification agents such as polyethylene glycol 400 (PEG 400) and non-solvent swelling agent, i.e. distillated water were added. Three different formulations were used, and the performances of the obtained membranes were tested for protein separation using a 2 mg/mL bovine serum albumin (BSA) solution. It was found that the membrane obtained from the formulation consisting of 20%wt cellulose acetate, 60%wt acetic acid, 10%wt PEG 400 and 10%wt distillated water gives a BSA rejection rate as high as 96.19%, which seems to be comparable with the commercial cellulose acetate dialysis membranes. However, testing the same membranes for separation of sucrose solution does not give satisfactory result as indicated by the low rejection rates of 50%.

Keywords: Dialysis membrane, cellulose acetate, rejection rate, bovine serum albumin (BSA), sucrose

Abstrak. Membran dialisis kepingan nipis dihasilkan dengan teknik fasa balikan menggunakan selulosa asetat sebagai polimer dan asid asetik sebagai pelarut. Polietelina glikol 400 digunakan sebagai agen modifikasi and air suling ditambah. Tiga formulasi yang berbeza telah dihasilkan dan prestasi membran dikaji dengan menggunakan 2 mg/mL larutan bovine serum albumin (BSA) yang mempunyai berat molekul 66 kDa. Daripada kajian ini, didapati bahawa formulasi dengan 20%wt selulosa asetat, 60%wt asid asetik, 10%wt PEG 400 and 10%wt air suling memberikan peratus penyingkiran larutan BSA sebanyak 96.19%. Berbanding dengan membran dialisis selulosa asetat komersial, didapati bahawa keputusan adalah memuaskan. Walau bagaimanapun, keputusan kajian dengan menggunakan 2 mg/mL larutan sukrosa tidak memberikan keputusan yang memuaskan di mana peratus penyingkiran adalah 50%.

Kata kunci: Membran dialisis, selulosa asetat, peratus penyingkiran, bovine serum albumin (BSA), sukrosa

1.0 INTRODUCTION

In the preparation of dialysis membranes by phase inversion, several variables can be adjusted to control membrane properties. Amongst these variables is the composition of the polymer such as the type and concentration of additives used.

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Although much research have been carried out on the effect of composition of polymer on membrane properties, most of the work involved reverse osmosis, ultrafiltration, microfiltration, and gas membranes. Besides the work of Henne and Dunwerg [1], Klein *et al* [2], Dunwerg *et al* [3], and Diamantoglou *et al* [4,5], not much has been reported regarding the effect of the polymer composition in dialysis membranes. Most of these mentioned works are patents and the parameters affecting the membrane preparation such as polymer concentration in the dope had not been systematically investigated.

In the recent years, investigations on dialysis membranes had been focused mostly on the characteristics as well as the properties of different commercial dialysis membrane such as sieving properties, flow misdistribution, diffusive permeabilities, and pore size distribution [2,6-9]. Middle molecule solutes from blood such as nucleic acid (C5a fragment), beta-2-microglobulin and cytochrome C have been used in some of the dialysis testing [1,4,5,10]. Albumin loss [11] and lowering of diffusive permeability caused by protein adsorption on dialysis membranes should be reduced in order to enhance dialysis adequacy of the patients. Dialysis membrane material serves to retain molecules larger than its material-related cut-off by hindering them from entering it due to sterical reason. Membranes with outstanding mechanical strength have low solute permeability, while highly permeable membranes are easily torn. Therefore, the most desirable dialysis membrane is one that stretches, light, thin and can achieve large water content while maintaining high mechanical strength [12].

Dialysis membranes can be distinguished by their material of fabrication such as cellulose acetate, poly-acrylonitrile (PAN), poly-methyl methacrylate (PMMA), ethylene vinyl alcohol (EVAL) copolymer, polysulfone (PS) and polyamide [10]. Off all, cellulose acetate is the most commonly used material for making dialysis membranes. This is due to its excellent properties such as biocompatibility, good desalting, high flux, and relatively low cost [13]. Dialysis membranes made of cellulose acetate have been known for almost five decades since Kolf during World War II [14].

Dialysis membranes are used clinically in the treatment of patients with renal failure. Its use is fast gaining importance due to the increase in the number of patients having kidney failure. According to the National Kidney Foundation of Malaysia, over 1000 people with kidney problems died in 2001 because they cannot afford dialysis treatment and recent statistics show that the number of kidney patients in the country increase by some 2,200 to 2,300 every year [15].

In view of this, locally used membranes must be developed so as to gain more insight into its fabrication process and reduce its cost. In this study, the dope formulation that produces dialysis membranes suitable for separation of BSA solutions is determined. The membrane preparation is carried out using the phase inversion process. A solution containing cellulose acetate or its derivatives, an organic carboxylic

acid, possible with the addition of the modification agents, and additives has been suggested [3]. In this experiment, three different dope formulations have been studied with the purpose of determining the most suitable composition to obtain dialysis membrane capable of protein (BSA) separation. The best membrane was then tested using sucrose solution. The performance of the membranes formed was evaluated in terms of percent rejection rate and flux flow.

2.0 EXPERIMENTAL

2.1 Materials

Cellulose acetate with the average molecular weight of 30000 Dalton was used as the membrane-forming polymer was purchased from Sigma-Aldrich. The solvent used was acetic acid (analytical purity of 99%) and distilled water was used as a non-solvent agent. Polyethylene glycol 400 (PEG 400) was used as a modifying agent.

2.2 Preparation Process

The polymer dope was prepared in a reaction vessel, where acetic acid was added in first, followed by cellulose acetate. The polymer dope was heated to approximately 70°C and a high stirrer speed was used to assist in the dissolution of polymers. The cellulose acetate was added in slowly, to ensure a complete dissolution. Finally, the PEG 400 and distilled water were added slowly to avoid any agglomeration in the polymer dope.

When the entire polymer was completely dissolved, as indicated by the clear solution obtained, it was cooled and poured into a storage bottle. Subsequently, the solution was degassed in an ultrasonic bath for about two hours to remove any micro bubbles present and kept away from direct sunlight to slow down its aging process. The three dope formulations prepared in this work are shown in Table 1.

Table 1 Formulations of three different dope solutions

Element	**Weight percentage, % wt**		
	A	**B**	**C**
Cellulose acetate	20	20	15
Acetic acid	70	60	70
Polyethylene Glycol 400	5	10	10
Distillated water	5	10	5
Total	100	100	100

2.3 Membrane Casting

The membranes were casted using a casting knife on a glass plate. The flat sheet membrane formed with thickness of 200 μm was sprayed with nitrogen gas for a few seconds. This is to allow the solidification and pre-orientation of the membrane skin by partial evaporation. Next, the membrane was immersed in a water bath to complete the phase separation, where exchange of phases occured between the solvent and water. Then, the membrane was transferred to another container containing glycerol for post-treatment to remove the excess acetic acid from the membrane. Eventually, the membrane was transferred to another container containing distillated water until it was used for permeation test.

2.4 Scanning Electron Microscopy Testing (SEM)

The membranes were snapped under liquid nitrogen, which gave a generally consistent and clean break. The membrane was then sputter coated with a thin film of gold. The membrane was mounted on a brass plate using double side adhesion tape in a lateral position. Images of cross sections of the membranes were obtained using a Phillip SEM Model XL-40 microscope.

2.5 Membrane Testing

2.5.1 Performance Evaluation Using Bovine Serum Albumin (BSA)

The performance of the dialysis membrane in terms of permeability and percentage rejection of BSA (Sigma-Aldrich, MWCO 66 kDa) was evaluated using the permeation cell as shown in Figure 1. The substance selected for the testing is BSA having a concentration of 2 mg/mL. The effective membrane area was 7.07 cm^2. The concentration of BSA in the permeate was analyzed using the Biuret Reagent [16] and its absorbance was tested using the spectrophotometer (Shidmazu UV-160) at a wavelength of 550 nm. The experiments were carried out at room temperature at 2 bar pressure.

2.5.2 Performance Evaluation Using Sucrose

The dialysis membrane with the best performance for BSA rejection rate was then used to test for 2 mg/mL sucrose (GCE, MWCO 342.30 Da) solution. The concentration of sucrose in the permeate was tested using a refractometer where standard curves have been plotted and shown in Figure 2.

The percentage retention rate can be calculated using the following equation:

$$R = \left(1 - C_p / C_f\right) \times 100\% \quad (1)$$

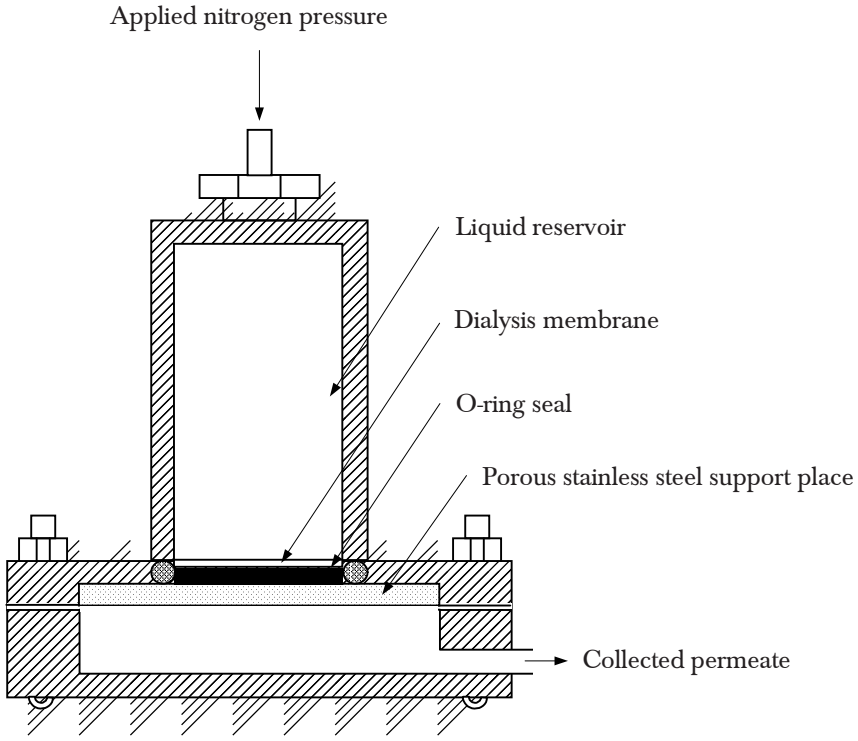

Figure 1 Schematic drawing of permeation cell

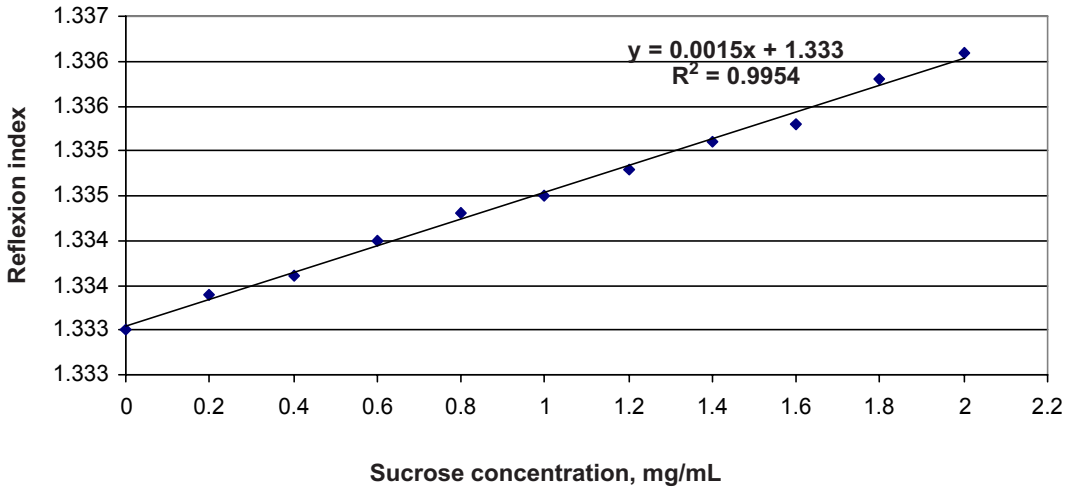

Figure 2 Sucrose standard curve

where R represents the retention rate, C_p represents the permeate concentration and C_f represents the feed concentration. The permeate's flux, J, can be calculated from its volume, V, collected during time, t. The equation below is used for the calculation of permeate's flux.

$$J = V / A.t \tag{2}$$

where A represents the effective membrane area. All experiments have been repeated three times to ensure reproducibility of the results. Results tabulated were the average values with an error of ± 3%.

3.0 RESULTS AND DISCUSSION

3.1 BSA and Sucrose Solutions Performance

Table 2 shows the retention rates of the BSA solution for three different flat sheet dialysis membranes casted. These values were plotted and shown in Figure 3. It is observed that membrane B has the highest BSA retention rate of 96.19%. The formulation B contains 20%wt cellulose acetate, 60%wt acetic acid, 10%wt PEG 400 and 10%wt distilled water. The reduced amount of the solvent acetic acid has a positive effect on the performance of the membrane. However, the amount of acetic acid must not be less than 50% to completely dissolve the polymer. The resultant membrane B has a higher packing mode of cellulose acetate in the membrane as can be observed in the SEM image in Figure 4. The higher packing density of the cellulose acetate results in smaller pore size structure. The smaller pore size results in higher BSA retention compared to the other two membranes (A and C). The presence of the additives (PEG 400) in the formulation of the membrane B is reduced or readjusts the dissolving power of the solvent (acetic acid) for the polymer and induces the formation of numerous porous polymer network entities with a finite size for each such entity (size of the super molecule polymer aggregate) [12]. The presence of a slightly higher amount of non-solvent (distilled water) accelerated the coagulation process from a solution to a gel when the casting solution was immersed in the coagulation bath [17]. This produces a thinner skin layer and a more uniform

Table 2 Results of dialysis test of BSA for various cellulose acetate membranes

Membrane	Retention rate (%)	Flux (ml/m^2.min)
A	42.51	292.374
B	96.19	420.590
C	90.19	943.140

*All the results are within ± 3 % error

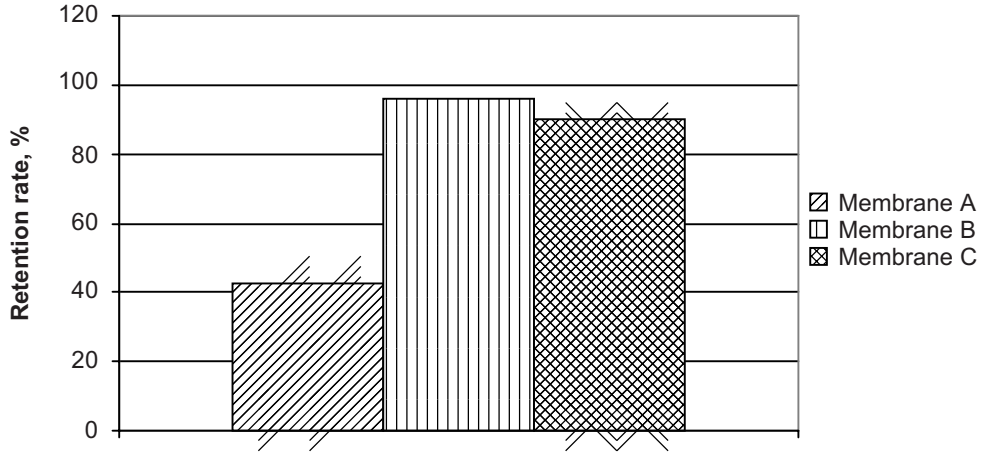

Figure 3 Retention rate of BSA for different cellulose acetate dialysis membranes

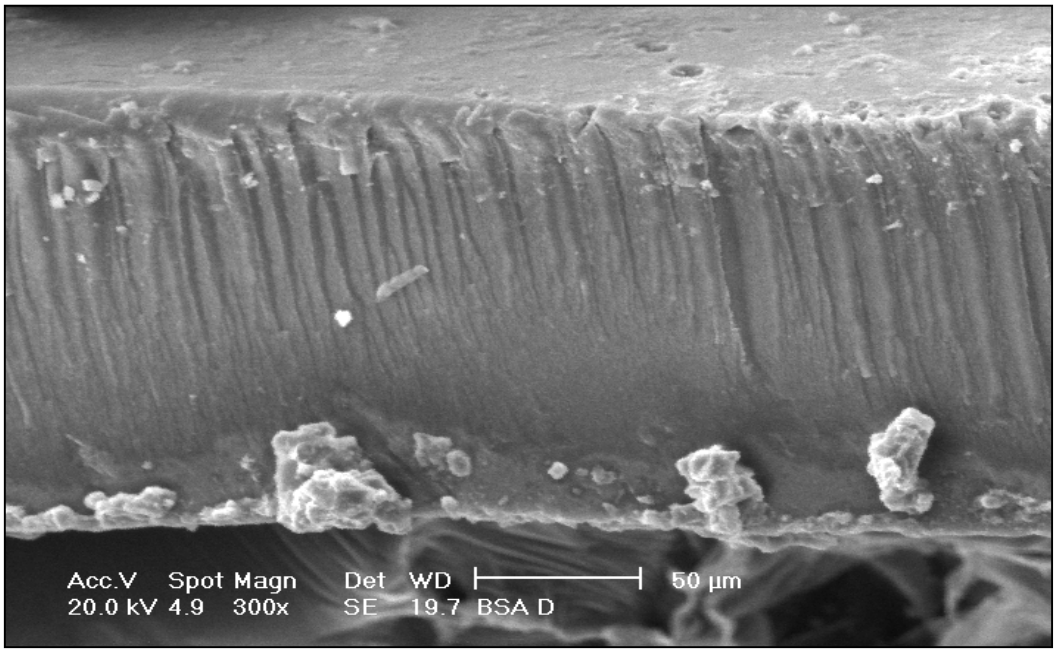

Figure 4 Micrograph cross section of membrane B

structure membrane as can be viewed from Figure 4, which gives a better separation performance [18].

The retention rate performances of membrane A and C are almost similar. The performance of membrane C is relatively higher than membrane A as shown in Figure 3. Apart from the higher retention rates of BSA, membrane B also has reasonable flux rates around 55% less than membrane C. Although the flux of

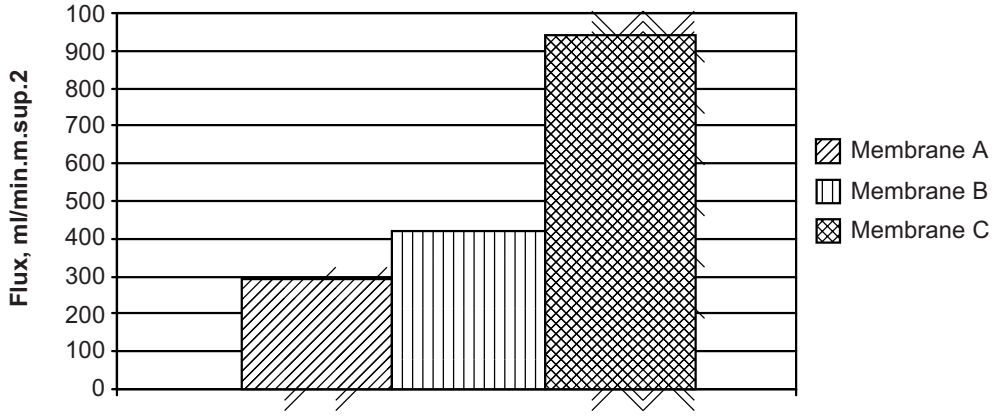

Figure 5 Flux of various cellulose acetate dialysis membranes

membrane C is the highest among all membranes as shown in Figure 5, its retention rate is less than membrane B. The high flux in membrane C could be explained by the lower amount of cellulose acetate polymer in the formulation. Thus, there are more voids (the holes among the polymer molecules) inside the membrane, which enhanced the speed of molecules passing through the membrane. This can be clearly observed in Figure 6. In brief, membrane with higher proportion of cellulose acetate polymer will give higher rejection rate but lower flux rate. Paradoxically, lower proportion of cellulose acetate polymer in a membrane formulation will give lower rejection rate but higher flux rate. As for membrane A and membrane B, the flux for membrane B is higher, as shown in Figure 5. The amount of non-solvent swelling agent (water) in formulation B is higher than formulation A, which results in membrane B being richer in hydrophilic factor and higher flux is obtained.

In conclusion, it could be stated that formulation B gives the best result in terms of protein (BSA) separation, while formulation C gives higher flux rate with slightly less retention rates. Since membrane B exhibits the best performance in terms of retention rate, it is used to separate sucrose solutions.

Table 3 shows the results obtained using membrane B for dialysis of sucrose solution testing in terms of retention rate and flux. It is observed that dialysis membrane with formulation B gives poor retention results in sucrose compared to its BSA retention of 96.19%. This behavior can be explained by taking the molecular weight of the solute into account. The BSA molecular weight cut off is 66 kDa whilst sucrose molecular weight cut off is 342 kDa respectively. Thus, the range of separation by membrane formulation B is wide enough due to its ability to separate up to 50% sucrose, which denotes as micro molecules. Upon comparison, it can be concluded that dialysis membrane made from formulation B is performing well for macro-molecules i.e. BSA separation but not suitable for micro molecules i.e. sucrose.

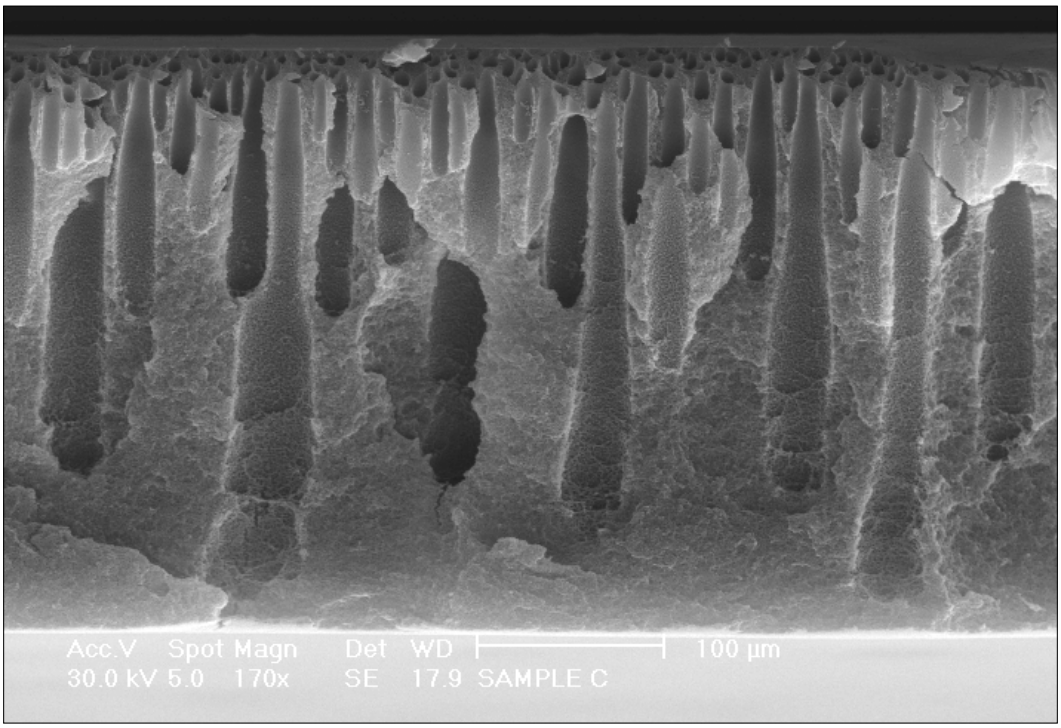

Figure 6 Micrograph cross section of membrane C

Table 3 Results of dialysis test for sucrose with membrane B

Test	Sucrose	
	Retention rate (%)	**Flux (ml/m^2.min)**
1	56.67	259.364
2	50.00	245.609
3	50.00	246.264

***** All the results are within ± 3 % error

3.2 Comparison of Self-made Membrane to Some Commercial Membranes

3.2.1 SEM Results

The structure of cellulose acetate dialysis membrane was examined using scanning electron microscope. All commercially available cellulose acetate membranes are structurally classified as asymmetric membranes; the dense surface skin membrane

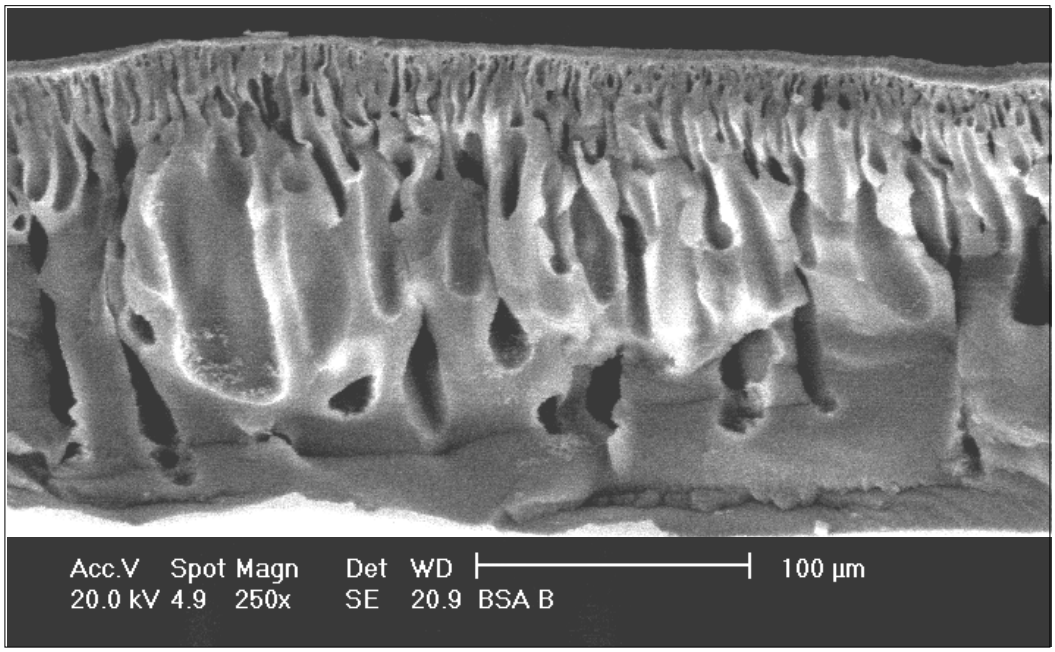

Figure 7 Micrograph cross section of membrane A

and the thick supporting under layer have the same composition, but are structurally dissimilar [19]. Upon analyzing the cross sections of the membranes produced under SEM, it was found to have an asymmetric structure as shown in Figures 4, 6 and 7, similar to the commercially available membranes.

These membranes consist of a thin dense non-porous skin layer supported by a coarse, sponge-like, macrovoid-free substructure [20]. In this study, phase inversion technique was used. During the desolvation process, the surface of membrane transformed rapidly from polymer solution to membrane (gel), resulting in a dense layer being produced. In the interior bulk region of the film, the polymer molecules aggregated and precipitated, giving a rise to a spongy porous mass underneath the surface layer [12].

3.2.2 Performance of Self-made Membrane to Commercial Membranes

Table 4 indicates the performance of protein retention for commercial Cell Sep® dialysis membrane available in the market. The membrane has an area of 14.5 cm^2 and the feed concentration for testing was 150 µg/ml. The Cell Sep® membranes exhibit 97% for the retention rate of protein [21]. Thus, the self-made membrane B prepared in this study with a 96.19% retention rate is almost comparable to the commercially available dialysis membrane in the market. In conclusion, the dialysis

Table 4 Comparison between the performance of self-prepared membrane and commercial membranes

	Retention, %
Cell-Sep	98.5*
Cell Ester	97.0*
Cellulose acetate	97.0*
Self made dialysis membrane	96.2

*Values taken from [21]

membrane B, which consists of 60% acetic acid, 20% cellulose acetate, 10% PEG 400 and 10% distillated water gave the best results in BSA separation.

4.0 CONCLUSIONS

Cellulose acetate membrane which is suitable for separation of BSA was prepared by phase inversion method. Varying the composition of the membrane formulation was found to affect its performance in terms of retention and flux to far extent. Results revealed that membrane B, which consists of 20%wt cellulose acetate, 60%wt acetic acid, 10%wt polyethylene glycol 400 and 10%wt of water gave good BSA separation. The membrane produced is an asymmetric membrane with a thin dense like skin layer and spongy tunnel like support layer underneath. However, the membrane produced is only suitable for macromolecule separation such as protein, but not for micro molecule separation like sucrose.

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