EFFECT OF SOLVENT/ADDITIVES RATIO ON CELLULOSE ACETATE DIALYSIS MEMBRANE FORMATION AND UREA CLEARANCE

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Abstract

Cellulose acetate flat sheet asymmetric dialysis membranes were fabricated via phase inversion process using acetic acid as the solvent. The effects of solvent to polymeric additives (polyethylene glycol; PEG 600) ratio on the morphology and urea clearance of the dialysis membranes were investigated. Dialysis membranes from six different formulations were casted using phase inversion method and were tested using single layer dialysis apparatus. The performances of the obtained membranes were tested using 1 mg/mL urea solution and the morphology of the membranes was characterized using scanning electron microscope. It was found that higher acetic acid/PEG ratio provide better urea clearance performance. Furthermore, the amount of additives strongly affects the membrane structure. Higher acetic acid/PEG ratio induced the formation of finger like structure macrovoids whilst dense spongy structures were found with lower acetic acid/PEG ratios. However, control experiment showed that the presence of PEG in the dope formulations is essential in order to increase the dialysis membrane performance.

1. Introduction

Dialysis first was reported in 1861 by Graham and became widely used as a laboratory technique for the solute purification. Latest research revealed that dialysis is fast gaining importance due to the increase in the number of patients having kidney failure. In fact, the total number of dialysis patients is increasing by about 8% per year in Germany and the patients receiving renal replacement therapy (RRT) increased about 20 % from year 1993 to 1995. Additionally, the total number of chronic dialysis patients in Spain is up to 17, 800, representing around 454 per million populations (pmp) [1]. Latest statistic shows that in Malaysia, intake of new dialysis patients increased 50 % from year 1980 to 2002 and the prevalent dialysis patients increased more than 100 % [2]. Thus, the developments in the field of dialysis become significantly important to reduce the mortality number shown.

There are various types of dialysis membranes found in the market these days such as cellulose acetate (CA), poly-acrylonitrile (PAN), poly-methyl methacrylate (PMMA), ethylene vinyl alcohol (EVAL) copolymer, polysulfone (PS) and polyamide [3]. Amongst the polymeric materials used, CA is commonly used as the basic material for dialysis membranes because they allow the diffusion of ions and low molecular weight solutes [4]. In addition, CA membranes also possess maximum uniformity, permselectivity and optimum physical properties [5] with very convincing characteristics like biocompatibility, good desalting, high flux and relatively low cost [6]. Several authors had carried out studies that revealed synthetically modified cellulose membranes, i.e. CA exhibits good properties and are highly comparable to other so called biocompatibility membranes, but at lower cost [7-12].

Many attempts were made by researchers to study the properties and characteristic of dialysis membrane such as sieving properties, flow maldistribution, mechanical properties, diffusive permeability and pores distribution [13-16]. Other works had also been performed to investigate the influence of different types of dialysis membrane to on hemodialysis patients [17-25]. However, most of the studies mentioned had focused solely on well developed dialysis membranes (markedly provided membrane) and rarely pin-point the self-produced dialysis membranes.

Various researches had done studies on the effect of additives to the membrane formation and membrane performance. Khayet *et al.* [26] reported that additional of non-solvent additives increases the molecular weight cut off of the membrane and enhanced the gas permeation flux. Seong *et al.* [27] also revealed that the addition of poly(vinyl pyrrolidone) (PVP) into different combination of solvent/polyamide system yield a totally different membrane morphology. On the other hand, Han and Nam [28] reported that increasing the PVP amount in polysulfone membrane would decrease the water permeate flux and diminish the formation of macrovoids. Although there had been much work carried out to investigate the membrane properties and morphology by using various additives and conditions [29-32], however, most of the above-mentioned works involved reverse osmosis, ultrafiltration, microfiltration and gas membranes. Not much had been said regarding the influence of the additives on dialysis membranes.

Therefore, the main objective of this study is to investigate the effect of solvent (acetic acid)/additives (PEG 600) to the cellulose acetate dialysis membrane with respect on urea clearance performance. The morphology of the dialysis membrane was also being discussed. In this experiment, the dialysis membranes produced were prepared from six different dope formulations with various ratios of acetic acid/PEG 600.

2. Experimental

2.1 Material

Cellulose acetate with the average molecular weight of 30,000 Dalton (Sigma-Aldrich) was used as the membrane-forming polymer. The solvent used was acetic acid (Acc) with analytical purity of 99% (Merck Co.) and distilled water was used as non-solvent agent. Polyethylene glycol (PEG) 600 (Merck Co.) was used as the additives. Experiments were performed using urea (60.02 MW) obtained from Sigma-Aldrich.

2.2 Preparation Process

Cellulose acetate of 20 %wt concentration was dissolved in mixtures of acetic acid and PEG of various ratios accept 25 %wt was used in dialysis membrane for control experiment that without additives. Polymerization temperature was maintained at 70°C with a high stirrer speed to assist in the dissolution of polymers. When the entire polymer is 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 air bubbles present and kept away from direct sunlight to slow down its aging process. All the dope formulations prepared in this work are shown in Table 1.

No.	**Ratio acetic acid/PEG**	**Acetic acid, %wt**	**Polyethylene glycol, %wt**	**Distilled water, %wt**	**Urea clearance percentage, -%**
X	-	65	-	10	20.84
1	4	56.00	14.00	10.00	26.43
2	6.5	60.67	9.33	10.00	22.71
3	9	63.00	7.00	10.00	20.43
4	11.5	64.40	5.60	10.00	25.64
5	14	65.33	4.67	10.00	28.99
6	16.5	66.00	4.00	10.00	35.48

***Table 1** : Formulation of six different dope solutions and urea clearance results*

2.3 Membrane Casting

The membranes were prepared 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 occurs 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 ready to be tested in the dialyser.

2.4 Membrane Testing

The performance of the dialysis membrane in terms of clearance was evaluated using the testing system shown in Fig 1. The total effective are of the membrane is 30 cm^2. The flow rate of the testing solution on the reservoir side is 50 mL/min whilst that on the pure water reservoir side is 100 mL/min. The temperature was maintained at 37 ± 2 °C using a Digi-sense temperature controller. Samples were collected at both reservoirs at 30 minutes intervals for a period of 210 minutes.

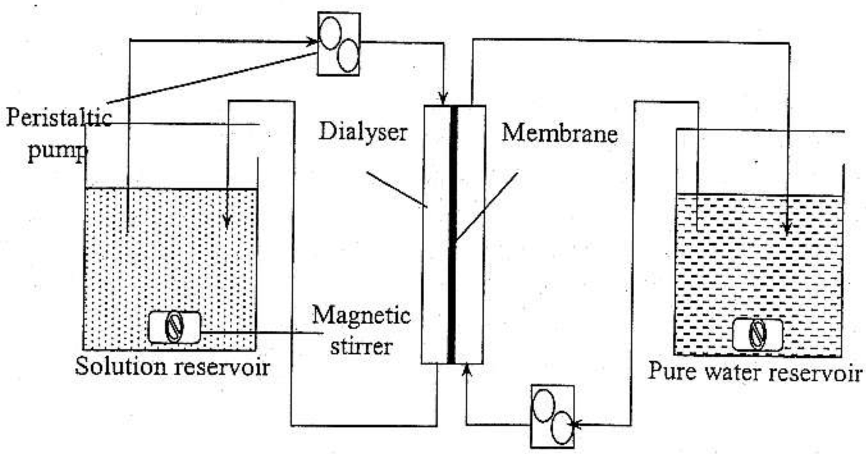


Fig 1: Schematic diagram of single membrane dialysis system

2.4.1 Testing using urea

The concentration of urea used in this experiment was 1 mg/mL, which approximately to the concentration level of kidney failure patients. The concentration of urea was evaluated using a commercial diacetyl method obtained from Eagle-Diagnostics. A 0.02 mL sample was added to 1.5 mL color reagent and 3.0 mL acid reagent and allowed to react. The urea concentration was determined from the difference in absorbance reading at 520 nm using UV spectrophotometer (UV-Spec Shidmazu UV-160). The urea clearance is calculated using the following equation: -

$$\text{Urea clearance percentage} = \frac{C_t - C_o}{C_t} \times 100 \quad , \qquad (1)$$

where C_t and C_o are the urea concentration in the testing solution reservoir at time t = 0 and t = 210 minutes respectively.

3. Results and Discussions

Table 1 showed the urea clearance percentage of the six dialysis membranes casted and Fig 2 depicted the results achieved. . In order to ensure reproducibility of the results, each of the membranes was tested three times and the average results were depicted in Table 1. Apparently, increasing of the acetic acid/PEG ratio enhanced the urea clearance efficiency for the dialysis membrane produced, membrane 6 with acetic acid/PEG ratio of 16.5 has the highest clearance percentage, 35.48 %. Higher ratio of acetic acid/PEG indicates a lower amount of polyethylene glycol in the dope solutions. Arthanareeswaran [33] reported that addition of hydrophilic additives (PEG 600) play a key role in changing the characteristics of cellulose acetate membrane in improving the permeability of proteins. The additional of PEG in our case certainly improve the solute clearance of the dialysis membranes produced. However, lower amount of PEG in the dope formulations seems to be favorable in our case.

Fig 2 depicted the plot of urea clearance percentage versus the ratio of acetic acid/PEG. It is clearly seen that the urea clearance increases as the acetic acid/PEG ratio increases, which indicate that lower amount of PEG was favorable. Urea clearance at the ratio point of 16.5 was found to be the highest amongst other ratio points and this showed that the

PEG amount needed in the formulation was less than 5 %wt. In fact, this result obtained is similar to the observation by Kim and Lee [34] in their latest findings that higher amount of PEG used in ultrafiltration membrane preparation, the flux decreased and the solute rejection increased. Moreover, our observation is in agreement with Torrestiana *et al.* [35] in their ultrafiltration membrane, which showed that lower PEG content in the membrane formulation improved its water flux and lower the lysozyme rejection.

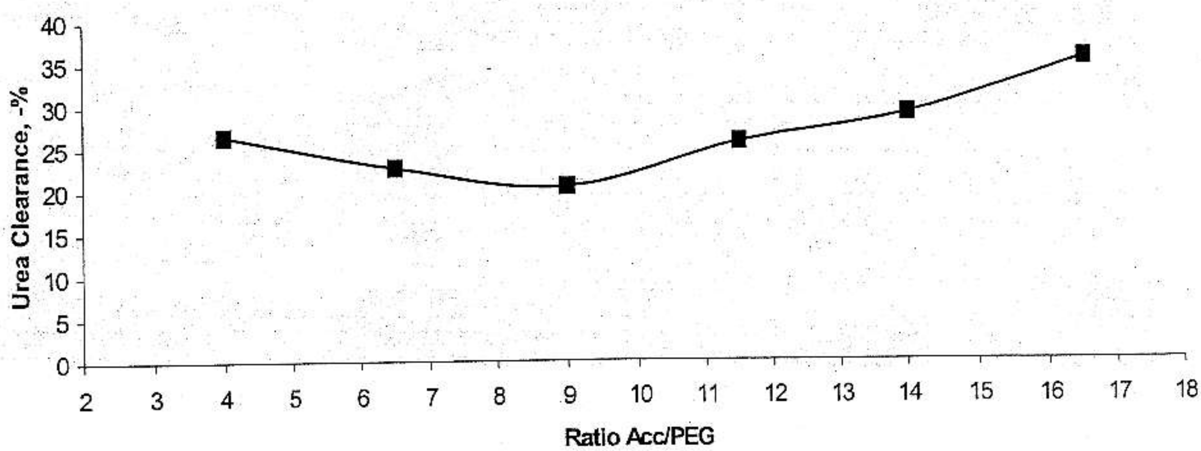
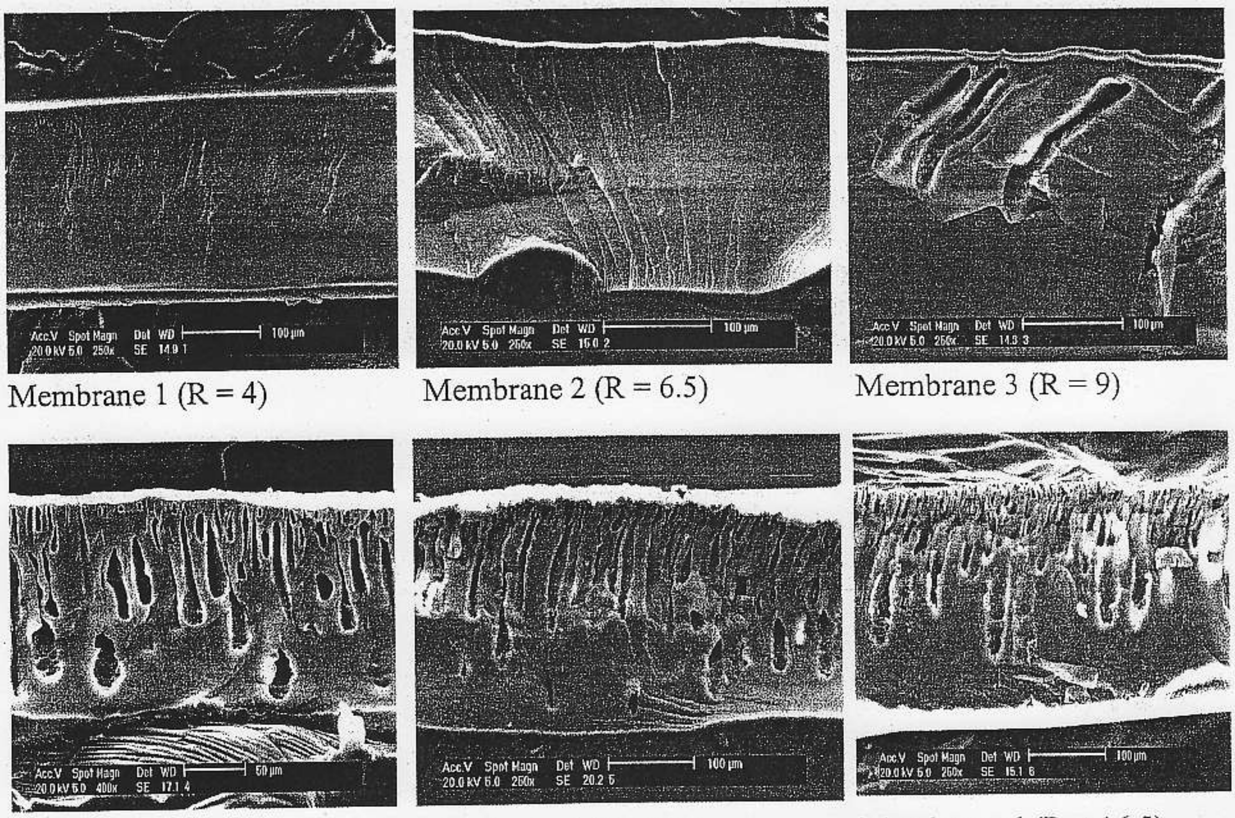


Fig 2 : Plot of urea clearance at different ratio acetic acid/PEG 600

SEM cross section images of each dialysis membrane produced at various ratios were depicted in Fig 3. It was found that the membrane morphology is significantly influenced by the variation of acetic acid/PEG 600 ratios. Dialysis membranes with lower ratio of acetic acid/PEG (membrane 1 and 2) were a dense sponge structure. As the acetic acid/PEG ratio increase, tunnel-like structure voids begins to form (membrane 3) and more were found in the dialysis membrane with higher acetic acid/PEG ratio (membrane 4, 5 and 6). In other words, lowering the additives amount, PEG in our case enhanced the formation of finger-like structure macrovoids in the dialysis membranes.

Finger like structure macrovoids are often observed in the asymmetric membranes made by phase inversion method. Smolders *et al.* [36] had reported that finger-like structure is formed during instantaneous demixing whilst delayed demixing promotes the spongy structure. However, it has been proven that appropriate amount of additives can shift the phase inversion system from delayed to instantaneous demixing but too much of the additives suppressed the macrovoids formation due to the inhibition by delayed demixing in the growth stage [36,37]. In addition, Frommer and Massalem [38] had also revealed that the presence of higher amount of additives would reduce the rate of precipitation and favor a more dense sponge structure.



Membrane 1 (R = 4) Membrane 2 (R = 6.5) Membrane 3 (R = 9)

Membrane 4 (R = 11.5) Membrane 5 (R = 14) Membrane 6 (R = 16.5)

Fig 3: *SEM cross section images of different dialysis membrane at various acetic acid/PEG 600 ratio (R)*

Finger-like structures are known suitable for ultrafiltration process and can be employed as support layers for composite membranes [36]. Higher solute removal (clearance) is desirable as the dialysis process objective is to remove uremic toxic out of the human blood. The formation of finger-like structure enable the solute to pass through the membrane easily whilst diminishing of the internodular void space would lead to an increase in solute rejection [5]. Thus, the finger like structure seems to be favorable with respect to urea clearance performance as can be seen from Fig 3. Membrane 6, which has the most finger-like structure, exhibits highest urea clearance of approximately 36 %.

Besides, too high amount of additives produced high viscosity dope solutions, which is difficult to cast. Han and Nam [28] revealed that the viscosity of dope solution would significantly increase when the amount of additives added is beyond 10 %wt and the flux decreased drastically. The increase in viscosity of casting solutions results in lower coagulation rates during immersion thus promotes delayed demixing to occur [39]. Slow coagulation rates encourage the dense spongy structure form. As mentioned earlier, formation of macrovoids favor the dialysis process that gives high urea clearance rate. This shows that lowering the amount of PEG in dope formulations will increases the urea removal percentage.

However, the dialysis membrane without PEG (membrane X), exhibits the urea clearance percentage (20.84 %) that is much lower than the other dialysis membranes produced. This shows that although only a small of PEG amounts is required, its absence will give a negative impact to the membrane performance. This result showed that additional of hydrophilic agent in the dope formulation improves the dialysis membrane performance and this seems to be in agreement with many authors [27, 34, 40].

Fig 4 shows the cross-section image of membrane X without the presence of hydrophilic agents, PEG. It exhibits a dense uniform structure without any macrovoids. The addition of suitable amount of PEG enhances the macrovoids formation as discussed earlier. The absence of PEG in the dialysis membrane promotes a dense spongy non-void structure. Seong *et al.* [27] also showed that the absence of hydrophilic agents in certain polymer/solvent system would give similar structure and result. As stated earlier, the formation of macrovoids was essential in the dialysis membranes as it improves the urea clearance performances. The nonexistence of the voids in membrane X definitely does not favor good membrane separation.

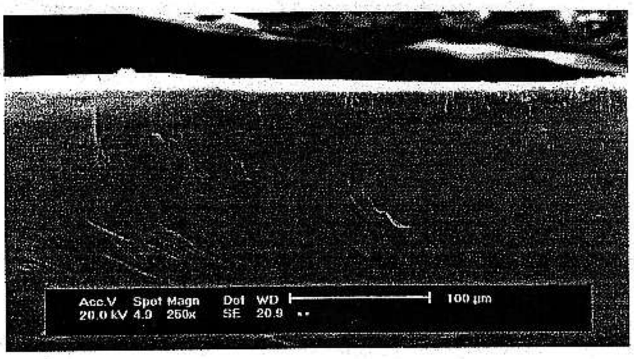


Fig 4: SEM cross section images of the dialysis membrane without PEG, membrane X

4. Conclusion

The cellulose acetate dialysis membrane produced by phase inversion method is an asymmetric membrane with a thin dense like skin layer and spongy structure underneath. Results revealed that dialysis membrane with higher acetic acid/PEG 600 ratios gives higher urea clearance percentage. The morphology of the dialysis membranes produced was strongly influenced by the acetic acid/PEG ratio. Higher ratio of acetic acid/PEG 600 ratio results in the formation of finger-like structure while lower ratio will give a dense spongy structure dialysis membrane. This study also revealed that finger-like structure is favorable to urea clearance. Although lowering the amount of PEG gives higher urea clearance performance, its absence in the dope formulations does not contribute to good urea clearance.

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