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Effect of Preparation Conditions on Morphology and Performance of Hemodialysis Membranes Prepared from Polyether Sulphone and Polyvinylpyrrolidone

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ABSTRACT

Flat-sheet hemodialysis membranes were prepared with 12% (wt) polyether sulphone (PES) and 2.8% (wt) polyvinylpyrrolidone (PVP) dissolved in dimethylacetamide (DMAc) by phase inversion method. The performances of membranes were investigated on the basis of direct removal of uremic toxins from blood serum. The membranes were put in contact with human blood serum in a batch dialyzer instrument and removal of urea, uric acid and creatinine were measured in a medical laboratory according to the standard methods. The effects of temperatures of coagulation bath and polymer solution on membrane morphology and hemodialysis performance were investigated. The SEM micrographs showed typical asymmetric channel-like structures, which their sizes and numbers change with different preparation conditions. Membrane structure depends on the diffusion rate of solvent and non-solvent molecules in the coagulation process. The temperatures of polymer solution and coagulation bath are among the most important parameters of the coagulation processes. The performance and morphology studies of hemodialysis membrane indicated that by increasing the temperature difference between coagulation bath and polymer solution temperatures, the sizes of channel- like structures were increased. A removal of 84% urea, 71% uric acid, and 53% creatinine was attained by a membrane prepared at 60°C coagulation bath and 23°C polymer solution temperatures. The L929 fibroblast cell culture test on PES membranes showed excellent biocompability.

Key Words:
membrane;
polyether sulphone;
morphology;
polyvinylpyrrolidone;
hemodialysis.

INTRODUCTION

The importance of polymeric membranes in separation processes such as sea water desalination, liquids purification, and gas separation is well established [1-4]. Some polymeric membranes also play an importante role in biological separation processes and medical devices such as blood hemodialysis instruments [5, 6].

Synthetic polymers such as polyacrylonitrile, polyvinylalcohol, polymethylmethacrylate, polyamide, polysulphone, poly-

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ethersulphone and cellulose-based materials are widely employed for preparation of hemodialysis membranes [7-11].

In the last two decades polyethersulphone as an engineering plastic by having good chemical and thermal resistance, mechanical strength, resistance to acids and alkalis, and excellent blood compatibility has been known as a favourable material for manufacturing of hemodialysis membranes [12-14]. Since, membrane preparation conditions affect structures and performances of hemodialysis membranes [15], it is considered to fabricate them in different preparation conditions.

In this study polyethersulphone (PES) was selected as the main polymer for manufacturing hemodialysis flat sheet membranes. Polymer solutions were prepared from polyethersulphone (PES) and polyvinylpyrrolidone (PVP) as a pore forming additive in *N,N'*-dimethylacetamide (DMAc) as solvent and pure water as non-solvent. Morphology and performance of the hemodialysis flat sheet membranes and the effects of polymer solution and coagulation bath temperature were investigated.

EXPERIMENTAL

Materials

Polyethersulphone (PES, Ultrason E6020, MW = 58000, flakes) supplied by BASF was employed as main polymer, polyvinylpyrrolidone (PVP, K90) provided by Fluka was used as additive. Dimethylacetamide (DMAc) (Merck) and distillated water were used as solvent and non-solvent, respectively.

Membrane Preparation

Details of flat sheet membrane preparations have been given elsewhere [16]. Homogeneous polymer solutions of membranes containing 12% (wt) PES and 2.8%(wt) PVP in DMAc were cast on a smooth glass plate by a casting rod with uniform speed. This composition had shown the best performance data among other polyethersulphone and polyvinylpyrrolidone compositions under the same preparation condition [16]. Immediately after casting, the polymer solutions on glass plates were immersed in the coagulation bath containing distilled water. The preparation conditions of flat sheet membranes were adapted by changing the coagulation bath and polymer solution temperatures. Three different coagulation bath temperatures (23, 40, and 60°C) and two polymer solution temperatures (23 and 43°C) were used for hemodialysis membrane production.

After coagulation was completed, the solidified flat sheet membranes were placed in fresh water at least for 48 h to leach out the water soluble components from them. Then, they were dried by placing each one between two sheets of filter paper at least for 48 h at room temperature.

Membrane Performance

A batch dialyzer instrument was used for determining the capability of prepared membranes to remove uremic toxins of high potential toxicity (i.e., urea, uric acid, and creatinine) from human blood serum. Details of dialyzer instrument and membrane performance calculation method have been given elsewhere [16].

The concentration gradient of each uremic toxins at two sides of the hemodialysis membrane is the driving force for the passage of each toxins from concentrated (i.e., blood serum) solution to dilute (i.e., water) side. The concentrations of urea, uric acid, and creatinine in human blood serum before and after the trails at 1,2,3 and 5 h from the begining of the experiments were measured by sampling and testing according to the standard methods in a medical laboratory.

The membrane performance i.e., concentration reduction (CR) of uremic toxins was calculated from the following eqn (1).

$$CR = 100 \times (c_1 - c_2) / c_1 \quad (1)$$

Where c_1 and c_2 are the uremic toxin concentrations in blood before and after the trails, respectively.

Scanning Electron Microscopy

The cross-sections of the prepared membranes were observed with a scanning electron microscope (Cambridge S360). For this purpose, the membranes were frozen in liquid nitrogen and then broken into two pieces. They were transferred into the microscope chamber with sample holder after sputtering with gold as conductive material. The SEM studies were carried out at room temperature and 10 kV with a magnification of 1000.

Cell Culture Assays

The mouse L929 fibroblast cells, according to ASTM F 813-83, are routinely cultured in Cell Culture Laboratory of Biomaterial Department of Iran Polymer and Petrochemical Institute and were used as a test model in this study. The cells were maintained in PRMI-1640 growth medium, supplemented with 100 IU/mL penicillin, 100 g/mL streptomycin (Gibco BRL Laboratories, Karslsruhe, Germany), and 10% fetal calf serum (FCS; Gibco BRL).

A routine subculture was used to maintain the cell line. The cells were incubated in a 95% humidified atmosphere with 5% CO_2 at 37ºC. After appropriate period of incubation, the monolayer was then harvested by trypsinization. The cell suspension of 4 10^5 cells/mL was prepared before seeding. The samples were sterilized at 121ºC for 15 min in an autoclave and duplicated samples were placed in a multi-well tissue culture polystyrene plate (Nunc, Denmark) with 1 mL cell suspension, with two wells kept as negative control, and then maintained in the incubator for 24 h.

After incubation, the samples were removed from the incubator and washed immediately in phosphate-buffered saline (PBS). The cells were fixed in graded alcohol (60, 70, 80, and 96%) and stained with 20% Giemsa for optical microscopic examinations.

RESULTS AND DISCUSSION

Membrane Performance

The performance of 12% (wt) PES and 2.8% (wt) PVP flat sheet membrane, prepared in polymer solution and coagulation bath temperatures of 23 C, is shown in Figure 1. The membrane efficiency is based on the removal of urea, uric acid, and creatinine from blood serum. A comparison is made among the 1 to 5 h data and it indicates that the removal of all uremic toxins from blood serum increases for longer periods. The 5 h duration corresponds to the hemodialysis time currently carried out for patients with kidney failure. However, there is not a pronounced difference between 3 and 5 h data. This is a basis for decrease in dialysis time, which provides more comfort for the patients.

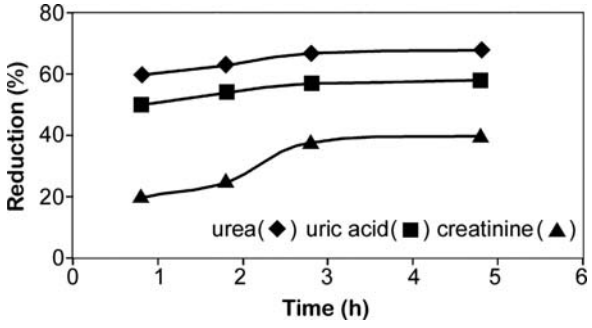


Figure 1. Reduction of uremic toxins as a function of time. Hemodialysis membrane prepared at 23°C polymer solution and coagulation bath temperatures.

Membrane Morphology

Morphology of the membrane prepared in quaternary system of (PES/ PVP/DMAc / water) at 23ºC for both polymer solution and coagulation bath temperatures is depicted in Figure 2. The typical asymmetric structure with a very thin and compact layer on the top (skin layer) and a porous and thicker layer as a support are shown in SEM micrograph. Figure 2 indicates a structure, which looks like channels or large open pores from top to the bottom of the membrane. This morphology is referred to as channel-like structure [17, 18].

Effect of Coagulation Bath Temperature on Membrane Morphology and Performance

The performance of membranes prepared at 23ºC polymer solution temperature in different coagulation bath temperatures is shown in Figure 3. For membrane with 23ºC coagulation bath temperature, the removal of urea was 60% after 1 h and 68% after 5 h. Uric acid dwindled 50% after 1 h and 58% after 5 h. Creatinine slaked around 20% after 1 h and 40 % after 5 h. When the temperature of coagulation bath increases to 40ºC, the per-

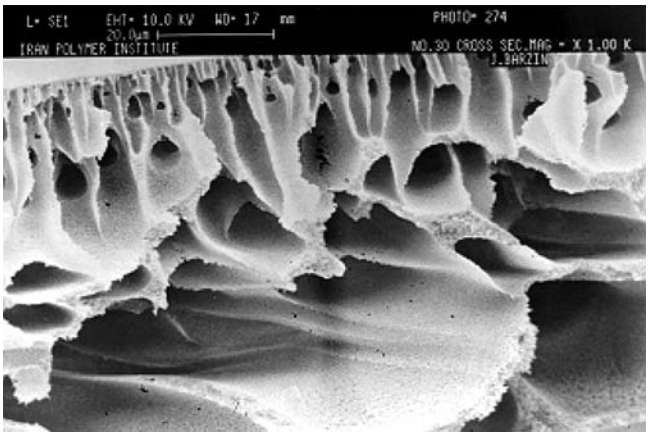


Figure 2. SEM Micrograph of cross-section of hemodialysis membrane prepared at 23°C polymer solution and coagulation bath temperatures.

formance i.e., uremic toxins removal is increased. The removal of urea was 61% after 1 h and around 70% after 5 h. Uric acid declined around 52% after 1 h and 62% after 5 h. Creatinine decreased around 35% after 1 h and 42 % after 5 h. For membrane prepared at 60ºC coagulation bath temperature, the urea removal was 84%, uric acid decreased up to 71% and creatinine was reduced up to 53 % after 5 h.

These results indicate that the membrane performance, i.e. uremic toxin removal, increases with increasing of the coagulation bath temperature. Abe et al. [15] reported the same results for flat membranes prepared from cellulose based material in water coagulation bath for hemodialysis application.

The diffusion rate of solvent and non-solvent molecules in coagulation process increased with increasing the coagulation temperature. Solvent-water exchange rates in coagulation process increase at higher coagulation bath temperature (40 and 60ºC) and coagulation phenomenon happens more rapidly compared to 23ºC coagulation bath temperature.

The morphologies of membranes prepared in different coagulation bath temperatures are presented in Figure 4. The comparison of micrographs a and b in Figure 4 implies that when coagulation bath temperature increases to 40 and 60ºC, the size and number of channels and finger-like voids are changed. For membranes prepared at 40ºC coagulation bath temperature (Figure 4a), the size of channel-like voids increases compare to the size of the similar voids in membranes prepared at 23ºC coagulation bath temperature (Figure 2). Similarly, for higher coagulation bath temperature (60ºC, Figure 4b), the size of channel-like voids increases in comparison with the size of voids in membranes prepared in coagulation bath temperatures of 23ºC (Figure 2) and 40ºC (Figure 4a).

These changes in membrane structures resulted in better performance i.e., passing of uremic toxins through those membranes which were prepared by increasing the coagulation bath temperature from 23 to 60ºC .

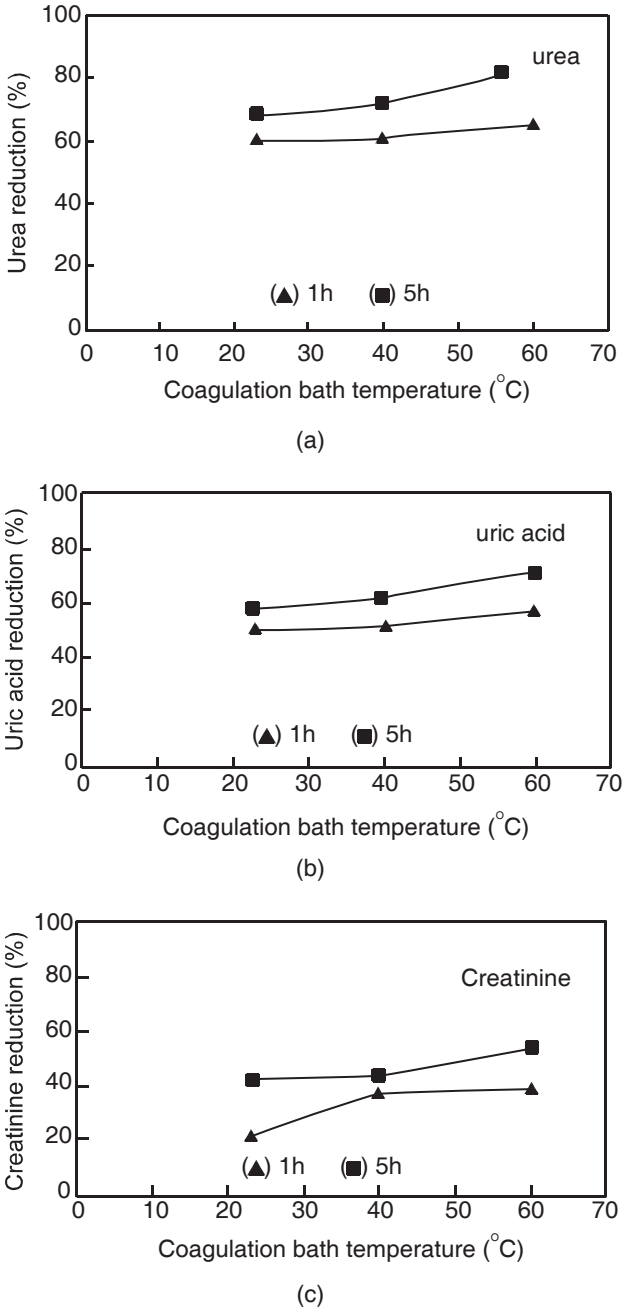
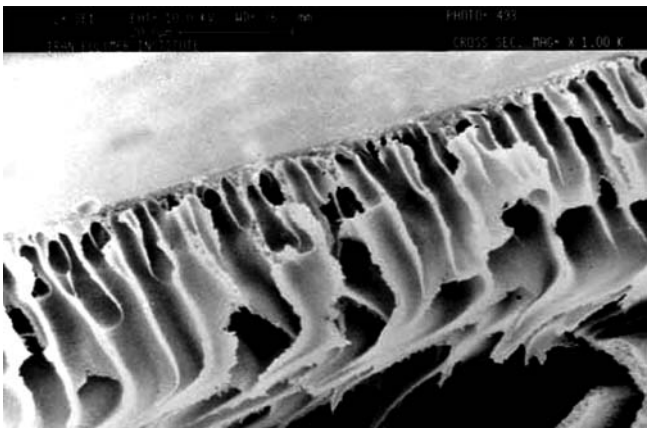


Figure 3. Reduction of uremic toxins as a function of coagulation bath temperature, using hemodialysis membranes prepared at 23°C polymer solution temperature, after 1 and 5 h, (a) urea, (b) uric acid, and (c) creatinine.

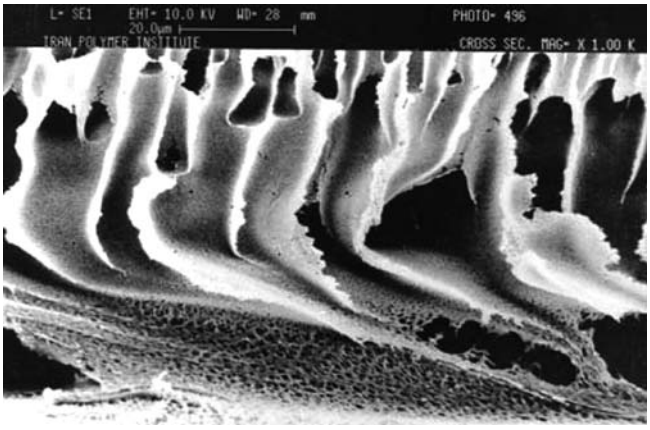
Effect of Polymer Solution Temperature on Membrane Morphology and Performance

To elucidate the effect of polymer solution temperature on membrane performance and morphology, the polymer solution temperature was raised to 43ºC and membrane preparation was carried out at different coagulation bath temperatures (23, 40, and 60ºC). The performance of membranes removal of uremic toxins are shown in Figures 5a-c.

For membrane, prepared at 23 C coagulation bath



(a)

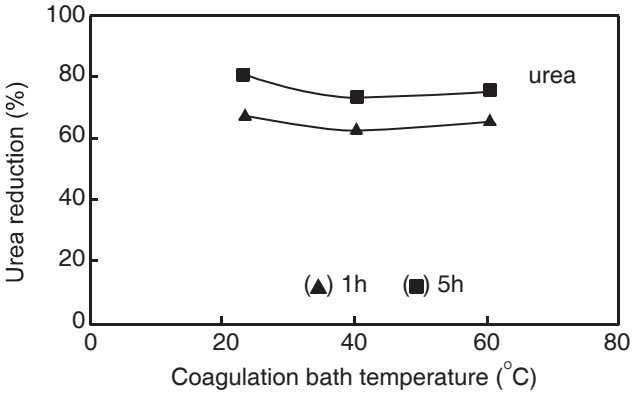


(b)

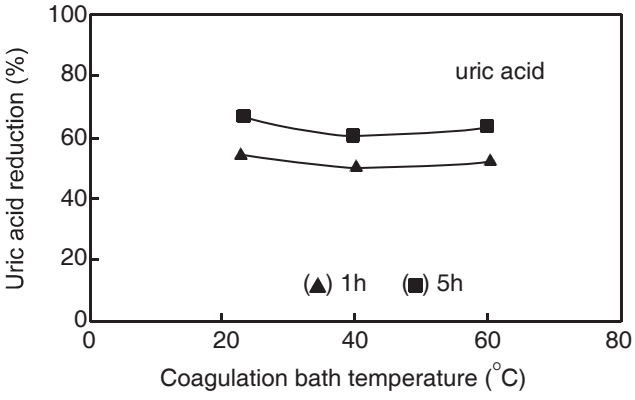
Figure 4. SEM Micrographs of cross-sections of hemodialysis membranes prepared at 23°C polymer solution temperature as a function of coagulation bath temperature: (a) coagulation bath at 40°C and (b) coagulation bath at 60°C.

temperature, the removal of urea was 64% after 1 h and 77% after 5 h. Uric acid decreased 54% after 1 h and 66% after 5 h. Creatinine was lessened around 33% after 1 h and more than 45 % after 5 h. For membranes prepared at 40 C coagulation bath temperature the removal of urea, uric acid, and creatinine, was around 70%, 60%, and 40%, respectively after 5 h.

By 60ºC coagulation bath temperature, urea removal was 62% and 72% after 1 h and 5 h, uric acid removal was up to 52 and 63% after 1 and 5 h, and creatinine was decreased up to 31% and 43% after 1 and 5 h tests, respectively. As the results show the membrane performance that is prepared at 43 C polymer solution and 23 C coagulation bath temperatures is better than the performance of membranes prepared at 40 C and 60 C coagulation bath temperatures, respectively.



(a)



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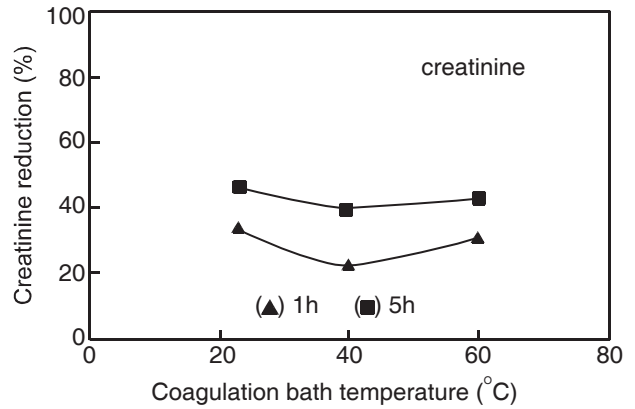
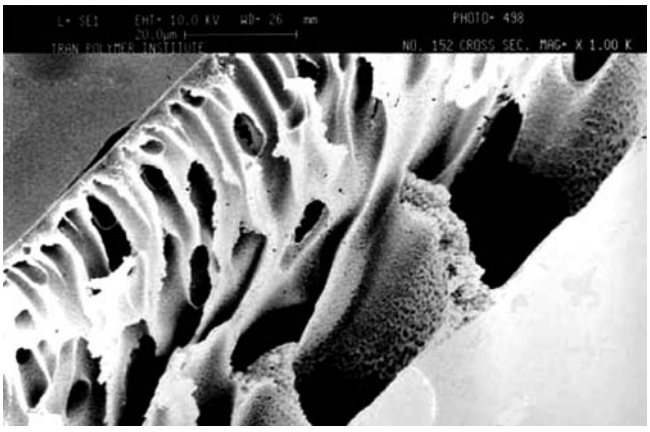


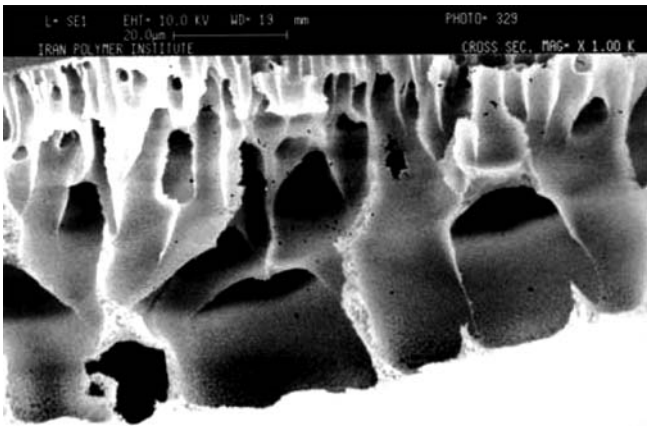
(c)

Figure 5. Reduction of uremic toxins after 1 and 5 h as a function of coagulation bath temperature, using hemodialysis membranes prepared at 43°C polymer solution: (a) urea, (b) uric acid, and (c) creatinine.

The morphology of membranes prepared at 43ºC polymer solution temperature is shown in Figure 6. These micrographs, similar to Figures 2 and 4, show channel-like structures. They indicated that when the



(a)



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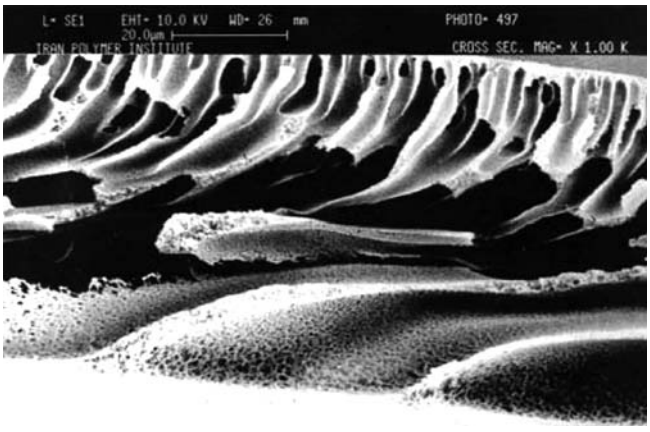
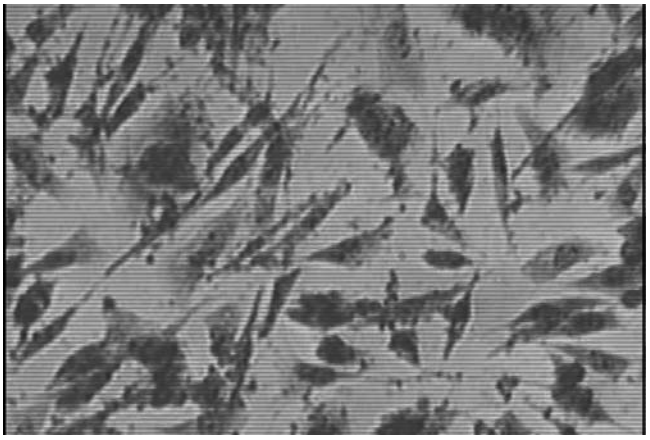
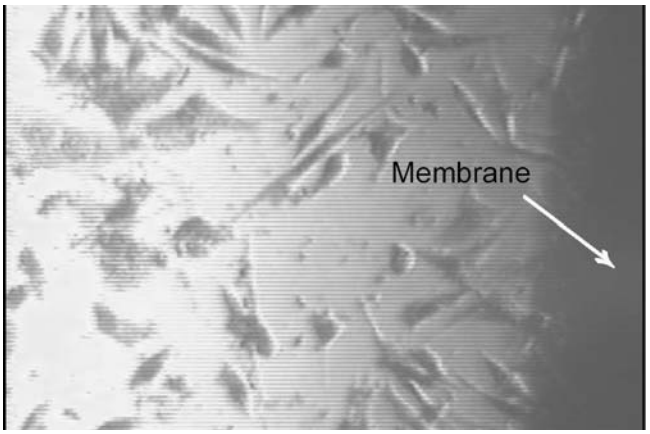


(c)

Figure 6. SEM Micrographs of cross-sections of hemodialysis membranes prepared at 43°C polymer solution as a function of coagulation bath temperature: (a) 23°C, (b) 40°C, and (c) 60°C.



(a)



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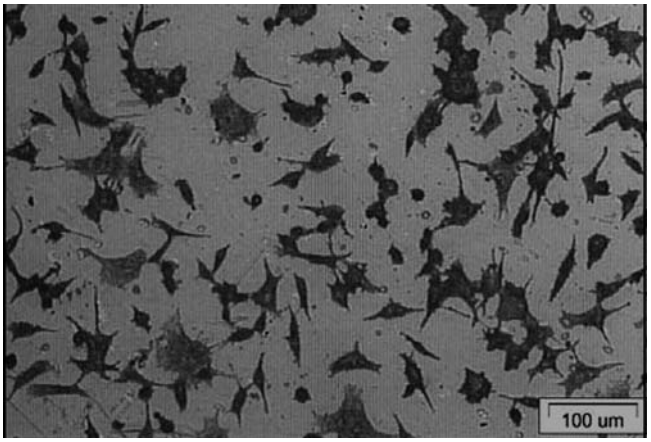

(c)

Figure 7. Optical micrograph of L-929 cells cultured: (a) negative control, (b) 12% PES and 2.8 % PVP membrane and adjacent area, and (c) 12% PES and 2.8 % PVP membrane surface.

temperature difference between coagulation bath and polymer solution decreases, the sizes of channel-like structures decrease. For example, the channels in membrane prepared at 43ºC polymer solution and 60ºC coagulation bath temperatures (Figure 6c), were smaller than those channels in membrane prepared in the same condition, but at the coagulation bath temperature of 23ºC (Figure 6a). In the coagulation process the sol-

vent molecules in the cast film and the non-solvent molecules in coagulation bath are exchanged. On the other hand, the diffusion rates of solvent and non-solvent molecules depend on the temperatures of polymer solution and coagulation bath. Therefore, an increase in the temperature difference of these conditions make this exchange takes place rapidly.

Cell Culture

Cell culture method as reported by Mirzadeh et al. [19] was used to evaluate cytotoxicity of the membranes. In the cell culture tests the cell behaviour in the presence of biomaterial is evaluated in comparison with a tissue cell culture polystyrene (TCPS) as a negative control. Cell attachment and spreading were evaluated by light microscopy.

As shown in Figure 7a the complete fibroblast growth contains adhesion and spreading of the cells, growth of filopodia, cytoplasmic webbing and flattering of the cell mass and ruffling of peripheral cytoplasm observed on the negative control. The results on 12% PES and 2.8% PVP membranes showed that cell proliferation and spreading were observed on the polyethersulphone membranes (Figures 7b and 7c). These results indicated that PES membranes cause no toxicity in the adjacent area and medium and the cell behaviour is the same as the negative control. PES is known as a biomaterial with no cytotoxicity [12-14]. Our results are consistent with these reports in the literature and shows that our process in this work has no effect on the PES biocompability.

CONCLUSION

The flat-sheet membranes prepared in quaternary systems of (PES/ PVP/ DMAc/water) by phase inversion method showed typical asymmetric channel-like structure. The removal of uremic toxins (i.e., urea, uric acid and creatinine) from human blood serum increased with time. Membrane performance i.e., removal of uremic toxins increased with increasing the temperature of coagulation bath. The size of membrane channels increases with increasing the coagulation bath temperature. Changing the temperature of the polymer solution showed that higher temperature difference between the coagulation bath and polymer solution temperatures causes faster coagulation with larger channel-like structure and therefore, a better hemodialysis performance takes place. Fibroblast cell culture improves the biocompatibility of PES membranes without cytotoxiticy.

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