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Research Paper

High-Performance Hemodialysis Membrane: Influence of Polyethylene Glycol and Polyvinylpyrrolidone in the Polyethersulfone Membrane

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Keywords

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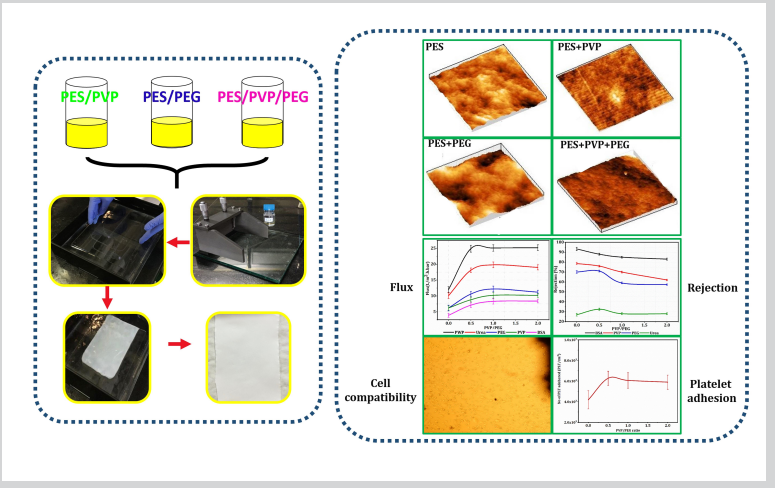
Highlights

- The simultaneous effect of PVP and PEG in the PES membrane
- Demonstrating better compatibility of PVP/PES rather than PEG/PES
- AFM study to evaluate mean pore size and surface roughness
- Maximum permeability of urea and maximum rejection of BSA for developed membranes
- Evaluation of blood compatibility with LDH method

Graphical abstract



Abstract

To the preparation of high-performance hemodialysis membrane, the effect of polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and also the simultaneous effect of both additives in the polyethersulfone (PES) membrane were investigated. Viscosity measurements demonstrated that PVP has better compatibility with PES, owing to the amorphous nature, closer glassy transition temperature (T_g), and solubility parameters rather than PEG (semi-crystalline and low T_g). This could lead to enhancement in the solution viscosity. SEM results revealed that membranes morphology was dependent upon casting solution viscosity and with increasing viscosity; the formation of macro-voids suppressed and achieving to a membrane with a smaller mean pore size would be possible. The results of the AFM study demonstrated that, with the addition of PVP, membranes with smooth surface were achieved. In contrast, the PEG addition led to a rougher membrane surface. The results verified that PEG had a tangible effect on the permeability of membrane rather than PVP or blend of PVP and PEG, which is owing to its impressive pore-forming role. The maximum pure water permeability (PWP) was achieved for MV4 (24.9 L/m^2.h.bar), MG2 (44.8 L/m^2.h.bar), MVG2 (25.2 L/m^2.h.bar), and MVG3 (25.1 L/m^2.h.bar). Rejection test showed that MV3, MV4, MG3, MG4, MVG2, and MVG3 had the best performance in terms of urea removal and maintaining other components, especially bovine serum albumin (BSA). In-vitro cytotoxicity demonstrated the biocompatibility of MV2, MG3, and MVG3 as representative of all membranes. The lactate dehydrogenase (LDH) test confirmed that PVP has a tangible effect on the reduction of platelet adhesion on the membrane surface.

1. Introduction

Hemodialysis recognized as one of the most common methods for the treatment of patients with end-stage renal disease (ESRD). National hemodialysis institute classified dialyzers into two categories: low flux and high flux dialyzers. Membranes using in the hemodialysis process are the main part of the dialyzer [1,2]. Membrane characteristics are strongly undergoing its performance. In the hemodialysis membrane, further separation of toxin materials, maintaining beneficial blood components like albumin is a vital issue. Therefore, a high-performance hemodialysis membrane should have the maximum separation of uremic toxins without removing beneficial blood substances. Many factors affecting membrane

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performance such as morphology, mean pore size, pore size distribution, hydrophilicity, etc. To achieve a high clearance of large molecular species in a short time, a dialyzer membrane with a high sieving coefficient for large molecules is required [3]. Although hemodialysis is an effective and applicable method for treatment of ESRD patients, according to the patient's condition, usually three times weekly for a period between 3 and 5 h require [4,5]. The characteristics and performance of membranes are the main determinative factors for the quality of the hemodialysis process. Generally, the membrane process is one of the most important and favorite techniques in separation industries. Phase inversion is the favorite and common way to produce membrane for various applications such as hemodialysis, gas separation, reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF). Membrane properties including morphology, porosity, permeability, pore size, and etc. have an impressive effect on the performance [6]. Membrane characteristics are dependent upon its preparation stage [7]. Various parameters such as the composition of casting solution, coagulation bath, and ambient conditions such as relative humidity and temperature, have a direct effect on the characteristics of the final formed membrane [8]. Hence, changing one or more variables will influence the structure and performance of the membranes. Investigations on these parameters have been reported repeatedly [6,9-11]. Various polymers were used in membrane industries. The shortcoming of some membranes such as cellulose-based membranes in hemodialysis led to the development of hemodialyzer based on the high-performance membranes such as polysulfone, PES, polyamide (PA), etc. [12]. PES is one of the most known and considerable candidates for this purpose. PES is a hydrophobic polymer and has high stability in harsh environments and biocompatibility as well [13-15]. Due to the outstanding properties such as high resistance against a wide range of pH and temperature, high T_g (225 °C), great durability against sterilization stage and excellent mechanical properties make PES as one of the considerable polymers for membrane industries [16], especially for blood purification processes such as hemodialysis, hemofiltration, hemodiafiltration and plasmapheresis [17-19]. Because of partial hydrophobicity, PES may undergo a fouling phenomenon. Applying hydrophilic additives are a common way for the improvement of membrane hydrophilicity. Various materials can be used for this purpose such as water-soluble polymers like PVP, PEG, polyvinyl alcohol (PVA), and inorganic additives like TiO_2, ZnO_2, SiO_2, and etc. [20-23]. Barzin et al. [13] applied PVP and PES for the preparation of the hemodialysis membrane and evaluated the effect of PVP on the characteristics and performance of the membrane. Boschetti-de-fierro et al. [24] used PVP-K85 and PVP-K30 in polyarylethersulfone (PAES) to adjusting molecular weight cut-off for the development of the hemodialysis membrane. Barzin et al. studies on the effect of PVP molecular weight on the morphology and performance of hemodialysis PES membrane [25]. Vatsha et al. [26] used PVP-K40 in the PES membrane for water purification to the improvement of antifouling property of the membrane. Chakrabarty et al. [27] blended PEG and polysulfone and their results demonstrated that with the addition of PEG, pore size, porosity, and water permeability would increase. Ghayeni et al. [28] used PEG in the PES membrane to adjusting morphology. Their results revealed that with the incorporation of PEG, reaching to a sponge-like morphology is possible.

Said et al. [29] in order to develop the hemodialysis membrane, incorporated Fe_2O_3 in the polysulfone membrane for separation of middle molecular weight of uremic toxins. Their results showed that with the addition of Fe_2O_3, hydrophilicity, and pure water permeability of the membrane were improved and showed an excellent separation for urea and lysozyme. Yu et al. [30] prepared a heparinized thin-film composite membrane with a sub-micron ridge structure for efficient hemodialysis and their results showed that the synergistic effect of modification with heparin and topography of sub-micron ridged surface improved the protein anti-fouling properties and its anticoagulant activity. Irfan et al. [31] used carboxylic functionalized multiwall carbon nanotube (MWCNT) and PVP-K30 in the PES membrane for preparation of the hemodialysis membrane. Their results confirmed the better blood compatibility and rejection capability rather than the pristine PES membrane.

Various approaches are applied to the development of hemodialysis membranes such as chemical modifications, but blending with hydrophilic materials is a practical and simple method for developing of the membrane. Usually, these materials are cost-effective and the properties of membranes are more tunable by using hydrophilic additives. Hence, the purpose of this study was based on the development of a high-performance hemodialysis membrane with a simple method of using for membrane manufactures. Although several works are reported around blending of PES and PEG or PVP, the current study has been focused on the influence of each additive and also the simultaneous effect of PEG and PVP to producing of a high-performance hemodialysis membrane by adjusting of membrane morphology, mean pore size, permeability, rejection capability, bio and blood compatibility. The best performance for the hemodialysis membrane is the maximum permeability of uremic toxins like urea and the maximum maintaining of other beneficial blood proteins such as albumin and coagulation factors. The precise evaluation has been done on the characteristics and performance of developed membranes and exhibited that achieving a high-performance hemodialysis membrane with tunable characteristics is possible by the use of PVP or PVP/PEG blend. For the first time, the better compatibility of PES/PVP rather than PES/PEG is verified via viscosity measurements and assigned to the intrinsic properties of each additive such as physical properties and solubility parameters. AFM analysis has been used to study the effect of PEG, PVP, and blending of both polymers on the membrane surface topography and roughness, mean pore size, water flux, and rejection. Also, the bioactivity of membranes assessed via cytotoxicity and platelet adhesion tests.

2. Experimental

2.1. Materials

PES (Ultrason E6020, Mw=58000 g/mol) as the main polymeric matrix of membranes was purchased from BSAF Co. (Germany). N, N-dimethylacetamide (DMAc, Analysis grade, purity > 99%) as solvent supplied from Merck Co. PEG 600 (Mw=600 g/mol) (Merck Co.) and PVP-K90 (Mw=360000 g/mol) (Fluka Co.) used for membrane preparation. PEG 35000 (Mw=35000g/mol), Urea (Mw=60.6 g/mol) and bovine serum albumin (BSA, Mw=66000 g/mol) which supplied from Merck together with PVP-K40 (Mw=40000 g/mol) (Fluka Co.) were performed for rejection tests.

2.2. Membrane preparation

Flat sheet membranes were prepared through the phase inversion process. For casting solution preparation, different ratios of PVP and PEG were mixed with DMAc in sealed glassy bottles on a magnetic stirrer. After dissolving, PES was added in a constant ratio of 15 wt.% (Table 1). Mixing was done until a homogenous solution was obtained. After degassing during overnight, prepared solutions were cast directly upon a glass plate by a film applicator adjusting at 300 μm air gap and then immersed in the non-solvent bath, where the exchange of solvent/non-solvent occurred. After separation of formed membranes from glass support, it transferred into fresh deionized water for 24 h to solvent removal completely. Then membranes were kept between two pieces of filter paper to dry at room temperature.

Table 1
Casting solution composition (wt.%) containing 15% PES

Membranes	PVP	PEG	DMAC
M0	0	0	85
MV1	1	0	84
MV2	2	0	83
MV3	4	0	81
MV4	6	0	79
MV5	10	0	75
MG1	0	2.5	82.5
MG2	0	5	80
MG3	0	7.5	77.5
MG4	0	10	75
MG5	0	20	65
MVG1	2	4	79
MVG2	3	3	79
MVG3	4	2	79

2.3. Viscosity measurement

Viscosities of the prepared casting solutions were measured by DV-II + Pro Digital viscometer (Brookfield, USA). For this purpose, the prepared solutions were poured in a special cell, where a spindle existed. A water bath

was applied to adjust the room temperature. To have reliable results, measurements were performed in 3 different times for each solution and average values were reported.

2.4. Membrane morphology

Cross-section morphology of the membranes was observed by scanning electron microscopy (SEM) (VEGA 3SBH\TESCAN, Brno, Czech). A piece of the membrane was immersed in liquid nitrogen for 30 min, then, it was broken in it. Membranes were placed on sample holders vertically and were coated by a thin layer of gold as a conductive material. The observation was performed at 20 kV with 500 x magnification.

2.5. Atomic force microscopy

A Dual Scope C-26 DME/ Denmark AFM instrument was applied to study the surface properties of the membranes, measuring of roughness parameter (S_a) and mean pore size estimation. The mean roughness parameter was calculated by equations 1:

$$S_a = \int\int_a |Z(x,y)| dxdy \quad (1)$$

Mean pore sizes were measured by visual inspection of the line profiles of different pores in various regions of the membrane surfaces. The sizes of 50 pores were measured and arranged in ascending order and the median rank was determined by equation 2 for all of the membranes [21].

$$\text{Median or 50\% rank} = [(j - 0.3) / (n + 0.4)] \times 100 \quad (2)$$

where j is the order number of the pore when arranged in ascending order and n is the total number of pores measured (50, in here) [21, 22].

2.6. Water contact angle measurement

The water contact angle was performed by a Kruss contact angle measuring System- G10 (Germany) based on the LBW02 method at 23±5 ˚C. For this purpose, the membranes were cut into dimensions of 2×2 cm^2. The upper side of each sample was thoroughly washed with water to remove all fats remained by hand touch. Then water droplets were placed on various parts of the samples by a syringe. Contact angle measurements were carried out in 5 points for each sample, and average values were reported. Also, Young-Dupre relation was used to estimation of surface free energy of membranes [32]:

$$\Delta G = (1+\cos\theta)\,\gamma_L^T \quad (3)$$

In Equation 3, θ is water contact angle between the water drop and the surface of the membrane, and γ^T representing water surface tension (72 mJ/m^2).

2.7. Permeability and rejection tests

Filtration was performed under constant transmembrane pressure (2±0.1 bar) at 25 ˚C (Scheme 1). The effective surface area of the membranes was 20 cm^2. Firstly, pure water was transferred to the membrane cell for measuring pure water permeability (J, L/m^2.h.bar). After 1 h and achieving a steady-state flow, water permeation was determined in a specific duration of time, by use of Equation 4. This procedure was repeated for the aqueous solutions containing urea (1000 ppm), PEG35000 (1000 ppm), PVP-K40 (500 ppm), and BSA (100 ppm). Each solution was used individually and their flux results were determined.

$$J = V/(A \times P \times t) \quad (4)$$

Where V (L); is the volume of permeate, P (bar); is transmembrane pressure, A(m^2); is membrane effective surface area and t (h); is the time of filtration.

After filtration, the concentration of feed and permeate samples were determined. For urea, standard methods in medical laboratories were applied. TOC analyzer (Multi N/C 3100, Analytic Jena, Germany) was used for PEG35000 [22] and UV spectrophotometry (CECIL 304, UK), was used for PVP-K40 and BSA [22, 23]. Then, the rejection was measured based on Equation 5.

$$R = (1 - C_p/C_f) \times 100 \quad (5)$$

Where C_p and C_f are the feed and permeate concentrations, respectively.

2.8. Membrane bioactivity

In-vitro cytotoxicity of membranes was assessed by cell culture method based on the ISO-10993-5-2009. For this purpose, firstly, L-929 mouse fibroblast cells transferred in a culture medium containing 100 IU/mL penicillin and 100 µg/mL streptomycins and seeded at 37 °C for 1 week in a humidified atmosphere containing 5% CO_2. Then a suspension of cells with a density of 4×10^5 cells /mL was prepared. On the other hand, Membrane samples were sterilized in an autoclave and then punched in a tissue culture polystyrene plate (TCPS). After that 1 ml of culture medium added to the microplate and placed in an incubator for 24 h. thereafter, membranes were washed with PBS and adhered cells were fixed with 60%, 70%, 80%, 90%, and 96% ethanol solutions. Then cells were colored with Giemsa solution (20 wt.%) and their morphology was observed under an optical microscope.

To evaluation of membranes blood compatibility, platelet adhesion was performed. For this purpose, the lactate dehydrogenase (LDH) method provides a quantitative determination of the number of platelets adhering to the membrane surface that is providing the pro-coagulant site. Firstly, membrane sterilized in an autoclave and then exposure with platelet-rich plasma (PRP) (supplied from blood transfusion organization). After incubating for 1 h at 37 °C, membranes washed with PBS slightly. To dissolving of adhered platelet, membranes were immersed in Triton X100 and incubated for 1 h. Finally, LDH activity was measured based on the enzyme method by use of the MPR1 kit (supplied from Roche Co). The obtained number has a direct relation with the adhered platelet on the membrane surface. Converting pyruvate to lactate was measured by investigation of its absorption at 340 nm and 37 °C. Also the initial concentration of platelets in PRP was measured by the use of a Coulter T890 cell counter.

3. Results and discussion

Membranes were prepared according to Table 1. In the current study, the effect of PVP, PEG, and PVP/PEG on the characteristics of the PES membrane with the name of MVi, MGi, and MVGi were evaluated, respectively. Viscosity measurements, morphology, surface roughness, mean pore size, water contact angle, permeability, and rejection capability of membranes with various aqueous solutions (Urea, PEG, PVP, and BSA), and also the cell and blood compatibility were evaluated to assess the best membrane for use in hemodialysis technique.

3.1. Viscosity of casting solution

The viscosity of the casting solution has an impressive effect on the thermodynamic and kinetic of phase inversion. As shown in Figure 1, weather addition of PVP or PEG, viscosity was increased. But the effect of PVP on the increasing solution viscosity was more tangible than PEG, so that for MV5 with 10 wt.% PVP, viscosity enhanced over than 21216 cP. Although PEG enhanced the viscosity of the casting solution, its effect wasn't the same as the PVP effect. The maximum value of viscosity obtained for MG5 (505 cP) that is much lower than MV5. Also, for MVG series, with increasing PVP/PEG ratio, viscosity was increased to 3100 cP for MVG3. Based on the obtained results for MV, MG, and MVG series, it can be concluded that PVP has the greatest effect on the increasing of casting solution viscosity. Also by blending of PVP and PEG, adjusting of solution viscosity is possible in the limited range.

PES is an amorphous polymer with a T_g of 225 °C [33]. On the other hand, PVP is also an amorphous polymer with 180 °C T_g, while PEG is a semi-crystalline polymer with T_g of -79 °C [34]. Also, the difference between the solubility parameter of PES/PVP is closer than PES/PEG (Table 2). Therefore, it is expected to have better miscibility of PES/PVP rather than PES/PEG. The better miscibility leads to the more physical entanglements of polymers chains and consequently, the viscosity of solution would increase in such a condition. As a consequence, the blending of both PVP and PEG with different natures can adjust final membrane properties based on the viscosity, morphology, mean pore size, and permeability.

3.2. Morphology of membranes

Membrane morphology is dependent upon the various parameters, but the viscosity of casting solution and kinetic speed of phase inversion has an impressive effect on the final structure [38]. According to Figure 2, membranes morphology has changed with the addition of PVP in the casting solution. M0 had a channel-like morphology with the appearance of macro-void at the bottom surface. With the addition of 1 and 2 wt.% PVP (Figure 2b and c), the size of macro-void was larger than M0. At lower concentrations, PVP has a strong pore former role in the membrane, then achieve to a

membrane with larger pore size and macro-void, is possible. For MV3 with 4 wt.% PVP, macro-voids were disappeared but larger channels were formed. Further addition of PVP led to a change in membrane morphology so that for MV5, the spongy structure was developed from the top region of the membrane. Extending of spongy structure for MV5 assigned to the severe increase of casting solution viscosity [39]. The effect of PEG was approximately the same as the PVP effect. With the addition of PEG, larger channels and macro-voids were obtained due to the impressive effect of PEG in the pore-forming (Figure 2g-i). More addition of PEG, produced a fully channel-like morphology with narrower and uniform channels without the formation of macro-voids due to the mild increase in casting solution viscosity, especially for MG5 (Figure 2f). For MVG1 (PVP/PEG=0.5) macro-voids formed at the bottom surface and with increasing PVP concentration, macro-voids disappeared due to the increase of casting solution viscosity and reducing phase inversion speed (Figure 2m and n). Although both PVP and PEG are water-soluble polymers and recognized as a pore former additives in the membrane, their effects on the membrane morphology aren`t the same as each other. Indeed, membrane morphology affected by the casting solution viscosity severely. As previously stated, PVP due to the more compatibility with PES and consequently more chain entanglement with polymeric chains, its viscosity will increase at a higher concentration of PVP. This higher viscosity leads to reducing phase inversion speed and finally, membranes with a structure containing macro-voids would turn to the channel, finger, and tear-like morphologies. With by more reducing of phase inversion speed, the formation of sponge-like morphology would be possible.

3.3. AFM study of membranes

According to Figure 3, the addition of PVP to 2 wt.% led to the formation of some new peaks with short heights (Figure 3b-c) which eventuated to the more surface roughness rather than M0 (Figure 6d). PVP is a hydrophilic additive, then, by immersion of casted film in the coagulation bath, PVP would leave the membrane toward its surface. Therefore, PVP immigrated to the surface easily and separated from the membrane surface in water, consequently, surface roughness increased in comparison with M0. By adding PVP to higher concentrations, peaks were disappeared (Figure 3d-f) and surface roughness reduced impressively. On the other hand, by increasing the PVP concentration, because of its high molecular weight and high solution viscosity, it cannot remove from the membrane perfectly, thus, the more concentration of PVP will be at the top region and surface of the membrane, in such a condition, PVP cover the surface and then membrane roughness will decrease (Figure 3d-f).

Similar to the PVP effect, PEG is a hydrophilic additive and acts as a pore former in the membrane. In this study, PEG (600 Da) was used in the preparation of membrane, thus, it is expected to remove from the surface of the membrane in the phase inversion process and finally led to an increase in surface roughness of membrane. As it is observed in Figure 4, small cones were formed at a low concentration of PEG. By increasing PEG content, the density of these cones became higher and they appear on the whole surface and then surface roughness was increased. According to the obtained results represented in Figure 6d, mean surface roughness (S_a) enhanced from 6.14 for MG1 to 13.3 for MG5 via increasing the PEG content.

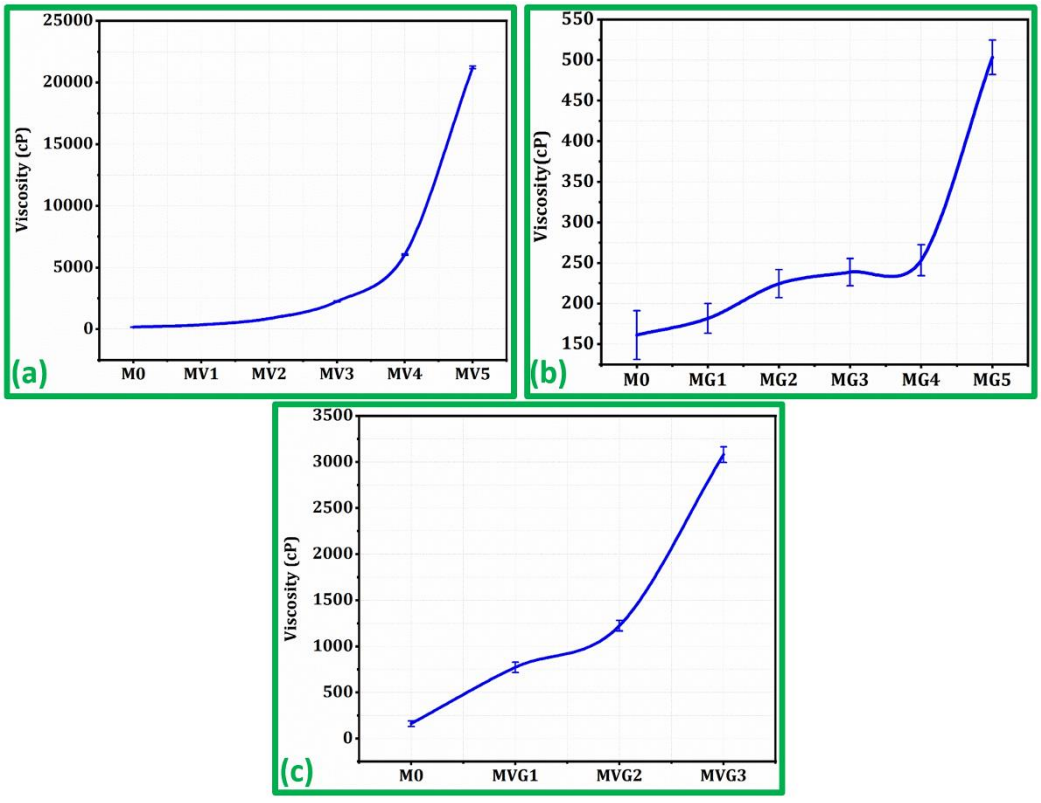


Fig. 1. Viscosity of polymeric solutions: (a) MVi, (b) MGi and (c) MVGi series.

Table 1
Physical characteristics and solubility parameters of PES, PVP, and PEG.

Polymer	Physical characteristics[33, 34]		Solubility parameters [35-37]		
	Crystallinity	T_g (°C)	δ_d ($MP^{1/2}$)	δ_p ($MP^{1/2}$)	δ_h ($MP^{1/2}$)
PES	Amorphous	225	19.6	10.8	9.2
PVP	Amorphous	180	18.8	13.4	7.5
PEG	Semi-crystalline	-79	17	10.7	8.9

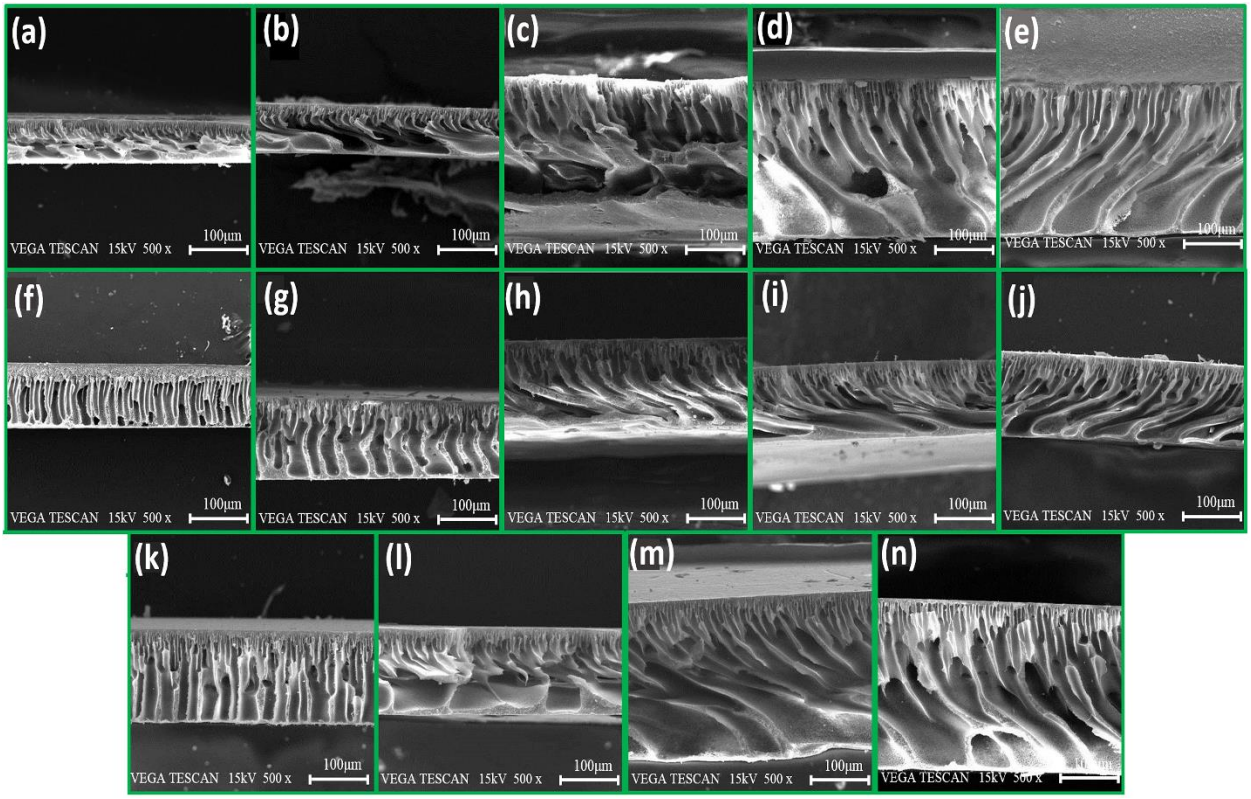


Fig. 1. SEM micrograph of membranes: (a) M0, (b) MV1, (c) MV2, (d) MV3, (e) MV4, (f) MV5, (g) MG1, (h) MG2, (i) MG3, (j) MG4, (k) MG5, (l) MVG1, (m) MVG2, (n) MVG3.

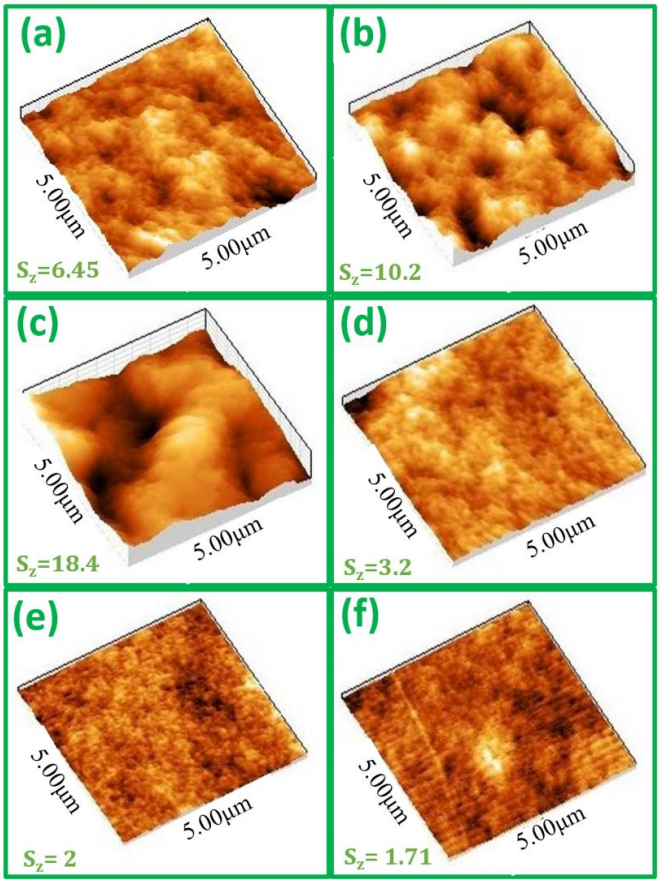


Fig. 3. AFM 3D images of membranes: (a) M0, (b) MV1, (c) MV2, (d) MV3, (e) MV4, (f) MV5.

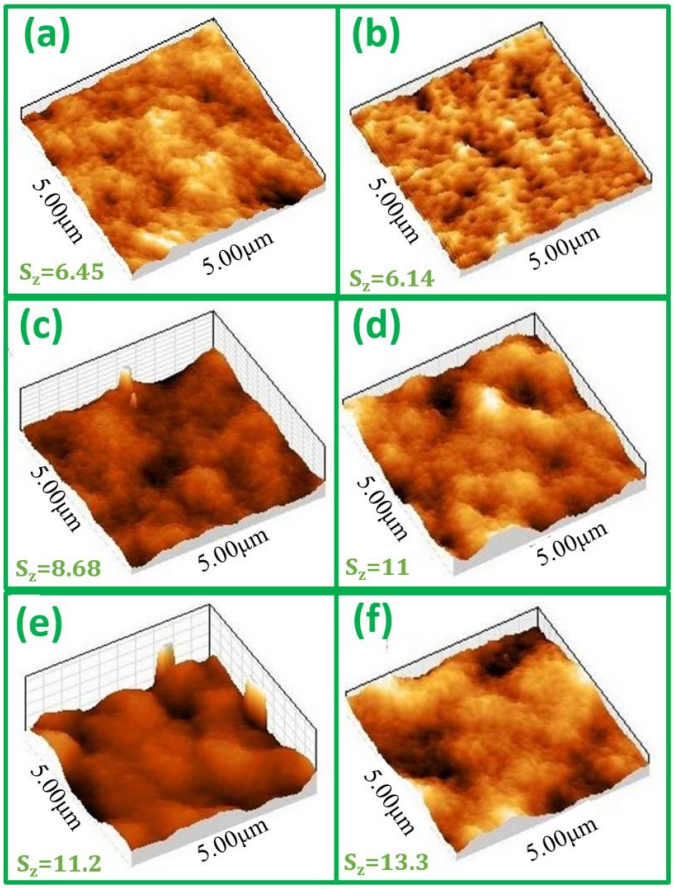


Fig. 4. AFM 3D images of membranes: (a) M0, (b) MG1, (c) MG2, (d) MG3, (e) MG4, (f) MG5

Although both PVP and PEG are hydrophilic polymers, however, their effect on the surface roughness of the membrane was different. Unlike PVP, with the addition of PEG, surface roughness was increased. This contradictory behavior can be attributed to the molecular weight and interaction of PVP or PEG chains with PES chains. Generally, PVP had higher compatibility with PES matrix due to the closer physical properties like amorphous nature and also the difference of solubility parameter. The more compatibility means the more entanglements between PVP and PES chains. Hence, the leach-out of PVP from the membrane surface would disappear by increasing physical chain entanglement. Thus, PVP remained in the top region and consequently lower surface roughness was obtained in comparison with the MG series. Figure 5 shows topographic images of the MVG series. The mean roughness parameter decreased from 29.9 for MVG1 to 3.39 for MVG3. For MVG1, due to the higher concentration of PEG, surface roughness was increased in comparison with M0. In MVG1, according to the PEG leach-out, surface roughness is higher (Figure 5b), while for MVG2 (Figure 5c) surface roughness reduced with more addition of PVP. For MVG3, with increasing of PVP/PEG ratio, roughness decreased effectively due to the higher concentration of PVP (Figure 5d).

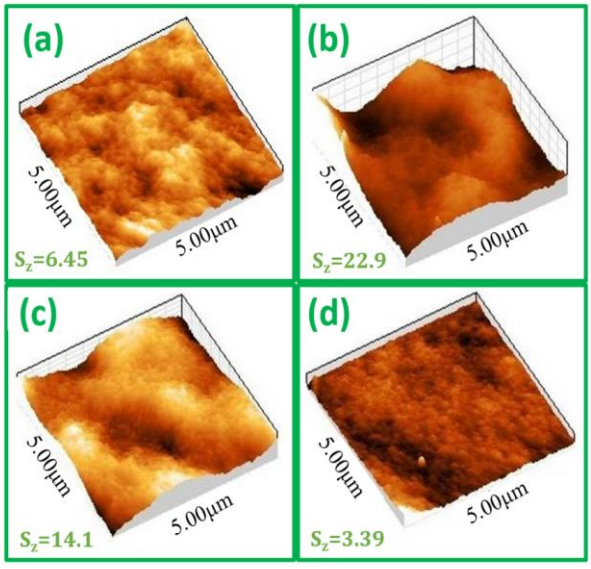


Fig. 5. AFM 3D images of membranes: (a) M0, (b) MVG1, (c) MVG2, (d) MVG3.

To calculate the mean pore size, AFM images were utilized. The observed pores are with different shapes: circular, rectangular, and elliptical. Hence, pore sizes were calculated by considering each pore's mean length and width. The sizes of 50 pores were measured on the surface. Sizes were arranged in ascending order and medians were determined for all the membranes. To obtain the probability density function diagrams, pore sizes were plotted on the abscissa, and y-axis involves pores with sizes less than the stated value. Figure 6a-c shows the lognormal- probability density function diagrams for all membranes. Mean pore sizes and surface roughness are presented in Figure 6d. As showed in Figure 6, mean pore size was increased with adding PVP from 53 nm for MV1 to 60 nm for MV4. While for MV5, the mean pore size was decreased again to 50 nm due to the denser morphology which affected from high viscosity of casting solution.

3.4. Membrane hydrophilicity

Membrane hydrophilicity is a considerable factor to resist against fouling occurring for the filtration of aqueous solutions. Moreover, for hemodialysis membranes, hydrophilicity is an important parameter in improving membrane biocompatibility and blood compatibility. As depicted in Figure 7, weather addition of PEG or PVP, the membrane contact angle was decreased but this effect for MV series was more tangible than the MG series. Surface free energy obtaining from the Young-Dupre equation was increased with the addition of PVP or PEG. The water contact angle was decreased from 72.8° for M0 to 63.6° for MV5 and accordingly, the surface free energy of membranes increased from 93.3 mJ/m^2 for M0 to 104.1 mJ/m^2 MV5. For the MG series, the water contact angle was decreased to 65.4° for MG5 and surface free energy achieved to 101.9 mJ/m^2 for this sample. With decreasing contact angle, the surface free energy of membranes would increase; therefore, membrane hydrophilicity would improve. Also for the MVG series, the same hydrophilicity with the PEG or PVP effect was obtained, but for MVG series, hydrophilicity was a little more than MV and MG series. As revealed in Figure 7c, the contact angle was recorded 65°, 65.4°, 64.8° for MVG1, MVG2, and MVG3, respectively. In accordance with surface roughness results, the better hydrophilicity of PVP-containing membranes can be attributed to the existence of this hydrophilic polymer at the top and surface regions of membranes. Because of water solubility nature, PVP would like to migrate to the coagulation bath in the phase inversion process. While due to the higher molecular weight of PVP (360 kg/mol) and better compatibility with PES chains rather than PEG which lead to more physical entanglement, this hydrophilic polymer cannot remove perfectly from the surface of the membrane, therefore more content of PVP, would be at the membrane tope region and finally hydrophilicity of membrane would improve in such a condition.

3.5. Permeability of the membranes

Permeability of pure water, urea, PEG, PVP, and BSA solutions from the membranes was evaluated via a crossflow filtration system which schematically represented in Scheme 1. Generally, the permeability of the membrane assigned to various parameters such as hydrophilicity, porosity, mean pore size, morphology, and thickness of the top layer [40,41]. In the hemodialysis process, excess bio-substances such as urea which recognized as toxins should be removed form blood. For this purpose, toxin materials should pass along membrane cross-section while beneficial blood components should have retained in blood [42]. Based on the data obtained for MV series (Figure 8a), with the addition of PVP to 6 wt.% (MV4), PWP was enhanced to 24.9 L/m^2.h.bar. This increase of PWP verified the pore former role of PVP in the membrane. With the addition of more content of PVP, membrane PWP was decreased due to the drastic increase of casting solution viscosity for MV5 that eventuated to a membrane with smaller mean pore size. This behavior of permeability was observed for all solutions. As revealed in Figure 8, after pure water; urea, PEG, PVP, and BSA solutions had higher permeability, respectively. This trend of permeability attributed to the molecular weight of each solute. On the other hand, for MG series membranes (Figure 8b), with the addition of PEG, PWP was increased. In comparison with MV series, water flux was higher for MG series. As discussed previously, PEG due to the lower molecular weight than PVP and lower compatibility with the PES matrix will leave the membrane faster than PVP. Also, the AFM study confirmed that surface roughness of MG series was increased due to the more leach out of PEG from the membrane. Hence, this higher PWP can be attributed to a significant role of PEG in pore-forming. For MG2 with 5 wt.% PEG, water flux reached 44.8 L/m^2.h.bar. The same behavior of membrane flux was recorded for urea, PEG, PVP, and BSA solutions. The sort of molecular weight for all solute is BSA>PVP>PEG>urea, thus it's a logical reason that urea solution had higher permeability than PEG, PVP, and BSA solutions. For MVG series, different behavior was recorded, thereby, for MVG2, maximum flux was achieved (25.2 L/m^2.h.bar). While with increasing of PVP/PEG ratio, PWP was decreased slightly due to the increasing of casting solution viscosity and harder leaching of PVP from membrane surface than PEG. Like MV and MG series, for MVG series higher flux was recorded by this sort: urea>PEG.PVP>BSA. This highest permeability of urea solution, especially for MV4 (16.9 L/m^2.h.bar), MG2 (42.5 L/m^2.h.bar) and MVG2 (19.8 L/m^2.h.bar) can be a hopeful factor for separation of urea in a short time.

3.6. Rejection test

The key role of the hemodialysis process is the separation of uremic toxins from the blood. Urea and creatinine are the main toxins that the kidney of ESRD patients is unable to separate from the blood. The membrane using in the hemodialysis process should have high rejection capability for the separation of toxins. Rejection ability of membranes assigned to the membrane features such as mean pore size and morphology. As mentioned before, with the addition of PVP as a pore former additive, the mean pore size was increased. Based on the rejection results which obtain for M0, 94% rejection was recorded for BSA (Figure 9). While with the addition of 1 wt.% PVP (MV1), BSA rejection didn`t change remarkably. For MV1 to MV4, due to the increase of mean pore size, BSA rejection was decreased slightly from 93.2% for MV1, to 85.7% for MV4. For MV5, BSA rejection increased again to 92% due to the decreasing of membrane mean pore size. BSA had 68 kDa molecular weight, and due to the lower molecular weight cut-off of MV series than BSA molecular weight, thus its rejection was high.

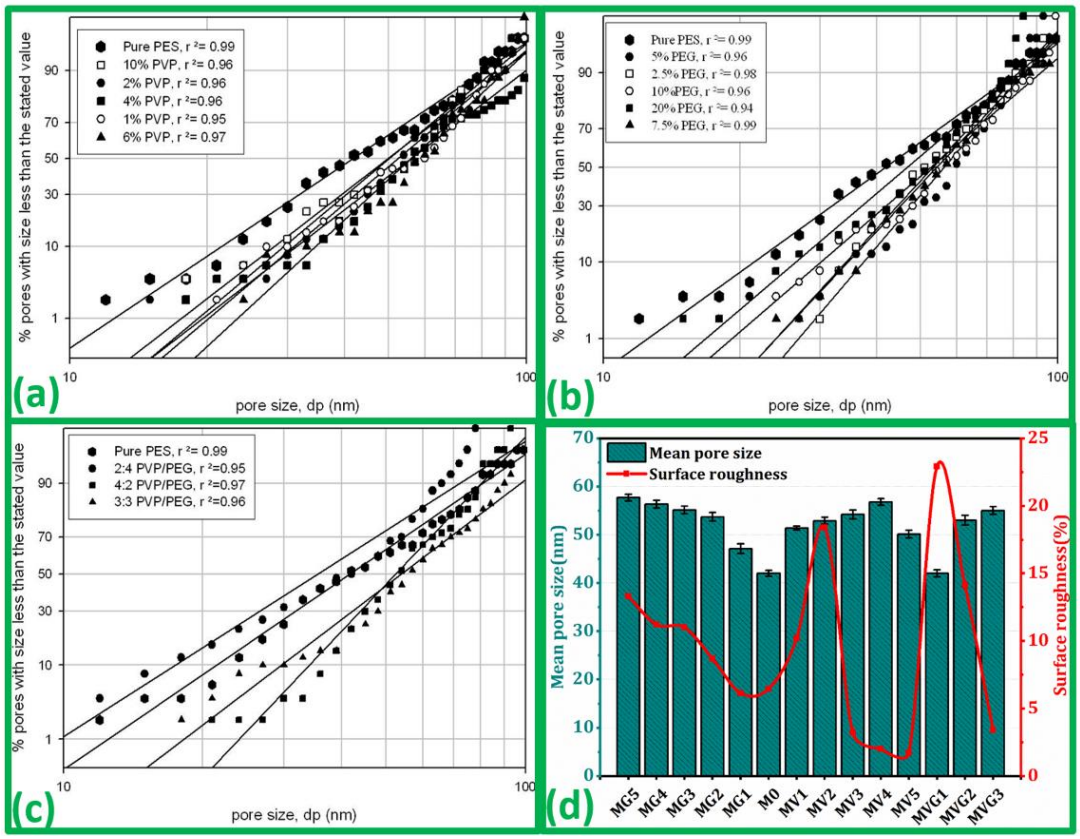


Fig. 6. Probability density function diagrams of (a) MV, (b) MG, and (C) MVG series. (d) representation of mean pore size and surface roughness of all membranes.

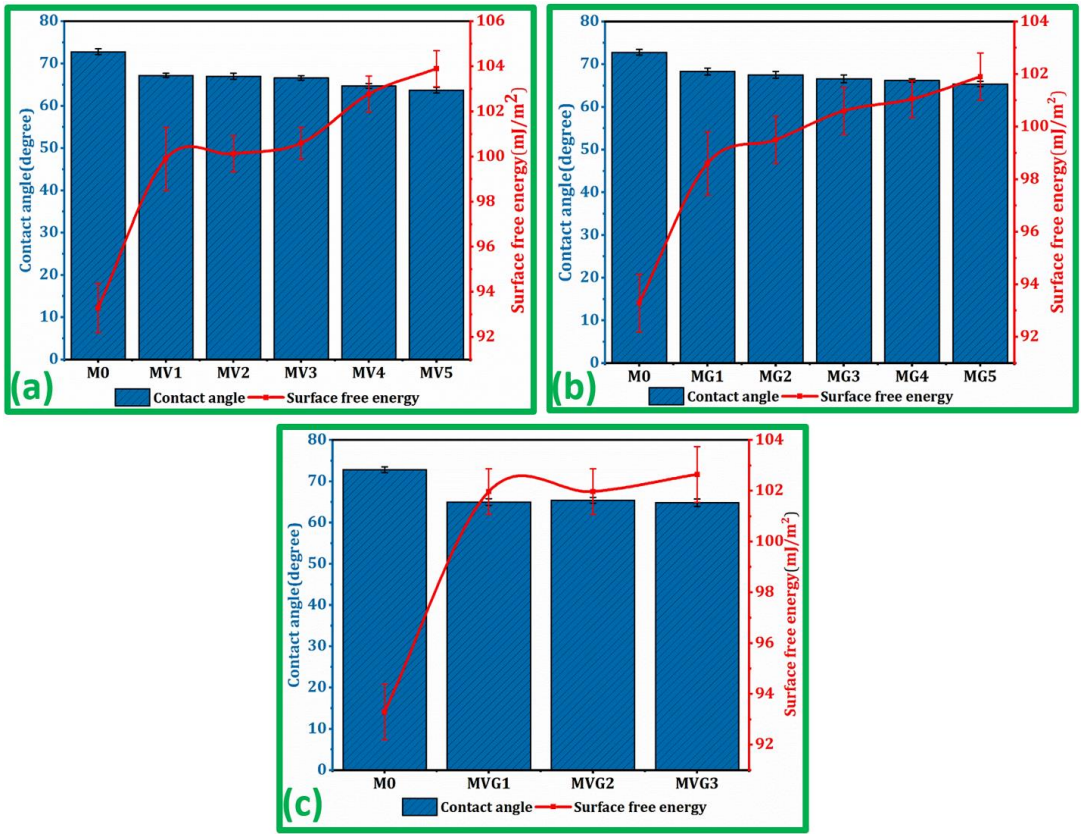
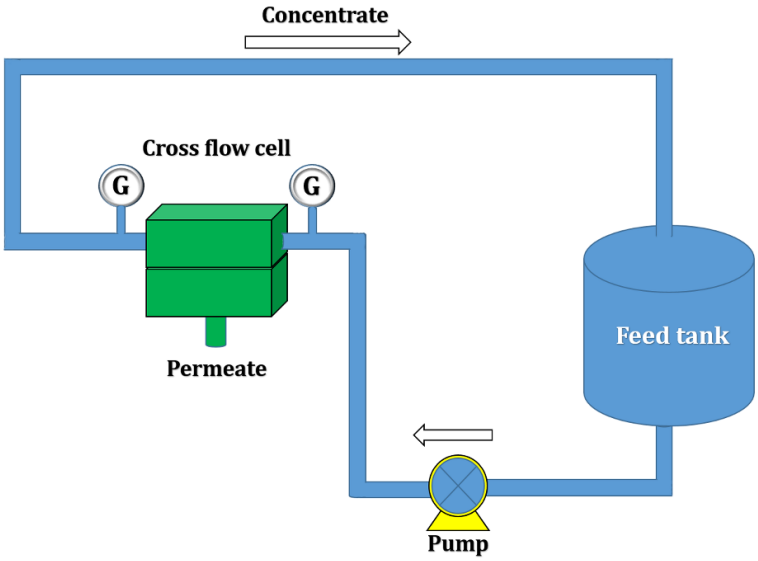


Fig. 7. Water contact angle and surface free energy of membranes: (a) MV, (b) MG and (c) MVG series.



Scheme 1. Schematic representation of filtration cross-flow system.

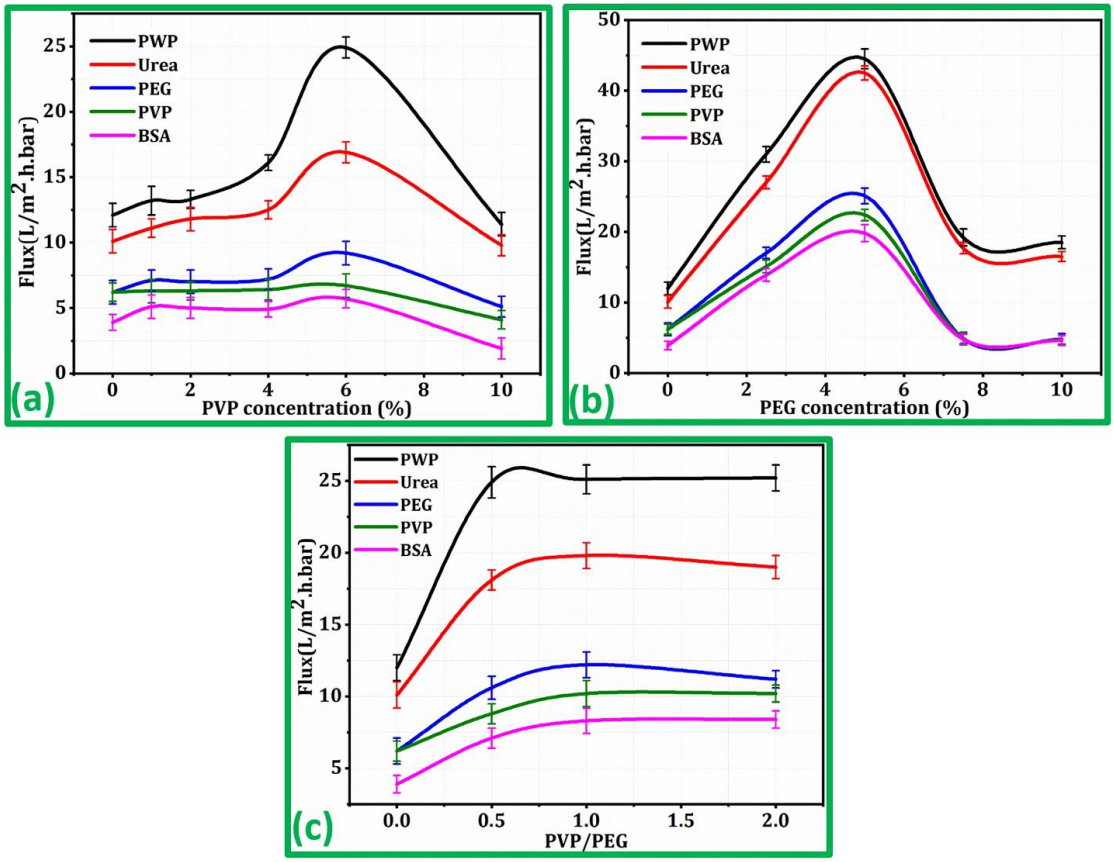


Fig. 8. Membranes flux with various solutions: water, urea, PEG, PVP, and BSA; (a) MV, (b) MG and (c) MVG series.

For PVP solution, rejection decreased from 78% for M0 to 70%, 67%, 62.2%, and 61% for MV1, MV2, MV3, MV4, respectively, and increased again to 81.3% for MV5. PVP-K40 with 40 kDa was used for this test. Therefore, it was expected that lower rejection has gained rather than BSA. The same trend was observed for PEG and urea solutions, but rejection percentage was lower than PVP and BSA solutions. For example, for urea solution, lower than 30% rejection was obtained for membranes because of the lower molecular weight of urea. For MV series, the urea rejection was 27.5% for M0 while with the addition of PVP, the rejection was decreased to 25%, 21%, 19.8%, and 19.7% for MV1, MV2, MV3, and MV4, respectively. While for MV5, due to the decreasing of mean pore size urea rejection was enhanced to 40.2 %. For MG series, BSA rejection was lower than 80% due to the more open morphology and larger mean pore size of membranes rather than M0 and MV series. For MVG series, because of the increasing mean pore size from MVG1 to MVG3, the rejection capability of membranes for all solute was decreased and this reduction in rejection was more tangible for MVG2 and MVG3. As a conclusion obtained from the rejection test, it is obvious that the rejection capability of membranes has a direct relation with membrane mean pore size. With increasing mean pore size, the rejection ability of membranes would decrease. As a conclusion, the desired condition for high-performance

hemodialysis process is the maximum clearance of toxins such as urea at the shortest time besides remaining of other blood components in various molecular weights. To give instances; albumin with 68 kDa is the main protein in the blood and is responsible for several duties like controlling of blood colloidal osmotic pressure [43], coagulation proteins in a wide range of molecular weights (50-340 kDa) [44], should be remained in blood during hemodialysis process. Rejection results revealed that MV3, MV4, MG3, MG4, MVG2, and MVG3 had the best performance in terms of urea removal and keeping other components especially, BSA.

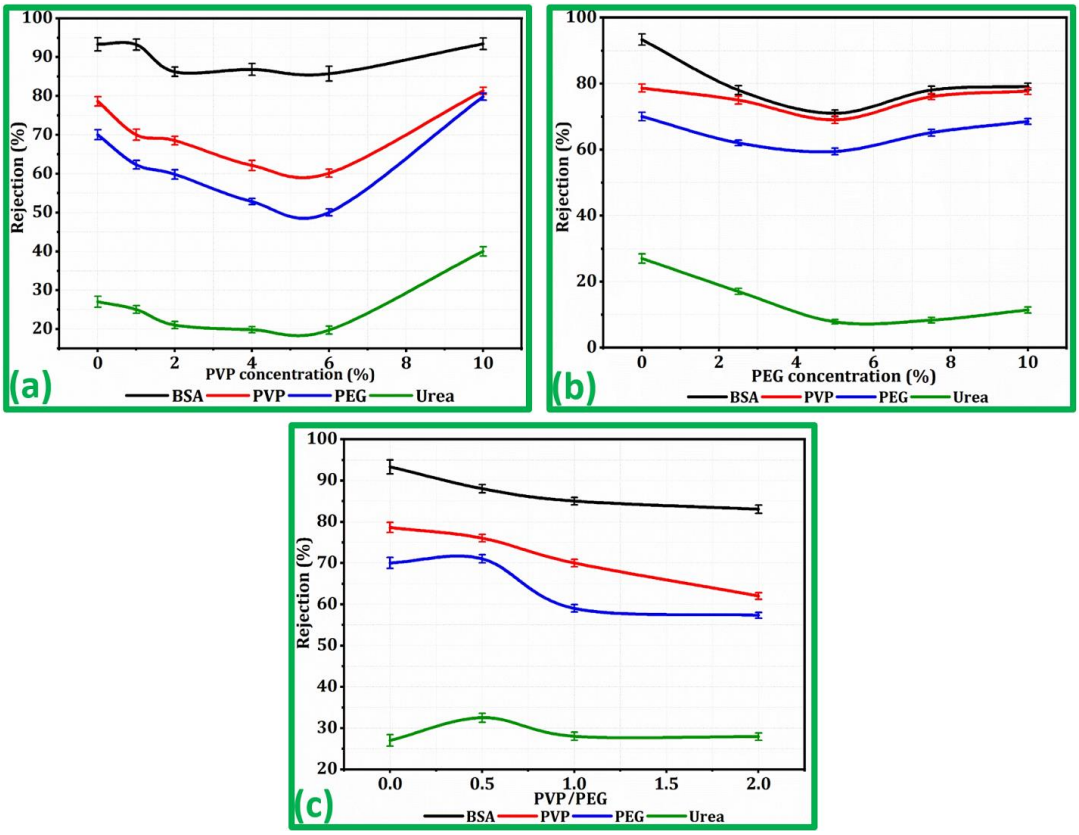


Fig. 9. Rejection capability of membranes with various solutions: water, urea, PEG, PVP, and BSA; (a) MV, (b) MG and (c) MVG series.

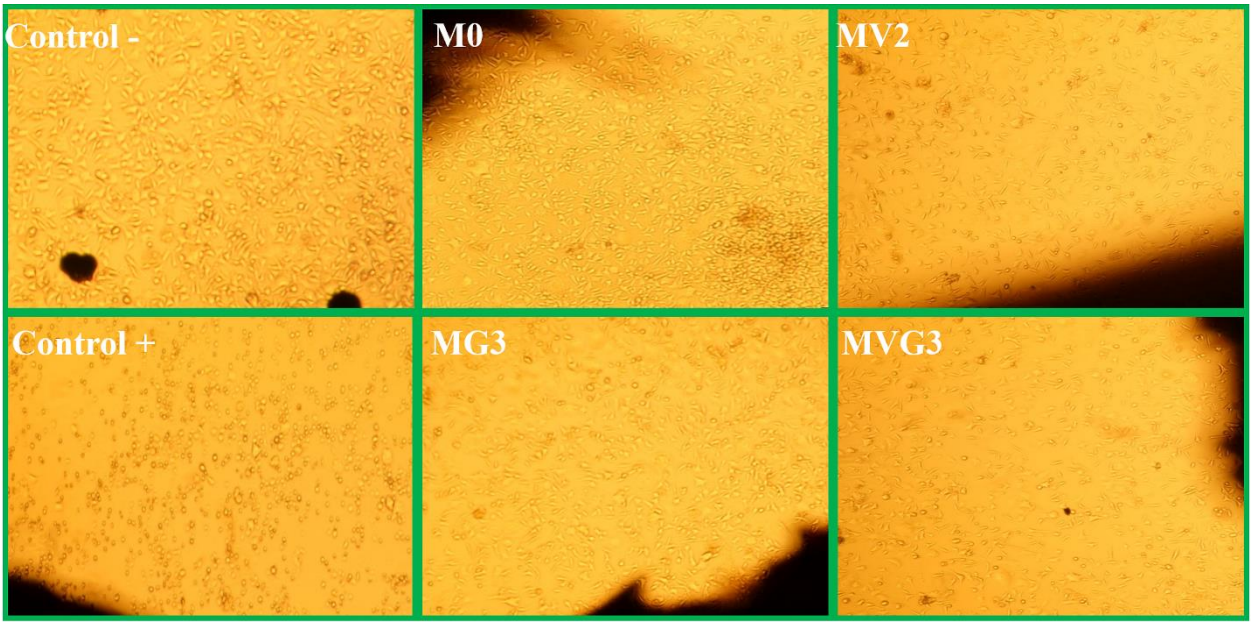


Fig. 10. Optical micrographs of cells morphology on the control samples and membranes.

3.7. Membranes bioactivity

Cell compatibility is a vital factor for biomedical tools and membranes used in the blood purification industry [45]. In this work, an in-vitro cytotoxicity assay was performed for the evaluation of membranes biocompatibility. M0, MV2, MG3, and MVG3 were selected as representative of other membranes. cytotoxicity of membranes compared with TCPS culture vessel and polyvinylchloride as negative and positive control samples, respectively. As revealed from Figure 10, cells morphology changed from a round shape to almost filopodia and in some cases turned to webbing and flatten sates especially for MV2, MG3, and MVG3, due to the more hydrophilicity of these membranes. This type of change in cell morphology is a criterion of membrane biocompatibility. Water contact angle analysis demonstrated that M0 had 93.3 mJ/m^2 surface free energy while these values were 100-101 mJ/m^2 for MV2, MG3, and MVG3. These data showed that with increasing surface free energy in a suitable range, desired interaction between cells and membranes is possible, thereby, membrane didn`t trigger the immune system in such a condition.

Another key factor about blood-contacting biomaterials like hemodialysis membranes is blood compatibility. Activation of cascade coagulation factors and platelets will lead to the formation of a blood clot. In the current study, adhesion of adhered platelets on the membrane surface evaluated with the LDH method. As shown in Fig. 11a, with the addition of PVP concentration to 2 wt.%, platelets adhered to the surface were increased while with the addition of more content of PVP, the number of platelets adhered to the membrane surface was reduced effectively. For MV5 this value decreased to 2008 PLT/cm^2. For the MG series, with the addition of PEG, platelet adhesion was increased so that for MG5, approximately 8100 PLT/cm^2 was achieved. For the MVG series, platelet adhesion on the membrane surface reduced slightly with increasing PVP/PEG ratio. According to the data of AFM and platelet adhesion analyses, can be concluded that, with increasing surface roughness of membrane, platelets adhered to the surface of the membrane would increase. The incorporation of PVP in the PES membrane, provides a smooth surface while the PEG addition led to a rougher surface. In consequence, MV series had better blood compatibility than MG series in terms of platelet adhesion. Also for MVG series, blood compatibility would improve with increasing of PVP/PEG ratio.

4. Conclusions

The performance of the hemodialysis process is strongly dependent upon the membrane characteristics. In the current study, attempts were based on the development of membrane with specific characteristics like cross-section morphology, surface roughness, mean pore size, permeability, rejection capability, hydrophilicity, cell, and blood compatibility. PVP, PEG, and the simultaneous effect of both additives were investigated in the PES ultrafiltration membrane. The results confirmed although both PEG and PVP had an obvious effect on the increasing casting solution viscosity, the effect of PVP was more tangible than PEG. PVP is an amorphous polymer with close T_g to the PES. While PEG is a semi-crystalline polymer with a huge difference of T_g (-79 °C) with the PES. Also, the solubility parameters of PVP and PES are closer than PEG and PES, therefore this cases will lead to better compatibility between PVP and PES rather than PEG and PES. This better compatibility caused more entanglement between polymeric chains and finally higher viscosity will obtain for blending solution (PES/PVP). The viscosity of solutions was tunable by the use of both PVP and PEG. Membranes morphology had a great dependency on the viscosity of casting solution, thus, with increasing viscosity, cross-section morphology without the formation of macro-voids was developed. AFM study showed that with increasing PVP concentration, a smoother surface was obtained while with increasing PEG, the rougher surface was obtained. Membrane performance including permeability with various solutions (water, urea, PEG, PVP, and BSA) demonstrated that MV3, MV4, MG2, MVG2, and MVG3 had the highest permeability among all membrane series. Actually, with increasing PEG, membrane with larger mean pore size and higher permeability was obtained, while the effect of PVP and PVP/PEG on the mean pore size and permeability was milder than the PEG effect. Also, rejection test verified that MV3, MV4, MG3, MG4, MVG2, and MVG3 had the highest performance in terms of high amounts of urea clearance and retaining of other components. As an explicit conclusion, PVP and PVP/PEG have a milder effect rather than PEG on the overall characteristics and performance of the membrane, hence, adjusting and optimization of a high-performance hemodialysis membrane is more possible by the use of PVP and PVP/PEG blend rather than just PEG. Therefore, according to the obtained results from all analyses, MV3, MVG2, and MVG3 are the best membranes for use in a high-performance hemodialysis membrane.

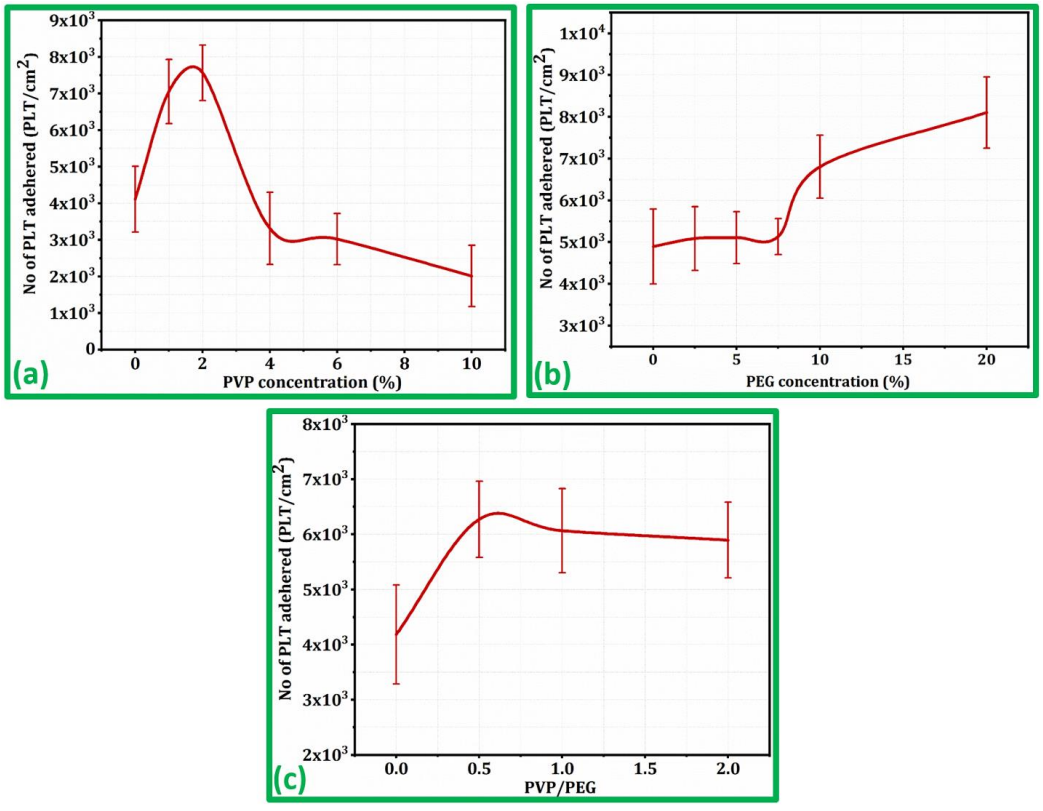


Fig. 11. Determination of platelet adhesion on the membrane surface by LDH method.

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