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INCORPORATION OF IMPRINTED-ZEOLITE TO POLYETHERSULFONE/CELLULOSE ACETATE MEMBRANE FOR CREATININE REMOVAL IN HEMODIALYSIS TREATMENT

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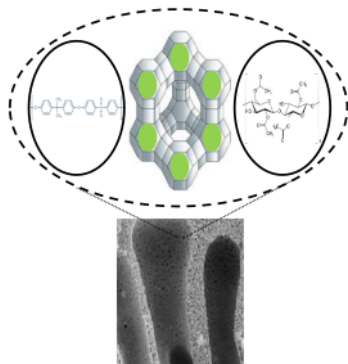
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Graphical abstract



Abstract

Polyethersulfon (PES) membrane has been widely used in the biomedical field especially in hemodialysis application. Many modifications of membranes have been applied into hemodialysis such as diffusion, adsorption, and mixed-matrix membrane. The main problem of those membranes is less selectivity to attract the uremic toxins. In this study, we report the modification of PES mixed with cellulose acetate (PES/CA) membrane as mixed-matrix membrane (MMM) using imprinted-zeolite (PES/CA/IZC) in order to increase the selectivity for targeted analyte. The hollow fibre membranes (HFM) were fabricated by dry-wet spinning technique. The successful zeolite A synthesised and was characterised by x-ray diffraction (XRD). The mixed-matrix membranes were characterised in terms of morphology using scanning electron microscopy (SEM), water contact angle (WCA), pure water flux (PWF), clearance of creatinine (CC), and BSA adsorption. In accordance with the results of characterisation, the synthesis of zeolite A, and imprinted-zeolite creatinine was successfully fabricated. The SEM results showed that the PES/CA/IZC membrane has uniform pores and fingerlike structure. The same result was obtained for PES/CA membrane, but not for PES/CA/ZA membrane. The WCA of the PES/CA; PES/CA/ZA; and PES/CA/IZC were 85.63; 84.98; and 77.53 (°), respectively. While the PWF were 22.84; 27.57, and 40.52 (Lm⁻² h⁻¹), respectively. The addition of imprinted-zeolite into the membrane improved creatinine removal up to 74.99%. It showed that PES/CA/IZC has succeeded in increasing the selectivity of membranes to attract the creatinine as target analyte. Compared to the PES/CA, the creatinine clearance of membranes improved and increased up to 5.2%. For protein rejection, the PES/CA/IZC rejected 79.05% of bovine serum albumin (BSA). Based on these results, it can be concluded that PES/CA/IZC can be considered as hemodialysis membranes.

Keywords: Hemodialysis membrane, polyethersulfon, cellulose acetate, imprinted-zeolite, creatinine

Abstract

Polietersulfon (PES) membran telah digunakan secara meluas dalam bidang bioperubatan terutamanya dalam permohonan hemodialisis. Jadi banyak

pengubahsuaian membran digunakan ke dalam hemodialisis seperti penyebaran, penyerapan, dan membran bercampur matriks. Masalah utama mereka adalah kurang pemilihan untuk menarik toksin uremic. Dalam kajian ini, kami melaporkan pengubahsuaian PES dicampur dengan selulosa asetat (PES/CA) membran sebagai membran bercampur-matriks menggunakan zeolite yang tercetak (PES/CA/IZC) untuk meningkatkan pemilihan untuk sasaran analit. Membran gantian geronggang (HFM) telah dipalsukan oleh teknik berputar kering-basah. Kejayaan sintesis zeolite A dicirikan oleh x-ray pembelauan (XRD). Membran bercampur-matriks dicirikan dari segi morfologi menggunakan mikroskop elektron pengimbas (SEM), sudut kenalan air (WCA), air fluks tulen (PWF), pelepasan kreatinin (CC), dan BSA penyerapan. Selaras dengan keputusan pencirian, sintesis zeolite A dan dicetak-zeolite kreatinin telah yang berjaya dipalsukan. Keputusan SEM menunjukkan bahawa membran PES/CA/IZC mempunyai liang seragam dan bentuk macam jejari. Keputusan yang sama untuk membran PES/CA dan bukan untuk membran PES/CA/ZA. Tambahan CA dan zeolite adalah untuk meningkatkan hidrofilik membran PES sekaligus meningkatkan keserasian dengan darah. The WCA daripada PES/CA; PES/CA/ZA; dan PES/CA/IZC adalah 85.63; 84.98; dan 77.53 (°) masing-masing. Manakala PWF suka 22.84; 27.57; dan 40.52 (Lm⁻² h⁻¹) masing-masing. Penambahan dicetak-zeolite ke dalam membran boleh meningkatkan pelepasan kreatinin sehingga 74.99%. Ia menunjukkan bahawa PES/CA/IZC berjaya untuk meningkatkan pemilihan membran untuk menarik kreatinin sebagai sasaran analit. Jika dibandingkan ke dalam PES/CA, pelepasan kreatinin membran meningkat sehingga 5.2%. Penolakan protein, PES/CA/IZC ditolak 79.05% daripada bovine serum albumin (BSA). Berdasarkan keputusan di atas, dapat disimpulkan bahawa PES/CA/IZC boleh digunakan sebagai membran hemodialisis dengan pemilihan yang baik.

Kata kunci: Membran hemodialisis, polietersulfon, selulosa asetat, zeolite yang tercetak, kreatinin

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1.0 INTRODUCTION

Membrane technology is growing very rapidly. At the beginning of the development of this technology, the membranes were not used commercially, but widely applied to support the development of science and technology both in terms of theoretical physics and chemistry. By knowing the benefits generated by the development of membrane technology, researchers began developing a membrane application for human survival. The application started from secondary human needs such as water filters [1] to primary needs such as application to substitute kidney organ function or artificial kidney [2, 3], like in hemodialysis treatment [4].

Hemodialysis is a high technology used as a replacement therapy in kidney function to remove the remnants of metabolism and uremic toxins particular of human blood circulation, such as water, sodium, potassium, hydrogen, urea, creatinine, uric acid, and other substances that cannot be released by urine [5]. There are three types of uremic toxins in the human body: (1) low molecule-weight water-soluble, (2) protein-bounded solutes, and (3) middle-molecular-weight molecules [6]. Many research have been tried to develop filtration of those uremic toxins in any type from the human body, such as diffusion membranes (DM) [7, 8, 9], adsorption membranes (AM) [10, 11], and MMM [12, 13]. Those membranes were developed in order to achieve adequate blood filtration during hemodialysis. Diffusion membrane focused on the filtration of low molecular-

weight water-soluble like urea and creatinine, while adsorption membrane like hemoperfusion was used to clean the protein-bounded of uremic toxins. Researchers had also developed MMM to get efficient blood purification devices. In some MMM, materials adsorption was embedded into the hollow fibre, so that diffusion and adsorption takes place at a time. But, the materials applied as adsorbent such as carbon nanotubes and activated carbon were not selective. This means that they adsorbed some protein and organic molecules that were still needed by the blood. Thus, the selective pore of the material has to be improved.

MMM has been widely developed, such as combining EVOH-zeolite [14], PMMA-zeolite [15], PES-HAP [3], PES-PVP [16], PES-MWCNT [17], and PAN-ZSM5 [18]. Zeolites are microporous crystalline aluminosilicates, composed of TO₄ tetrahedral (T=Si, Al) with atoms connecting neighboring tetrahedral. Zeolites is proven as potential material, and has been chosen by researchers as adsorption material [18], and also combined into MMM [13]. The crystalline nature of the framework of zeolite, ensured that there was uniformity in the pore opening, so that the pores are able to absorb uremic toxins from the blood. In addition to the resulting pores, zeolite is also nontoxic and nondegrade under physiological condition, making zeolites a good material to be combined with other polymer applied into hemodialysis application.

This paper explored a new treatment to modify the micropores of zeolite to be more selective pore. Some strategies for modification of mesopores

zeolites such as assembly methods like hard and soft templating, and indirect templating; demetallisation methods like dealumination and desilication, and the mixed methods [19]. By adopting those mechanisms, the micropores can also be modified. This study tried to modify the micropores zeolite by soft templating using molecularly imprinting technology. The first step was synthesis zeolite A. There are two steps to produce it, there are aging and hydrothermal step. The materials used in the synthesis of Zeolite A are sodium aluminate (NaAlO_2) as a source of alumina and sodium oxide. Silicon dioxide (SiO_2) was source of silica and distillate water as a solvent. The imprinting process occurred at the stage after hydrothermal before crystallisation. The creatinine as a template was added before neutralised the pH of zeolite, so that the pores would be printed by the template.

2.0 METHODOLOGY

2.1 Materials

Cellulose acetate was purchased from Sigma Aldrich ($M_w = 60,000$), and was used to increase the hydrophilicity of PES; PES (Radel A-300A Resin) was purchased from Solvay Advanced Polymers; NMP (99.5%; $M_w=99.1$ g/mol) was purchased from Acros Organics; LUDOX HS-40 colloidal silica (40 wt. % suspension in H_2O , $M_w=60.08$), Sodium Aluminate Anhydrous, Creatinine, and bovine serum albumin (BSA) of >98% purity were obtained from Sigma Aldrich.

2.2 Preparation of Imprinted-Zeolites-Creatinine (IZC)

IZC was synthesised by zeolite A [20]. There were 2 steps in the synthesised, namely aging and hydrothermal. In the aging step, the composition of mixture was $\text{SiO}_2:\text{Al}_2\text{O}_3:\text{Na}_2\text{O}:\text{H}_2\text{O} = 1.8:1.4:270$ until white colloidal was formed, then with the hydrothermal step. In this step, the colloidal was heated in an oven at 100°C for 48 hours. After the hydrothermal process was obtained, precipitate on the bottom, and clear solution on the top was obtained. The precipitate subsequently neutralized to eliminate remnants of alkaline solution because it can interfere with the analysis process. Neutralisation was done using distilled water by centrifugation at 3000 rpm, then dried at 80°C and formed a zeolite A. In order to synthesise the IZC, creatinine was mixed with zeolite A for 1 hour under magnetic stirrer. After that the mixture was allowed to stand for 3 hours to condition the imprinted zeolite by creatinine. Finally, the creatinine trapped in the pores of zeolite was cleansed with warm water.

2.3 Fabrication of PES/CA/IZC

Fabrication of PES/CA/IZC began with dissolving of PES into N-methylpyrrolidone (NMP) by slowly stirring,

and heating at 50°C . Powder of CA and IZC was added to the solution of PES that had a homogenous dope, and kept for 1 hour with stirring for homogenous, and 5 hours to remove air bubbles. To get the optimum condition for mixture of PES, CA, NMP, and IZC need to be optimised. The composition of those materials can be seen in Table 1. The hollow fibre membranes were spun at room temperature (30°C) by dry-wet spinning technique. The dope solution passed through a spinneret with 0.4mm internal diameter, and 0.8 mm outer diameter, with bore fluid flow rate $1\text{ cm}^3/\text{min}$; spinning pressure using nitrogen gas for 1 bar; air gap was 50 cm; and take up speed was 5.4 Hz. The coagulant bath for the system used tap water. After the spinning process, the hollow fibre membranes were treated using tap water for 1 day to remove the remaining solvent, and then dried for 1 day under aerated.

Table 1 Composition of membrane dope solution

Membrane	Composition (%)					Viscosity mPa.s
	CA	PES	NMP	Zeolite A	IZC	
PES/CA	1	17	82.0	-	-	4929
PES/CA/ZA	1	17	81.5	0.5	-	6971
PES/CA/IZC	1	17	81.5	-	0.5	7919

2.4 Membrane Characterization

2.4.1 X-Ray diffraction (XRD)

To make sure that Zeolite A was successfully synthesised, characterisation using XRD (Rigaku-Ultima, 40 kV, 30 mA, D/teX Ultra 250 detector) was measured. The result was compared to the Standard International Zeolite Association (IZA). A typical peak of zeolite was seen on their peak in each 2θ . It was concluded that the type of zeolite synthesised was successfully formed.

2.4.2 Scanning Electron Microscopy (SEM)

The SEM (Hitachi, model: TM3000 tabletop microscope) was used to observe the morphology of PES/CA, PES/CA/ZA, and PES/CA/IZC casted membranes. Samples of hollow fibre membrane were coated with gold to provide electrical conductivity. The outer surface and cross-sections of each hollow fibre membrane were analysed.

2.4.3 Water Contact Angle (WCA)

WCA was used to measure the hydrophilicity of the hollow fibre membrane produced. This characterisation used contact angle goniometer (Kruss Gambult, Germany). $0.2\ \mu\text{L}$ of water was dropped on the surface of the hollow fibre membrane sample.

2

2.5 Membrane Performance

2.5.1 Pure Water Flux (PWF)

The hollow fibre membranes separation was investigated using PWF. In PWF, the membranes were subjected to pure water estimation at a trans-membrane pressure of 1 bar under cross-flow filtration. The permeability was measured under steady-state flow. PWF and CF were calculated as follows:

$$J = \frac{V}{A \times \Delta t}$$

where V is the quantity of permeate collected (L), and A is membrane area (m^2). Δt is the sampling time (h), so J is pure water or creatinine flux ($L.m^{-2}.h^{-2}$).

2.5.2 Clearance of Creatinine

The clearance of creatinine was to determine how much creatinine can be removed from the blood to the dialysis solution. In this step, creatinine standard 50 ppm was inserted into a steady-state flow of HF. Then the creatinine outcome from the pores of HF for 2 hours under spectrophotometer UV-Vis under wavelength 490 nm was calculated. The concentrations of creatinine used as calibration curve were 10, 20, 30, 40, and 50 ppm. It was then used to calculate the creatinine after treatment using developed HF for 2 hours.

2.5.3 BSA Rejection

The function of this test was to foul assessment of the membranes. BSA 500 ppm solution was used; and then concluded the effect of zeolite addition to the membrane hydrophilicity. The percentage of rejection was calculated as follows:

$$R (\%) = \left(1 - \frac{C_p}{C_f}\right) \times 100\%$$

where R is rejection in percent value. C_p and C_f are the BSA concentration of permeates and feed, respectively.

3.0 RESULTS AND DISCUSSION

3.1 Characterisation of IZC

The aim of characterisation using XRD was to determine the crystallinity of the zeolite A. This characterisation was done at wave number $2\theta = 5-50$ using Cu Ka radiation. Successful synthesis of zeolite A on the result of XRD is shown in Figure 1(a), while diffractogram of zeolite A compared with International Zeolite Association (IZA) standard is shown in Figure 1(b).

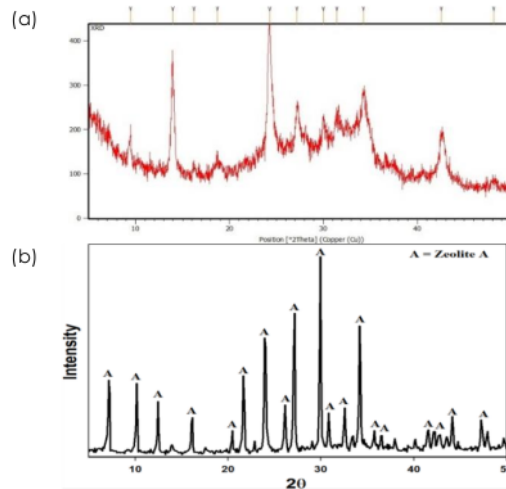


Figure 1 XRD diffractogram of (a) Zeolite A synthesised and (b) International Zeolite Association (IZA) standard

As can be seen in Figures 1(a) and (b), the zeolite structure synthesised that was formed had a crystalline structure. Based on the diffractogram of zeolite A synthesised, besides the specific peak of zeolite A, it also formed the peak $2\theta = 9.4543$, 13.9414 , and 18.70350 . This indicates that the product synthesised not only zeolite A, but may be also zeolite X [21].

3.2. Characterisation of PES/CA/IZC

3.2.1 Morphologies of Membranes

Increasing the concentration of polymer solution enhanced the solution viscosity. This phenomenon affected the precipitation process more slowly, and thickness of the membrane became dense and thick [22, 23]. Based on the quantification of viscosity measured by Cole Parmer model 98965-40, the viscosity of each HF studied is shown in Table 1. The results showed an increase in the viscosity of dope solution added zeolite A and IZC. Normally, the thickness of hollow fibre membrane from the mixing of main polymer with zeolite A and IZC were more dense and thick, but not in the IZC. The results for morphologies of each condition in cross-section of SEM are shown in Figure 2.

The thickness of additional IZC in the main polymer was less than the main polymer and additional of zeolite A. This caused the outer diameter of this membrane to be smaller than the other membranes. The greater the solution viscosity, the faster the dope solution fell into the coagulant bath, which affected the outside diameter of hollow fibre and made it smaller [24]. These conditions provide benefit for hemodialysis treatment. In this case, a smaller internal diameter increased the opportunity of blood to be in direct contact with the inner surface of HF membrane, so that the diffusion

process became better. Moreover, thin membrane thickness resulted in faster of target distribution of target analyte distributed from blood inner of HF membrane to the dialysis solution in the outer HF membranes.

The SEM images in Figure 2 show the cross section of HF membranes. Based on these results, the HF membranes studied had an asymmetrical structure with compact spongy sub layer, selective layer, and fingerlike macro-pores. As can be seen in these figures, the structure of the main polymer embedded with IZC in Figure 2(c) was the same as the structure of the main polymer in Figure 2(a). They had asymmetric and fingerlike structures. In Figure 2(c), it can be seen that there are any IZC particles in the cross section figure. This indicated that the IZC can spread to the inside of the pore until the inner surface of HF. Based on the quantification of the outer and inner diameters; the thinnest wall membrane was PES/CA/IZC. These membranes also had good asymmetric structures compared with PES/CA/ZA and similarity with PES/CA. In Figure 2(b) it can be seen that the asymmetric structure was not uniform, and not fingerlike, so would be effect into dialysis. It may have been that zeolite A was not distributed evenly or the solution dope was not homogenous.

As shown in Figure 3, it appeared that the pores of the mixture between PES and CA were very uniform and similar with the embedded IZC. In contrast to after the additional of zeolite A, consequent to the incorporation of zeolite A and spinning using phase inversion, the pores decreased in number and size

(23–25 nm), different from pore size of PES/CA and PES/CA/IZC which were 1–2 μm and 1–4 μm , respectively. This was because zeolites A inhibited the formation of pores, and covered the original main polymer pores. This situation hampered the diffusion process of uremic toxin from blood inner the HF to dialysis solution outer of HF. Unlike the one embedded with IZC, the pore size was uniform and not covered with a lot of main polymer pores. It may cause the size of imprinted zeolite to be smaller than the original zeolite A, and can break through into the main polymer pores. The main focus on the addition of imprinted zeolite was to withdraw target analyte (creatinine) to be more adhering into the inner surface of HF in order to be quickly attracted by the dialysis solution outer the HF. So, the clearance of creatinine was increased.

3.2.2 Hydrophilicity of PES/CA/IZC Membrane

Hydrophilicity is an important result for the development of membranes used as hemodialysis. It caused the hydrophobic membranes to hamper the uremic toxins soluble in water such as urea, creatinine, uric acid, oxalate and many others, to come out of blood, and undergo the cleaning process. According to the result of contact angle measurement, the contact angle for PES/CA, PES/CA/ZA, and PES/CA/IZC were 85.63; 84.98; and 77.53, respectively (see Table 2).

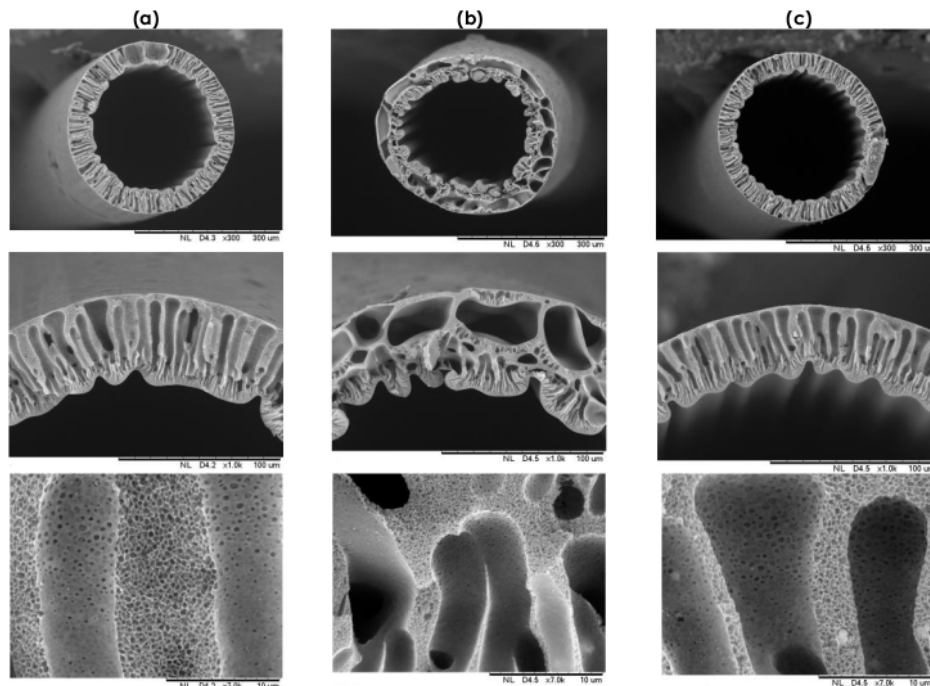


Figure 2 The cross sectional SEM images of (a) PES/CA, (b) PES/CA/ZA and (c) PES/CA/IZC

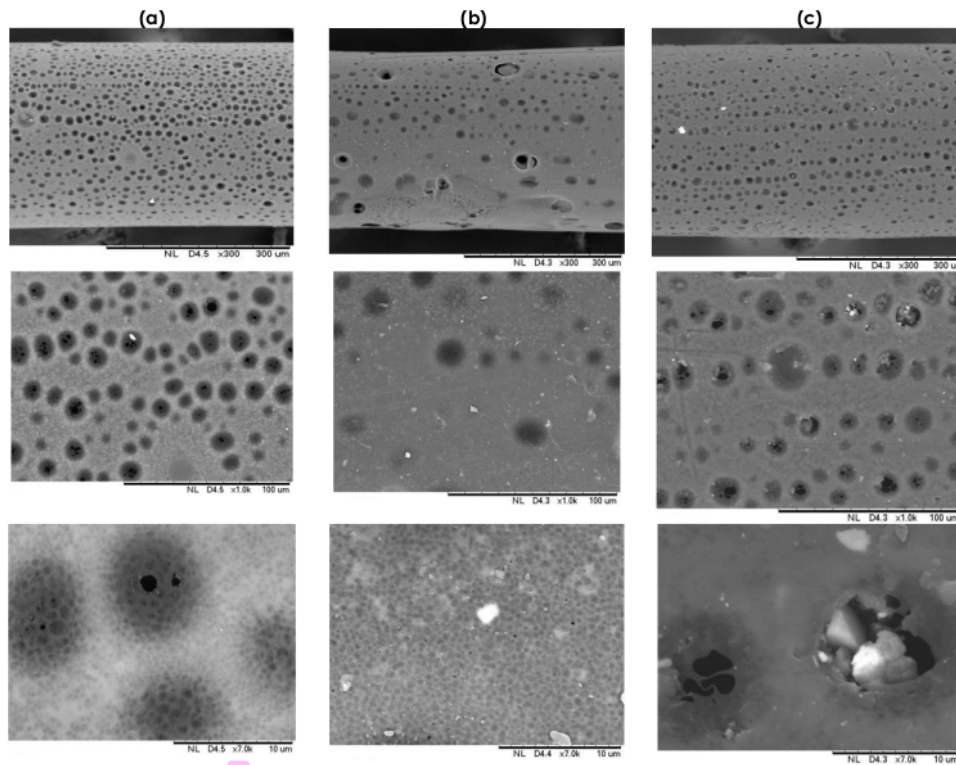


Figure 3 The SEM images for outer surface of (a) PES/CA, (b) PES/CA/ZA, and (c) PES/CA/IZC

This indicates that zeolites and imprinted zeolites increased the hydrophilicity of membrane which allowed better attraction with water molecules. So, the diffusion process increased using PES/CA/IZC to the removal of uremic toxins especially creatinine. To support this result, the value of water and creatinine flux should be considered.

Table 2 The value of pure water flux, contact angle and clearance of creatinine on each HF studied

HF	Pure water flux ($\text{Lm}^{-2}\text{h}^{-1}$)	Contact angle ($^{\circ}$)	Creatinine clearance (%)
PES/CA	22.84	85.63	69.80
PES/CA/ZA	27.57	84.98	74.19
PES/CA/IZC	40.52	77.53	74.99

3.2.3 Pure Water Flux (PWF) and Creatinine Clearance

Besides hydrophilicity, pure water flux and clearance of creatinine has to be proved to make sure, and conclude which is the best membrane for hemodialysis application has been improved. Pure water flux describes the ability of water to through of membrane and perform the dialysis process. The

value of PWF was similar with contact angle measurement. It was found that the more hydrophilic membrane, the greater the PWF. This resulted in faster removal of target analyte. Results of PWF and creatinine clearance are shown in Table 2. PES/CA/IZC had the highest PWF value of $40.52 \text{ Lm}^{-2}\text{h}^{-1}$. The PWF for PES/CA was $22.84 \text{ Lm}^{-2}\text{h}^{-1}$; it means that the PES/CA still had hydrophobic membrane, so there are limitations for the diffusion.

In clearance of creatinine, the quantity of creatinine comes from creatinine at the inner side of HF into the dialysate solution at the outer side of HF. The addition of IZC besides to increase hydrophilicity to the membrane, also increased the selectivity of membrane to attract target analyte (creatinine) from blood from the inner to outer surface of membrane in order to accelerate the dialysis process. Normally, the mechanism of creatinine clearance is diffusion. This study tried to increase the clearance of creatinine using selective adsorption of zeolite. As the creatinine was more attracted to the zeolite, it became entrapped by dialysate solution at the outer side of the membrane. The interaction between creatinine and zeolite was physical bonding, where creatinine that was bounded by zeolite was easily attracted to the dialysate solution. As can be seen in Table 2, the percentage of

creatinine clearance of PES/CA/IZC was 74.99%. This calculation was for a 2-hour dialysis. Thus, time required for a patient to do dialysis process can be calculated, and it should be faster than commercial HF (4 hours). It can be concluded that adding IZC into the main membrane increased the selectivity of target analyte during the hemodialysis process. The clearance of uremic toxins depended on the concentration of uremic toxins in patients' blood. The adequacy of dialysis was also considered.

3.2.4 BSA Rejection

Protein rejection is the one of characterisations for hemocompatibility test. It is an important factor as it makes sure of pores blocking by protein from blood [25, 26]. This research used BSA as a protein. According to the contact angle result, PWF in Table 2, can be seen that the hydrophilicity of PES/CA; PES/CA/IZA, and PES/CA/IZC increased, and it affected BSA rejection. The more hydrophilic, effect in BSA rejection also higher. It caused the BSA to have a nonpolar side, so that it was easy to adsorb with hydrophobic substances. The BSA rejection results for this research were 50.47, 51.60, and 79.05% for PE/CA, PES/CA/IZA, and PES/CA/IZC, respectively. It means that by using PES/CA/IZC there was less protein loss during the hemodialysis process. The protein rejection in this study was lower than those in previous studies which were 90% [27], and 88.2% [28]. It may instigate the pores of hollow fibres to enlarge. Several factors that affected the interaction between protein and membrane were hydrophilicity, roughness, protein type, solution environment, and surface charge [29].

4.0 CONCLUSION

PES is an important material and is used in any application as separation membranes. Mixed-matrix membrane using PES combined with CA as additional hydrophilic side and zeolite that impregnated by target analyte (creatinine) has been done successfully. The IZC had successfully increased the selectivity of hollow fibre membrane applied into the hemodialysis process, and significantly reduced the creatinine up to 74.99%. The morphologies for this mixed-matrix membrane (PES/CA/IZC) asymmetrical structure with compact spongy sub layer, selective layer, and fingerlike macro-pores. Future work should explore the improvement of the selectivity for the removal of protein-bounded uremic toxins such as p-cresol and indoxyl sulfate by using imprinted-zeolite. Based on the results obtained in this study, the future suggestion on the modification of membrane for hemodialysis application is focused on the enhancement of selectivity membrane. The nanoparticles such as zeolites can be further studied as selective nanoparticles mixed to the polymer.

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