Application of Molecularly Imprinted Polymer Technique on Chitosan Membranes for Increasing Creatinine Transport Effectiveness

Eka Sulistyaningsih
Industrial Engineering Department, Technology Engineering Faculty, Institut Sains and Teknologi AKPRIND Yogyakarta, INDONESIA
sulistyaningsih@akprind.ac.id

Abstract
This work aims to investigate the mechanism of creatinine transport across Molecule Imprinted Polymer (MIP) membrane as well as the mechanical properties of membranes. The material used for making membranes is chitosan, and the molecule used as a printer is creatinine. MIP techniques are used to introduce target sites in order to increase the effectiveness of creatinine transport. Membrane transport effectiveness was tested by modeling the hemodialysis kinetics.

The results indicate that transport percentage on MIP chitosan membrane is lower than the chitosan membrane, because not all creatinine was lost during the covering of the membrane pores. However, the membrane flux value of MIP chitosan membrane with a ratio of 0.05:0.0183 was higher compared to other membranes. This indicates that this membrane is the easiest and most convenient for the transport process. The chitosan membrane has a higher water absorption capacity because it has more hydrogen bond allowing for more water binding.

Keywords: Chitosan, creatinine, leaching, membrane, transport.

Introduction

The use of chitosan has been applied in many different fields because it is easily molded and modified in terms of its chemical properties. One of the uses of chitosan is for membrane making in the health field as a hemodialysis membrane. Hemodialysis membranes used are cellulose-based membranes with various substitutions (cf. copper), especially acetate, such as cellulose acetate, diacetate and triacetate. These are less inflammatory to the host and are able to be produced with slightly larger pore sizes, especially in the triacetate form. Nevertheless, the problem of bio incompatibility was not eliminated, and the search for improved membranes continues[1].

Chitosan has a similar chemical structure to cellulose, so it is expected to improve the quality of cellulose-based membranes. This is a natural polysaccharide obtained from chitin deacetylation. Chitosan was suggested as a material for membrane due to its suitable solute permeability and mechanical strength. Chitosan has the ability to form thin film membranes with strong mechanical properties[2].

A number of techniques can be used for membrane preparation. The technique used in the membrane-making process in this study is template-leaching (melting-leaching), referred to as Molecularly Imprinted Polymer (MIP). In the past few years, the preparation of novel membranes using stable MIPs having a specific synthetic receptor structure resulted in an effective method that can be a possible solution for this challenge. Molecular imprinting is the most applicable method for the introduction of molecular recognition properties in synthetic polymer in response to the presence of template species during the formation of a three-dimensional structure of the highly cross-linked polymers[3].

Molecular Imprinting technology is an approach for designing the introduction of molecular sites on artificial materials, such as on antibodies and receptors. MIP has been widely applied in medicine and in the separation of biological compounds and purification derivatives, chemical sensors, and catalysts.

MIPs are inexpensive, strong, resistant to the effects of temperature and pressure, and are inert, compared to other biological systems[4].The principle of the MIP technique is printing the molecule to the membrane, which interacts with a covalently or non-covalently bonded membrane (such as ionic, hydrophobic and hydrogen bonds). After polymerization, the printed molecules are removed from the polymer, and the mold is formed in the polymer[5].

The printed molecule used in this study is creatinine. Therefore, it is expected that the resulting membrane has a pore fit to creatinine. Those membranes transport creatinine well. The resulting membranes were characterized and then used to transport creatinine. The mechanism of creatinine transport across MIP membranes was also investigated. The membrane transport effectiveness was tested by modeling the hemodialysis kinetics.

Material and Methods

Materials: The materials used in this research are chitosan from crab shells with 78% Deacetylation Degree (DD), creatinine, sodium hydroxide, picric acid, phosphoric acid, boric acid, acetic acid, distilled water, and double distilled water.

Methods

Membrane Preparation: The preparation of the MIP membrane was performed using the compositions of chitosan: creatinine 0.050 g :0.0000 g; 0.050 g: 0.0183 g; and 0.050 g: 0.0368 g. The membrane-forming component was dissolved into 10 mL 5% acetic acid in a Petri dish and stirred for 48 hours. The solution was then stirred at 80 °C for about 10 hours to form a membrane.

Leaching of Membrane: Before the MIP membranes were used, they were first immersed in 0.4 mol/L NaOH. The leaching process was done by immersing the membrane into 100 mL of 0.4 mol / L NaOH in a beaker glass, and then stirring for 6 hours. Sampling was done every 1 hour by taking 1 mL of the sample solution and then diluting up to 10 times. The measurement of the sample concentration was performed by reacting the sample with dye reagent, then measuring by a UV-Vis spectrophotometer. After leaching, the membranes were carefully removed from inside the Petri dish and washed with distilled water. Finally, they were washed with ethanol. The washed membrane was dried at room temperature.

Determination of water absorption ability of membranes: The dry membrane was weighed, and then soaked in 10 mL of distilled water for 24 hours. It was then dried with tissue and weighed again. The mass changes were recorded, and the percent of water absorption was computed by the following formula[6]:

$$\varphi = \frac{m_h - m_d}{m_d} x100\% \quad (1)$$

where m_h = mass of membrane after hydration and m_d = mass of dry membrane.

Determination of tensile strength and strain of membrane: The tensile strength and strain of the ready-made membrane are measured by Universal Testing Machine at a speed of 10 mm / min.

Creatinine transport with various concentration of source phase: The creatinine solution with 75 ppm 50 mL concentration as a phase source was placed into transport chamber pipes, and another chamber pipe was filled with 50 mL of distilled water as the acceptor phase was stirred by a magnetic stirrer for 28 hours at room temperature. The pH condition of the acceptor and source phase solution was 7.40. The sampling of the acceptor and phase source solution was performed by taking 1 mL of the solution, then diluting up to a dilution factor of 10. Sampling was taken at 2, 4, 6, 22 and 28 hours. Measurements of creatinine concentration in the sample were taken by a UV-Vis spectrophotometer at λ 490 nm. The transport of concentration of creatinine was 100 ppm and 125 ppm and had the same steps as in the transport of creatinine of 75 ppm.

The use of such concentrations is based on the maximum concentration of normal creatinine in the body. Males have normal creatinine levels of about 0.6-1.2 milligrams / deciliters (mg / dL) or 6-12 ppm, whereas the normal creatinine values in women are between 0.5-1.1 mg / dL or 0.5-11 ppm. Dialysis is recommended when creatinine levels reach 10.0 mg / dL or 100 ppm.

Determination of creatinine concentration in sample (source and acceptor phase): The determination of creatinine concentration was based on Jaffe reaction[7]. 2 mL of the sample solution of source and acceptor phase was added to the dye reagent in the volume ratio of 1:1. The solution was then shaken. After that, the samples were allowed to stand at room temperature, and measured at λ 490 nm.

Membrane Effectiveness Test: The effectiveness test of the membrane was calculated based on hemodialysis kinetics modeling by involving the flux value of the membrane. The value of flux is calculated based on the percent of transport obtained[8]. The following formula was used:

$$\ln = \frac{[C_D]t - [C_R]t}{[C_D] - [C_R]} = \beta t$$

$[C_D]t$ = concentration of source/donor phase at the certain time (t); $[C_R]t$ = concentration of recipient/ acceptor phase at the certain time (t); $[C_D]$ = concentration of source/donor phase at the initial time (t); $[C_R]$ = concentration of solution transported at certain time and (t) = phase recipient/ acceptor concentration at the certain time (t)

Based on the formula, one obtains:

$$y = mc + c$$

where m=slope

$$\text{Slope} = -\left(\frac{A}{Vs}\right) xPs \;,\; Ps = -slope\left(\frac{Vs}{A}\right)$$

where A = Sectional area of chamber mouth (m^2) ; Vs = Source volume (m^3); Ps = membrane permeability coefficient (m/s); Slope = s^{-1}; A = $\pi.r.r$; A=3,14*0,77*0,77 = 1,86 m^2; Vs = 50 mL = 5 x 10^{-5} m^3 and $Ps = -slope\left(\frac{Vs}{A}\right)$

Flux: Flux is measured based on the optimum time:

J = Ps x Cs

where J = flux (g/m^2s), Ps= permeability coefficient (m/s), Cs= phase *recipient*/ acceptor concentration at the optimum and time (t) ($mg/dm^3 = g/m^3$)

Results and Discussion

Membrane Preparation: Acetic acid was used as a solvent because of the influence of acid that donates H^+, causing the breaking of the bond between the chitosan polymer and the amine group ($-NH_2$) protonated becomes $-NH_3^+$, so the

polarity is increased and is soluble in water. The membranes that have been created are leached first before being used for releasing creatinine, so that pores suit the creatinine size. The percentage of membrane leaching is calculated by the following formula:

$$\text{Percent of leaching} = = \frac{Ct}{Co} x100\% \quad (2)$$

where Ct= solution concentration at the time and Co= solution concentration at initial time.

The percentage of membrane leaching is shown in figure 1. Figure 1 shows that the leaching process could not release all creatinine molecules, because they are large. Chitosan is difficult to be released when entered and interacts with the chitosan molecular membrane. The interaction occurs between chitosan and creatinine to form hydrogen bonds. This hydrogen bond is partially interrupted during heating and leaching. The most likely interaction between chitosan and creatinine is the hydrogen interaction. Thus, the chitosan membranes made with MIP techniques contain intramolecular chitosan hydrogen bonds and the bonds between creatinine and chitosan.

The composition of membrane with Chitosan: creatinine 0.05: 0.0183 has a less leaching percentage than chitosan: creatinine 0.05: 0.0366 membrane. This means that creatinine is only partially released from the membrane. This is due to the amount of creatinine being less laid out. Creatinine in chitosan membrane: creatinine 0.05: 0.0366 g does not fully react with chitosan active group, so it is likely to just stick to the surface of the chitosan passages and become easily lost during the leaching process. After the leaching process, the chitosan-based membranes were measured for their thickness for calculating the flux (creatinine transport effectiveness). The thickness of the membrane affects the flux. The thickness of the membrane with varying creatinine compositions is shown in table 1.

Membrane Characterizations

Water Absorption of Membrane: Water absorption of the membrane is the average number of water molecules present in each functional group on the membrane treatment[6]. The water absorption capacity of the membranes was measured before the membrane was used for transport, as it relates to the membrane's ability to transport creatinine. The water absorption percentage of the membranes is shown in figure 2. The results demonstrated that the MIP membrane has a lower water absorption percentage than the chitosan membrane, because the activated groups on chitosan bind to creatinine, so those which bind with water are less. The more creatinine are printed, the less is the water absorption in the membrane. The chitosan membrane without creatinine has the highest percentage of water absorption capacity.

Chitosan membranes have more free hydrogen bonds (less binding to creatinine), so it is relatively easy to bind with water to form hydrogen bonds. H ions on the amine group make chitosan available to readily interact with water through hydrogen bonds. The hydrogen bonds formed are among chitosan monomers and between chitosan and creatinine. The high-water absorption is also caused by the formation of hydrogen bonds between the (-OH) groups owned by the membrane with the -H or -OH group are owned by water. The more water are absorbed by the membrane, the more active groups (-OH) form hydrogen bond with the target compound.

Tensile Strength and Strain of Membrane: Tensile strength and strain test is among the techniques to test the mechanical properties of membranes. The characterization of mechanical properties needs to determine the strength of the membrane against the force that comes from the outsides which can damage the membrane. Tensile strength is defined as the force required to break the specimen or cause complete separation of the constituents in a linear direction[9]. The closer membrane structure means the distance among the molecules in the membranes is tighter, so that it has a strong tensile strength. The tensile strength of the membrane can be seen from the tensile value. The strain of a material indicates the length of the material that can be pulled to the breaking point, referred to as the elasticity. The value of tensile strength and membrane strain are shown in figures 3 and 4.

Figures 3 and 4 show that the tensile strength and strain value of membranes before being used for transport were lower than after transport. This indicates that the membrane molecular density after transport is greater because the pores of membrane are filled with creatinine molecules during transport. Chitosan membrane has a greater value of tensile strength and strain than other membranes because it has more hydrogen bonds with a regular structure. This is in contrast to the MIP membrane because the chemical chain arrangement becomes irregular, so it breaks easily when tested by tensile strength.

Creatinine Transport Mechanism: The hemodialysis membranes principle is that it includes a porous membrane facilitated with a very small pore size[10]. The creatinine transport is based on the diffusion and facilitated membrane principle (the presence of active groups on the membrane). Diffusion is a molecular displacement that occurs due to the high concentration difference between the source and the acceptor phase. The source phase contains high concentrations of creatinine, while the acceptor phase does not contain creatinine. Creatinine molecules at the source phase move into the acceptor phase across the membrane. In facilitated membrane transport (a membrane in the presence of active groups), the carrier compound has an important role.

Creatinine from the source phase enters into the membrane pores due to differences in the solution concentrations between the source and the acceptor phase. After entering into the membrane pores, creatinine is carried by the active groups present on the membrane to pass through the

membrane by traveling a certain distance (the thickness of the membrane). The active groups on the membrane interact through the hydrogen bonds with water, and the water interacts through the hydrogen bond with creatinine so that creatinine passes through the membrane.

The creatinine molecules are carried to the surface of the membrane which is closer to the acceptor phase. Those are then released into the acceptor phase, because there is pressure from the source phase. This process occurs continually as long as the membrane contacts the source solution. The creatinine transport percentage with varying source phase concentrations is shown in table 2. The effect of time on creatinine transport results is shown in figures 5-7. Based on observation, the percentage of creatinine transport reaches maximum transport at the 28th hour. This is because the longer is the contact time between the solute and the carrier group (water), the greater is the percentage of transport. Molecular displacements during transport stop after reaching a relatively balanced state on both phases. Variation of creatinine solution concentrations has no effect on the trend of transport percentage.

Membrane Effectiveness Test: Testing the effectiveness of the membrane involves the flux value of the membrane. Measurements of flux values are performed to determine the ability and effectiveness of the membrane in passing a certain amount of feed volume. Flux is the standard for evaluating membrane performance. The concentration of the membrane-forming polymer greatly influences the character of the formed membrane. The higher polymer concentration membrane component makes the membrane denser, so this decreases the membrane flux[11].

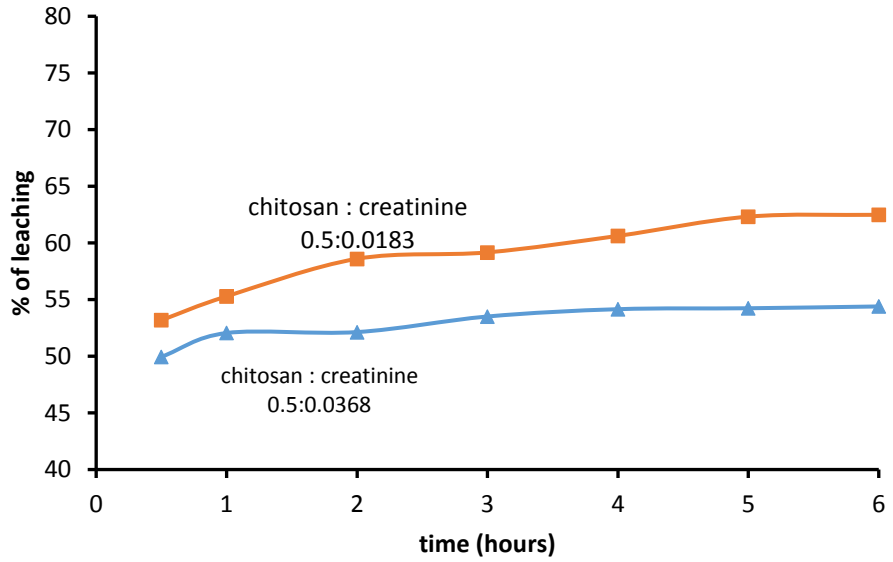

Figure 1: Percentage of Membrane Leaching

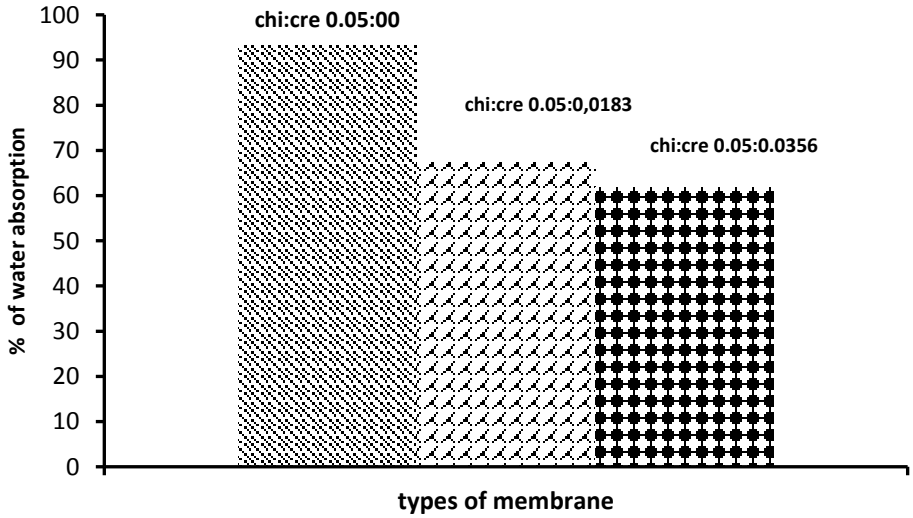

Figure 2: Percentage of Membrane Water Absorption

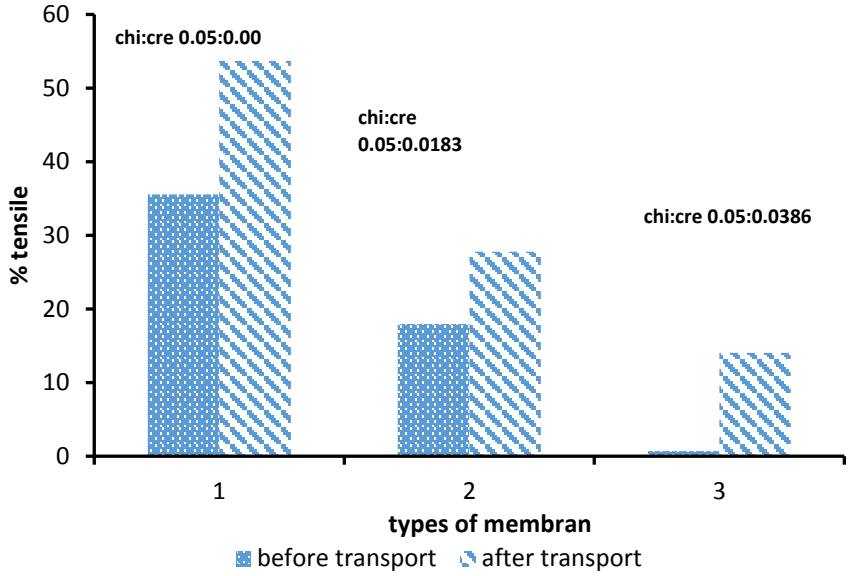

Figure 3: The tensile strength value of membranes

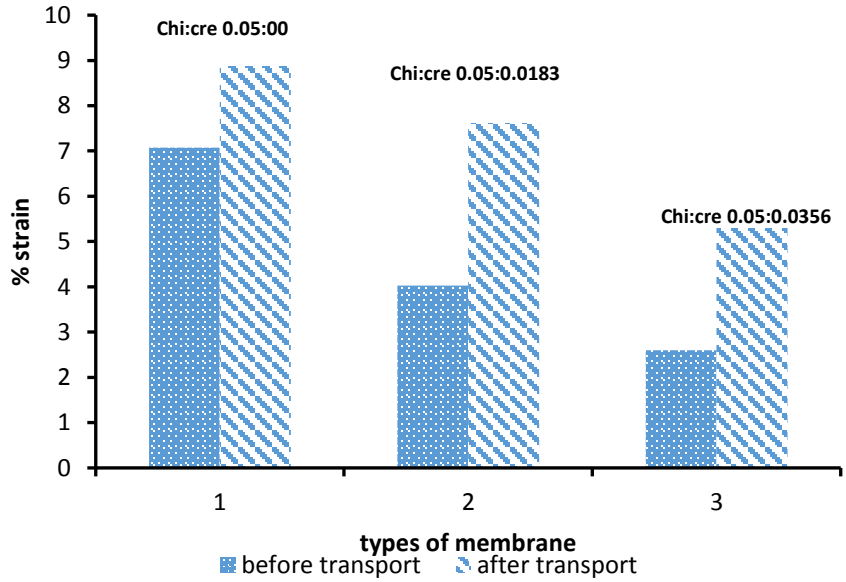

Figure 4: The strain value of membranes

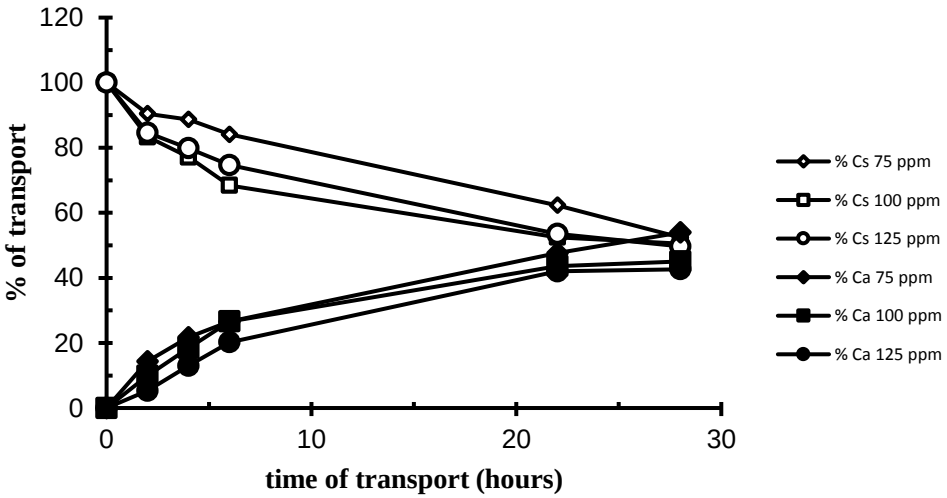

Figure 5: Effect of time on transport percentage using chitosan: creatinine 0.05:0.00 membrane

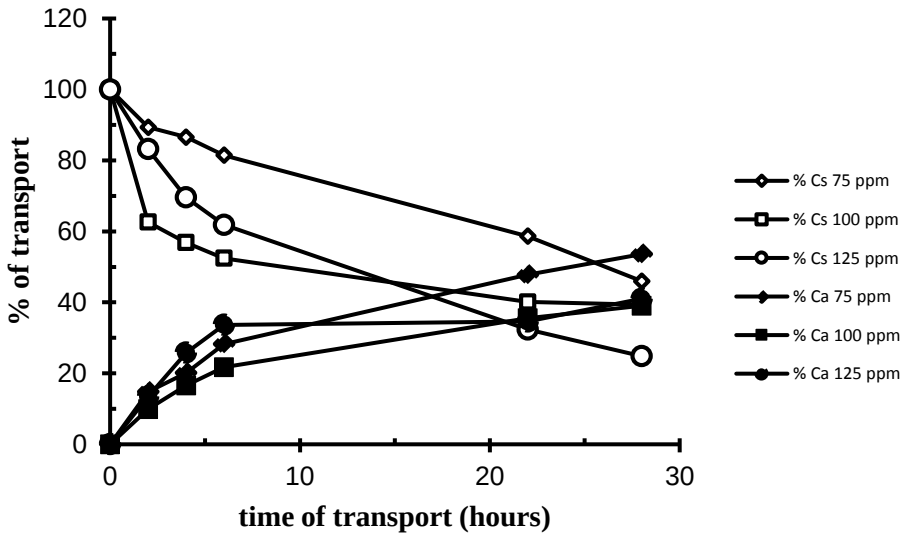

Figure 6: Effect of time on transport percentage using chitosan: creatinine 0.05:0.0183 membrane

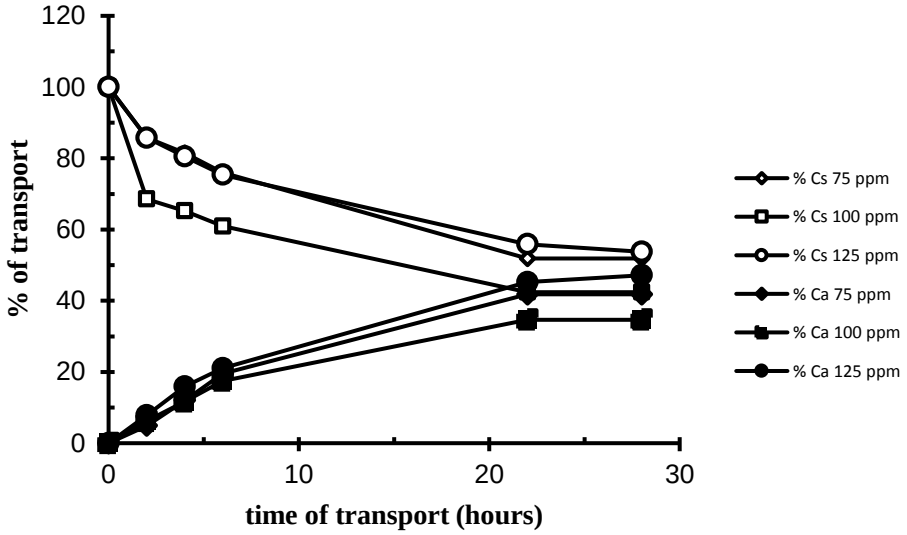

Figure 7: Effect of time on transport percentage using chitosan: creatinine 0.05:0.0356 membrane

Table 1
The thickness of membranes

Chitosan membrane with Variation of Chitosan: Creatinine (g)	Thickness of membrane ($x10^{-5}$ m)		
	75 ppm	100 ppm	125 ppm
0.05 : 0	5.10	5.00	5.10
0.05 : 0.0183	5.10	4.80	3.10
0.05 : 0.0368	5.30	5.50	5.70

Table 2
The creatinine transport percentage with varying source phase concentrations

Chitosan membrane with Variation of Chitosan: Creatinine (g)	% Transport		
	70 ppm	100 ppm	130 ppm
0.5 : 0	53.94	45.04	42.63
0.5 : 0.183	53.59	39.06	41.03
0.5 : 0.368	41.81	34.65	47.15

Based on observation, the MIP membrane with a creatinine ratio of 0.05: 0.0183 g had the highest flux value. This means that the membrane is easiest and faster to transport creatinine than other membranes, although in the end the value of the transport percent is slightly lower than that of the chitosan membrane. The low percent of transport at the end of the transport is due to a great amount of creatinine retained in the membrane pores. The chitosan membrane with creatinine ratio 0.05: 0.0356 g has lowest flux value. The thickest membrane was the chitosan membrane with creatinine ratio 0.05: 0.0356 g; creatinine covers the pores of membrane make it difficult to pass by the feed.

Conclusion

1. The results show that the percentages of transport using MIP membrane are lowest than chitosan membrane because not all creatinine could be released and then covered the pores of the membrane.

2. The flux value of MIP membrane with ratio chitosan 0,05:0,0183 is higher than others. This shows that the membrane is easiest and fastest for transport.

3. The chitosan membrane has the highest water absorption than others because it has more hydrogen bonding.

Acknowledgement

The author thanks Kopertis V Yogyakarta for funding this research and Mr. Dwi Siswanta for the valuable discussion.

References

1. Kerr P.G. and Huang L., Membranes for haemodialysis, *Nephrology*, **15(4)**, 381-385 **(2010)**

2. Amiji M.M., Permeability and blood compatibility properties of chitosan-poly (ethylene oxide) blend membranes for haemodialysis, *Biomaterials*, **16(8)**, 593-599 **(1995)**

3. Scorrano S., Mergola L., Di Bello M.P., Lazzoi M.R., Vasapollo G. and Del Sole R., Molecularly imprinted composite membranes for selective detection of 2-deoxyadenosine in urine samples, *International Journal of Molecular Sciences*, **16(6)**, 13746-13759 **(2015)**

4. Trotta F., Biasizzo M. and Caldera F., Molecularly imprinted membranes, *Membranes*, **2(3)**, 440-477 **(2012)**

5. Xia S.L., Wang H.Y., Sun H., Liu Y.K. and Cao S.K., Preparation of molecular imprinted copolymer membrane for uracil recognition, *Chinese Chemical Letters*, **14(8)**, 794-796 **(2003)**

6. Li Y.S., Zhao T.S. and Yang W.W., Measurements of water uptake and transport properties in anion-exchange membranes, *International Journal of Hydrogen Energy*, **35(11)**, 5656-5665 **(2010)**

7. Narayanan S. and Appleton H.D., Creatinine: a review, *Clinical Chemistry*, **26(8)**, 1119-1126 **(1980)**

8. Mahliçli F.Y., Preparation and Characterization of Hemodialysis Membrans, Thesis, Graduate School of Science and Technology, Institute of Technology İzmir, İzmir **(2007)**

9. Morrilton, Why Determine Tensile Strength and Percent of Elongation?, Bitumen Technology, 3–4 **(2000)**

10. Ulbricht M., Advanced Functional Polymer Membranes, *Polym. Bull*, **47**, 2217-2265 **(2006)**

11. Mulder M., Basic Principles of Membrane Technology, 2nd ed., Kluwer Academic Publisher, Dordrecht **(1996)**.