The effect of leaching agent on molecularly imprinted membrane urea transport process based on polyeugenoxo acetic acid

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Abstract. Research on membrane synthesis as a hemodialysis membrane candidate has been carried out with molecular imprinting technique using MIM (Molecularly Imprinted Membrane) based on polyeugenoxo acetic acid. Polyeugenoxo acetic acid was obtained from the synthesis of eugenol derivatives. This research aims to produce a selective urea membrane based on polyeugenoxo acetic acid for hemodialysis process and to know the effect of different leaching agents in the template release in the transport test. Polyeugenoxo acetic acid that has been bound with urea was mixed with Poly Vinyl Alcohol (PVA), a crosslinking agent for Poly Ethylene Glycol Diglycidyl Ether (PEGDE), and N-Methyl 2 Pyrrolidone solvent (NMP) to form the hydrogel. The hydrogel was then dissolved by NMP to make a membrane. The process of releasing the urea template used ethanol and HCl to obtain urea MIM (Molecularly Imprinted Membrane) and transports was done to find out the difference in membrane transportability. The polymer base and urea MIM obtained were characterized using FTIR, SEM-EDX, and TGA / DTA, while urea MIM was used to transport urea, creatinine, and vitamin B12 through the transport process and was analyzed by UV-VIS spectrophotometer. The results obtained are that MIM has better transports results than NIM. The percentage of transport of urea, creatinine, and vitamin B12 by MIM membranes is 45.02%; 33.63%; 14.28%, and the percentage of transport of urea, creatinine, and vitamin B12 by NIM membranes was 43.36%; 27.42%; 9.9%. In addition, there is an influence of eluent variations (leaching agent) on the transport of urea, creatinine, and vitamin B12, where the optimum transport results are shown in washing using HCl eluents. Percentage of urea transport by membrane washed with eluent variations of HCl, Ethanol, and Aquadest was 45.02%; 40.12%; 39.17%, the percentage of creatinine transport in HCl, Ethanol, and Aquadest variations was 47.03%; 44.2%; 36.6%, and the percentage of vitamin B12 transport in the HCl, Ethanol and Aquadest variations respectively was 8.87%; 18.27%; 3.65%.

1. Introduction
Chronic kidney failure (CKD) is a global public health problem with an increased prevalence and poor prognosis. According to the Global Burden of Disease 2010 results, CKD was the 27th leading cause of death in the world in 1990 and increased to 18th in 2010 [1]. The data showed that the prevalence of CKD is going to increase by about 1.8 % over a year [2]. Currently, hemodialysis is a biomedical method that is used to replace kidney function in removing urea and creatinine from the body that was...
found in the blood [3]. In general, the high levels of urea and creatinine indicate the kidney inability in disposing these toxic compounds.

Hemodialysis use a semipermeable membrane to selectively dispose the harmful component from the kidney. A good semipermeable membrane should not pass proteins or substances that are still beneficial for the body, instead it should be able to pass urea molecules in the blood, have good strength and mechanical resistance, and should also be biocompatible. Hemodialysis membrane based on cellulose, chitosan, and polysulfone have some disadvantages such as causing an inflammatory response in patients, only the small molecules can pass through, lack of active sites, low mechanical strength, and adsorption of blood proteins to the surface. [4-6]. Therefore, in this study, the latest clean material used was eugenol, where eugenol has three functional groups which capable of being functional monomers that are potential for selective adsorption processes with molecular imprinting technology, and hemocompatibility properties, to minimize the weaknesses of the basic ingredients of hemodialysis membrane, and also improve the properties of hemodialysis membrane.

MIM (Molecularly Imprinted Membrane) is a membrane that contains introduction sites to molecules made with molecular printing techniques. This printing technique can give the membrane the ability selectively recognizing target molecules in solution during the transport process [7]. An important step in the imprinted technology concept is the release of target molecule templates, which will produce areas that capable of recognizing the target molecule or target analyte. Djunaidi (2016) has conducted research on the adsorption of selective phenol molecules with different eluent solvents which are methanol, water, ethanol, and chloroform [8]. Another research by Lee et al (2008), described the molecularly imprinted creatinine membrane (MIM) using poly (ethylene-vinyl alcohol) material with eluent used to release (leaching) target molecules from the template such as ethanol and water deionization [9]. However, the above research does not explain its effect to the transports applications results. Therefore, in this research, variations of leaching agents such as aquadest, HCl, and ethanol were used to find the optimal eluent to release the template molecules, to know its effect on the transport of urea, creatinine, and vitamin B12, and hopefully, to increase the selectivity of MIM (molecularly imprinted membrane) when compared to NIM (Non-Imprinted Membrane).

2. Materials and method

2.1. Materials
The materials purchased from SIGMA-Aldrich were Eugenol and BF3-diethylether while other reagents were purchased from Merck, NaOH, chloroacetic acid, Chloroform, Methanol, Poly vinyl alcohol (PVA), urea, creatinine, vitamin B12, PEGDE (Polyethylene glycol diglycidyl ether), 1-Methyl 2-pyrrolidone and Diethyl ether. Demineralized water was purchased from Bratachem.

2.2. Instrumentation
The instruments used were FTIR Spectrophotometer (Shimadzu Prestige 21), analytical balance (Ohaus), UV-Vis Spectrophotometry (LW-V-200-RS), pH meter (Trans Instrument), SEM-EDX (Phenom Pro X Desktop with EDX), and TGA (DTA/TG Exstar SII 7300).

2.3. Method
2.3.1. Synthesis of polyeugenol
5 grams of eugenol were added to a three-neck flask and then added with 1 mL of BF3-diethyl ether. The mixture was stirred using a stirrer for 4 hours and once every 1 hour the addition of 0.25 mL BF3-diethyl ether was done. After the reaction lasts for 4 hours, the polymerization was stopped by adding 1 mL of methanol. The formed gel was then dissolved with diethyl ether and washed with distilled water until it reached a neutral pH. The solution was then dried by adding Na2SO4 anhydrous. After being completely free of water, the solution was evaporated at room temperature. The formed
precipitate was then dissolved with distilled water, dried and weighed. The obtained result was analyzed by FTIR.

2.3.2. Synthesis of polyeugenol acetic acid
A total of 5 g of polyeugenol was put into a boiling flask, then 33% NaOH solution (33 grams NaOH in 100 mL) as much as 17.5 mL was added. Subsequently, the mixture was stirred for approximately 30 minutes and added 12.5 mL of 50% chloroacetic acid solution (50 g in 100 mL water) little by little with a dropper pipette while continuing to stir. The mixture was heated in a water bath with a temperature of 80–90°C. Heating was carried out for 2 hours, then let cooled and acidified with 6 M HCl to pH = 1. Then extracted with diethyl ether as much as 3 times, 50 mL in each extraction. The ether extract was combined and extracted with 5% w/v sodium bicarbonate 3 times 30 mL each, then the water layer was acidified with 6 M HCl to pH = 1. Then filtering, drying and weighing were carried out. The result obtained was analyzed by FTIR.

2.3.3. Synthesis of MIM in-situ
A total of 1 g polyeugenol acetic (PA) acid was contacted with 1000 ppm urea as much as 20 mL. The results are then dried. PA which has been contacted with urea coupled with 0.5 g PVA dissolved in 2.5 mL of NMP added with 0.1 M sodium hydroxide as a catalyst and PEGDE and heated at 120–140°C for 20–30 minutes and allowed to stand overnight to form a gel. Gel with a weight of 0.35 grams for membrane thickness was then added with NMP solvent and heated to obtain the right viscosity to do the casting process. The casting membrane was then heated at 80°C until the membrane is dry and molded. It was then soaked with 2 M KCl until the membrane is detached from the petridish. The membrane sheet was then cut to the size of the diffusion cell. The removal of urea with HCl and ethanol for 24 hours produced the MIM urea membrane. Then the obtained membrane is mounted on the diffusion cell tool.

2.3.4. Synthesis of NIM (Non-imprinted Membrane)
NIM was synthesized in the same way as MIM urea but without the binding stage of urea first. The PA was used without contacting the urea first.

2.3.5. Urea transport
Transport urea with membrane produced using a diffusion cell apparatus, in the feed phase (feed, urea) is 300 mg/L of urea and in the receiving phase is phosphate buffer pH 7.4, each 50 mL and then stirred for 24 hours. Sampling was done every one hour until 8 hours and followed by sampling at the 24 hours.

2.3.6. Creatinine transport
Creatinine transport with the membrane produced using a diffusion cell device, in the feed phase (feed, creatinine) was 25 mg/L creatinine and in the receiving phase was a phosphate buffer at pH 7.4 each 50 mL and then stirred for 24 hours. Sampling was done every one hour until 8 hours and followed by sampling at the 24 hours.

2.3.7. Vit B12 transport
Transport of vitamin B12 with a membrane produced using a diffusion cell apparatus, in the feed phase (feed, urea) is 20 mg/L of vitamin B12 and in the receiving phase is a phosphate buffer at pH 7.4, each 50 mL and then stirred for 24 hours. Sampling was done every one hour until 8 hours and followed by sampling at the 24 hours.

2.3.8. SEM-EDX analysis
SEM-EDX analysis was performed on the MIM membranes before and after the 24 hours template removal.
2.3.9. TGA-DTA Analysis
TGA-DTA analysis was carried out on polyeugenol polymers, and polieugenoxy acetic acid, as well as the MIM membrane washed with HCl and ethanol, and unwashed MIM.

3. Results and discussions

3.1. Synthesis of polyeugenol and polyeugenoxy acetic acid
It appears in the FTIR analysis (figure 1) that the loss of eugenol vinyl spectra at wavenumbers of 915 cm\(^{-1}\), 997 cm\(^{-1}\) and 1648-1638 cm\(^{-1}\) indicates that the polymerization has occurred into polyeugenol. The C-O alcohol group (1600 cm\(^{-1}\)) transforms into an acid carbonyl group which is indicated by the peak at 1720 cm\(^{-1}\). The alcohol group (O-H) of eugenol and polyeugenol turn to acids at around 3400 cm\(^{-1}\).

![Figure 1. Graph of eugenol IR spectra and their derivatives.](image1)

3.2. Transport of 300 ppm urea with the variation of template release eluent
The performance of the membrane can be seen by transporting 300 ppm urea. It appears in figure 2 that the MIM shows good transport performance, especially at the beginning of transport (up to 8 hours of transport), both in the receiving phase and the feed phase with transport capability almost...
half of the initial concentration up to the 8th hour. Thus, it is possible that the presence of the imprinted area on the membrane gives a large percentage of transport value in the urea transport process, and also shows the difference in eluent template release at urea transport performance.

In figure 2, a high yield percentage of transport using a 1 M HCl as eluent was indicated by the surface of the membrane that have a more regular and uniform pore as stated by the results of the characterization with SEM instruments and indicates that the release using HCl can improve the process of releasing the urea template because of its ability to dissolve the urea template is better than ethanol.

3.3. Transport of 25 ppm creatinine with a variation of the template release eluent

Figure 3 shows a high yield of transport percentage using a 1 M HCl eluent. This is indicated by the surface of the membrane having a more regular and uniform pore as stated by the results of the characterization with the SEM instrument and indicates that the release using HCl can improve the template release process better than ethanol.

![Figure 3. Creatinine transport on MIM with eluent variation release template.](image)

3.4. Transport of 20 ppm vitamin B₁₂ with a variety of template release eluents

Figure 4 shows that the membrane with HCl eluent cannot transport Vitamin B₁₂ properly (not in large quantities) which can be seen from the graph. The feed phase (FP) has a high percentage of transport indicating that the HCl eluent has the ability to maintain vitamin B₁₂ during the transport process and is not able to transport properly due to the template or the imprinted area when compared with ethanol.

![Figure 4. Vitamin B₁₂ transport on MIM with eluent variation release template.](image)
3.5. Urea, creatinine and vitamin B\textsubscript{12} transport

It appears in figure 5 that the MIM is also capable of transporting creatinine and vitamin B\textsubscript{12} even though it is not as large as urea, and is very small in transporting vitamin B\textsubscript{12}. This is caused by creatinine and vitamin B\textsubscript{12} sizes which are larger than urea. Creatinine has a size of (60 and 113 daltons) [10] while vitamin B\textsubscript{12} has a size of 8,5 A or 1.35 kDa [11].

![Figure 5. Transport of creatinine, vitamin B\textsubscript{12} and urea with MIM membrane.](image)

3.6. Membrane characterization using SEM-EDX

Table 1 below shows that washing with HCl for 24 hours can reduce the level of urea which is characterized by the composition of element N after leaching proved by EDX with an element composition of 0% and also shows that the composition of the element N in NIM is 0% because there is no urea template in the NIM. So, in this case, it shows that the urea MIM has been successfully formed and the levels of urea in the feed and receiver phases are not derived from the leaching process but indeed from the urea transport from the feed phase.

| Element | Weight Concentration |
|---------|----------------------|
| Number  | Symbol | Name          | MIM before and after washing HCl | NIM after washing HCl |
|         |        |               | Before washing | After washing | After washing |
| 6       | C      | Carbon        | 45.07          | 63.83        | 65.40        |
| 8       | O      | Oxygen        | 26.32          | 36.16        | 34.59        |
| 7       | N      | Nitrogen      | 28.57          | -            | -            |

While table 2 below showed that washing with ethanol for 24 hours, the composition of element N after leaching on EDX with an elemental composition of 32.62%. So, in this case, the level of urea present in the feed and receiver phases may come from the leaching process.

| Element | Weight Concentration |
|---------|----------------------|
| Number  | Symbol | Name          | MIM before and after ethanol washing |
|         |        |               | Before washing | After washing |
| 6       | C      | Carbon        | 45.07          | 44.75         |
| 8       | O      | Oxygen        | 26.32          | 22.62         |
| 7       | N      | Nitrogen      | 28.57          | 32.62         |
3.7. SEM analysis

Figure 6 shows that the obtained membrane is porous with a size of about 1–5 µm. The size of the pore membrane is suitable for use as hemodialysis membranes [12]. The appearance surface morphology in NIM showed that there are no pores and it showed differences in surface morphology when compared to MIM. Thus, the presence of templates gives a different effect on membrane surface morphology, and will also affect the transport performance.

![MIM before washing](image)

![MIM after ethanol washing](image)

![NIM after HCl washing](image)

![MIM after HCl washing](image)

**Figure 6.** Comparison of SEM results before and after washing.

3.8. Thermal analysis with TGA-DTA

From the results of the analysis of a weak exothermic peak at a temperature range of 50–100°C it is possible to have a dehydration reaction or release of water from water molecules that are absorbed in the material [13–14]. Endothermic peak at no temperature range 303–424°C (figure 7 (A)) and temperature range 321–446°C (figure 7 (B)), the temperature of 220°C and a wide endothermic peak at a temperature of 350–390°C (image C and D in figure 7), and temperatures 275–501°C (figure e) significantly further decomposition with respect to the TGA curve caused by the double bond on the benzene aromatic ring which breaks into aliphatic chains due to high temperatures [15]. A decrease in mass of about 20–60% (image A and B in figure 7), a decrease in mass of about 20–80% (image C and D in figure 7), and a decrease in mass of about 10–80% (figure e) is due to the breakdown/depolymerization of the benzene ring which is abundant in both of the polymers [13].

The material polyeugenol and polyeugenoxo acetic acid only showed two steps: dehydration and polymer decomposition. This is different from MIM and NIM, because their composition has been cross-linked with PEGDE, PVA, and polyeugenoxo acetic acid polymers, showing that many stages occur at higher temperatures. The increase in chemical stability is due to the crosslinking and also caused by the bonding between molecules which requires more energy for decomposition [16] and the chemical structure that becomes more stable when crosslinked [17-19]. In the TGA-DTA result of polymer polyeugenol and polyeugenoxo acetic acid, it showed that the change of group in the polymers are capable of increasing the thermal resistance of the polymer. Also, the results of MIM and NIM TGA-DTA analysis showed that the presence of template urea in the membrane has no significance influence in the membrane thermal resistance (stability).
4. Conclusions
Membrane synthesis with active compounds derived from polyeugenol in the form of polyeugenoxi acetic acid has been successfully synthesized. Molecularly imprinted urea membrane has been successfully synthesized. The urea imprinted membrane is unable to transport creatinine and vitamin B$_{12}$ properly. The eluent variations of HCl 1 M, Ethanol 10 % for template release produce differences in the results of urea, creatinine, and vitamin B$_{12}$ transport. The HCl 1 M template release effluent can optimize the results of the urea, creatinine and vitamin B$_{12}$ transport percentage. Changing the polymers group from originally polyeugenol into polyeugenoxo acetic acid increases its thermal resistance and there is no significant difference in thermal resistance on the imprinted and the non-imprinted membranes.
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