Indones. J. Chem., 2016, 16 (2), 144 - 150

Preparation of Citric Acid Crosslinked Chitosan/Poly(Vinyl Alcohol) Blend Membranes for Creatinine Transport

Retno Ariadi Lusiana[1,*], Dwi Siswanta[2], and Mudasir[2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Diponegoro, Jl. Prof. Soedarto, Tembalang Semarang 50275, Indonesia*

[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*

Received July 28, 2015; Accepted March 16, 2016

ABSTRACT

Preparation of membrane using crosslinking reaction between chitosan and citric acid showed that functional group modification increased the number of active carrier groups which lead to better transport capacity of the membrane. In addition, the substitution of the carboxyl group increased creatinine permeation of chitosan membrane. The transport capacity of citric acid crosslinked chitosan membrane for creatinine was found to be 6.3 mg/L. The presence of cyanocobalamin slightly hindered the transport of creatinine although compounds did not able to pass through citric acid crosslinked chitosan/poly(vinyl alcohol) blend membrane, as compounds no found in the acceptor phase.

Keywords: *hemodialysis; crosslinked; active carrier; transport; membrane*

ABSTRAK

Reaksi taut silang kitosan-asam sitrat menunjukkan bahwa modifikasi gugus fungsional telah dapat meningkatkan jumlah gugus aktif pembawa sehingga berimplementasi pada peningkatan kemampuan transpor membran. Di samping itu, substitusi gugus karboksil juga telah terbukti meningkatkan permeasi kreatinin melewati membran. Kapasitas transpor membran kitosan tertaut silang asam sitrat terhadap kreatinin adalah sebesar 6,3 mg/L. Keberadaan sianokobalamin sedikit mengganggu transport kreatinin namun senyawa tersebut tidak mampu melewati paduan membran kitosan tertalut silang asam sitrat-poli(vinil alkohol) dengan tidak terdapat serapan sianokobalamin pada fasa akseptor.

Kata Kunci: *hemodialisis; taut silang; sisi aktif; transpor; membran*

INTRODUCTION

Excessive blood creatinine concentration (above 2.5 mg/dL) is an indication of a kidney disease [1-2], which is usually treated with hemodialysis therapy. Hemodialysis is a process acting as an artificial kidney to remove the metabolism wastes (such as urea, creatinine) from the blood diffusion mechanism. The main element of hemodialysis is the high capacity semipermeable membranes capable of efficiently transporting low molecular weight toxic metabolites (urea, creatinine) from blood as well as restraining protein plasma and cells with high molecular weight. Due to the importance of this role, the membranes used for hemodialysis have to be mechanically strong, resistant to leakage, able to remove/transport the waste compound rapidly, selective, and unable to adsorb protein on the membrane surface.

Membrane transport process is influenced not only by the membrane pore size but also by the presence of reactive groups on the membrane which enable the interaction between membrane with the target compound to occur. According Lusiana et al. (2013) specific interaction between urea and creatinine with reactive groups membrane is dominated by hydrogen bonds. In addition, appropriate ratio of hydrophilic and hydrophobic groups as well as the reactive groups charge orientation are among important factors in hemodialysis membranes [3-4].

Chitosan, a non-toxic, inert and compatible polymer, began to be used as the base material for membrane synthesis due to its ability to dissolve in dilute acid and form a thin layer film [4]. However, the functional groups available in chitosan are not reactive enough to provide sufficient interaction with the target compound [5-6]. Primary amine groups in chitosan causes the material to be positively charged at low pH,

* Corresponding author. Tel/Fax : +62-87739443843
Email address : lusianaretno@yahoo.com

which could initiate the adsorption of protein on membrane surface [7]. Protein adsorption occurring when blood is in direct contact with a foreign substance (such as membrane) is a serious problem in hemodialysis process. The adsorption is mainly triggered by the electrostatic forces between charged reactive groups in the membrane with the opposite charge in blood protein. This will result in the decrease of membrane permeability which may lead to blood coagulation [8-9].

To overcome the disadvantages of the nature of pure chitosan, chemically modifying chitosan can be an effective technique. Some of the common strategies for this modification include changing the charge of the active sites, attaching active groups onto the membrane as well increasing membrane hydrophilicity. The three modifications can be carried out reached by grafting, crosslinking s as well as blending with other polymers [3,7,11-12].

To increase the reactivity and selectivity of chitosan, the modification of functional groups of chitosan through crosslinking reaction using a carboxylic group of citric acid had been done in the present study. Citric acid has been widely known as a natural blood anticoagulant [9]. Negatively charged carboxylate group from this acid will replace the positive charge of the amine group in chitosan and reduce the electrostatic forces with the blood protein, thus minimizing protein adsorption. In addition, carboxylic group is a reactive group able to form hydrogen bonds with urea and creatinine, so that its use as an active side will increase the ability of the membrane to capture the permeates, which will allow for an effective permeation process. The purpose of this study was to increase the number of functional groups that can be used as the active side of the membrane in a way to synthesize membrane material through crosslinking reaction of chitosan and citric acid.

The mechanical strength of the membrane represents its ability to not become fragile and leak during the transport process. Not all polymers and biopolymers can be used as membrane, as only polymers capable of forming flexible thin layer films and swelling can be used as the base material for membrane synthesis. In order to improve the mechanical strength of the citric acid crosslinked chitosan membrane, in this study we made blends materials with more hydrophilic polymers such as poly(vinyl alcohol) [3,10,14]. This blends membranes were investigated by tensometer, thickness meter, EA and FTIR. All of the results showed the successful blending improves the mechanical strength as well as the flexibility of chitosan membrane by regulating balanced ratio of hydrophobic and hydrophilic groups in the material.

EXPERIMENTAL SECTION

Materials

Chitosan flakes (MW ~40.000 Da, 87% deacetylation degree) were obtained from Biotech Surindo, Cirebon, Indonesia. Citric acid (anhydrous powder), acetic acid (glacial, 96.6%), sodium hydroxide (pellets ACS reagent ≥97.0%), hydrochloric acid (extra pure 32%), cyanocobalamin were purchased from Merck (Germany). Poly(vinyl alcohol) (PVA) was purchased from Fisher Scientific (ON, Canada). Creatinine, albumin, ethanol, p-dimethylamino benzaldehyde (DAB), potassium dihydroxyphosphate, potassium hydroxyphosphate were also obtained from Merck (Germany). Picric acid (ACS reagent ≥99.5%) was purchased from Sigma Aldrich.

Instrumentation

The instruments used in this study included laboratory glassware, balance (Mettler Toledo AB54-S), hot plate with magnetic stirrer (E-scientific), petri dish (Iwaki), oven, shaker, pH-meter (Hanna) and a set of transport experiment apparatus.

To quantify the chemical composition membrane film before and after crosslinking. Infrared Spectrophotometer (Shimadzu FT-IR 8201 PC) was conducted between 500-4000 cm-1. The thickness of membrane was determinate using Mitutoyo thickness meter. Tensometer (Shimadzu, AG-I-250 KN) was done for measured the mechanical strength of membrane.

Procedure

Synthesis of citric acid crosslinked chitosan

Synthesis of citric acid crosslinked chitosan is based on Gohil methods with some adjustments. At the beginning, 1.5g chitosan, 100 mL of acetic acid 1%, citric acid (different mole ratio with chitosan), 5 mL hydrochloric acid a catalyst as were added to 250 mL one neck round flask. The system was prevented under reflux at 50-70 °C for 24 h under magnetic stirring.

Preparation of citric acid crosslinked chitosan/poly(vinyl alcohol) blend membrane with different thickness

Citric acid crosslinked chitosan 1.5% (w/v) solution was blended with 1.5% (w/v) PVA solution in a petri dish with different volume ratio. The blend solution was stirred for 20 h and then put into the oven to remove the solvent at 50-70 °C for approximately 24 h. When the membrane was visibly dry, 10 mL 1M NaOH solution was poured into the petri dish to help remove

the membrane from the mold. The membrane obtained was then washed with distilled water and subsequently air dried.

Swelling measurements

All of the membranes were allowed to dry at room temperature to a constant weight. The membranes were immersed in phosphate buffer solution of pH 7.4 at room temperature for 1-6 h. Every 1 h the membrane samples were removed, swabbed with tissue, and weighed. The swelling percentage was calculated using the following expression:

$$\text{Swelling (\%)} = \frac{\text{Weight of swollen membrane}}{\text{Weight of dry membrane}} x100\%$$

Membranes characterization

To determine the properties of the membrane, the analysis will be done on the material before and after the crosslinking. Functional groups were obtained using Shimadzu FTIR spectroscope. The mechanical strength of the blend crosslinked membranes were measured using Tensometer (Shimadzu, AG-I-250 KN tester). Mitutoyo thickness meter was used to measure the thickness of membranes. Thickness measurements performed at 3 different points of the membrane. The percentage atomic composition is calculated using elemental analysis (XPS, Shimadzu).

Permeation study

The transport study was carried out using a solution with single solute (15 mg/L creatinine) in a 100 mL phosphate buffer (pH 7.4) in the feed phase, and phosphate buffer without creatinine as the acceptor phase. Transport experiments were also carried using mixture solutions of the following metabolites: Urea (500 ppm), creatinine (15 ppm) and cyanocobalamin (20 ppm) in 100 mL of phosphate buffer solution in the feed phase, and phosphate buffer without a metabolite into the acceptor phase using magnetic stirrer in each phase. The concentrations of permeate used in the experiments were determined based on the maximum creatinine concentration that can still be tolerated in blood. The permeability of the solutes was determined using UV-Vis spectrophotometry (772 Spectrophotometer) at time intervals of 0-6 h. For the purpose of the spectrophotometric analysis, creatinine was complexes with picric acid and NaOH to form colored complex which absorbs visible radiation at 486 nm (Jaffe method). The pink colored cyanocobalamin solution can be analyzed without complexation and analyzed directly at 361 nm.

RESULT AND DISCUSSION

Synthesized Citric Acid Cross-linked Chitosan (CA.cl.CS)

In the first reaction occurred protonation of $-NH_2$ on chitosan by proton derived from acetic acid, this reaction takes place quickly. Consequently N atoms become positively charged and are easily attacked by a lone pair owned by the -OH group of citric acid (phase 2). In the second stage of the reaction, forming a citric acid cross-linked chitosan which is illustrated by the type of reaction that intermolecular cross-link reaction that occurs on two chitosan chains and intramolecular cross-links which cross-links occur in the one chitosan chain. The second stage of the reaction is slowly. Types of reactions occur which can not be explained with certainty (Fig. 1).

To success of the cross-linking reaction was verified by FT-IR spectrophotometry (Fig. 2). The specific differences between the spectra of chitosan and modified chitosan can be observed as a sharp absorption band at the wave number of 1597.06 cm^{-1}, which indicates the modification of to the amine group in chitosan, from primary amine to a secondary amine groups. Another difference is observed as a sharp absorption in the region 1690-1750 cm^{-1} which is the characteristic absorption of C=O group in citric acid [13]. The blending between CS.cl.CA and PVA also resulted in the widening of -OH absorption band at 3448 cm^{-1}. This indicates that the integration of the two materials did not change the type of functional groups in backbone compound, but only increases the percentage of hydroxyl groups [10,14]. The result confirmed the successful crosslinking of citric acid on amine group of chitosan.

Further analysis of the modified compound was conducted by elemental analysis to obtain more detailed information regarding the amount/percentage of each element in the material. The results from the analysis were then compared to the theoretical percentage calculated by assuming that crosslinking reaction occur between 2 chitosan monomers and 1 citric molecule as displayed in Table 1. The data displayed in Table 1 indicates the increase of surface carbon elemental concentration from CS to CA.cl.CS indicated that the crosslinking reaction has occurred between chitosan and citric acid [15].

Membrane Characteristics

The CA.cl.CS membranes were prepared using phase inversion method, in which the liquid phase is converted to solid phase by evaporating the solvent by gradual heating at a temperature range of 50-70 °C for

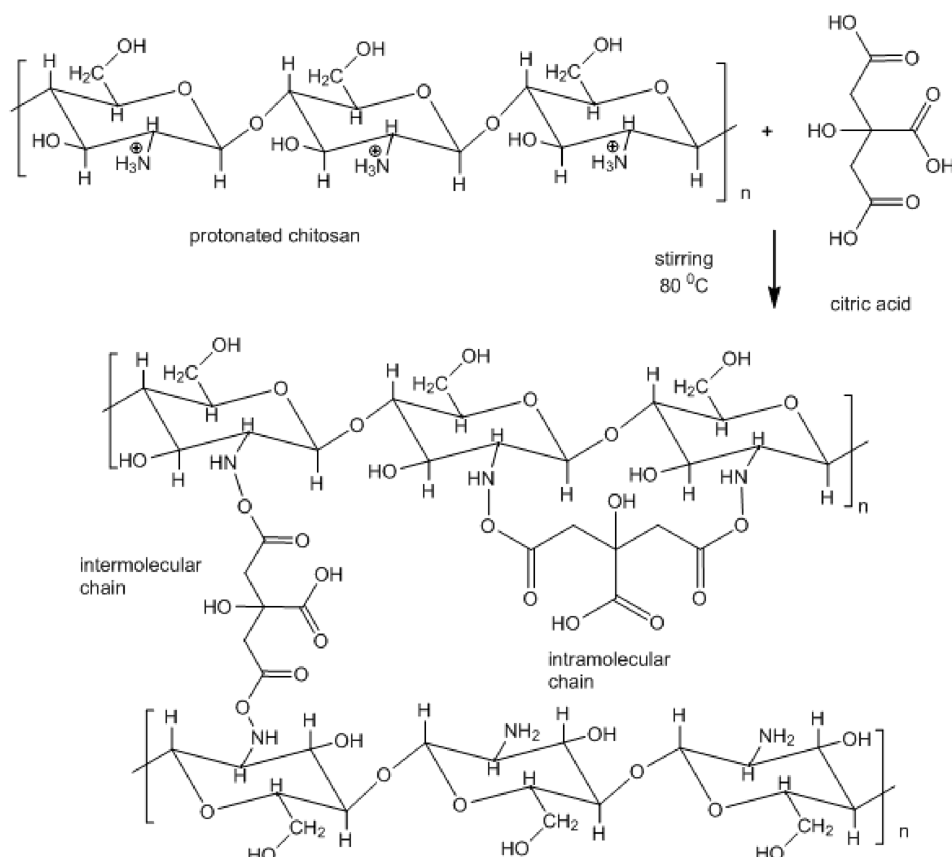

Fig 1. Mechanism crosslinked reaction

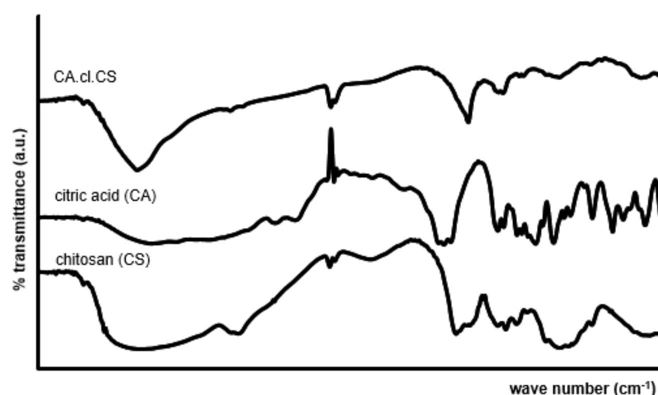

Fig 2. FTIR Spectra of the membranes

Table 1. Atomic composition of sample surfaces measured by XPS

Sample suface	Atomic composition		
	C%	H%	N%
Chitosan	40.11	7.03	7.79
CA.cl.CS	42.20	6.00	5.45
CA.cl.CS/PVA	42.65	5.47	5.47

24-48 h. The gradual heating was proved to produce asymmetric CA.cl.CS membranes.

Water absorption of the membranes

For materials contacting human blood, the balance between hydrophilic and hydrophobic was important [17]. Water absorption of the membrane depends on the strusture and compotition of the polymer membrane [14]. The wáter absorption variation of chitosan membranes before and after modification was calculated and shown in Table 2. In this study, membranes were made in three different thickness: 50 μm (A), 100 μm (B) and 180 μm (C). The water absorption indicated that membranes with the surface area (diameter = 5 cm, L = 78.57 cm^2) shows that the least thickness of membrane (A) has the highest water absorption capacity, which amounted to 220%. This value relates to the ability of water to diffuse to all parts of the membrane. In other words, in the thin membrane, all parts of the membrane were able contact/interact rapidly with water in a certain period of time, so that the wetting of the membranes occured not only on the surface but also the inside part of the membrane [3,12,16]. Water absorption test was performed to predict the size of the substance that can diffuse through the membrane material. When the membrane expands, the mobility of the polymer chains increases, thus facilitating solvent penetration to fill the

Table 2. Physicochemical properties of the membranes

Membrane	Thickness (mm)	Water absorption (%)	Mechanical properties	
			Strength (MPa)	Strain (%)
CS	0.25	90	1.50	16.40
CA.cl.CS	0.30	247	0.24	24.73
CA.cl.CS/PVA (A)*	0.50	219	18.68	74.72
CA.cl.CS/PVA (B)*	1.00	174	24.75	120.50
CA.cl.CS/PVA (C)*	1.80	142	29.76	151.85
CA.cl.CS/PVA (A)**	0.50	220	8.10	25.14

Notes: CA.cl.CS/PVA (A)* = crosslinked blend membrane with thickness of 50 µm unused, (B)* the 100 µm membrane thickness (C)* the 200 µm membrane thickness, and (A)** the 50 µm membrane thickness after used for permeation creatinine and cyanocobalamin

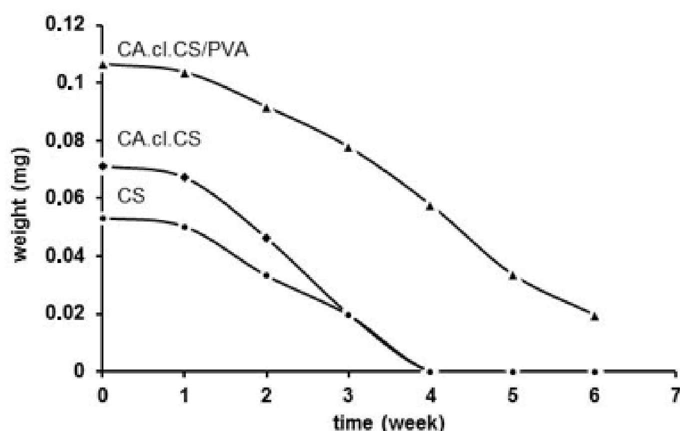

Fig 3. Decomposition of membranes by bacterial

empty spaces in the membrane interphase [11,15]. The presence of -COOH groups in the cross-link structure led to increased interaction between the water with polymers. The blend membranes usually exhibited a higher wáter absorption degree then the CS membrane, indicating a more flexible membrane structure [14,16].

Strength and strain of the membrane

A successful blending should lead to intermolecular interaction between two component polymers, thereby improving mechanical strength of the blend [Hyder et al., 2009]. Mechanical testing gives an indicating of the strength and elastibility of polymers membranes [14]. The tensile strength measurement of the membrane, performed by performing strain and stress strength test, was done to study the tensile strength as well as elasticity of the membrane. The measurement result is displayed in Table 2. Data indicates that unused membranes are generally more elastic than the used ones, as exemplified by the elongation of the membranes after they are used for transport. It was also obtained that the cross-linking reaction between amine groups in chitosan with the carboxyl groups in citric acid was able to increase membrane elasticity by 8-16 fold. The blending with PVA also results in an enormous improves the tensile strength and elongation of chitosan film. Crosslinked blend membranes show a higher mechanical strength compared uncrosslined blend samples [16].

Biodegradable test

Biodegradation test was conducted to determine the ability of the decomposition of the membrane by the environment. The process can be done in anaerobic and aerobic. In this study the decomposition process conducted under aerobic conditions with the help of bacteria and fungi found in the soil. Membrane is cut and weighed, dumped in the ground with the same depth and left for 6 weeks. Each week, the test specimen is taken, cleaned and weighed. Value decomposition process is shown in Fig. 3. It appears that the membrane can easily be broken down by bacteria in the soil. Within 6 weeks, almost all of the membranes have been exhausted decomposed by bacteria.

Creatinine permeation

The thickness and modification group effect. One of the factors that affect the transport process is the membrane thickness. The thickness of the membrane is determined by the mass of chitosan, citric acid and PVA are added in the same unit area. The more the mass of compounds added, then the resulting membrane will thicken. The result indicates, The longer contact time, causing transport creatinine increased. The membrane transport capacity lies in the ability to allow compounds with low molecular weight (urea, creatinine) to pass through the membrane and at the same time inhibit large molecular weight compounds (vit B12) from passing through. In the present study, three different sets of experiments were performed to study the transport capacity of CA.cl.CS/PVA. The first experiments was to study the ability of the membrane to transport creatinine when disolved in a solution as a single permeate. The second transport experiment was done with the the presence of urea, cyanocobalamin together with creatinine in the feed phase.

The transport creatinine as a single permeate was performed on three membranes with different

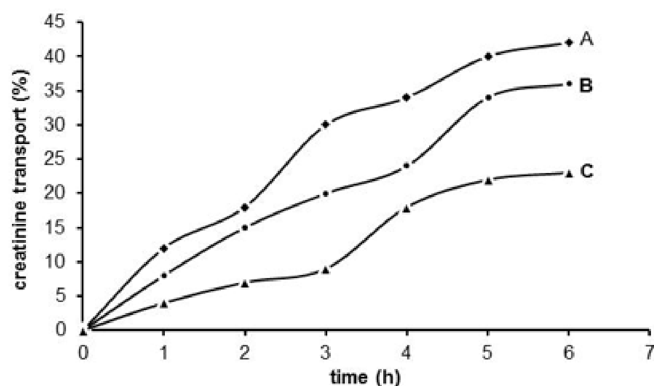

Fig 4. The effect of membrane thickness on CA.cl.CS/PVA, A = 50 μm, B = 100 μm, C = 180 μm

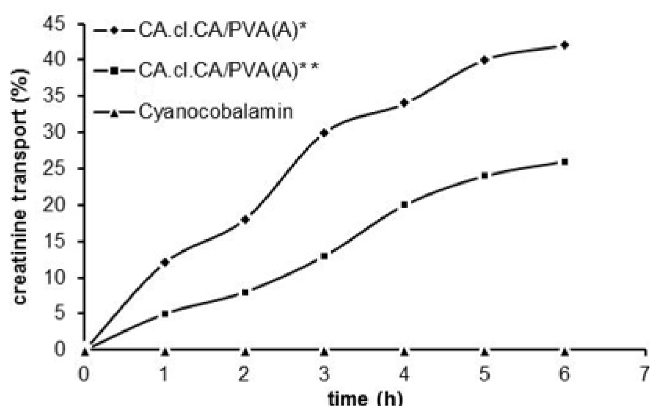

Fig 5. The effect of cyanocobalamin in th creatinine transport of membrane, CA.cl.CS/PVA(A)*: unused membrane, CA.cl.CS/PVA(A)** : membrane after used for cyanocoabalamin permeation

thicknesses. The different in membrane thickness represents the different amounts of the compounds constituting the membranes for the same unit area. Fig. 4 shows that the creatinine transport percentages decreased with increasing thickness, with the values of 42, 36 and 23%, for the membranes with the thickness of 50, 100 and 180 μm, respectively. The results show that the membrane transport creatinine 50 μm provides the best transport percent. Creatinine diffusion becomes faster on thin membranes.

Based on Table 2 was found that the thin membrane showed a large water uptake value. It is noticeable that the thinness of the membranes influential in the transport process. From the data obtained that the thin membrane has a water uptake is highest among the other membrane. Apparently, the water absorption capability comparable to the ability of the membrane to transporting creatinine. In fact, the high water uptake associated with the increase in transport capability of the membrane.

Chitosan-citric acid cross-linking reaction showed that functional group modification increased the number of carrier compound active groups which led to the increase of membrane transport capacity. This is due to the replacement of the $–NH_2$ group by –COOH groups. Thus, we have seen that the creatinine permeability of the chitosan modification was higher than the virgin chitosan membranes.

The effect of cyanocobalamin. The effects of cyanocobalamin in the feed phase on the transport percentage of creatinine by the membrane are represented in Fig. 5. Transport experiments was carried out using the membrane with a thickness of 50 μm for 6 h. It can be seen from Fig. 5 that the presence of cyanocobalamin together with creatinine decreased the transport percentage of creatinine by 28.5%. The size of cyanocobalamin molecule (1355 g/mol) is 10 times larger than the size creatinine so that it was able to block creatinine from entering the pores of the membrane. In addition, cyanocobalamin has several C=O groups able to form hydrogen bonds with the the active sites of the membrane. The hydorgen bonds indirectly interfere with the binding process of creatinine by the active sites of the membrane [3,10]. In other words, there is a competition between cyanocobalamin and creatinine when approaching the membrane surface, which is likely to be the cause for the decrease of the transport percentage of creatinine. From the analysis on the receiving phase, and there was no cyanocobalamin which can pass through the membrane. Cyanocobalamin is only interfere with the process of transport without being able to pass through the membranes.

CONCLUSION

Crosslinking reaction between chitosan and citric acid with tricarboxylic functional groups increased the hydrophilicity and the number of active group that serves as the active sites and thereby increasing membrane permeation. We demonstrated here that the blend of the poly(vinyl alcohol) to chitosan chain improves the flexibility and mechanical strength backbone. The resulting membrane material has been used for the transport of creatinine although cyanocobalamin did not pass through CA.cl.CS/PVA membrane.

REFERENCES

1. Lee, M.H., Tsai, T.C., Thomas, J.L., and Lin, H.Y., 2008, *Desalination*, 234 (1-3), 126–133.
2. Lacson, E.Jr., and Brunelli, S.M., 2011, *Clin. J. Am. Soc. Neprol.*, 6, 2522–2530.

3. Lusiana, R.A., Siswanta, D., and Mudasir, 2013, *Indones. J. Chem.*, 13 (3), 262–270.
4. Pandele, A.M., Ionita, M., Crica, L., Dinescu, S., Costache, M., and Iovu, H., 2014, *Carbohydr. Polym.*, 102, 813–820.
5. Lin, W.C., Liu, T.Y., and Yang, M.C., 2004, *Biomaterials*, 25 (10), 1947–1957.
6. Balan, V., and Verestiuc, L., 2014, *Eur. Polym. J.*, 53, 171–188.
7. Hoven, V.P., Tangpasuthadol, V., Angkitpaiboon, Y., Vallapa, N., and Kiatkanjornwong, S., 2007, *Carbohydr. Polym.*, 68 (1), 44–53.
8. Ma, L., Su, B., Cheng, C., Yin, Z., Qin, H., Zhao, J., Sun, S., and Zhao, C., 2014, *J. Membr. Sci.*, 470, 90–101.
9. Taji, S., Nejad-Sadeghi, M., Goodarznia, I., 2014, *J. Supercrit. Fluids*, 95, 603–609.
10. Lusiana, R.A., Siswanta, D., Mudasir, and Hayashita, T., 2013, *Int. J. Chem. Eng. Appl.*, 4 (4), 229–233.
11. Dash, M., Chiellini, F., Ottenbrite, R.M., and Chielline, E., 2011, *Prog. Polym. Sci.*, 36, 981–1014.
12. Li, L., Cheng, C., Xiang, T., Tang, M., Zhao, W., Sun, S. and Zhao, C., 2012, *J. Membr. Sci.*, 405-406, 261–274.
13. Hyder, M.N., and Chen, P., 2009, *J. Membr. Sci.*, 340 (1-2), 171–180.
14. Huangfu, P., Gong, M., Zhang, C., Yang, S., Zhao, J., and Gong, Y.K., 2009, *Colloids Surf. B*, 71 (2), 268–274.
15. Sagnella, S., and Mai-Ngam, K., 2005, *Colloids Surf. B*, 42 (2), 147–155.
16. Gao, A., Liu, F., and Xue, L., 2014, *J. Membr. Sci.*, 452, 390–399.