Preparation, Characterization, and *In Vitro* Hemocompatibility of Glutaraldehyde-Crosslinked Chitosan/Carboxymethylcellulose as Hemodialysis Membrane

Khabibi Khabibi[1,2], Dwi Siswanta[1], and Mudasir Mudasir[1*]

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

[2]*Department of Chemistry, Faculty of Sciences and Mathematics, Diponegoro University, Jl. Prof. H. Soedarto, S.H., Tembalang, Semarang 50275, Indonesia*

* ***Corresponding author:***

email: mudasir@ugm.ac.id

Received: November 27, 2020
Accepted: August 8, 2021

DOI: 10.22146/ijc.61704

Abstract: *This study aims to examine the manufacture, characterization, and* in vitro *hemocompatibility of glutaraldehyde-crosslinked chitosan/carboxymethyl cellulose (CS/CMC-GA) as a hemodialysis membrane. The CS/CMC-GA membrane was prepared using the phase inversion method with 1.5% CS and 0.1% CMC. The chitosan was crosslinked with glutaraldehyde in various monomers ratios, and the membranes formed were characterized by FTIR, SEM, and TGA. Furthermore, the hydrophilicity, swelling, porosity, mechanical strength, and dialysis performance of the membranes against urea and creatinine were systematically examined, and their in-vitro hemocompatibility tests were also conducted. The results showed that the CS/CMC-GA membranes have higher hydrophilicity, swelling, porosity, mechanical strength, and better dialysis performance against urea and creatinine than chitosan without modification. In addition, the hemocompatibility test indicated that the CS/CMC-GA membranes have lower values of protein adsorption, thrombocyte attachment, hemolysis ratio, and partial thromboplastin time (PTT) than that of pristine chitosan. Based on these results, the CC/CMC-GA membranes have better hemocompatibility and the potential to be used as hemodialysis membranes.*

Keywords: *chitosan; CMC; membrane; hemodialysis*

■ INTRODUCTION

The incidence of chronic renal disease is significantly increasing globally, with 3 million recorded cases in 2012 [1]. Presently, hemodialysis is the most common treatment recommended for chronic renal failure patients in the ESRD (End-Stage Renal Disease) phase. The function of hemodialysis is as an artificial kidney that can separate toxic substances in the blood, such as urea and creatinine, from materials still needed by the human body, such as vitamins, hormones, albumin, and blood cells fibrinogen. In hemodialysis, a semi-permeable membrane is the most vital component responsible for the separation of substances. The early generation type of this membrane was made of acetic cellulose, which then has been replaced by a synthetic polymer, such as polysulfone (PSF), polyethersulfone (PES), polyvinylpyrrolidone (PVP), polymethyl-methacrylate (PMMA), and polyacrylonitrile (PAN) [2-5]. Although the early generation and the synthetic polymer types have small pores and are good in transporting urea and creatinine compounds, there are still some problems concerning their permeability and biocompatibility [6]. Therefore, considering that the use of natural materials yields biocompatible products, the utilization of a semi-permeable membrane made of natural biopolymeric resources is very promising and necessary to be further explored and developed [7].

One of the promising biopolymeric materials as hemodialysis abundantly available in nature is chitosan, which is obtained from chitin deacetylation. This biopolymer contains a hydroxyl (–OH) and amine ($–NH_2$) and dissolves in an organic acid to form a thin

film, biodegradable, inert, non-toxic, easy to modify, and safe for the human body [8-11]. However, chitosan is not ready to be used as a membrane without some modifications. Its performance still requires improvement, especially in the number of active groups, permeability against urea and creatinine, mechanical strength, hydrophilicity, porosity, and biocompatibility [12]. Some studies have also suggested the necessary modification of these membranes to improve their performance as hemodialysis membranes [12-15]. Also, previous studies have reported that chitosan membranes have been modified by increasing their active groups and improving their mechanical strength [16]. The modification was achieved by blending the membrane with active-sites-rich polymer and crosslinking reactions [14,16-17]. This blend gives rise to the change in their hydrophilicity-hydrophobicity balance, mechanical strength, stability, porosity, and influences the effectiveness of the membrane dialysis towards urea and creatinine and their biocompatibility [7,14].

In this study, the active sites of the chitosan membrane are enriched by blending it with carboxymethyl cellulose (CMC), while its mechanical strength is improved by crosslinking it with glutaraldehyde (GA). It has been previously proved that the CMC has a more effective active group of carboxyl ($–COO^-$) in their interaction with urea and creatinine [16] by hydrogen bonds [18]. Also, it has been reported that CMC modification gives more biodegradable and non-toxic compounds [19]. Furthermore, a carboxyl group in the CMC influences the hydrophilicity-hydrophobicity balance and dialysis capability of the membrane and its interaction with blood [20]. The preparation of chitosan membrane using CMC as a blending agent and glutaraldehyde as a crosslinking candidate have been conducted in prior studies [19,21-23]. However, most of these products have not been applied to hemodialysis membranes. The modification of the chitosan membrane crosslinked with glutaraldehyde (CS/CMC-GA) in this study is expected to improve its mechanical strength and membrane porosity and influence the hydrophilicity-hydrophobicity balance. The obtained CS/CMC-GA membranes were characterized using FTIR, SEM, TGA, and their properties were determined, including hydrophilicity, swelling, porosity, and mechanical strength. The membrane permeability was also tested against urea and creatinine, while its biocompatibility was examined *in vitro* by establishing its protein adsorption, hemolysis ratio, thrombocyte attachment, and partial thromboplastin time (PTT).

■ EXPERIMENTAL SECTION

Materials

Chitosan (MW ~40,000 Da with DD 87%) was obtained from Biotech Surindo, Cirebon, Indonesia. Carboxymethyl cellulose (low viscosity, DS 70%) and picric acid (ACS reagent 99.5%) were produced by Sigma Aldrich. Glutaraldehyde (ACS reagent 25%), glacial acetic acid (96.6%), sodium hydroxide (ACS reagent 97.0%), creatinine, *p*-dimethylaminobenzaldehyde (DAB), sodium citrate, potassium dihydrogen phosphate, and potassium hydroxy phosphate were obtained from Merck (Germany).

Instrumentation

This study used instruments that include: FTIR spectrophotometer (Shimadzu FT-IR 8201 PC), scale (Mettler Toledo AB54-S), hot plate with a magnetic stirrer (E-scientific), petri dish (Iwaki), oven, centrifuge (PLC Gemmy), pH-meter (Hanna), glassware and dialysis equipment set, TGA (Perkin Elmer), UV-Vis Spectrophotometer (Shimadzu), SEM (JSM 6360 LA) and Tensometer (Shimadzu, AG-I-250 KN).

Procedure

Preparation of glutaraldehyde crosslinked CS/CMC

In this study, the membranes were prepared using a phase inversion system. Chitosan solution (1.5% w/v) was prepared by dissolving chitosan powder in 100 mL of 1% glacial acetic acid and stirred for 24 h. The variations of glutaraldehyde (54.4, 27.2, 18.2, and 13.6 mg) were respectively added to 50 mL chitosan to obtain membrane cross-linked at 40, 80, 120, and 160 monomers of chitosan (CS/CMC-GA40, CS/CMC-GA80, CS/CMC-GA120, CS/CMC-GA160). The number of monomers 40, 80, 120, and 160 indicated the possibility of repeating the crosslinked chitosan monomer by GA.

The solution was stirred at 60 °C. Then, 50 mL of 0.1% CMC was added to each resulting solution by steadily dropping and stirring for 24 h. The mixed solution (10 mL) was poured into a Petri dish, and the solvent was evaporated at 40–50 °C for 24 h. Then 1.0 M NaOH solution was added until the membrane was detached from the petri dish and washed using mineral-free water until neutral and dried.

Membrane characterization

The resulting membrane was characterized using an FTIR instrument (Shimadzu Prestige 21) to determine its functional group. In addition, its surface morphology was analyzed by SEM (Phenom Pro X), and its thermal characteristics were assessed with TGA (Perkin Elmer).

Mechanical strength. The membrane's mechanical strengths were tensile strength and strain (%), measured by tensile strength tester (Zwick/Z05) and expressed in MPa. The membrane was cut into 4 × 6 cm^2 dimensions and given a load of 5 N at the moving speed of 5 mm/min.

Hydrophilicity and porosity tests. The hydrophilicity test was carried out by establishing the water contact angle and swelling. The contact angle measurement was conducted with static water sessile drops using the Axisymmetric Drop Shape Analysis Profile (ADSA-P) approach. The water contact angle was established using drop water from the pipette perpendicularly to the flat membrane. The angle between the water curve surface and the flat area of the membrane was recorded every 20 sec. The swelling test was carried out by soaking the 2 × 2 cm^2 dimension membrane in phosphate-buffered solution (PBS) pH 7.4 for 5 h. Subsequently, the membrane surface was dried using filter paper and weighed. The following equation was used to establish the swelling power:

$$\text{Swelling}(\%) = \frac{W_1 - W_0}{W_0} \times 100\% \quad (1)$$

where W_1 and W_0 were the wet and dry weights, respectively.

The porosity test was carried out by soaking the 3 × 3 cm^2 dimension membrane in the doubled-distilled water for 5 h, and the membrane surface was dried. The following equation was used to establish the porosity:

$$\varepsilon(\%) = \frac{W_2 - W_1}{V\rho} \times 100\% \quad (2)$$

where ε = porosity, W_2, and W_1 = the membrane weight after and before soaking, V = volume of the membrane, and ρ = density.

The average pore radius rm (m) was determined using Guerout–Elford–Ferry equation:

$$rm = \sqrt{\frac{(2.9 - 1.75\varepsilon) \times 8\eta tQ}{\varepsilon A \Delta P}} \quad (3)$$

where η is the water viscosity at 25 °C, t is the membrane thickness (m), Q is the volume of the permeate water per unit time (m^3/s), A is the effective area of the membrane (m^2), and ΔP is the operational pressure (Pascal). Pore diameters (pore size, nm) of the membrane are calculated by multiplying rm by 2 [24].

Dialysis performance

The permeable capability of the membrane was evaluated by determining urea and creatinine dialysis. The membrane was inserted into the dialysis apparatus between two compartments with an effective diffusion area of 3.14 cm^2. The source compartment was filled with 30 mL of PBS solution containing urea and creatinine. The experiment was conducted in a single solution, and the concentrations of the urea and the creatinine were respectively 500 ppm and 20 ppm in PBS of pH 7.4. Then, 50 mL of each solution was poured into the feeding phase, and a phosphate-buffered solution was filled at the receiving phase. Each of these was stirred for 5 h. The dialyzed urea was determined every hour by spectrophotometer UV-Vis using *p*-dimethylaminobenzaldehyde/DAB (Ehrlich's reagent) in an acid condition. The complexing agent of picric acid in alkaline conditions (Jaffe method) was used to determine the dialyzed creatinine. The following equation was employed in determining the percentages of the transported urea and creatinine:

$$t(\%) = \frac{C_t}{C_o} \times 100\% \quad (4)$$

where t = permeated percentage, C_t = dialysate concentration, and C_o = initial concentration at feeding phase.

Hemocompatibility study

Protein adsorption. The blood sample was centrifuged at 3000 rpm for 15 min to obtain two layers

of the solution. The top part contains less thrombocyte/platelet-poor plasma (PPP), while the bottom is enriched with thrombocyte/platelet-rich plasma (PRP). Then, 2 × 2 cm^2 membrane was washed using PBS buffer, and 1 mL of PPP was poured into it and incubated for an hour at 37 °C. Subsequently, the membrane was sprayed with PBS solution, washed with 2% sodium dodecyl sulfate, and flushed with doubled-distilled water. The resulting solution was used to determine the concentration of the adsorbed protein using UV-Vis spectrophotometry and the biuret methods.

Thrombocyte attachment. After washing the 2 × 2 cm^2 membrane using the phosphate-buffered solution (PBS) of pH 7.4, it was poured into 1 mL of PRP and incubated for an hour at 37 °C. The concentration of the thrombocyte before and after soaking the membrane was analyzed using a hemocytometer. The following equation was used to establish the number of thrombocytes attached to the membrane:

$$\text{Thrombocyte attachment} = \frac{C_t}{C_o} \times 100\% \quad (5)$$

where C_t and C_o = concentration of the thrombocyte after and before the soaking.

Hemolysis ratio. The membrane cut with the 2 × 2 cm^2 dimension was washed using doubled-distilled water and 0.9% NaCl, then soaked quickly in the mixture of 5 mL of 0.9% NaCl and 20 mL of a blood sample for 30 min. The blood sample was centrifuged at 1500 rpm for 10 min. The absorbance of the upper layer of the blood (plasma) was measured using a UV-Vis spectrophotometer at 545 nm. Double-distilled water was used as a positive control, while 0.9% NaCl was used as the negative. The following equation was used to establish the hemodialysis ratio (HR):

$$HR = \frac{AS - AN}{AP - AN} \times 100\% \quad (6)$$

where AS = sample absorbance, AN = negative control absorbance and AP = positive control absorbance.

Partial thromboplastin time (PTT). The membrane dimension of 0.5 × 0.5 cm was soaked in 0.5 mL of PPP and incubated at 37 °C for 10 min. Then, 250 µL of 0.025 M $CaCl_2$ solution that has been heated at 37 °C for 10 min with constant stirring was added to the PPP mixture. Once fibrin fibers were formed, the time was recorded as the PTT [25].

■ RESULTS AND DISCUSSION

The CS, CS/CMC, and CS/CMC-GA membranes in this study were prepared using the phase inversion method, in which the liquid phase was converted to a solid phase by evaporating the solvent by gradual heating at 40–50 °C for 24 h. The CS/CMC-GA membrane was prepared from the chitosan with a deacetylation degree (DD) of 87% for more accessible treatment. The higher DD of 1.5% chitosan quickly formed a gel, and it was difficult to mold the membrane. The stability of the CS/CMC-GA was tested in water and PBS pH 7.4 for 6 h. After the soaking process, both in water and PBS, the chitosan membrane and the CS/CMC crosslinked with glutaraldehyde showed no dissolution (stable) and reduction, meaning that the membranes were stable. Meanwhile, a small amount of CS/CMC dissolution was observed. These findings indicated that CS and CS/CMC-GA membranes were applied to blood plasma at pH of 7.4, while CS/CMC was not feasible.

Membrane Characterization

FTIR was used in this study to characterize the functional groups of the chitosan and CS/CMC-GA, and the results were shown in Fig. 1. Both types of chitosan showed a wide adsorption band of about 3400 cm^{-1}, indicating the presence of –OH overlapping with the –NH group. Furthermore, the chitosan amide group was

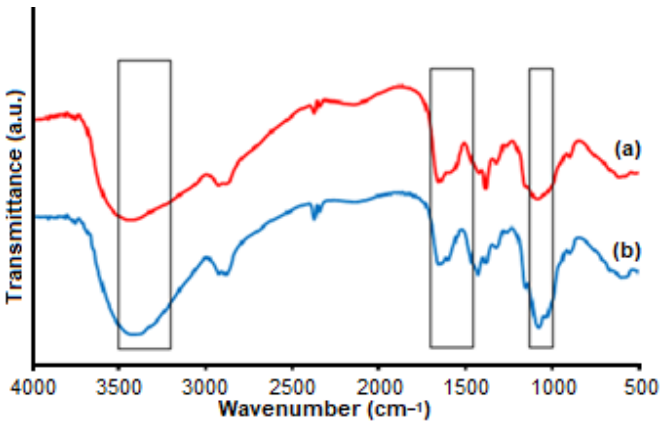

Fig 1. FTIR spectra of (a) chitosan and (b) CS/CMC-GA

observed in the absorption of 1650 cm^{-1}, and the intensity of this peak increased in the CS/CMC-GA membrane. This observation was consistent with previous studies by Oyrton et al. [26] and Beppu et al. [27], which stated that glutaraldehyde-crosslinked chitosan shows the vibration absorption at 1655 cm^{-1} due to the formation of a new N–C bond, formed by N atom from the chitosan and C atom of glutaraldehyde. The crosslink formation between the chitosan and the glutaraldehyde was also proved by the intensity decrease of a peak of 1100 cm^{-1}, belonging to an amino group (–NH_2). Regarding the interactions between CS and CMC, no new vibration was observed because, according to Wang et al. [21], this interaction does not involve chemical bonds but through electrostatic formation only.

Further characterization of the membranes has been carried out by the TGA method. The thermograms of the TGA analysis of chitosan and glutaraldehyde-crosslinked chitosan/CMC were shown in Fig 2. The membrane was heated from 30 to 1000 °C at an elevated 5 °C/min, and the thermogram was recorded. As observed from the figure, the first decrease in weight occurred at 30–165 °C for chitosan and the CS/CMC-GA chitosan, indicating the discharge of the water trapped in the membrane through evaporation. However, the percentage decrease in the CS/CMC-GA (13%) weight was more significant than that of the chitosan (9%), suggesting that the CS/CMC-GA contains more water and is more hygroscopic than the chitosan. The second decrease in the weight for chitosan occurred at 250–425 °C, while that of CS/CMC-GA occurred at 250–645 °C. The weight decrease indicated that the destruction of the bonds other than the glucopyranose ring produced volatile compounds, such as CO_2, H_2O, and CO. The higher temperature and longer time required for the second weight decrease and bond breaking in the CS/CMC-GA indicated higher membrane stability resulting from the crosslink reaction. The third weight decrease in the chitosan was observed at 425–565 °C, while that of the CS/CMC-GA was found at 645–750 °C. This range of temperatures corresponded with breaking the remaining bonds available in the membranes, including the polymerization bond of the chitosan chain and the opening of glucopyranose and pyrolytic rings. The more complex bonds available in the CS/CMC-GA required a higher temperature than the chitosan to break the bonds.

The surface morphology of chitosan and CS/CMC-GA membranes have been characterized by SEM analysis, and the results are shown in Fig. 3. From this figure, the surface of the chitosan membrane is flat and homogeneous (Fig. 3(a)). It is also observed that the surface morphology of the CS/CMC-GA is relatively homogeneous coarser and is a little bit amorphous. This

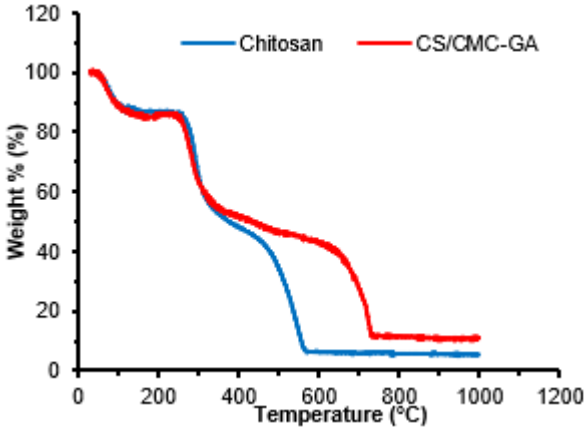

Fig 2. TGA curves of chitosan and CS/CMC-GA

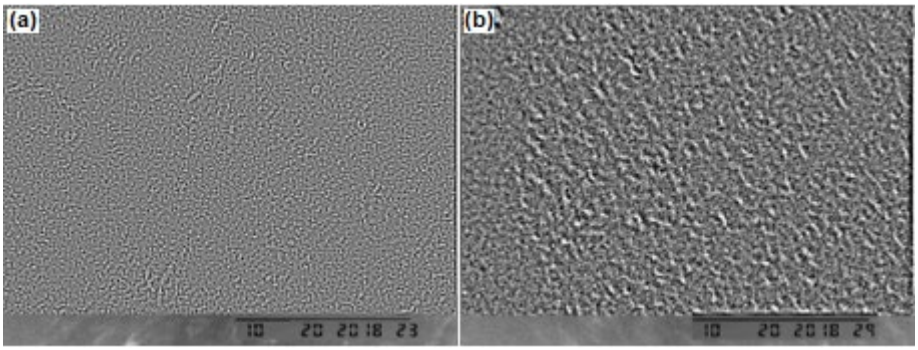

Fig 3. SEM image of chitosan and CS/CMC-GA

morphology causes the membrane porosity to increase as well as the surface contact area of the dialysate.

Hydrophilicity Measurement

The degree of hydrophilicity-hydrophobicity of the membrane surface has been observed by measuring its water contact angle. The narrow water contact angle indicated that the membrane surface has high hydrophilicity and vice versa. The measurement results of the water contact angle of the membrane surface of the chitosan (CS), CS/CMC, and CS/CMC-GA were summarized in Fig. 4. In general, the surfaces of the three membranes are hydrophilic, and the longer they are in contact with water, the narrower the angle obtain. Fig. 4 clearly shows that the CS/CMC membrane has the narrowest water contact angle, suggesting it was the most hydrophilic. This fact can be explained from the points that the CS/CMC has –OH and $–NH_2$ hydrophilic groups of its chitosan structure and that CMC is rich in carboxyl groups that can form hydrogen bonds with water. Therefore, it is easily understood that the crosslink reaction, as in CS/CMC-GA, significantly contributes to the decrease in its water contact angle, i.e., its hydrophilicity is significantly reduced. This phenomenon happens because the hydrophilic groups in the chitosan and the CMC will partially bind to the glutaraldehyde during the crosslinking reaction. This result was in agreement with the study reported by Beppu et al. [27] suggesting that more addition of glutaraldehyde as crosslinked solvent gives rise to the lower hydrophilicity of the chitosan obtained.

In addition to the water contact angle, the hydrophilic characteristics of the membranes have also been observed by measuring their swelling ability, which is one of the main features of hemodialysis membranes. The swelling measurements of the chitosan, CS/CMC, and crosslinked-CS/CMC membranes were summarized in Fig. 5. It was observed from the figure that the blending membrane of chitosan and CMC has the highest water absorption than that of chitosan. Meanwhile, the introduction of the crosslinking agent (glutaraldehyde) leads to a decrease in the water adsorption, and the higher the addition of GA, the lower the swelling obtained. The

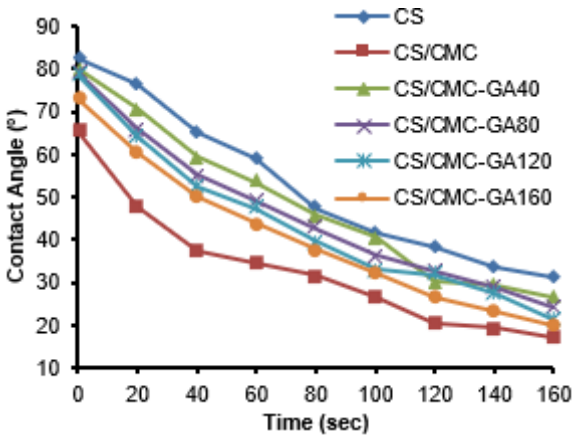

Fig 4. Contact angle curves of chitosan, CS/CMC, and CS/CMC-GA

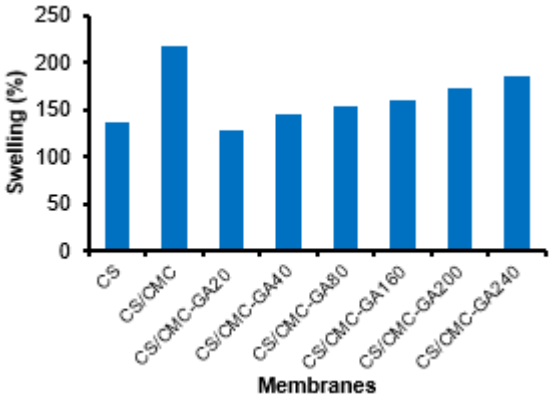

Fig 5. Swelling curves of chitosan, CS/CMC, and CS/CMC-GA

decrease in membrane swelling is possibly due to the chitosan and CMC hydrophilic groups' binding with glutaraldehyde during the crosslinking reaction. Similar to the water contact angle parameter, the high swelling property of the membrane does not guarantee that the layer is suitable for hemodialysis because some other aspects should also be considered, such as mechanical strength and hemocompatibility. Nevertheless, a suitable hemodialysis membrane generally has high hydrophilic properties.

Porosity Analysis

Porosity is one of the membrane characteristics, which is referred to the number of cavities present. Therefore, the membrane with high porosity indicates a large number of cavities that play an essential role in the dialysis process. The membrane porosity measurement

of the chitosan and the CS/CMC-GA were summarized in Fig. 6. The addition of CMC and GA to the chitosan membrane was able to increase its porosity. However, the little glutaraldehyde addition decreased the porosity as the excess GA in the membrane results in more crosslinked chitosan monomers, leading to a denser structure.

On the contrary, the smaller number of GA addition gave rise to the farther distance of the crosslinked monomer, also affecting the membrane porosity. Therefore, the combination of chitosan membrane with CMC and its crosslinking has increased the porosity of the modified chitosan. The membrane's pore size has also been determined using the Guerout–Elford–Ferry equation and gives the results that the pore size of the CS/CMC-GA40 and CS/CMC80 membrane are 9.72 8.37 Å, respectively. This pore size is larger than the urea and creatinine molecules, which are 5.6 and 3.2 Å, respectively [28]. Therefore, this membrane pore size allows urea and creatinine to pass through the membrane, while much larger proteins than the membrane's pore cannot be transported.

Tensile Strength Analysis

The tensile strength and strain measurement of chitosan and the crosslinked CS/CMC were summarized in Fig. 7. It was observed that the combination of chitosan and the crosslinked CMC, in general, increased the tensile strength and strain of the membrane (Fig. 7(a)). The CS/CMC membrane crosslinked by GA at every 80 monomers has the strongest tensile strength. However, its strain was shorter than that of CS/CMC membrane crosslinked by GA at every 40 monomers. This result was consistent with the study of Beppu et al. [27], suggesting that GA is able to stabilize the membrane. However, its excess introduces a brittle surface. Thus, the tensile strength and the strain play an essential role in the flat membrane, especially in the dialysis process.

Dialysis Performance

One of the main characteristics of the hemodialysis membrane is its capability to dialyze urea and creatinine, representing the largest quantity of toxic substances that should be separated from blood plasma. The dialysis process of urea and the creatinine using CS and crosslinked CS/CMC were shown in Fig. 8. It was observed that the crosslinked CS/CMC membrane showed better dialysis capability than that of the chitosan. The result was consistent with the fact that the

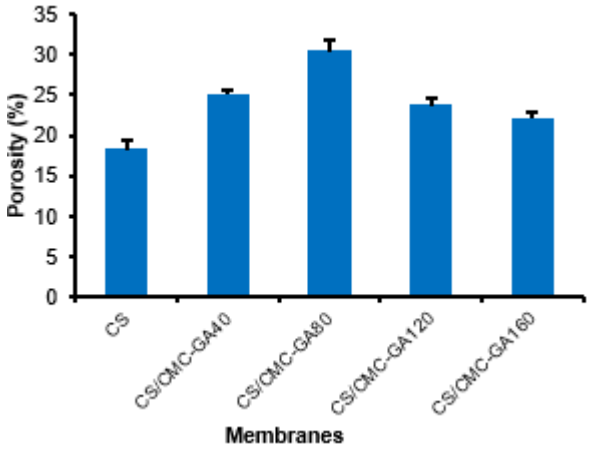

Fig 6. The porosity of chitosan and GA-crosslinked CS/CMC

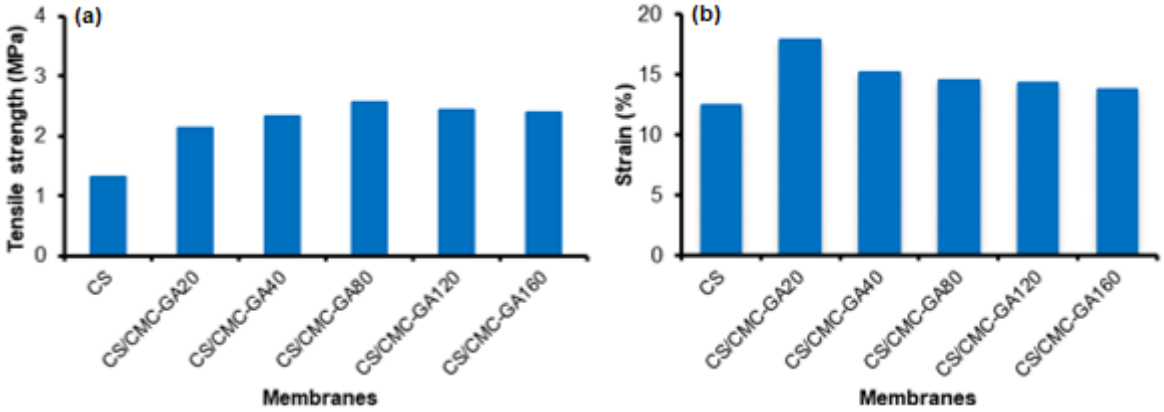

Fig 7. Tensile strength (a) and strain (b) of chitosan and CS/CMC-GA membranes

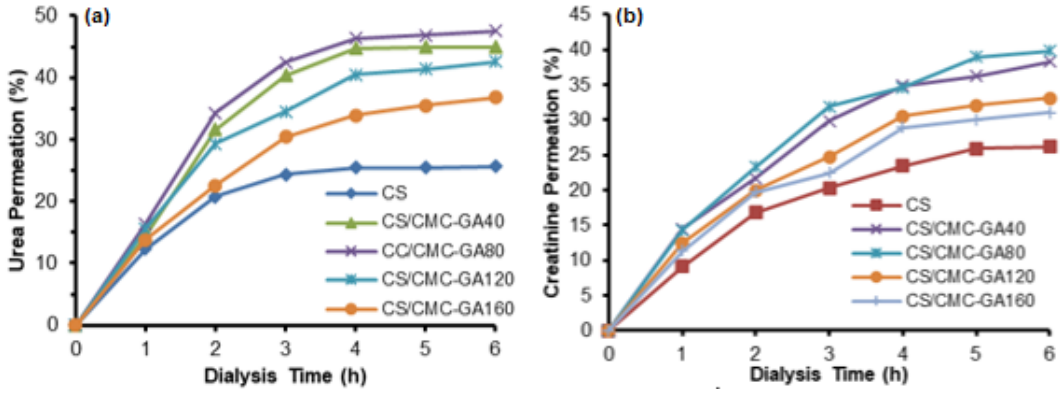

Fig 8. Dialysis performance against (a) urea and (b) creatinine of chitosan and CS/CMC-GA membranes

hydrophilicity and porosity of the crosslinked CS/CMC membrane were better than those of the chitosan. The better dialysis performance of the crosslinked CS/CMC compared to the other membranes was due to the presence of the excess carboxyl group compared to that of the chitosan. The CMC also possesses more carboxyl groups. Therefore it has better dialyzing ability than the initial amine and hydroxyl of the chitosan [22]. The best performance of the crosslinked CS/CMC membrane was shown by those bonded to every 80 chitosan monomers because the crosslinking to every 80 chitosan monomers leads to relatively better porosity and hydrophilicity than its chitosan membrane. The dialysis percentages of the CS/CMC-GA membrane against urea and creatinine were 46.5% and 33.8%, respectively. According to Amri et al. [29], the dialysis performances of the acetic cellulose membranes against urea and creatinine were 51.2% and 31.2%, respectively. Therefore, the dialysis performance of the synthesized membrane was comparable to that of acetate cellulose, known as conventional hemodialysis membrane. Table 1 showed the detailed comparison of the dialysis performance in this study with the recently reported literature.

The findings that the crosslinked CS/CMC membrane has better performance than the other types were also supported by the data indicating that the flux of CS/CMC-GA membrane in dialyzing the urea and creatinine were larger than that of its chitosan, producing the best rate of 2.417 mg cm^{-2} h^{-1} for the urea and 0.683 mg cm^{-2} h^{-1} for the creatinine. The detailed data of urea and creatinine fluxes for CS and GA-crosslinked CS/CMC membranes were summarized in Table 2.

In Vitro Hemocompatibility Test

Protein adsorption and thrombocyte attachment

The ideal hemodialysis membrane does not adsorb excess proteins that cause fouling on the surface layer and hampers the dialysis of urea and creatinine. The experimental results of protein adsorption in the chitosan and crosslinked CS/CMC membranes were shown in Fig. 9(a). Based on these data, it was observed

Table 1. Comparison of membrane dialysis performances of CS/CMC-GA with the recently reported literature

Membranes	Urea Dialysis (%)	Creatinine Dialysis (%)	Ref.
Pure PVDF	± 10.0	± 2.0	[30]
PVDF/FMCNT	± 9.5	± 2.5	[30]
PVDF/PEG	± 46.0	± 21.0	[30]
PES/CNT	± 10.0	± 12.0	[3]
Pure PES	± 9.5	± 7.5	[31]
CA	51.2	31.2	[29]
Present work	46.5	33.8	

Table 2. The flux of urea and creatinine across chitosan and GA-crosslinked CS/CMC membranes

Membrane	Urea Flux (mg cm^{-2} h^{-1})	Creatinine Flux (mg cm^{-2} h^{-1})
CS	1.934 ± 0.18	0.452 ± 0.045
CS/CMC-GA40	2.314 ± 0.36	0.561 ± 0.054
CSCMC-GA80	2.417 ± 0.87	0.683 ± 0.043
CS/CMC-GA120	2.178 ± 0.62	0.548 ± 0.039
CS/CMC-GA160	2.065 ± 0.14	0.583 ± 0.048

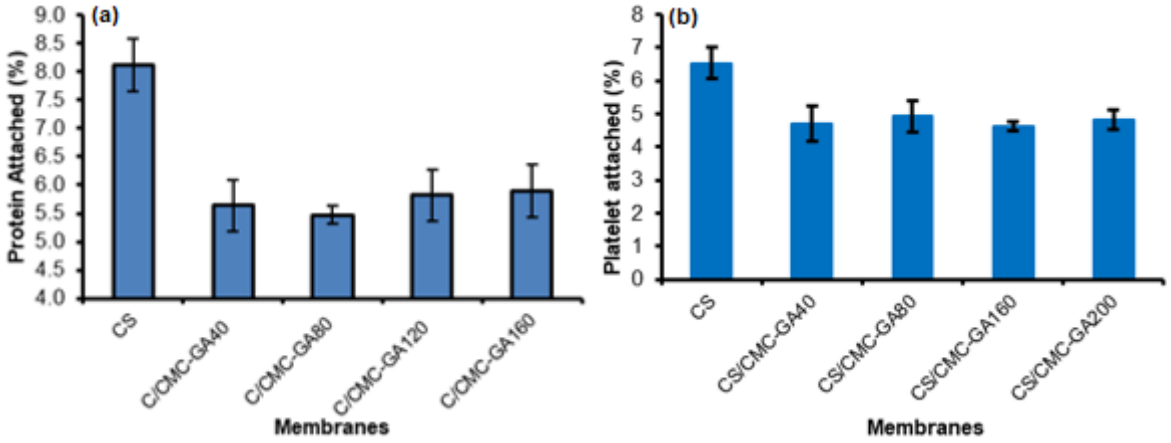

Fig 9. Membrane hemocompatibility: (a) protein adsorption and (b) thrombocyte attachment

that protein adsorption was much lower in the crosslinked CS/CMC membrane than in the chitosan. This result was consistent with the study of Ren et al. [32], stating that the CMC with a negative charge of carboxyl group and protein repelled each other, decreasing protein adsorption. Thus, the crosslinked-CS/CMC membranes investigated in this study have relatively equal protein adsorption performance regardless of its variation of crosslinking. In comparison, the membrane with crosslinking at every 80 monomers showed the lowest adsorption.

In addition, the thrombocyte test is one of the essential analyses for determining the hemocompatibility characteristics of the hemodialysis membrane. The thrombocyte with a complex structure plays an initial role in forming a thrombus that functions in blood coagulation. The experimental results of thrombocyte attachment on the membrane surface of the chitosan and the crosslinked CS/CMC were shown in Fig. 9(b). It was observed that the thrombocyte attachment of crosslinked CS/CMC membrane was lower than that of chitosan. Similar trends of thrombocyte attachment have also been reported by Ren et al. [32] and Tang et al. [33], suggesting that the negatively charged group of carboxyl causes a decrease in the adsorption of thrombocytes increases hemocompatibility of the membranes.

Hemolysis ratio and PTT

The hemolysis ratio is defined as the number of damaged erythrocytes when blood is in contact with a particular material. Therefore, it is necessary to measure the hemolysis ratio of the layer when interacting with the blood to evaluate the membranes' performance. The results of this study were shown in Fig. 10(a). Based on these data, it was observed that the crosslinking of CS/CMC membrane significantly decreased the blood

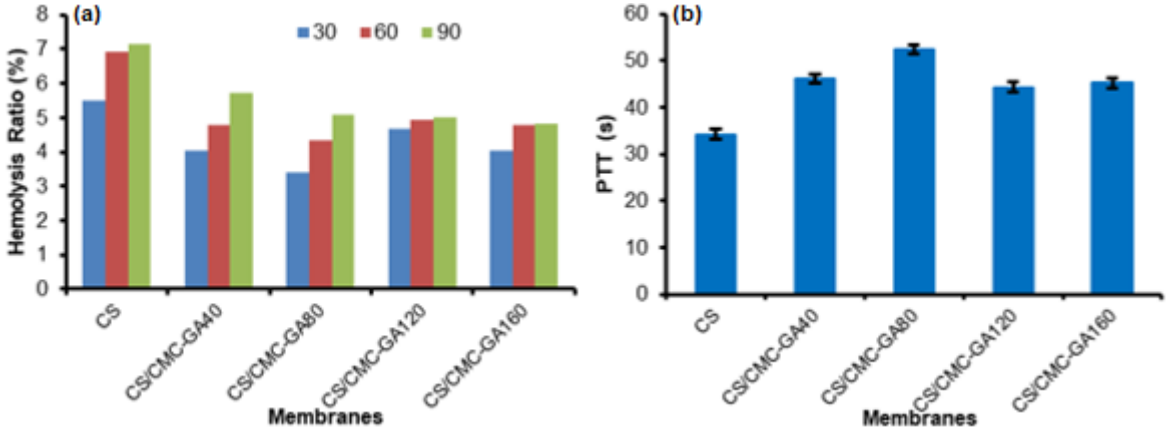

Fig 10. Membrane hemocompatibility: (a) hemolysis ratio and (b) PTT

hemolysis ratio from 6.53% for CS to 4.62% for CS/CMC-GA in the soaking process for 60 min. In addition, the negatively charged membrane of the crosslinked CS/CMC also decreased the number of damaged erythrocytes, suppressed platelet attachment, and blood coagulation.

Another parameter of hemocompatibility was partial thromboplastin time (PTT), which is the time required by blood plasma to coagulate and is influenced by factors such as calcium availability. The presence of calcium accelerates the formation of fibrin fibers and *vice versa*. According to Gao et al. [25], when coagulation factor VII is activated, thrombin produced from thrombinogen triggers fibrin fibers from fibrinogen. In this study, the PTT test results were shown in Fig. 10(b). The crosslinked CS/CMC membranes were found to inhibit blood coagulation or increase the PTT values compared to that of chitosan. This result also correlated with the studies of Gao et al. [25] and Li et al. [34], stating that the additional carboxyl group contributed by CMC improves anti-coagulation and lengthens the PTT period of the blood.

■ CONCLUSION

The glutaraldehyde-crosslinked CS/CMC membranes were successfully synthesized in this study. It was observed that they have better characteristics than that of the chitosan, including their hydrophilicity (contact angle and swelling), porosity, mechanical strength (tensile strength and strain), and dialysis performance against urea and creatinine. Furthermore, based on *in vitro* hemocompatibility tests consisting of protein adsorption, thrombocyte attachment, hemolysis ratio, and PTT parameters, it was shown that the synthesized crosslinked-CS/CMC membranes were more compatible with the blood (have higher hemocompatibility) than that of chitosan. Based on these results, it is recommended that the synthesized products are further developed to be applied and used as hemodialysis membranes in the future.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Ministry of Education and Culture, the Republic of Indonesia, through Doctoral Dissertation Research Grant (PDD 102-21/UN7.P4.3/PP/2018). Khabibi also thanked the Directorate General of Higher Education, Ministry of Education and Culture, the Republic of Indonesia for doctoral programs through the BPPDN scholarship scheme.

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