

Blend membrane of succinic acid-crosslinked chitosan grafted with heparin/PVA-PEG (polyvinyl alcohol-polyethylene glycol) and its characterization

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Abstract. Crosslinking and grafting reactions are required to modify the functional groups on chitosan to increase the number of its active groups. In this study, crosslinking reaction of succinic acid and grafting reaction of heparin on chitosan were conducted to produce a membrane as a candidate of a hemodialysis membrane. The mole ratio between chitosan and succinate acids was varied to obtain the best composition of modified materials. By blending all the material composition with PVA-PEG, the blend was transformed into a membrane. The resulted membrane was then characterized by various test methods such as tests of thickness, weight, water uptake, pH resistance, tensile strength and membrane hydrophilicity. The results showed that the best composition of the membrane reached in the addition of 0.011 gram of succinic acid proved by its highest mechanical strength compared to the other membranes.

Keywords: chitosan; membrane; cross-linking; grafting.

1. Introduction

Hemodialysis is an important process in medical treatment aiming to remove toxic substances from the blood of a patient with acute renal failure. The main component of hemodialysis equipment is semi-permeable membrane which can selectively transport permeates having low molecular weight, such as urea and creatinine from the blood [1]

The hemodialysis membrane should be compatible, porous, hydrophilic and has functional groups that are capable of capturing permeates through the formation of hydrogen bonds. Chitosan, a natural polymer, is developed into a raw material of membrane because of its ability to be dissolved in a dilute acid to form a thin-film membrane [2]. Furthermore, Hoven, et al., [3] stated that chitosan is biodegradable, inert, non-toxic and biocompatible. The content of -OH and -NH₂ groups of chitosan makes this material becomes reactive, which means it can be easily modified with the other groups to obtain a material suitable for the application needs. Lusiana, et al., [2] used three methods to modify chitosan, i.e. grafting, cross-linking and blending. These three modifications improved the strength of



the chitosan membrane by increasing its number of functional groups and porosity, and reducing the negative charge on the membrane surface. Mitra et al., [4] also modified chitosan with succinic acid/collagen by cross-linking. The result showed that the -COOH group at both ends of the succinic acid increased the active side of the chitosan membrane.

To improve the mechanical strength of the membrane, it is blended with synthetic polymer like PVA (polyvinyl alcohol). PVA is hydrophilic, elastic, non-toxic, non-carcinogenic and biocompatible with human body [5] Polyethylene glycol (PEG) is also excellent for chitosan blend since it is a water-soluble polymer and less toxic. Zen, et al.[6] examined the modification of chitosan with PEG. The investigation showed that modification using PEG increased the porosity and the membrane mechanical properties. The modification of membrane with PVA/PEG blends is needed to increase the elasticity and porosity of the membrane so that it can be used in the hemodialysis process which takes approximately 4-6 hours.

To reduce red blood clots due to the incompatible membrane to the blood, heparin is added as a grafting agent. Heparin is anticoagulant in blood and it is non-toxic. Chitosan modification using heparin was performed by Badr, et al., [7]. They showed that the resulted membrane was selective against sodium and an increase in its compatibility to blood was observed. The purpose of this study is to investigate the results of the synthesized chitosan membrane in terms of its physical properties through various physical characterization tests.

2. Experimental method

2.1. Materials

Materials used in this study were chitosan (Surindo Bioteck, Cirebon, DD 87%), succinic acid, polyvinyl alcohol (PVA), polyethylene glycol (PEG), heparin, disodium phosphate (Na_2HPO_4), phosphoric acid (H_3PO_4), acetic acid (CH_3COOH) and sodium hydroxide (NaOH) 2 M. All materials were pro analysis and purchased from Merck.

2.2. Tools

The physical characterization tests of the resulted membrane included OHAUS analytical balance to measure the membrane weight, thickness meter (Mitutoyo) to measure the thickness of the membrane, and tensile strength analyzer (Zwick 2.0,5) to measure the tensile strength of the membrane. Moreover, pH resistance was measured using pH meter (pH-009 type), while water uptake and membrane hydrophilicity were determined using contact angle method.

2.3. Procedures

2.3.1. The synthesis of succinic acid crosslinked chitosan membranes (CS-Succ)

CS-Succs with eight different compositions were prepared by dissolving 1.5 gram of chitosan in 100 ml of acetic acid 1%, and then added with succinic acid (Table 1) with a constant stirring for 4 hours at 60°C to achieve an optimal ratio and then added with 5 ml of acetic acid 10% during the reaction process. The next step was pouring 10 ml of the mixed solution into a petri dish and dry it in an oven at $50\text{-}70^\circ\text{C}$ for 24 hours. After the printed membrane was dry, NaOH 2 M was added to remove it from the dish. Subsequently, it was washed using distilled water until pH 7 or neutral was achieved. The synthesized membrane was characterized to identify the quality of the membrane.

Table 1. Ratio of chitosan monomer and succinic acid.

Material type	Chitosan (g)	Succinic acid (g)	Chitosan mol (3.75×10^{-5} mmol)	Succinic acid mol (3.75×10^{-5} mmol)
CS-Succ1	1.500	0.037	1.000	8.333
CS-Succ2	1.500	0.028	1.000	6.250
CS-Succ3	1.500	0.022	1.000	5.000
CS-Succ4	1.500	0.018	1.000	4.167
CS-Succ5	1.500	0.016	1.000	3.571
CS-Succ6	1.500	0.014	1.000	3.125
CS-Succ7	1.500	0.012	1.000	2.778
CS-Succ8	1.500	0.011	1.000	2.500

2.3.2. Blending CS-Succ membrane with PVA/PEG

The CS-Succ compound fabricated in the previous reaction was then added with 32 mL of PVA and 18 mL of PEG and stirred with a constant stirring for 2 hours. After that, the membrane was produced by pouring 10 ml of the mixture into the petri dish and dried in an oven at 50-70 °C. The dried membrane was removed from the petri dish using NaOH 2 M and washed using distilled water until pH 7 or neutral was achieved. The resulted membrane was then physically tested.

2.3.3. Grafting of CS-Succ/PVA-PEG membrane with heparin

The CS-Succ/PVA-PEG solution was added 1 ml of Heparin 100 iu and stirred for 2 hours. After that, the membrane was produced by molding the 10 mL of the mixture into the petri dish. The mold was then dried in an oven at 50-70 °C. To remove the mold from the dish, the dried membrane was added by NaOH 2 M. The resulted membrane was then washed using distilled water until pH 7 or neutral was achieved.

2.4. Membrane characterisation

2.4.1. Thickness and Weight Tests

The thickness of all membranes synthesized from different compositions was measured using a thickness meter, while the weight of the membranes was determined using an analytical balance. Every membrane was measured before and after the use for the next process.

2.4.2. pH Resistance Test

Before testing, the membrane was weighed (w_1) and immersed in various solutions of pH 3, 5, 7, 9 and 11. They were kept for 6 hours, after that being weighed using the analytical balance (w_2).

2.4.3. Water Uptake Test

At the beginning of the process, the membrane was weighed using an analytical balance and immersed in water for 6 hours. After immersing, the membrane was weighed again. The weight difference taking from the weight before and after the immersion was used to determine the water uptake adjusted according to the following formula:

$$\% \text{ water uptake} = \frac{W_t}{W_o}$$

W_o = the weight of the initial membrane

W_t = the weight after the immersion

Tensile Strength Test

The tensile strength test was performed using the membrane with a size of 15 x 1.5 cm. The both ends of membrane were clamped in a mechanical test equipment, then pulled them up to break the membrane and its tensile strength was observed.

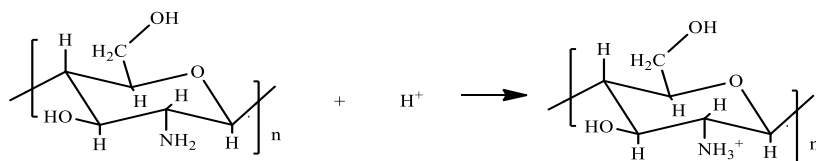
3. Results and discussion

3.1. The synthesis of CS-Succ compounds

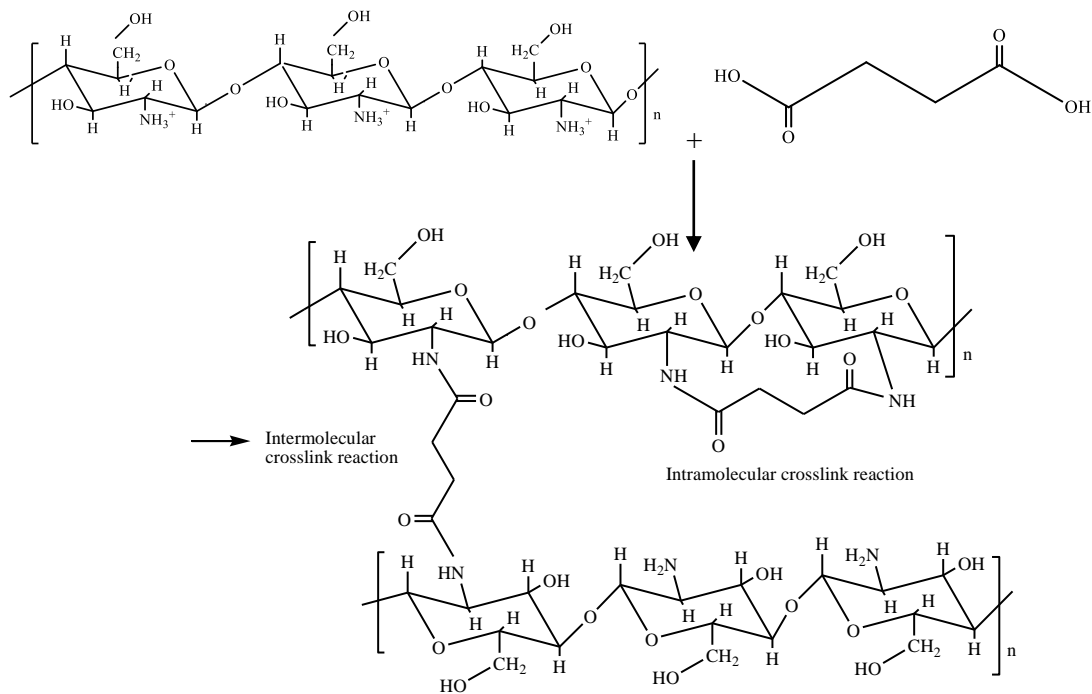
Crosslinking reaction was undertaken using an acid catalyst to facilitate a proton transfer to the succinic acid molecules. Heating of the mixture at 50°C aimed to accelerate the crosslinking process. The mechanism of the crosslinking reaction using an acid catalyst usually begins with transferring protons from or into the substrate molecules. In an acidic condition, $-\text{NH}_2$ groups of chitosan underwent protonation resulting in $-\text{NH}_3^+$, which was then easily attacked by free carboxylic groups, whereas the hydroxyl groups initiate an esterification with succinic acid [8]. The hydroxyl groups would subsequently react with the carboxylate group of succinic acid. The mechanism of the crosslinking reaction of chitosan with succinic acid can be explained by two-stage reactions: 1) chitosan underwent protonation due to the acidic condition coming from acetic acid (Scheme 1); 2) the $-\text{OH}$ group of succinic acid molecules was bound to the protonated chitosan by hydrogen bonding with the amine group of chitosan (Scheme 2). This two-stage reaction mechanism is shown in Figure 1.

The success of the crosslinking reaction between chitosan and succinic acid was proved by the FTIR spectra as are shown in Figure 2. The FTIR spectra indicated the difference between pure chitosan and succinic acid cross-linked chitosan. The FTIR spectrum of chitosan membrane showed the vibration modes at 3425 and 2877 cm^{-1} attributed to $-\text{OH}$ and $-\text{NH}$ groups, respectively. Twin peaks at 1651 and 1597 cm^{-1} indicated the presence of $-\text{NH}_2$ group. In the mean time peaks at 1427, 1381 and 1327 cm^{-1} are represented as C-O group.

FTIR spectrum of succinic acid cross-linked chitosan membrane showed the appearance of a peak at 3448 cm^{-1} assigned to $-\text{OH}$ group. However, the twin peaks attributed to $-\text{NH}_2$ group as present in pure chitosan disappeared. Nevertheless, a single peak at 1720 cm^{-1} attributed to C=O group of carboxylates appeared. This shift indicated that there was a change of $-\text{NH}_2$ group from primary to secondary or tertiary due to the inclusion of the $-\text{COOH}$ group from succinic acid. It can be concluded that the crosslinking reaction of succinic acid to the chitosan was carried out properly.



Scheme 1



Scheme 2

Figure 1. The cross-linking reaction mechanism between chitosan and succinic acid.

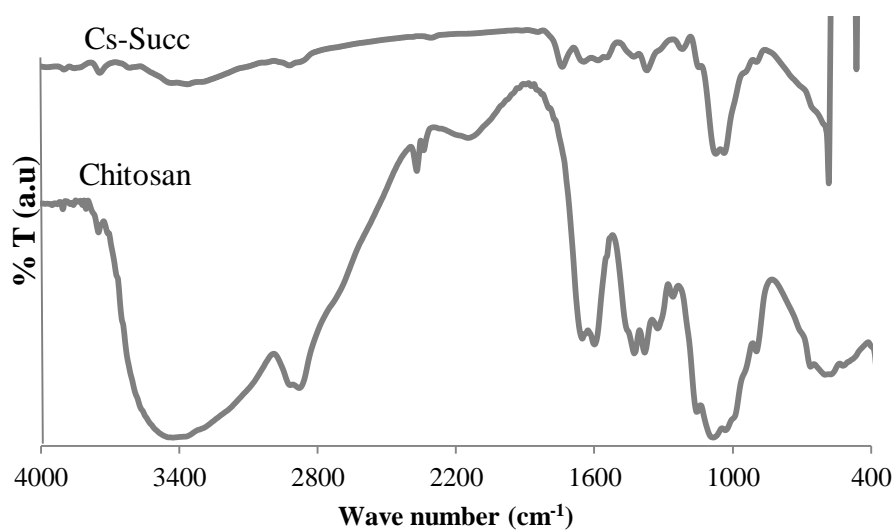


Figure 2. The FTIR spectra of chitosan and CS-Succ.

3.2. Characterization

3.2.1. Tests of Membrane Thickness and Weight

Membranes have different weights and thicknesses for each type of membranes indicating the mass content of the substances of the membranes. Membranes having high mass content would increase their weight and thickness as is shown in Table 2.

Table 2. The results of measurement of thickness and weight on various membranes.

Material Type	Weight (gram)	Thickness (mm)			
		I	II	III	Average
CS-Succ1	0.147	0.550	0.570	0.620	0.580
CS-Succ2	0.145	0.380	0.330	0.340	0.350
CS-Succ3	0.139	0.330	0.290	0.260	0.290
CS-Succ4	0.136	0.360	0.420	0.410	0.400
CS-Succ5	0.134	0.280	0.200	0.220	0.230
CS-Succ6	0.134	0.310	0.290	0.440	0.210
CS-Succ7	0.132	0.180	0.170	0.220	0.190
CS-Succ8	0.132	0.190	0.140	0.180	0.170

3.2.2. pH Resistance Test

The membrane resistance to pH is shown in Table 3 and Table 4. The results showed that the membrane was not resistant to acid pH indicated by significant decrease in the membrane mass at pH 3 and 5, while at basic pH membrane, there was no significant change in membrane mass at pH 7, 9, 11 and 13.

Table 3. pH resistance test of CS-Succ1 membrane.

pH	CS-Succ1		CS-Succ1/PVA-PEG		CS-Succ1/ PVA-PEG/Hep	
	W ₀	W _t	W ₀	W _t	W ₀	W _t
3	0.0236	0.0003	0.0424	0.0035	0.0360	0.0003
5	0.0236	0.0031	0.0424	0.0165	0.0360	0.0005
7	0.0236	0.0259	0.0424	0.0428	0.0360	0.0366
9	0.0236	0.0265	0.0424	0.0419	0.0360	0.0368
11	0.0236	0.0240	0.0424	0.0442	0.0360	0.0363
13	0.0236	0.0252	0.0424	0.0424	0.0360	0.0375

Table 4. pH resistance test of CS-Succ8 membrane.

pH	CS-Succ8		CS-Succ8/PVA-PEG		CS-Succ8/PVA-PEG/Hep	
	W_0	W_t	W_0	W_t	W_0	W_t
3	0.0248	0.0067	0.0384	0.0146	0.0386	0.0064
5	0.0248	0.0024	0.0384	0.0132	0.0386	0.0162
7	0.0248	0.0261	0.0384	0.0379	0.0386	0.0391
9	0.0248	0.0248	0.0384	0.0395	0.0386	0.0396
11	0.0248	0.0281	0.0384	0.0398	0.0386	0.0393
13	0.0248	0.0230	0.0384	0.0384	0.0386	0.0396

3.2.3. Water Uptake Test

The water uptake test aimed to determine not only the ability of the membranes to adsorb water but also the membrane hydrophilicity. The results of the water uptake test of all membranes can be seen in Figure 3.

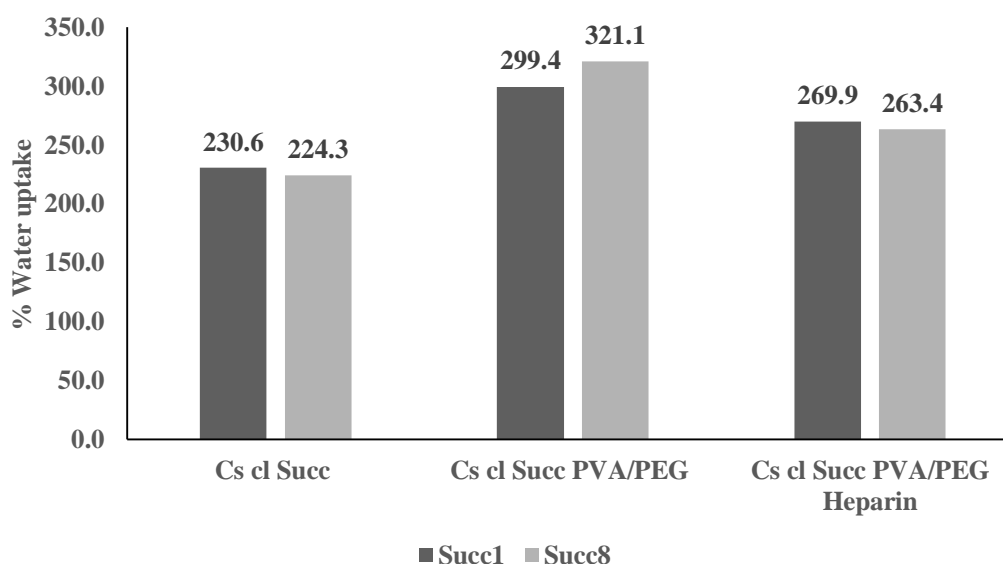


Figure 3. Water uptake of various membranes

Membrane blended with PVA and PEG showed the highest percentage of water uptake. This is because the membrane is more hydrophilic than others which may be due to the presence of PVA which is also hydrophilic [8].

3.2.4. Tensile Strength Test

The measurement of the tensile strength of the membranes with different composition showed the strength and elasticity of the membranes as reflected by the measurement of tensile strength presented in Figure 4.

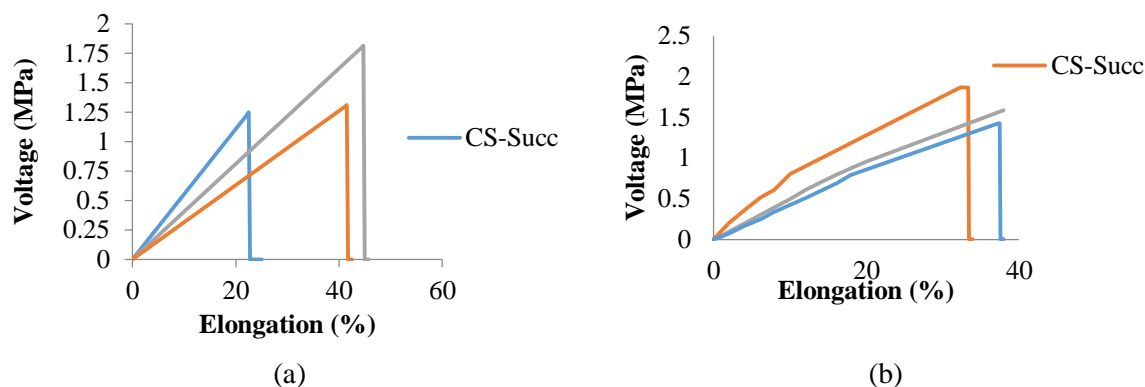


Figure 4. Tensile strength of (a) CS-Succ1 and (b) CS-Succ8.

The CS-Succ/PVA-PEG membrane had the highest elasticity when compared to other membranes. The CS-Succ blended with PVA/PEG increased the level of membrane elasticity. This is due to the presence of PVA/PEG which is more elastic and mechanically robust when compared to the chitosan polymer itself. By increasing the mechanical properties, it is expected that the resulting membranes can withstand the pressure when being used for transport processes. Further, it is open a way to apply this membrane for hemodialysis.

4. Conclusion

The crosslinking reaction of chitosan with succinic acid was carried out to increase the number of active groups of chitosan, while blending the membrane with PVA/PEG was conducted to improve membrane elasticity. The results showed that the best composition of the membrane occurred in the addition of 0.011 gram succinic acids with the highest mechanical strength compared to other membranes.

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