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IOP Conf. Series: Materials Science and Engineering **509** (2019) 012121 doi:10.1088/1757-899X/509/1/012121

The influence of grafted heparin on chitosan/poly (ethylene glycol) blend membrane and it's application for creatinine and urea transport

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Abstract. Modification of membrane using grafting reaction between chitosan and heparin showed the functional group on grafting agent reduced the positive charge on the membran surface and increased the active carrier groups which lead to increasing the membrane transport. The optimum concentration of heparin modified chitosan was determined by creatinine and urea transport studies. With the optimum concentration, the membrane was used for the transports of creatinine, urea and as well as a mixture of 5 species (creatinine, urea, Vitamine B_{12}, albumin, and mineral salts). Characterization of the membrane was done using FT-IR Spectrophotometer and SEM. The results showed that the best concentration of heparin at 100 iu in the percent transports of creatinine and urea were 31.83% and 41.99%, respectively, for 6 hours of transport times. The other species in the transport membrane were proposed to disturb the creatinine permeation through the membrane. Addition of polyethyleneglycol (PEG) also increased the mechanical and porosity of the membrane in transport process.
Keywords: chitosan, heparin, grafting reaction, creatinine and urea

1. Introduction

Excessive blood creatinine and urea concentrations (above 2.5 mg/dL) and (above 5-20 mg/dL, respectively) is as an indication of kidney disease [1], which is usually treated with haemodialysis therapy. Haemodialysis is a method that acts like an artificial kidney to remove the metabolism wastes such as creatinine and urea from the blood system with diffusion mechanism through the semipermeable membrane.

Membrane is a selective and semipermeable thin layer located between two phases, a source phase containing entrained components, and acceptor phase containing the components that can pass through the membrane. The membrane can block some species which size are larger than the pore size of the membrane, and pass through the other with smaller size. Membrane for transport process is also influenced by reactive groups on the membrane surface which enable to form hydrogen bonds [2, 3]. The membranes used for hemodialysis should be mechanically strong, resistant to leakage, able to remove the waste compounds rapidly, selective, and unable to adsorb protein on the membrane surfaces. Chitosan is an inert biopolymer that can be used as haemodialysis membrane. However, the functional groups available in chitosan are not reactive enough to interact with target compounds [4-6]. Chitosan is hydrophobic in water but in acid condition, the amine groups on chitosan backbone ($-NH_2$) is protonated and causes the material to be positively charged, which could adsorb proteins to the

membrane surface and enhances the haemodialysis process. However, it will decrease the membrane permeability and lead to blood coagulation [7].

Chemical modification is an effective technique to improve the chitosan property. Modification of the membrane surface can be done by grafting reaction with addition of functional groups or a polymer through $–NH_2$ of the chitosan and blending with other polymers to increase the membrane mechanical properties [8].

One of the biocompatible polymers used as a grafting agent is heparin with a reactive groups $–OSO_3$, -COOH or $NHSO_3^-$ which can bind with the active groups of chitosan ($-NH_3^+$) and form an electrostatic interaction on the membrane surface [9]. The reaction neutralizes the positive charge on membrane surface and leads to increase of the membrane permeation. By grafting modification with heparin, it also can improve the membrane biocompatibility. The purpose of this study was to increase the number of functional groups as the active sites of the membrane by synthesizing through the reaction of chitosan and heparin as a grafting agent.

The second strategy is by blending with other polymers that are more hydrophilic to increase the membrane elasticity, strength and no fragile during the transport process. The polymer used is PEG with –OH groups that can form hydrogen bonds with chitosan. The role of PEG is to increase the mechanical strength, improve the uniformity of membrane porosity as well as to set the balance of hydrophilicity, so that it can lead to increase the membrane permeability [10].

2. Materials and Method

2.1. Materials

Chitosan (MW 40.000 Da, 87% deacetylation degree) were obtained from Biotech, Surindo, Cirebon, Indonesia. Acetic acid (glacial, 96.6%), sodium hydroxide (pellets ACS reagent ≥97.0%), hydrochloric acid (extra pure 32%). Poly (etylen glycol) were purchased from Fisher Scientific. Creatinine, urea, vitamine B12, albumin, ethanol, p-dimethylaminobenzaldehyde (DAB), potassium dihydroxyphosphate, were obtained fromMerck (Germany). Picric acid (ACS reagent ≥99.5%) was purchased from Sigma Aldrich.

2.2. Experimental in details

2.2.1. Synthesis of heparin grafted chitosan/poly (ethylene glycol) blend membrane. Heparin grafted chitosan (Hep.g.CS) with three different compositions was prepared by dissolving 1.5 g chitosan in 90 mL acetic acid 1%. then, there different concentrations of heparin 10 mL and poly(ethylene glycol) 0.0375 g (constant mole ratio with chitosan) as shown in table 1 were added to the solution with constant stirring at 24 h, including 4h in 60℃. The resulted materials were analysed using FTIR to analyse the functional groups.

Table 1. Composition of heparin grafted chitosan/PEG blend membranes.

Type of materials	CS(g)	Heparin (iu)	PEG(g)	Mole ratio of CS/PEG
CS.g.Hep/PEG A	1.5	50	0.0375	4:1
CS.g.Hep/PEG A	1.5	100	0.0375	4:1
CS.g.Hep/PEG A	1.5	200	0.0375	4:1

2.2.2. Preparation of heparin grafted chitosan/poly(ethylene glycol) blend membrane with different thickness. The blend solution in Petri dish with two different volume ratios (5 and 10 mL) was put in oven at 50℃ for approximately 24 h to remove the solvent. When the membrane was dry, NaOH solution was added to remove the membrane from Petri dish. Then, the membrane was washed with distilled water and subsequently air dried.

2.2.3. Water uptake measurement. All the dry membranes were weighed. Afterwards the membranes were immersed in distilled water at room temperature with the same volume ratio for 1-6 h intervals. Every 1 h the membrane samples were removed, swabbed with a tissue, and weighed. The water uptake percentage was calculated using the following expression:

$$\text{Water uptake (\%)} = \frac{Wwet - Wdry}{Wdry}\ x\ 100\%$$

2.2.4. Hydrophilicity of membrane measurement. All the dry membranes with a flat surface were dropped with a drop of water from the membrane surface. Then the contact angle was determined based on the resulted image to calculate the hydrophilicity of the membrane.

2.2.5. Membranes characterization. To determine the properties of the membrane, the analysis was done before and after the grafting modification. Functional groups were obtained with Shimadzu FTIR spectrophotometer. The mechanical strength of the blend of grafted membranes was measured using Tensometer (Shimadzu, AG-I-250 KN tester). Thickness measurements were obtained using Mitutoyo thickness meter at 3 different points of the membrane. Membrane surface image was characterized using scanning electron microscopy (SEM) Shimadzu U8000.

2.2.6. Permeation study. It was performed using a transport device with the membrane located in the middle of the device. The feed phase contained 50 mL either creatinine 15 ppm or urea 500 ppm standard solutions in buffer phosphate pH 7.4, while the acceptor phase only contained 50 mL phosphate buffer solution. The transport tests were carried out for 6 h by measuring the acceptor phase concentration every 1 h using UV-visible spectrophotometer.

2.2.7. Study of membrane transport capacity for creatinine, urea, albumin and vitamin B_{12}. Transport process experiments were done by using the following metabolites: creatinine 15 ppm, urea 500 ppm, vitamin B_{12} and albumin 20 ppm and 0.01 mole of mineral salts in 50 mL of dissolved phosphate buffer solution in the feed phase and 50 mL phosphate buffer only in the acceptor phase. Solute permeability passing through the membrane was determined by analysing the concentrations of each solution in the acceptor phase with a UV-Vis spectrophotometer at 0–6 h intervals. The creatinine was complexed with picric acid and NaOH to form coloured complex that absorbs a visible radiation at 486 nm (Jaffe method). Urea was complexed with 4-dimetilaminbenzaldehida (4-DAB) in acidic condition and measured at 430 nm. The pink vitamin B_{12} solution was measured at 361 nm without using any complexing agent. A mixture of NaOH, $CuSO_4$, KNa-tartart and KI was as complexing agent of albumin, then measured at 535 nm.

3. Results and Discussion

3.1. Synthesis of heparin grafted chitosan/poly (ethylene glycol) blend membrane

In the first step, $–NH_2$ protonation of chitosan with $–H^+$ of acetic acid as a catalyst occurred. its step takes place quickly and N atoms become positively charged and easily attacked by a lone pair of $–OSO_3$ groups from heparin as a grafting agent. Heparin grafted chitosan is illustrated with intermolecular grafting reaction that occurs at the two chitosan chains with a slow reaction process.

The grafting reaction was confirmed by determining the functional groups of the resulted reaction using FT-IR spectrophotometer (Fig. 1). The specific different between chitosan and modified one was observed as a sharp absorption band at wavenumber of 1638 cm^{-1}, which is indicated as the modification of amine groups of the chitosan, from a primary to a secondary amine groups. The other absorption bands at wave numbers of 1275 and 1414 cm^{-1} are as characteristic of $–OSO_3$ of heparin [11]. The blending between CS.g.Hep and PEG also resulted the widening of –OH absorption band at 3466 cm^{-1}, this proves that the integration of the two materials did not change the type of functional groups in the backbone compound, but only increased the percentage of hydroxyl groups [12, 13]. The result confirmed the success of heparin grafting of amine group of chitosan.

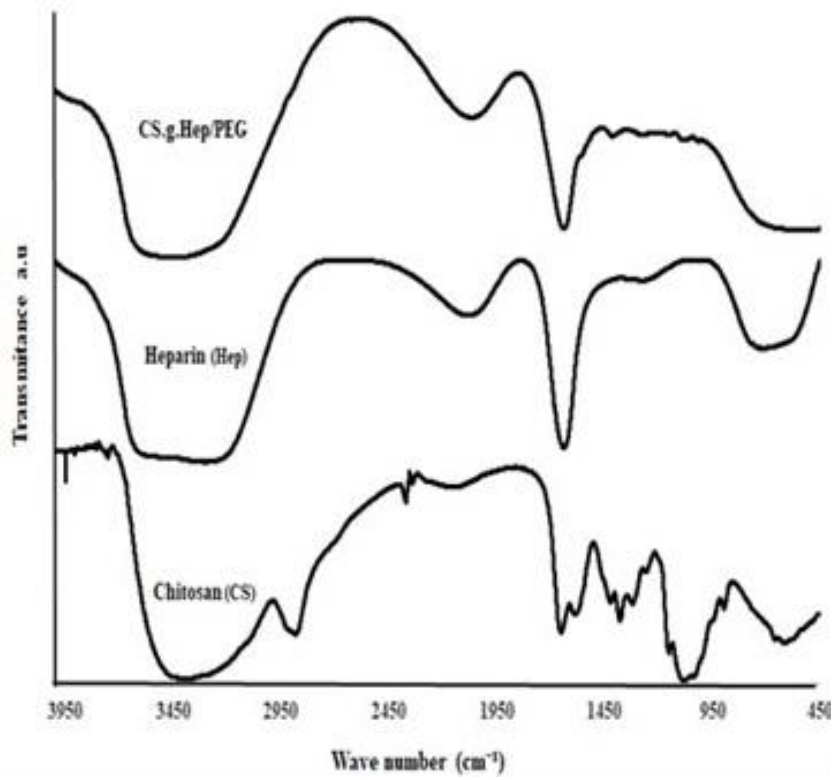

Figure 1. FTIR spectra of types of membranes.

3.2. Water uptake of membranes

Materials that contact to the blood system, it is related with the important balance between hydrophilic and hydrophobic [4]. The best blend of hydrophilic groups and hydrophobic membrane is resulted in a certain hydrophilicity values with optimum performance. The water uptake variation of chitosan membranes before and after modification is shown in table 2. The water uptake indicated that CS.g.Hep/PEG A, CS.g.Hep/PEG B and CS.g.Hep/PEG C membranes have water uptake of 179%, 191.16%, and 195%, respectively. It indicates that with the increasing of heparin concentration will increases the water uptake percentage. The increasing of the water uptake percentage is caused by the addition of PEG which that has a hydrophilic –OH groups. Likewise, with the addition of heparin concentration through the grafting modification will increase in the water uptake (%) because its abundant hydroxyl groups and other electronegative groups that are capable for hydrogen bonding, thus increasing the hydrophilicity of the modified membrane increases its water absorption capacity.

Table 2. Physochemical properties of the membranes.

Membrane	Thickness (mm	Contact angle (°)	Water uptake (%)	Mechanical properties	
				Strength (MPa)	Strain(%)
CS	0.0613	83.96	69	0.0675	5.19
CS.g.Hep/PEG A	0.0643	58.28	179	1.9066	12.00
CS.g.Hep/PEG A	0.0616	48.43	191	2.6773	22.66
CS.g.Hep/PEG A	0.0780	39.25	195	2.8879	24.27

3.3. Hydrophilicity of Membranes

Membrane surface hydrophilicity was evaluated by measuring the water contact angle. The surface material is very hydrophilic when the contact angle on the surface is smaller than 30°. The contact angle between 30°-89° is called partially wet, while the contact angle greater than 90° is called hydrophobic.

Based on the data obtained (table 2), all the membranes synthesized are partially wet membrane or are somewhere in between hydrophilic or hydrophobic with different hydrophilicity levels. Therefore, by addition of greater heparin concentration, the membrane's hydrophilicity increases and the contact angle decreases.

3.4. Strength and the strain of the membranes

A successful blending should lead to intermolecular interaction between the two components oh polymers, thereby improving mechanical strength of the blend [14]. Tensile strength of the membrane was performed to indicate the strength and the strain as well as to study the elasticity of the membrane. The measurement of the results is displayed in table 2. The data indicate that the modified chitosan membranes are more elastic than chitosan membrane. It was obtained by the grafting reaction between chitosan and $–OSO_3$ groups of heparin that was able to increase the membrane elasticity by improving the tensile strength and elongation of the membrane.

3.5. Permeation Study

Creatinine or urea transport was used to study the ability of the membrane to transport creatinine or urea when dissolved in solution with single permeation. The result indicates, the longer contact time, causing transport percent increased.

The study of creatinine or urea as single permeation was done with three types of membrane in different thicknesses. The results of creatinine and urea transport membranes are shown in Fig. 2 and Fig. 3.

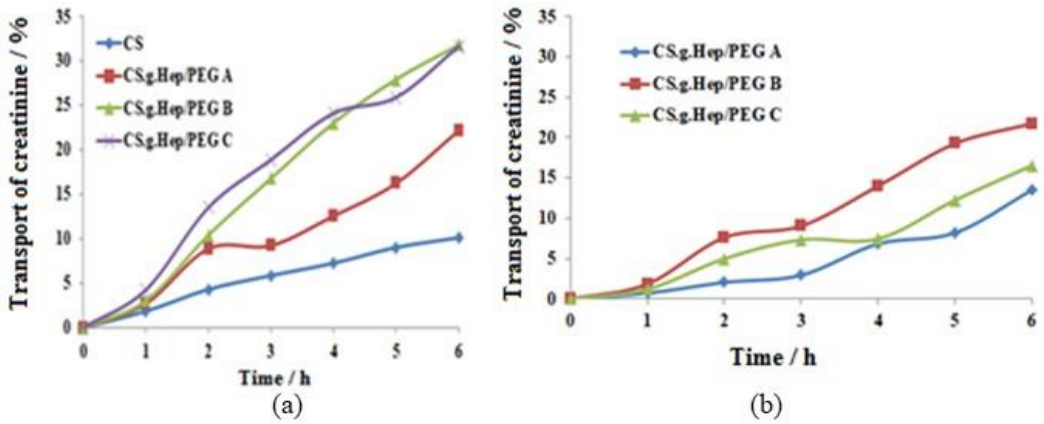

Figure 2. Creatinine transport of membranes with different thickness: 0.0616 mm (a); 0.1210 mm (b).

Based on the graphic of Fig. 2, the membrane composition of CS.g.Hep/PEG B with concentration of heparin 100 iu in both (a) ratio volume 5 mL with thickness 0.0616 mm and (b) ratio volume 10 mL with thickness 0.1210 mm has the highest transport percentage of 31.83% and 21.74%. In Fig. 3, the composition of CS.g.Hep/PEG has the best urea transport percentage too, namely membrane (a) and (b) were 41.99% for the former and 28.13% for the later. It indicates that the thickness membrane effects on the creatinine permeation, meaning the creatinine diffusion through the membrane becomes faster on the thin membrane. Chitosan has the lowest creatinine transport (10.12%). The significant increase

indicates that the grafting reaction with heparin was able to increase the active side of the membrane surface to form hydrogen bond that was able to permeate and bring it into the acceptor phase.

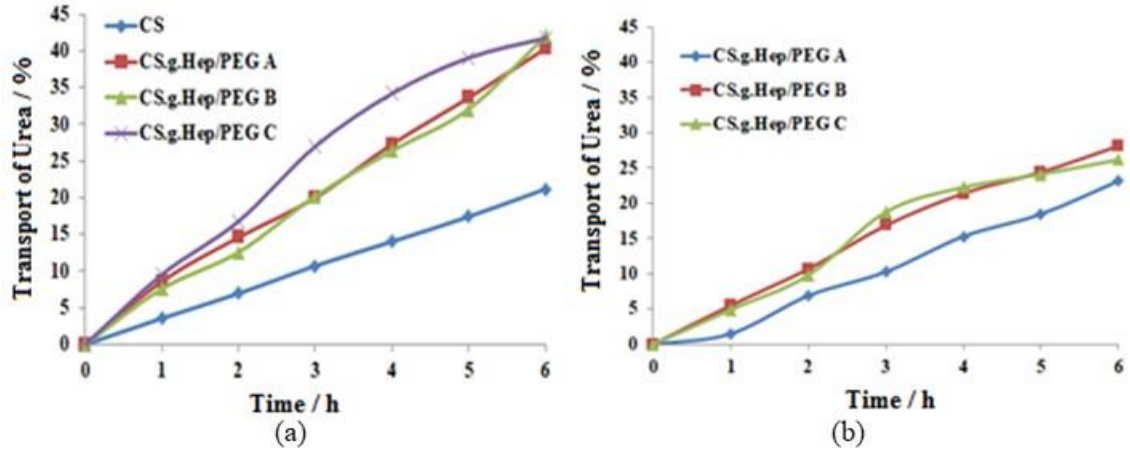

Figure 3. Urea transport of membranes with different thickness: 0.0616 mm (a); 0.1210 mm (b).

Based on Fig. 2 and Fig. 3, the percent transport of urea was higher than creatinine transport in all membrane compositions. This is due to the smaller molecular weight of urea than creatinine that the urea mobility was faster than creatinine and entered the membrane through hydrogen bonds on the membrane surface producing the highest transport percentage.

3.6. The Effects of vitamin B_{12} and albumin on the transport of creatinine

The effects of vitamin B_{12}, albumin and mineral salts were studied by combining three species in the source phase. The transport of a mixture of four species was done using the membrane with the best volume ratio (5 mL) with the best composition of membrane modification.

Vitamin B_{12}, albumin, and also mineral salts are present in the blood together with creatinine. Vitamin B_{12} and albumin (with large molecular weights of 1355 and 66.500 g/mol) and some moles of mineral salts can inhibit the transport process of creatinine.

The transport percentage of creatinine in three species mixture was lower than when it was transported as a single permeation. The transport of creatinine percentage decreased from 31.83 to 23.50% when the permeates mixed together. Thus, that much large molecular size of vitamin B_{12}, albumin and salts have a significant influence on the creatinine permeation. The transport of three species mixture was resulted no transport of vitamin B_{12} and albumin. It seems the molecular sizes of the both are too large to pass through the membrane pores [3]. The competition of the three metabolites to approach the membrane surface may also be caused by additional collision between the molecules, so that it is difficult for creatinine to enter the membrane pores. The presence of vitamin B_{12} and albumin that cover the pores might also be one of the reasons for the decline in the transport percentage of creatinine, since the molecules were able to block creatinine from passing through the membrane.

4. Conclusion

The grafting reaction between chitosan and heparin was done in order to incorporate the reactive $–OSO_3$ groups of heparin to the backbone chains of chitosan through the electrostatic interaction. This reaction was to increase the active sides on the surface of chitosan membrane so that creatinine and urea were transported via hydrogen bond formation, as well as the blending reaction was to increase the porosity and improve the hydrophilicity balance of the membrane. From the all data, transport of both creatinine and urea increased linearity to the addition of heparin through grafting reaction. The appropriate

proportion of hydrophobic and hydrophilic groups allows the membrane to have a higher transport capacity. The presence of vitamin B_{12} and albumin in the source phase reduced the creatinine transport.

Acknowledgment
Thanks to the Faculty of Science and Mathematics, Diponegoro University for providing research funding**.**

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