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In-Vitro Study of Polysulfone-polyethylene glycol/chitosan (PEG-PSf/CS) Membranes for Urea and Creatinine Permeation

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Abstract

High concentrations of creatinine and urea in the blood can be removed by dialysis using semipermeable membranes that are selective for certain species and hold other species through diffusion processes. This ability requires a membrane that has an active side, which functions as a targeted species identifier. The membrane must be biocompatible because the membrane will be in direct contact with the body's biological systems. The membrane material that is made must be acceptable to the blood system so that there is no rejection from the body and have a large contact area to obtain an effective diffusion process. For this reason, a hollow fiber membrane (HFM) is needed. One of the synthetic polymers used as the base material for HFM is PSf. PSf has mechanical strength, heat resistance, and is easily formed into HFM. However, PSf has disadvantages such as lack of active side and less compatible with blood due to its hydrophobic properties. Modification using PEG and chitosan will reduce the hydrophobicity of the PSf. Membrane results were analyzed the physical, chemical, and transportability for urea and creatinine. The results of functional group characterization by FTIR show that the modification reaction was successfully carried out on polysulfone to produce PEG-PSf/CS. The modification succeeded in making the PSf membrane more hydrophilic, as evidenced by a decrease in the contact angle from 69.4° (PSf) to 53° (PEG-PSf/CS). Water uptake capability increases to 609%, and membrane porosity increases porosity increased from 72 to 83%. The water flux is also increased. Creatinine clearance ability increases from 0.09 mg/dl to 0.25 mg/dL. Urea clearance ability increases from 2.3 mg/dL to 3.07 mg/dL. The SEM image showed that the modification makes the membranes more porous.

1. Introduction

Increased levels of urea and creatinine in the blood indicate a decrease in kidney function as a compound filter system in the body. If the situation continues, it needs equipment outside to dispose of excess levels of residual metabolic [1, 2]. The main components of the system filter (dialysis) consist of a semipermeable membrane that is biocompatible to blood, selective, high flux, and non-toxic [3, 4].

Usually, dialysis membranes have a shape of a hollow fiber membrane (HFM). This type of membranes has a high surface contact area, making it effective in the process of permeation [5, 6, 7]. Polysulfone, polyethersulfone, cellulose acetate are some of the most common materials used to make HFM. This hollow fiber membrane is made by phase inversion technique. This process is a popular technique for making asymmetrical membranes. In this process, a membrane solution (dope) is inserted into a hollow membrane molding device. A hollow fiber membrane that comes out of the syringe

directly dipped in a coagulation bath containing non-solvents (water). The precipitation process starts when dope is in contact with non-solvent, where the solvent will diffuse into the non-solvent, which causes a phase transition from a dope solution to a dense membrane layer [5, 7]. From the diffusion that occurs, non-solvent will pull the solvent out of the dope system so that the polymer will lose the solvent and form a solid membrane. The main advantage of hollow fiber membrane modules is the extensive surface area in a closed volume, which is useful for increasing the separation process's efficiency.

PSf is the most widely developed material because it has mechanical strength, thermal stability, and inert to chemical compounds. At room temperature, polysulfone is not reactive/difficult to react with other compounds. It requires temperatures above 60℃ and strong reagents, such as sulfuric acid and nitric acid, to change its structure. However, the PSf is hydrophobic, low compatibility to blood, only soluble in organic solvents, and non-biodegradable [5, 8]. Modification of PSf with polyethylene glycol has been studied to increase hydrophilicity and biocompatibility but is still inadequate [9, 10, 11].

Other materials can be used as a membrane is chitosan. Chitosan has properties of degradable, non-toxic, and biocompatible, easily modified because it has two active groups: amine (-NH_2) and hydroxyl (-OH) groups. Besides, chitosan has excellent hydrophilicity and can bind to urea and creatinine through the formation of hydrogen bonds to increase the ability of permeation of urea and creatinine. However, chitosan is difficult to build-in hollow fiber membranes, especially during solidification [12].

Based on the chitosan properties, modified chitosan to PSf is interesting to study for the dialysis membrane. The inclusion of PEG in the structure of PSf makes PSf more hydrophilic. PEG is a hydrophilic additive and has the same properties as non-solvents (water) to influence the mechanism of membrane formation. PEG will enter the structure, together with the polymer, dissolve homogeneously in dope. When the membrane compaction process occurs, a diffusion process occurs between the solvent and the non-solvent. Hydrophilic PEG will interact with non-solvent (water), which causes the anesthetic system to become thermodynamically unstable and accelerate diffusion between solvent and non-solvent, thereby leaving pore traces on the membrane [13]. As more pore traces in the membrane causing an increase in the value of water flux in the membrane, it means that the membrane will absorb more water so that the hydrophilicity value of the membrane increases [14]. While entering chitosan in the membrane framework structure, it will increase the number of active sides that function in the permeation process. It will facilitate the permeate that will pass through the membrane. Thus, permeation becomes more effective. It is hoped that this modification can produce a PSf membrane with good physicochemical characteristics and high permeability so that it can be used as a membrane in the dialysis process. However, modifications are having problems on the solubility of both systems are quite different. Chitosan is only soluble in weak aqueous acid, such as acetic acid. However, dilute acetic acid has a high-water content, a non-solvent system of PSf in the solidification process. In dilute acid, chitosan is protonated to form -NH_2 positive charge -NH_3^+ [15]. To form a bond between the two compounds, the PSf should be modified to form polyanion. One way to convert PSf to polyanions is through a sulfonation reaction, an electrophilic substitution reaction. It replaces the H atom on one of the aromatic rings with a benzene sulfonate group, which in turn will be the aromatic ring electrophilic negatively charged.

2. Methodology

2.1. Materials and equipment

Materials used were PSf (Udel-P1700) from Solvay Advanced Polymers (Molecular weight average = 69,500 g/mol), N-methyl-2-pyrrolidone (NMP; purity = 99.5%; MW = 99.1 g/mol; Merck) , polyethylene glycol (PEG; average molecular weight = 1,200 g/mol; Merck), distilled water, chitosan (Biotech Surindo, DD 87%, MW = 40,000 D), glacial acetic acid (≥99%, ACS, Sigma-Aldrich), creatinine (MW = 113.11 g/mol, Merck), urea (MW = 60.06 g/mol, Merck), NaOH (MW = 40 g/mol, Merck), HCl, (MW = 36.5 g/mol, Merck), H_3PO_4 (MW = 97.994 g/mol Merck), Na_2HPO_4 (MW = 141.96 g/mol, Merck), picric acid (MW = 229.11 g/mol, Merck), dimethyl amine benzaldehyde (MW = 149.19 g/mol). Several equipment which use in this research were set of glassware (Pyrex), magnetic stirrer (Biomega and Jenway), mixer, digital balance (OHAUS), Hollow Fiber Membrane Making Apparatus (MMA-1500A-HF), pH Meter (PH-009 (I) A PEN TYPE). The instruments used for characterization were FTIR (Perkin Elmer/Frontier), UV-Vis Spectrophotometer (LW-V-200-RS) and SEM (Shimadzu).

2.2. Preparation of PSf and PEG-PSf Hollow Fiber Membrane

Polysulfone was dried in an oven for 1 hour for removal of absorbed moisture. NMP was used as a solvent for dope preparation. The dope compositions used for HFM preparation were 17/83 (PSf/NMP) determined as HP and 17:3:80 PSf/PEG/NMP determined as HPP in the manuscript. The dope was poured on the hollow fiber membrane chamber engine, and then distilled water was poured on the chamber bore fluid. HFM spinning apparatus was used for making fibers.

2.3. Modification of prepared PEG-PSf/CS composite membrane

The sulfonation of prepared PEG-PSf/CS hollow fiber membrane using conditions reported by Teotia *et al.* [5]. The membranes were then soaked in 20, 40, and 60 wt.% sulfuric acid for 4 h. After that, the membranes were immersed in a 1.5% chitosan solution made for 4 hours. The membranes were washed using distilled water and hang at 50°C for 2 hours.

Table 1. Code of membranes

	Composition
HP	PSf
HPP	PSf-PEG
HPPs1	PSf/PEG + 20 % H_2SO_4 + Chitosan
HPPs2	PSf/PEG + 40 % H_2SO_4 + Chitosan
HPPs3	PSf/PEG + 60 % H_2SO_4 + Chitosan

2.4. Functional groups analysis of membrane

Test the functional groups that were carried out by FTIR. Firstly, some samples of the membrane were formed into pellets. The pellets entered on the tablet holder coated KBr and recorded with the infrared wavenumber of 400-4000 cm^{-1}.

2.4.1. Water uptake test

At the beginning of the process, the membrane was weighed using the analytical balance. Then the membrane was soaked in water for 24 hours. Then the soaked membrane was weighed again. The difference in weight between before and after the immersion was used to determine the water uptake [12].

$$\% \text{ water uptake} = \frac{W_t - W_0}{W_0} \text{ x } 100\% \quad (1)$$

where w_t is the weight of wet membrane, w_o is the weight of the dry membrane

2.4.2. Hydrophilicity

The membrane was placed on the glass, and then water was dropped on the membrane surface. Pictures of water droplets on the surface were taken using a digital microscope. To determine the contact angle of the membrane, the water droplet images were processed using Microsoft PowerPoint, and then the arcus tangent method was calculated to measure the angle of contact.

2.4.3. Morphology test

The membranes were dried with N_2 gas stream for 10 seconds to dry, then lift using tweezers and cut with a size of 0.5×0.5 cm^2. Pieces of the membrane were then coated with Pt, and analyzed by SEM.

2.4.4. Permeation Test

Permeation test was performed using a transport machine. The donor phase contained a 200 mL standard solution of creatinine 1.5 mg/dL in distilled water, and a dialyzer contained distilled water. Then the transport process was carried out for 1 hour. For every 15 minutes, as much as 2 mL samples of donor phase and dialyzer were taken, then were complexed using picric acid and analyzed using a UV-Vis spectrophotometer at a wavelength of 486 nm.

For Urea permeate, the donor phase was made of 200 mL standard solution of urea 30 mg/dL in distilled water, and dialyzer contained distilled water. For every 15 minutes, 2 mL samples were taken from the donor and acceptor phase, which were then complexed using picric acid and analyzed using a UV-Vis spectrophotometer at a wavelength of 430 nm. The permeation of membrane is calculated using Equation (2) [15]:

$$\text{Clearance permeate} = C_0 - C_t \quad (2)$$

where C_0 = initial concentration solute in feed dan C_t = concentration solute in feed at the time feed

2.4.5. Flux Test

Flux test was measured by passing water, creatinine, and urea in crossflow performed for 3 minutes and repeated three times. Then water, creatinine, and urea, which successfully passing through the membrane were measured the volume. The water flux was calculated by the following:

$$Flux\ (Lm^{-2}h^{-1} = \frac{V}{A\Delta t} \quad (3)$$

Where: $[V]_t$ = volume permeate (L), A is the total surface area (m^2), while Δt is the permeation time. For the rejection test, 500 ppm urea and 15 ppm creatinine solution were used. The percent rejection was calculated by Eq (4) [16, 17]:

$$R\ (\%) = \left(1 - \frac{C_p}{C_f}\right) \text{x } 100\% \quad (4)$$

Where Cp and Cf are concentration (urea or creatinine) of the permeate and feed. The concentration solution was measured using UV-Vis.

3. Results and Discussion

3.1. Functional groups analysis using FTIR

The FTIR image is displayed in Figure 1. The spectra for polysulfone FTIR shown characteristics peaks for C-S at 690 cm^{-1}, stretching -SO_2- at 1300 cm^{-1}, and –C-O-C- at ~1240 cm^{-1}. The other significant bands for PSf were observed at 1700-1500 cm^{-1} owing to –C=C- and at 3000 cm^{-1} due to aromatic –CH [5]. Similar spectra were obtained when there was mixing using PEG, and sulfonation reactions were carried out. The sulfonation process produced only a slight spectral difference, at 1320-1200 and at 2370 cm^{-1}, which showed the -SO_3 groups. The specific spectra PSf-PEG/CS (HPPs) membrane was shown by a peak in the wavenumber of 1320 cm^{-1} to show C-N's absorption. Furthermore, absorption at 3780 - 3400 cm^{-1} indicated the strain of –O-H of chitosan, overlapping between –N-H and –O-H, strength peak of 1582 cm^{-1} indicates amide –N-H and –C-OH could be observed at 2300 cm^{-1} [15].

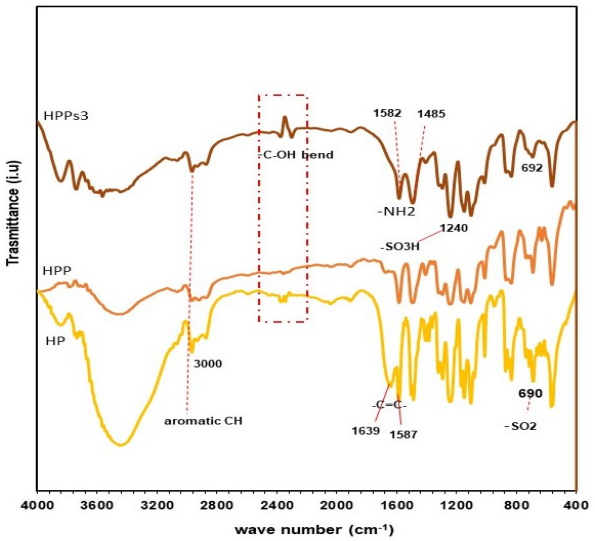

Figure 1. FTIR spectra of the membranes

Table 1. Interpretation of IR spectra on various membranes [5, 15, 18]

Functional groups	Wavenumber (cm^{-1})		
	HP	HPP	HPPs3
-CS	690	690	690
$-SO_3-$	-	1240	1240
$-SO_2-$	1360	1350	1320
-C-O-C-			-
-C=C-	1639, 1587	1600	
$-NH_2$	-	-	1582, 1485
-C-OH	-	-	2400

3.2. Hydrophilicity Test

Characterization of membrane surface hydrophilicity aims to determine the degree of membrane hydrophilicity to water. Hydrophilicity is determined by measuring the angle of contact of the membrane against water droplets. The smaller the angle formed; the membrane is more hydrophilic because the membrane pore more absorbs the droplets of water molecules. According to Elimelech's theory, hydrophilic membranes can form a hydration layer on the surface, which is very useful for improving the membrane's anti-fouling performance [16]. Figure 2 observed that the modification of PSf with PEG and chitosan could reduce the amount of water contact angle on the membrane surface. In the HP membrane, the highest contact angle is 64.4°, indicating that this membrane has the lowest hydrophilicity level. The HPP membrane results in lower contact angles. The addition of several -OH groups from PEG allows the formation of hydrogen bonds with water on the membrane surface, which increases membrane hydrophilicity [18, 19]. Further modification with chitosan also shows a tendency to decrease the membrane contact angle. According to Lusiana *et al.* [15], the size of the contact angle produced is due to surface tension between the membrane and water contact angle.

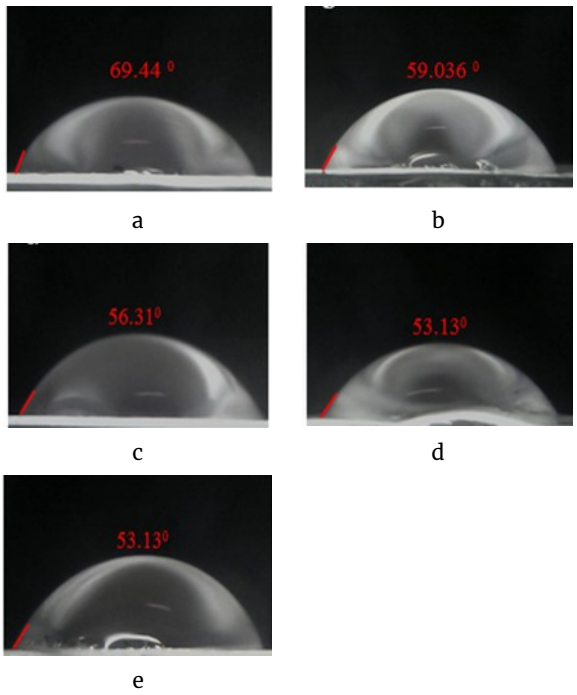

Figure 2. The contact angle of membranes HP (a) HPP (b) HPPs1 (c) HPPs2 (d) HPPs3 (e)

3.3. Membranes Water Uptake

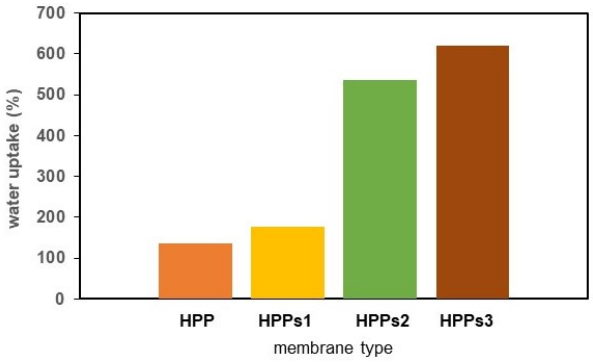

Figure 3. Water uptake of membranes

Figure 3 shows the modification of polysulfone significantly increasing the ability of water absorption by the membrane. Judging from the SEM image (Figure 4 and 5), the right modification with PEG followed by chitosan through the sulfonation process as an intermediate increase the area of membrane contact. Like an adsorbent, the wider the contact area will increase the ability to contact substances to the environment outside (water). The depletion of the upper layer and the enlargement of the inner void of the membrane cause the inner part to be connected to the outer membrane. With the pore tunnel's connection, it will cause more and more water to pass through it.

3.4. Morphology of membranes

The surface morphology and the finger layer of the membrane PEG-PSf (HPP) shown in Figure 4.

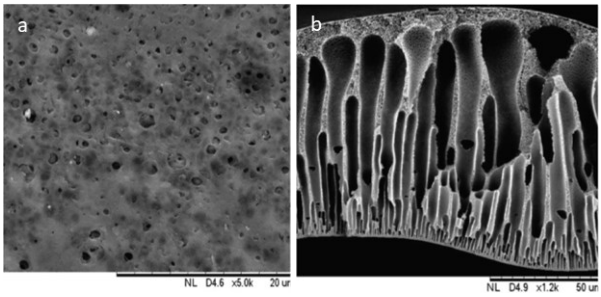

Figure 4. SEM images of surface morphology (A) dan finger layer (B) of HPP

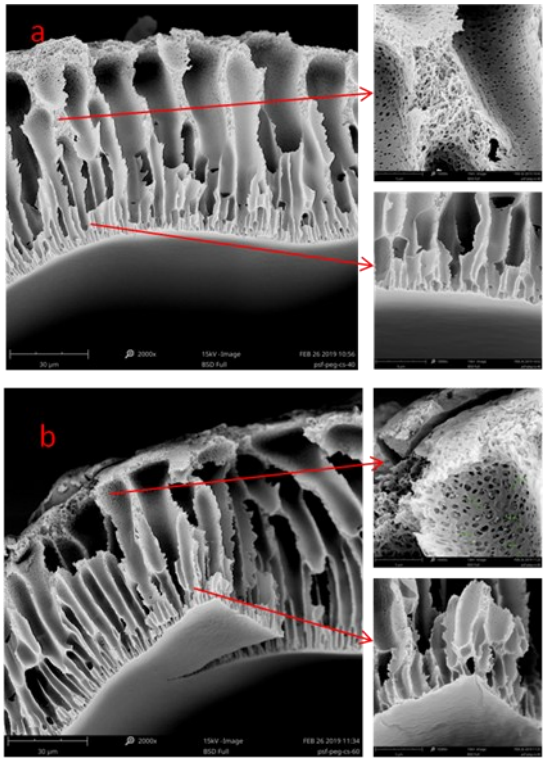

Figure 5. SEM images of *cross-section* membrane HPPs2 (a) HPPs3 (b)

In general, the modified PSf and PSf membranes have the same morphological structure, consisting of a dense top layer and a lower layer in the form of finger-shaped voids. An essential difference from the membrane morphology is the number and distribution of pores in the void wall. In pure PSf, there are not as many pores as there are pores in voids, such as the HPPs2 and HPPs3 membranes. HPPs2 and HPPs3 membranes are made from PSf, which has been mixed with PEG and chitosan through sulfonation reactions as intermediates. As a result of FTIR, the structure of HPPs compounds will consist of PSf polymers, -OH groups from PEG and -SO2 -NH groups from chitosan sulfonation. These additive groups are electronegative, facilitating the diffusion of water into a polymer solution (dope). During the HPPs membrane solidification process, the water (non-solvent) diffuses into the dope solution is large enough to facilitate the formation of membrane pores during membrane formation. In this case, the added additive compound (PEG and chitosan) is a pore-forming agent compound to help the formation of membrane pores.

Another morphological difference is the thickness of the upper skin layer. In pure PSf membranes (HP), the upper layer is dense with a thickness higher than the top layer on the membrane HPPs (Figure 5). This structure will affect the filtration performance of the membrane. The difference between these two morphological structures can explain the significant increase in the ability of water uptake on the modified PSf and PSf membranes. This data is in line with the results of the membrane's porosity measurements, as shown in Figure 6. From that figure, a modification of PSf with PEG and chitosan is shown to increase membrane porosity. These results are in accordance with research [20], where the addition of additives will increase membrane porosity because the additives can diffuse completely and form larger pore sizes.

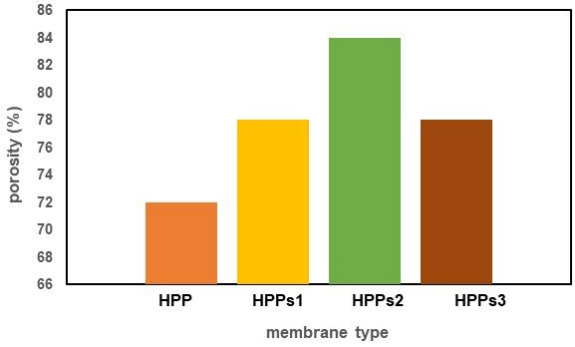

Figure 6. Membrane porosity test results

3.5. Membrane Permeation

The most important properties in the dialysis membrane are permeability. The results of the creatinine membrane permeation shown in Figure 7.

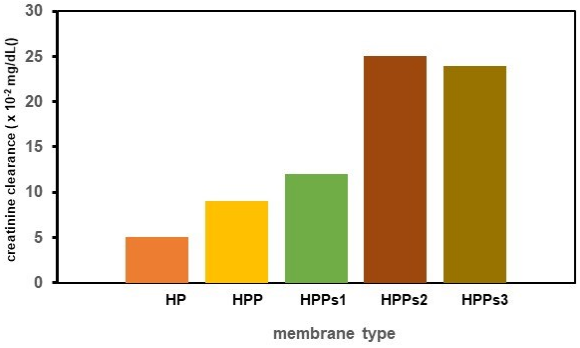

Figure 7. Creatinine transport of membranes

Pure PSf membrane has a creatinine clearance ability of 0.05mg/dL. PSf/PEG (HPP) membranes have a permeation ability of 0.09 mg/dL and increase with the entry of sulfonated chitosan to 0.12-0.23 mg/dL. This result correlates with porosity data and membrane morphology structure. Increasing porosity due to the modification process of both PEG and CS also plays an essential role in determining the membrane's ability to transport permeate. The increase in porosity makes the number of the open cavity in the membranes increase. This situation will facilitate the flow of permeate through the membrane [15, 21].

Table 3. The membrane transport percentage

Membrane	Creatine permeation (mg/dL)
HP	0.05
HPP	0.09
HPPs1	0.12
HPPs2	0.25
HPPs3	0.23

3.6. The flux of water dan creatinine

Flux is the amount of permeate volume that passes through one-unit area of membrane in a particular time, with the pressure thrust that enters the ultrafiltration cell device [22]. Table 4 shows the addition of PEG additives and chitosan to the PSf membrane causing an increase in membrane flux values. This correlates with the number of pores and morphological shape of the membrane before and after modification, as seen in the SEM image and porosity values. In membranes that have undergone modification, the contact area is increased by increasing the number of pores in the voids. The formation of pores in the membrane makes it easier for water molecules to pass through the membrane, resulting in higher flux values. The flux data shows that the flux of water > creatinine because of the size of water < creatinine [2, 8].

Table 4. The flux of water, and creatinine of membranes

Membrane type	Flux ($Lcm^{-2}h^{-2}$)		Rejection (%)
	water	creatinine	creatinine
HP	90.4	26.8	75.2
HPP	106.5	95.0	93.3
HPPs1	95.4	31.8	98.5
HPPs2	203.0	98.2	100.0
HPPs3	166.9	39.7	88.8

4. Conclusion

Polysulfone membrane has been successfully treated with chitosan with the evidence using FTIR to show specific wavenumbers on the membrane PSf/PEG/CS. The best membrane is the PSf/PEG membrane treated with 40% H_2SO_4/chitosan with a contact angle value of 53.13, 0.25 mg/dL of creatinine transport, 3.07 of urea transport.

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