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Research Paper

Glutaraldehyde Cross-Linked Chitosan Polylactic Acid Membrane for Creatinine and Urea Dialysis Applications

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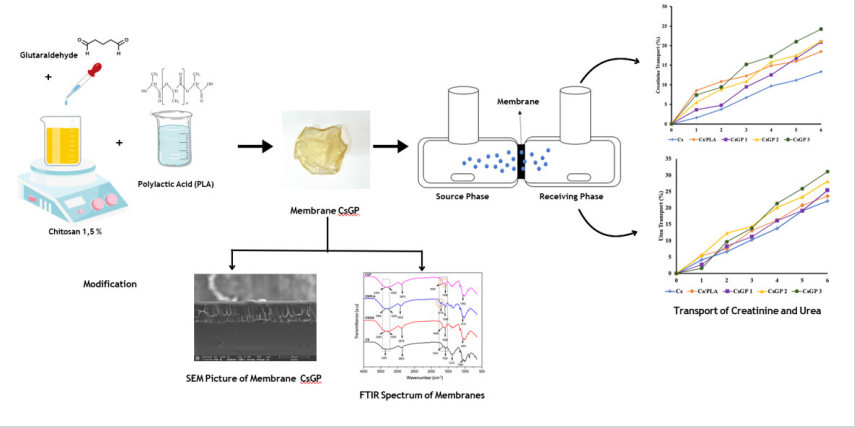
Highlights

- Chitosan membrane modified by glutaraldehyde and PLA (CsGP) made chitosan more effective as a urea-creatinine dialysis.
- Modification increases hydrophilicity, porosity, swelling, water uptake and tensile strength
- The optimized CsGP3 membrane increased creatinine and urea transport by 81% and 40%, respectively, compared to pure chitosan.

Graphical abstract



Abstract

This study synthesized and characterized glutaraldehyde cross-linked chitosan membranes with a blend of polylactic acid (CsGP) and determined the dialysis performance for creatinine and urea. This research was conducted to evaluate the impacts of various glutaraldehyde concentrations (1 [CsGP1], 2 [CsGP2], and 3% [CsGP3]) combined with polylactic acid (at a mass ratio of chitosan to polylactic acid of 1:0.2, on both the physical and functional attributes of the membrane. The physical and chemical characterization of the membrane, including weight and thickness test, swelling, porosity, water uptake, hydrophilicity, tensile strength, flux, FTIR, SEM, and membrane transport for creatinine and urea, was also investigated. The FTIR results demonstrated the successful completion of the CsGP membrane, as evidenced by the appearance of C=N groups resulting from the reaction of chitosan with glutaraldehyde and a shift in the absorption of -OH groups suggests that hydrogen bonds exist between chitosan and polylactic acid. Characterization of the modified membrane showed that glutaraldehyde and polylactic acid could increase the weight and thickness, swelling power, porosity, water absorption, hydrophilicity, and membrane transport ability. The transport of creatinine and urea increased by 81% and 40%, respectively, compared with pure chitosan. These findings suggest that the CsGP membrane may represent a viable option for dialysis and biomedical applications.

1. Introduction

Kidney failure is indicated in a person if the creatinine level exceeds 2.5 mg/dl and the urea levels range from 5 to 20 mg/dl. Patients with kidney failure typically receive hemodialysis [1]. Hemodialysis is a medical procedure utilized for patients diagnosed with acute or chronic kidney failure that is used globally as a replacement for kidney function, facilitating the removal of metabolic waste and uremic toxins in human blood, including urea and creatinine [2]. The hemodialysis process primarily involves a semipermeable membrane that acts as a selective transport mechanism for low-molecular-weight toxic compounds, such as creatinine and urea, within the bloodstream while maintaining the concentration of plasma proteins and cells [3], [4].

Biopolymers have been crucial in advancing various biological and medical applications, such as enhancing medical devices, revolutionizing tissue engineering, and transforming drug delivery systems [5]. Therefore, the development of biopolymers as hemodialysis membrane materials is essential. Currently, commercial membranes such as polysulfone (PSf) and polyethersulfone (PES), which are synthetic polymer-based, are widely used in hemodialysis due to their mechanical strength and stability [6, 7]. However, the hydrophobic nature of synthetic polymers may decrease biocompatibility and increase the tendency for biofouling to occur, both on the surface and in the membrane pore, which ultimately reduces flux and separation efficiency [8]. These limitations are driving innovation in developing alternative membrane materials that effectively remove toxins and are sustainable.

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Chitosan is a biopolymer that can form a thin film membrane, giving it potential as a material for hemodialysis membranes [9]. Furthermore, chitosan is biodegradable, non-toxic, inert, compatible, and has the capacity for chemical modification due to the presence of two key functional groups, namely, amine (-NH_2) and hydroxyl (-OH) [10]. Due to their small number, the active groups in chitosan do not react effectively with the target compounds. Therefore, it is necessary to modify the membrane to enhance its physical and selective characteristics, thereby improving dialysis efficiency [11]. Some studies have indicated that crosslinking reactions can alter chitosan membranes, enhancing their efficacy as hemodialysis membranes. Additionally, their functionality can be increased by blending chitosan membranes with polymers rich in active sites [12].

One of the crosslinking agents that can increase the stability and mechanical strength of chitosan membranes is glutaraldehyde [9]. Glutaraldehyde has an aldehyde group structure at both ends, which allows it to bind two different polymer layers to form the desired material. The chemical modification of chitosan and glutaraldehyde changes the morphology and structure of the membrane, causing the matrix to become closer. The crosslinking reaction occurs through a Schiff base [13].

Previous studies have indicated that crosslinking chitosan with glutaraldehyde significantly enhances the mechanical strength of membranes [14], [15]. Additionally, chitosan-carboxymethyl cellulose (CS-CMC) crosslinked with glutaraldehyde has been shown to improve the hemocompatibility of membranes [16]. Crosslinking with glutaraldehyde (GA) offers superior mechanical strength compared to other agents like citric acid and tripolyphosphate (TPP). GA's high binding affinity allows it to form strong covalent bonds, resulting in a more robust structure [17] [18].

The following modification is blending chitosan with other polymers to optimize its performance. Polylactic acid as a biodegradable polymer is flexible, thermo-stable, and mechanical strength [19]. Chitosan and PLA chains may form hydrogen bonds to expand the membrane's active surface area, enabling better interaction with solutes [20]. Based on research [20] reports that pure PLA membranes have a higher pore density, more excellent hydrophilicity, and enhanced antimicrobial activity compared to CS/PLA membrane blends. These properties make the membranes promising candidates for biomedical applications.

In this study, a chitosan membrane was synthesized, crosslinked with glutaraldehyde, and combined with polylactic acid to enhance the membrane's mechanical strength, porosity, and dialysis ability for potential application in dialysis. The research investigates the effect of varying glutaraldehyde concentrations (1-3%) on the physical and functional properties of the membranes. Although glutaraldehyde has been extensively studied as a crosslinking agent for chitosan, the addition of PLA to enhance mechanical strength and transport performance has rarely been reported in the literature. Therefore, the hypothesis is that modifying chitosan membranes using a combination of glutaraldehyde and polylactic acid will result in more effective membranes for dialysis of creatinine and urea.

2. Material and Methods

2.1. Materials

The materials utilized in this study are chitosan (BM = 40,000 g/mol, DD = 88.5%) (Cv. Bio Chitosan Indonesia), distilled water, acetic acid p.a (BM = 131.11 g/mol) (Merck), sodium hydroxide (NaOH)(Merck), glutaraldehyde (25% Aqueous solution) (BM = 100.12 g/mol) (Merck), polylactic acid (grade 2002d, BM 3000-5000 g/mol) (NatureWorks LLC), chloroform p. a (Merck), creatinine (BM = 113 g/mol) (Merck), picric acid (BM = 229.11 g/mol), urea (BM = 60 g/mol) (Merck), (Merck), 4-dimethylamine benzaldehyde (DMAB) (BM = 149.19 g/mol) (Merck), ethanol p. a 96% (Merck), HCl 37% (Merck), Buffer phosphate solution (Na_2HPO_4 and NaH_2PO_4) (Merck).

2.2. Instrumentation

The tools used in this study include standard research glassware (Herma and Pyrex), filler pipettes, dropper pipettes, disposable pipettes, stirring rods, watch glass, Petri dishes, magnetic bar, magnetic stirrer, hotplate, oven, ruler, stopwatch, digital analytical balance (OHAUS, Model PA323), portable pH meter (OHAUS Starter 400 Series), thickness meter (QITCO), dialysis set, Tensile strength analyzer (Brookfield CT), UV-Vis spectrophotometer (Shimadzu UV-1280, serial A120660), FTIR spectrometer (Agilent Cary 630), Scanning Electron Microscope (SEM) (Thermo Scientific Quattro S).

2.3. Procedures

2.3.1. Synthesis of Chitosan Membrane (Cs)

To synthesize the chitosan membrane, 1.5 g of chitosan was dissolved in 100 mL of 1% acetic acid, and chitosan was slowly added while stirring with a magnetic stirrer for 24 hours until homogeneous. The chitosan solution was diluted to 5 mL and printed onto a Petri dish. The membrane was dried using an oven until the chitosan membrane was dry; once dry, the membrane was released by soaking in 1M NaOH and washed with distilled water until neutral.

2.3.2. Chitosan/Glutaraldehyde/Polylactic Acid (CsGP) Membrane Synthesis

The chitosan/glutaraldehyde/ polylactic acid membrane synthesis process begins with the crosslinking of chitosan with glutaraldehyde and then continues with the fusion with polylactic acid. The synthesized glutaraldehyde crosslinked chitosan membrane was prepared according to Khabibi et al. 's method [16] with modifications [16] based on variations in glutaraldehyde concentration according to Arianita et al. 's method [21].

The chitosan membrane was crosslinked with glutaraldehyde by preparing a 1.5% chitosan solution in 79 ml of 1% acetic acid, which was stirred for 24 hours and then added with glutaraldehyde solution with a level (crosslinking) made fixed at 1:80 as much as 1.1 mL with various concentration variations of 1%, 2%, and 3%. The addition of glutaraldehyde was accompanied by stirring with a magnetic stirrer for 5 hours and heating at 60-80°C. The chitosan/glutaraldehyde solution was then allowed to stand until it was no longer hot, and the alloying process with polylactic acid was continued.

This study involves the synthesis of polylactic acid (PLA) using a modified method based on Han's technique [20]. Polylactic acid was solubilized in chloroform at 20 mg/mL and then mixed with various concentrations of chitosan/glutaraldehyde. PLA did not fully dissolve in Chitosan; a chitosan/ PLA mass ratio of 1:0.2 was selected due to preliminary experimental results indicating that PLA dissolves completely in chloroform at this ratio. In contrast, PLA did not fully dissolve in chloroform at a higher ratio of 1:0.5.

2.4. Characterization

2.4.1. Fourier transform infrared (FTIR) analysis

The functional groups were identified by examining membranes with an Agilent Cary 630 FTIR spectrophotometer at Diponegoro University's Integrated Laboratory. The FTIR spectra of each sample were taken across a wave number range of 500–4000 cm^{-1}.

2.4.2. Scanning electron microscopy (SEM) analysis

The cross-sectional morphology of chitosan and its modification was examined using a Thermo Scientific Quattro S at BRIN Lampung. The membranes used were ruptured and affixed to the sample holder, then coated in platinum metal and evaluated at an energy level ranging from 10-20 kV.

2.4.3. Weight and Thickness Test

Membrane weight measurements for each variation were weighed using an OHAUS analytical digital balance for five repetitions and then averaged. The thickness of the membrane was measured using a thickness gauge with five repetitions at different points and averaged.

2.4.4. Swelling Test

The swelling test measured the diameter of the initial chitosan membrane with a ruler and dry modified chitosan in three parts: horizontally, diagonally, and vertically. The membrane was immersed in 10 mL of distilled water for a duration of 24 hours, ensuring complete immersion of the surface. The diameter of the wet membrane was measured again after soaking [22]. The test consists of three repetitions.

$$Swelling = \frac{lt}{lo} x\ 100\% \quad (1)$$

2.4.5. Porosity

A porosity test was conducted by immersing the membrane in distilled water until the entire surface was immersed, allowing it to stand undisturbed for 24 hours. The membrane was weighed on an analytical digital balance for three repetitions, resulting in the final weight data. The membrane was subsequently dried and reweighed for three repetitions, yielding the initial membrane data. The membrane volume was calculated by weighing 10 mL of solution before printing using a 10 mL volumetric flask [4].

$$\text{Porosity} = \frac{Ww - Wd}{v.\rho w} x\ 100\% \quad (2)$$

2.4.6. Water Uptake

The water uptake test was initiated by weighing the chitosan and modified chitosan membranes on an analytical digital balance. The membranes were soaked in distilled water for six hours. They were taken out of the water at hourly intervals, dried, and then reweighed [4]. The test consists of three repetitions.

$$Water\ Uptake = \frac{Ww-Wd}{Wd} x\ 100\% \qquad (3)$$

2.4.7. Hydrophilicity

The hydrophilicity test was conducted using the sessile drop method. This involved placing the membrane on a flat surface and then applying a drop of distilled water with three different points just above the surface of the membrane. The resulting images were then captured using a camera. The hydrophilicity can be observed in the image and quantified using the contact angle measurement tool in CorelDRAW.

2.4.8. Tensile Strength Test

A tensile strength test was conducted on chitosan and modified chitosan membranes with dimensions of 5 x 1 cm. The membrane is secured at both ends to a tensile strength meter and subjected to a tensile force until it reaches its maximum elongation, at which point it will fracture. The resulting tensile strength value and percent elongation are then produced.

2.4.9. Calibration Curve of Creatinine and Urea

Standard solutions of creatinine were prepared at various concentrations (3, 6, 9, 12, 15, and 18 ppm) from a 20 ppm stock solution. Creatinine solution absorbance was measured at a maximum wavelength of 486 nm. For urea, standard solutions were prepared from a 500 ppm stock solution to achieve various concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm). A wavelength of 431 nm was used to measure the absorbance of the urea solutions. To estimate the model and quantify the concentrations of creatinine and urea during transport through membranes, a linear regression analysis was conducted.

2.4.10. Flux Test

The flux test was calculated using the creatinine and urea transport results of chitosan and modified chitosan membranes. This calculation aimed to evaluate the membranes capacity to allow the permeation of fluids. [23].

$$\text{Flux} = \frac{W}{At} \qquad (4)$$

2.4.11. Transport Test of Creatinine and Urea

The creatinine and urea transport tests were conducted using a system comprising two chambers connected by a membrane. The transport mechanism on the membrane is comprised of two distinct phases: the source phase, which contains 50 mL of a 15 ppm creatinine solution and 500 ppm urea, and the acceptor phase, which contains 50 mL of a phosphate buffer solution. Two milliliters of solution were withdrawn from the source and acceptor phases every hour during the six-hour transport. Subsequently, a complexing solution was added to the creatinine samples, complexed with picric acid, and to the urea samples, complexed with p-dimethylbenzaldehyde, in amounts of up to 2 mL. The absorbance of creatinine was measured at 486 nm, while that of urea was measured at 431 nm.

2.4.12. Statistical Analysis

The mean ± standard deviation is utilized to display experimental data. Using excel software, a one-way ANOVA was employed to assess statistical differences among groups. Statistically significant results are defined as those with P values below 0.05.

3. Results and Discussion

3.1. Synthesis of CsGP Membrane

This research developed a chitosan membrane through crosslinking glutaraldehyde. The crosslinking reaction is initiated by a Schiff base reaction between the aldehyde group (-CHO) of glutaraldehyde and the amine group (-NH_2) of chitosan [24]. The mechanism of the crosslinking reaction between chitosan and glutaraldehyde involves two distinct stages. The initial stage is the nucleophilic addition of an amine to a partially positively charged carbonyl carbon, followed by releasing protons from both nitrogen and oxygen. The second stage involves the protonation of the -OH group, which may subsequently be released as water during an elimination reaction. Upon completion of the reaction, an imine (C=N) group is formed. The C=N group formed indicates crosslinking of chitosan with glutaraldehyde, as evidenced by a change in membrane color from transparent yellow to dark yellow or orange [25].

The following step involves the incorporation of polylactic acid into the chitosan-glutaraldehyde alloy. When chitosan and polylactic acid are alloyed, hydrogen bonds are formed between the two polymers. Additionally, the carboxyl group of polylactic acid can interact electrostatically with the amine group of chitosan. The CsGP membrane has a physical appearance of yellow, transparent, slightly stiff, and not easily damaged. Fig.1 illustrates the research results, presenting the shape of the modified membrane.

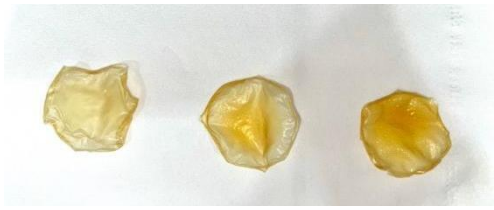


Fig.1. Physical Shape of Membrane (a) CsGP1, (b) CsGP2, (c) CsGP3.

3.2. Characterisation Results

3.2.1. FTIR

The FTIR spectra of chitosan, chitosan/glutaraldehyde, chitosan/polylactic acid, chitosan/glutaraldehyde/polylactic acid are shown in Fig. 2.

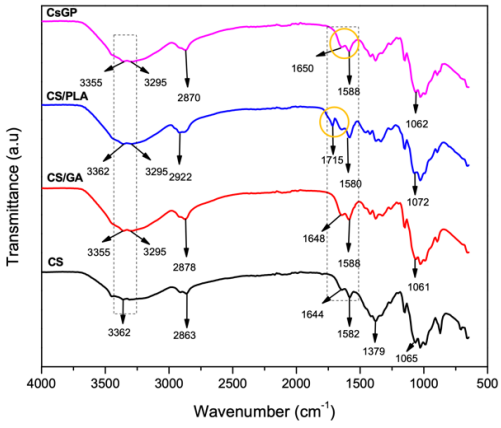


Fig. 2. FTIR Spectrum of Cs, Cs/GA, Cs/PLA, CsGP membranes.

Infrared spectroscopy demonstrates that adding glutaraldehyde to chitosan changes the functional group peaks seen in the infrared spectrum. There has been a shift in the wavenumbers of the O-H and N-H bands overlapping peaks, as indicated at 3355 cm^{-1} and 3295 cm^{-1}. The absorption observed at 2878 cm^{-1} suggests a change in the aliphatic C-H group. The peak at 1645 cm^{-1} shifts to 1648 cm^{-1}, indicating the interaction between the amino functional group (NH_2) of chitosan and the aldehyde of glutaraldehyde. As observed in previous studies [26], the C=N peak appears at wavenumbers between 1620 and 1660 cm^{-1}. The FTIR results reveal that no new peaks indicated the presence of C=N bonds, which can be attributed to the overlapping signals from the -NH groups of chitosan. The absorption observed at 1588 cm^{-1} signifies a shift in N-H bending vibrations within the amide group. Similarly, the absorption at 1061 cm^{-1} spectra indicates a C-O shift. The reaction between chitosan and glutaraldehyde is approximately illustrated in Fig. 3.

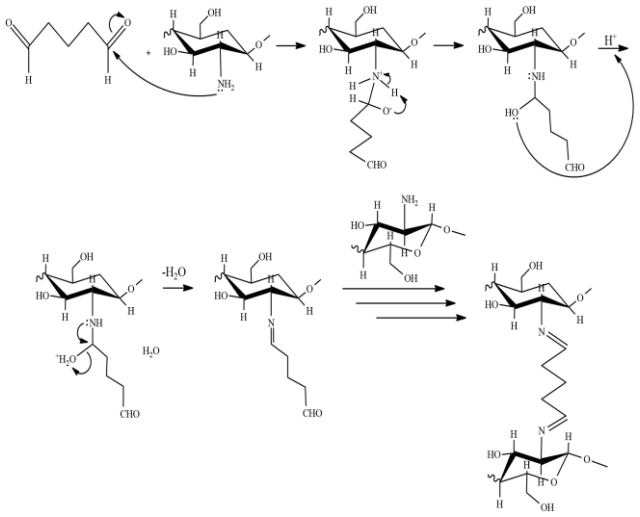


Fig. 3. Reaction chitosan with glutaraldehyde.

The results of FTIR characterization of the Cs/PLA membrane exhibited a slight difference in absorption at wave numbers 3362 cm^{-1} and 3295 cm^{-1}, indicating the presence of overlapping O-H and N-H strain shifts. The 1715 cm^{-1} absorption peak exhibits a distinctive carbonyl group (C=O) characteristic of polylactic acid; following previous research, the carbonyl group peak appeared at 1710 cm^{-1} [27].

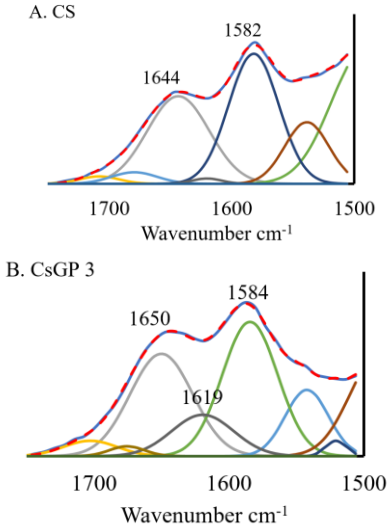


Fig. 4. Deconvolution Spectra of A. Cs, B. CsGP at wavelengths of 1750-1500 cm^{-1}.

The infrared spectra of the chitosan/glutaraldehyde/polylactic acid blend membrane exhibit a change in the position of the peaks. Adding glutaraldehyde and polylactic acid shifts the peak of the -OH stretch vibration to a lower and wider wave number, specifically 3355 cm^{-1}. According to the research conducted by Han et al., 2018 [28] [20], the shift in wave number of the -OH stretch to a reduced value serves as an indication of the interaction between the chitosan chain and polylactic acid, resulting in the formation of hydrogen bonds. The -NH group is responsible for the absorption at 3295 cm^{-1}. The absorption at 2870 cm^{-1} indicates a shift in the C-H stretch to a higher wave number. The 1650 cm^{-1} absorption demonstrates a change from the wave number 1644 cm^{-1}. Based on Fig. 4, the deconvolution results for the CsGP membrane, 1619 cm^{-1} is the wavenumber where a new peak occurs. These results indicate that glutaraldehyde was successfully derivatized with chitosan, forming a new N-C bond between the N atom from the chitosan and the C atom from glutaraldehyde, which produced a Schiff base and an imine bond (C=N). Based on previous research, an imine bond (C=N) is characterized by new peaks appearing at a wave number of 1559 cm-1 [29] and 1660 cm^{-1} [30]. The absorption at 1588 cm^{-1} indicates the formation of an N-H group, while the absorption at 1062 cm-1 demonstrates a shift in the C-O group. Fig. 5 illustrates the approximate interaction between chitosan, glutaraldehyde, and polylactic acid.

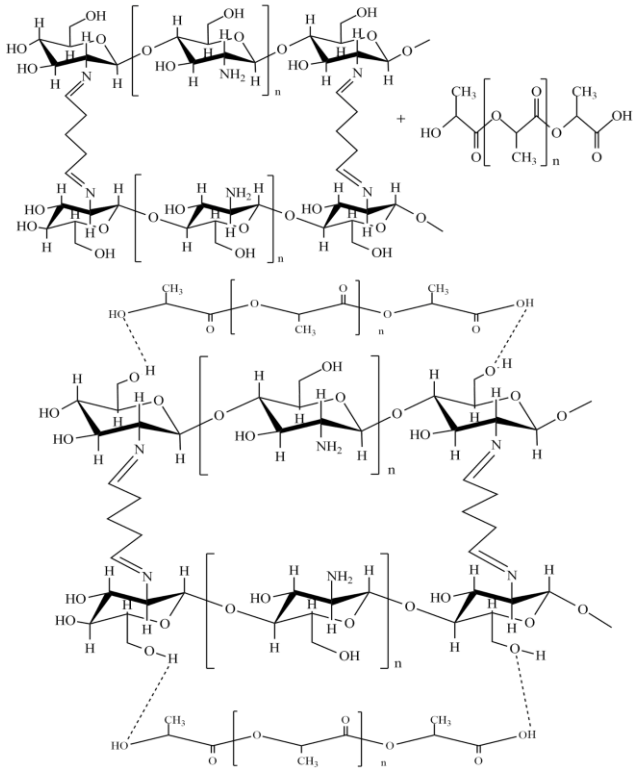


Fig. 5. Approximate interaction of CsGP membrane.

3.2.2. Weight and Thickness Membranes

The weight and thickness of membranes depend upon the basic materials utilized in their construction and the structural configuration of the membrane itself. Table 1 presents the measurements of the weight and thickness of the resulting membrane.

Table 1
Weight and Thickness Membranes.

Membranes Variation	Weight (g)	Thickness (mm)
Cs	0,074 ± 0,002	0,042 ± 0,008
Cs/PLA	0,083 ± 0,009	0,052 ± 0,000
CsGP 1	0,093 ± 0,002	0,086 ± 0,004
CsGP 2	0,094 ± 0,001	0,091 ± 0,005
CsGP 3	0,096 ± 0,002	0,113 ± 0,006

As shown in Table 1, the weight and thickness of the chitosan and modified chitosan membranes have increased. This indicates that the mass of the substance impacts its weight and thickness. It can be observed that as the mass of the substance increases, the weight and thickness also demonstrate a corresponding increase. The concentration of glutaraldehyde also affects the weight and thickness of the membranes, as glutaraldehyde forms cross-links between polymer chains and interacts with amine groups in chitosan, thereby causing structural changes that increase the membranes weight and thickness.

3.2.3. Swelling

The development of membranes depends upon the penetration of solvents into the membrane pores. The development value will be affected as the membranes pore count increases. The results of the swelling test are displayed in Fig. 6.

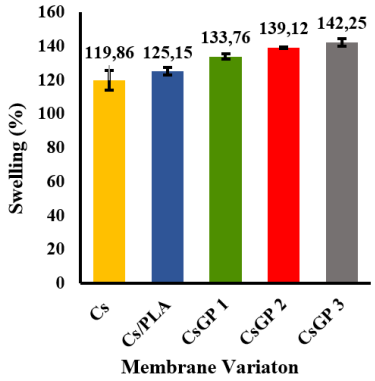


Fig. 6. Swelling Ratio (%) of Chitosan-Glutaraldehyde- PLA Membranes with Varying GA Concentrations.

The modified results of this study demonstrated an increase in development. This is made possible by the crosslinking reactions, specifically the concentrations of glutaraldehyde and the addition of polylactic acid alloys. Adding polylactic acid to chitosan can facilitate the formation of active groups, as polylactic acid contains a polar carboxyl group (-COO-) that can form hydrogen bonds with water molecules. This leads to an improved development of the membrane.

3.2.4. Porosity

Porosity is the ratio of the volume of the pores volume to the membranes total volume. It is a membrane characteristic directly related to the number of voids it contains. The presence of numerous voids within a membrane with high porosity is a crucial factor in the effective functioning of the dialysis process. The results of the porosity test on the modified membrane are presented in Fig. 7.

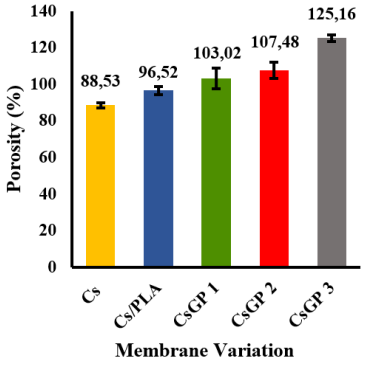


Fig. 7. Porosity Ratio (%) of Chitosan-Glutaraldehyde- PLA Membranes with Varying GA Concentrations.

Based on Fig. 7, the porosity measurement results of the modified membrane show an increase compared to pure Cs. The CsGP 3 membrane produces the highest percentage of membrane porosity, 125.16 %. The increased porosity indicates that the membrane has sufficient and regular void spacing so that creatinine and urea can easily pass through the membrane during diffusion.

Adding glutaraldehyde as a crosslinking agent can create a monomer spacing that affects the membrane's porosity and produces well-arranged pores. The increase in porosity is also caused by carboxyl groups (-COO-), which are hydrophilic and derived from alloying with polylactic acid. They can influence the membrane to be more hydrophilic. Correlated with membrane hydrophilicity, the membrane porosity is also higher if the membrane has high hydrophilicity.

3.2.5. Water Uptake

The water uptake test was evaluated the membranes capacity to absorb the water. The results of the water uptake test on the membranes are presented in Fig. 8.

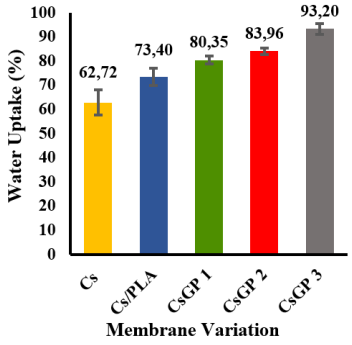


Fig. 8. Water Uptake Ratio (%) of Chitosan-Glutaraldehyde- PLA Membranes with Varying GA Concentrations.

As illustrated in Fig. 8, the Cs membrane exhibits the lowest percentage of water uptake, 62.7%, while the highest rate is obtained by the CsGP 3 membrane, with a percentage of water uptake of 93.2%. The addition of polylactic acid causes an increase in water uptake, which has a hydrophobic carboxyl group (-COO-), which has a hydrophobic carboxyl group (-COO-), so it interacts well with water to increase water uptake. In the CsGP 3 membrane, the high concentration of glutaraldehyde causes the monomer distance between the chitosan to be further apart, forming a void that can affect water absorption in the membrane. The membrane porosity value also affects the rise in water absorption; if the membrane has a good porosity value, the resulting water absorption value will also be better.

3.2.6. Hydrophilicity Test

The hydrophilicity test of the membrane surface can be determined by measuring the resultant water contact angle value. As illustrated in Table 2, the results of the membrane hydrophilicity measurement are presented.

The surface of the whole membrane is hydrophilic. The polar interactions of hydrogen bonds between water and chitosan-polylactic acid enable the combined chitosan chain to absorb water more rapidly, thus increasing the hydrophilicity of the membrane [31]. The CsGP 3 membrane has the lowest contact angle of 52.50°. An increase in the concentration of glutaraldehyde leads to a reduction in the contact angle value. This is because increasing the concentration of glutaraldehyde can increase the membrane's pore size so that water absorption is increasingly supported by the presence of active groups in polylactic acid, making the membrane more hydrophilic.

Table 2
Contact Angel Membranes

No	Membranes Variation	Contact Angel (°)
1	Cs	77,91
2	Cs/PLA	69,79
3	CsGP 1	58,13
4	CsGP 2	56,16
5	CsGP 3	52,5

3.2.7. Tensile Strength

The tensile strength test will yield two values: the tensile strength value, which represents the strength of the membrane at the point of rupture, and the elongation at the break value, which represents the strain strength value produced by the membrane at rupture. Table 3 presents the results of tensile strength measurements conducted on various membranes.

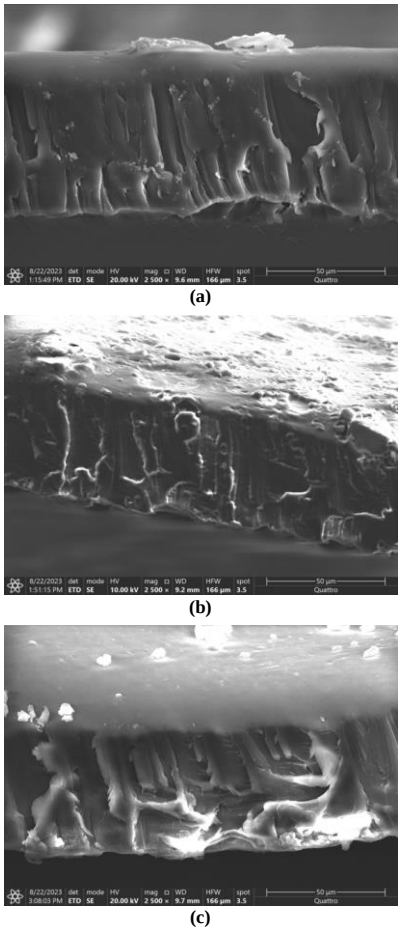
Table 3
Tensile Strenght Test on Various Membrane.

Membranes Variation	*Tensile Strength* (MPa)	*Elongation at break* (%)
Cs	27,23	16,8
Cs/PLA	26,92	8,4
CsGP 1	32,76	24,8
CsGP 2	36,85	9,2
CsGP 3	32,76	10

3.2.8. Scanning Electron Microscope (SEM)

Characterization was conducted on the Cs, Cs/PLA, and CsGP membranes to identify any distinctions in membrane structure morphology observed at the pore or cavity level. Fig.9 presents the results of the cross-sectional morphology analysis at 1000x magnification.

As illustrated in Fig. 9(a), the SEM analysis of the pure chitosan membrane reveals a dense and tight cross-sectional structure with only a few discernible membrane pores. Fig. 9(b) illustrates a cross-sectional image of the Cs/PLA membrane, which exhibits a cavity. This indicates that the Cs/PLA membrane is superior to the Cs membrane. The incorporation of polylactic acid results in an augmentation of the pore count, although the resulting structure becomes less regular. Fig. 9(c) indicates that the CsGP membrane has a greater number of pores, thereby demonstrating greater potential for use as a dialysis membrane for the transport of creatinine and urea due to its enhanced capacity for water absorption, which correlates with the observed increases in porosity and hydrophilicity of the membrane.



(a) **(b)** **(c)**

Fig. 9. Cross-section morphology of membrane (a)Cs, (b)Cs/PLA, (c)CsGP3.

2.2.9. Flux

This experiment's results can explain the membrane's potential in the dialysis process. The results of flux values in chitosan and modified chitosan membrane transport are shown in Table 4.

Table 4
Flux Values of Urea and Creatinine.

Membranes Variation	Flux (mg cm^{-2} h^{-1})	
	Creatinine	Urea
Cs	0,033	2,459
Cs/PLA	0,034	2,557
CsGP 1	0,053	2,857
CsGP 2	0,059	3,064
CsGP 3	0,065	3,561

As illustrated in Table 4, the modified chitosan membrane, with the highest concentration of glutaraldehyde, exhibits enhanced urea and creatinine transport capabilities compared to pure chitosan and chitosan/polylactic acid. The CsGP 3 membrane exhibited a flux value of 0.065 mg cm^{-2} h^{-1} for creatinine and 3.561 mg cm^{-2} h^{-1} for urea. This evidence supports the conclusion that CsGP 3 exhibits the optimal porosity and hydrophilicity.

3.3. Membrane Transport

3.3.1. Creatinine Transport

Creatinine has a molecular weight of 113 g/mol. The analysis used UV-Vis, which has a wavelength of 486 nm, to measure transported creatinine compounds using picric acid complexing. The reaction of picric acid with creatinine occurs in an alkaline atmosphere. The deprotonation of picric acid and the subsequent formation of a negatively charged species allows for the combination with creatinine, producing a red-orange-colored molecule [32]. The results of creatinine transport across the membrane are shown in Fig. 10.

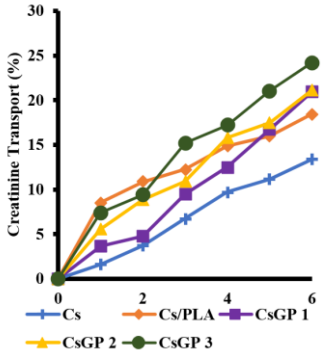


Fig 10. Graph of Creatinine Transport Results in Various Membranes.

Based on the graph, the highest creatinine transport result was observed in the CsGP 3 membrane. The creatinine transport on the CsGP 3 membrane increased by 81% compared to the pure chitosan membrane. The crosslinking of chitosan with glutaraldehyde and alloying with polylactic acid on the membrane demonstrated enhanced dialysis capabilities. Improvements in the dialysis process result from the effects of increased glutaraldehyde concentration on the membrane pores. Additionally, introducing polylactic acid carboxyl (-COO-) groups enhances the membrane's active sites, reducing the formation of hydrogen bonds with urea and creatinine, thereby increasing the transport rate of these substances through the membrane. These findings are directly correlated with the hydrophilicity, water absorption, and porosity of CsGP 3 membranes, which exhibit superior performance compared to pure chitosan membranes.

3.3.2. Urea Transport

Urea is a metabolic byproduct generated during protein breakdown and primarily excreted by the kidneys via urine. The transport of urea was measured by forming a complex with 4-dimethylamino-benzaldehyde and using UV-Vis spectroscopy at a wavelength of 431 nm. The reaction between the aldehyde and urea initiates with the protonation of the amino dimethyl group, resulting in a loss of charge on the carbonyl carbon and rendering it susceptible to nucleophilic attack from the urea moiety. Fig. 11 illustrates the outcomes of urea transport.

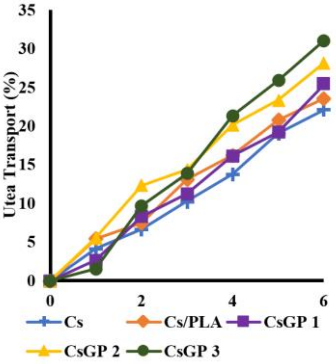


Fig. 11. Graph of Urea Transport Results in Various Membranes.

Fig. 11 shows that the CsGP3 membrane exhibits optimal permeability, as evidenced by the findings related to porosity, water uptake, and hydrophilicity. The percentage of urea transport is elevated compared to creatinine transport due to the lower molecular weight of urea (60 g/mol) relative to creatinine (113 g/mol). The smaller size of urea allows for faster dialysis than other metabolites, resulting in superior immobilization [33].

The increase in creatinine and urea transport suggests that adding functional groups from polylactic acid (PLA) enhances the membrane's interaction with water. This increases the hydrophilic groups on the surface, improving its ability to bind and transport creatinine and urea. Furthermore, glutaraldehyde, as a crosslinking agent helps create uniform pores, enhancing transport efficiency. There were statistically significant differences in urea transport among the five membrane types ($p < 0,05$). A comparative analysis of dialysis performance in this study and recently published literature is presented in Table 5.

Table 5
Comparison of membrane dialysis performances.

Membranes	Creatinine Dialysis (%)	Urea Dialysis (%)	Ref
Pure PES	± 9.5	± 7,5	[34]
Pure PVDF	± 2.0	± 10.0	[35]
CS/GA/CMC	33.8	46.5	[16]
Present Work	24.2	31.04	

4. Conclusion

The synthesis of a glutaraldehyde-crosslinked chitosan membrane with a polylactic acid alloy was successfully carried out with variations in glutaraldehyde concentration (1%, 2%, 3%) and a comparison of the outcomes membranes. The synthesized membrane has been shown to exhibit enhanced characteristics compared to pure chitosan membrane, including increased weight and thickness, enhanced hydrophilicity, augmented porosity, and augmented mechanical strength. Modifications to the membrane were demonstrated to improve its performance in dialysis against urea and creatinine. The CsGP 3 membrane exhibited the most optimal dialysis performance, as evidenced by its superior physical properties and the elevated percentage of transport outcomes attained in the final hour. Specifically, creatinine transport increased by 81% in comparison with the pure chitosan membrane, while urea transport increased by 40%.

In conclusion, this research indicates that the CsGP membrane developed here is viable for hemodialysis membranes. Nevertheless, additional research is required to further explore the potential of polylactic acid (PLA) in enhancing chitosan-based membranes for hemodialysis applications. In particular, future studies should focus on evaluating the long-term stability and cytotoxicity of the developed membranes. Furthermore, comprehensive hemocompatibility tests and real blood filtration performance assessments are necessary to determine the clinical feasibility and safety of CsGP membranes as hemodialysis membranes.

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Data availability

The data and materials utilized are all included in the text.

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Declaration of Competing Interest

The authors state that none of their personal affiliations or financial conflicts could affect the work presented in this paper.

Credit authorship contribution statement

N.A.I. Aanisa: Conceptualization; Data Curation; Formal Analysis; Investigation; Methodology; Validation; Visualization; Writing – Original Draft; Writing – Review & Editing

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