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Synthesis of Chitosan Derivative Compounds Through Chloroacetic Acid and Heparin Grafting and Their Application as Membrane Materials with Polyvinyl Alcohol (PVA)

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Abstract

A chitosan membrane modified with chloroacetic acid with heparin (hep) and polyvinyl alcohol (PVA) has been successfully prepared. Chitosan was modified with chloroacetic acid through a nucleophilic substitution reaction to form N-carboxyl methyl chitosan (*N*-CMC) and then combined with PVA. *N*-CMC/PVA grafting with heparin was conducted using the immersion method and produced *N*-CMC/PVA.*g*.Hep membrane. This study aims to obtain a membrane with the best chemical and physical characteristics and the highest creatinine transport. Membrane characterization includes water absorption test, tensile strength, thickness, biodegradation, resistance to pH, and transport of creatinine and vitamin B12. Chemical characterization of active groups and morphology using FTIR and SEM. The characterization results show that the reaction of grafting chitosan using chloroacetic acid produces N-carboxymethyl chitosan (*N*-CMC). The *N*-CMC/PVA membrane has a creatinine transport capacity of 19.61%. The N–CMC/PVA.*g*.Hep membrane has a creatinine transport capacity of 24.81%. Supporting PVA improves the hydrophilicity and mechanical strength of the membrane.

1. Introduction

Membranes are a thin layer that has selective and semi-permeable properties. According to Lusiana *et al.* [1], membranes can pass species with small sizes and reject species that are larger than the pore size of the membrane, thus making membranes usable in the separation process. One of the biopolymer materials that can fulfill these properties is chitosan. Chitosan is degradable, permeable, biocompatible, non-toxic and reactive material [2]. Chitosan has two functional groups: the amine group (-NH_2) and the hydroxyl group (-OH) [3, 4]. The positive charge of the amine groups of chitosan can cause circulation disorders in plasma proteins which cause protein adsorption on the membrane surface and reduce the reactivity of chitosan [1, 5]. In addition, the chitosan-based membrane has only a few permeate-trapping active groups and poor mechanical strength. For that reason, in order for the chitosan membrane to be utilized in the membrane transport process, it must therefore be modified to eliminate its lack of characteristics.

Methods that can be used to modify chitosan include modification of membrane surface groups through grafting and cross-linking reactions with other groups and alloys with synthetic polymers to change the chemical composition and mechanical properties of chitosan membranes [6, 7]. Different group modifications will generate chitosan with distinct properties; hence group modifications must consider the intended usage of chitosan [8].

This research aims to modify chitosan through a graft reaction using chloroacetic acid, which has an active group -COOH, a graft reaction with heparin which has an active group -SO_3, and integration using a synthetic polymer polyvinyl alcohol (PVA). Modification of the -COOH and -SO_3 groups will add an active site that can function as a permeate (creatinine) catcher by forming hydrogen bonds, allowing creatinine to be

transported rapidly and efficiently across the membrane. Integration with PVA aims to increase the mechanical strength of the modified membrane.

2. Methods

2.1. Materials and Instrument

Chitosan, isopropanol, polyvinyl alcohol (PVA), chloroacetic acid ($ClCH_2COOH$), acetic acid (CH_3COOH), heparin, distilled water, sodium hydroxide (NaOH), phosphate buffer, ethanol. The instruments used were the Oswald viscometer, FTIR Spectroscopy (IR Tracer-100), SEM (JEOL), thickness gauge (Mitutoyo), pH Tester (pH-009), analytical balance (OHAUS), Strength Tester (Gester).

2.2. Synthesis of Chloroacetic acid-grafted Chitosan (*N*-CMC)

Mixture I was prepared by dissolving 3 g of chitosan and 35 mL of isopropanol, stirring for 30 minutes, adding 10 mL of 15% NaOH, and then stirring for 30 minutes. In another beaker, mixture II was prepared by adding chloroacetic acid dropwise to 15 mL isopropanol with different mole ratios (Table 2) and stirring until evenly mixed. Mixture I was mixed with mixture II and stirred for 20 hours. After stirring, 50 mL of 95% ethanol and 2.5 mL of 10% acetic acid were added to the final mixture and stirred for 30 minutes. The precipitate formed was filtered and dried at 50-70°C. The formed material was characterized using FTIR to identify the functional groups.

Table 1. Composition of chloroacetic acid-grafted chitosan

Material	Chitosan (g)	Chloroacetic acid (g)	Chitosan (mol)	Chloroacetic acid (mol)
N-CMC1	1	1.77169	1	1
N-CMC2	1	3.54338	1	2
N-CMC3	1	7.08676	1	3

2.3. Manufacture of *N*-CMC /PVA Membrane

A number of grams of *N*-CMC were mixed with PVA with various ratios (Table 2), dissolved in 100 mL of 5% acetic acid, and stirred for 4 hours. The solution was poured into a petri dish and dried in an oven at 50-70°C for 24–48 hours. The dried membrane was removed from the petri dish by adding 1 M NaOH and washed with distilled water until the pH was neutral.

Table 2. Composition of *N*-CMC and PVA

Membrane	*N*-CMC (g)	PVA (g)
N-CMC/PVA1	1	2
N-CMC/PVA2	2	2

2.4. Determination of Optimal *N*-CMC/PVA Ratio

The resulting *N*-CMC membrane was used for creatinine transport for 6 hours, and the initial and final creatinine concentrations were measured using a UV-Vis spectrophotometer at a wavelength of 483 nm. The membrane with the largest percentage of creatinine transport is the optimal *N*-CMC/PVA ratio.

2.5. Grafting of *N*-CMC/PVA/Hep Membrane

The *N*-CMC/PVA membrane with optimum ratio was immersed in heparin at 100 IU for 24 hours. The *N*-CMC/PVA membrane grafting with heparin was done under acidic conditions using 5% acetic acid.

2.6. Creatinine Transport

Creatinine transport was performed by placing *N*-CMC/PVA.*g*.Hep in the middle of the transport device, as shown in Figure 1. The device has two phases: the source phase containing 50 mL of 25 ppm creatinine standard solution and the acceptor phase containing 50 mL phosphate buffer. Transport was conducted for 6 hours, taking 2 mL of the sample every hour from the source and acceptor phases complexed using picric acid and 0.4 M NaOH. The results were analyzed using a UV-Vis spectrophotometer at a wavelength of 483 nm.

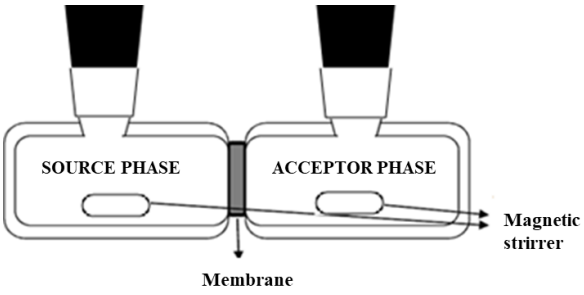

Figure 1. Illustration of a transport device

2.7. Membrane Transport Assay of a Mixture of Creatinine and Vitamin B12

N-CMC/PVA.*g*.Hep was placed in the middle of the transport device (Figure 1). The transport device has two phases: the source phase containing 50 mL of 25 ppm creatinine standard solution and 20 pm vitamin B12, and the acceptor phase containing 50 mL phosphate buffer. Transport was performed for 6 hours, taking 2 mL of the sample every hour from the source and acceptor phases complexed using picric acid and 0.4 M NaOH. The results were analyzed using a UV-Vis spectrophotometer at a wavelength of 483 nm.

2.8. Membrane Morphology

The *N*-CMC/PVA membrane was dried by flowing N_2 gas for 4 hours, then removed and cut into a size of 0.5 x 0.5 cm using tweezers at both ends. The membrane pieces were then coated. The surface and cross-section were analyzed using SEM.

2.9. The resistance of the *N*-CMC/PVA Membrane to pH

The *N*-CMC/PVA membrane was weighed and immersed in a solution with different pH (3, 5, 7, 9, 11,

and 13), which was adjusted using HCl or NaOH solution. The membrane was left at 25°C for 24 hours, then dried in an oven at 400°C and weighed.

2.10. Swelling Ratio

The *N*-CMC/PVA membrane was weighed for dry weight, then soaked in distilled water for 6 hours. Every hour the membrane was removed, wiped with a tissue, and weighed. The difference in weight between before and after immersion was used to calculate membrane swelling.

2.11. Biodegradation Assay

The membrane was weighed and then planted in compost soil to a depth of 25 cm and left for several days in the soil to observe its ability to be decomposed by microbes. Unloading was conducted every week to re-weigh the specimens.

3. Results and Discussion

3.1. Synthesis and Characterization of Chloroacetic acid-grafted Chitosan

N-CMC was synthesized by modifying the amine group of chitosan by substituting a carboxylic group in an alkaline state. The main purpose of this reaction is to convert primary amines into secondary or tertiary and add active sites to chitosan. The mechanism for the synthesis of *N*-CMC was carried out in an isopropanol solvent which caused swelling of chitosan, allowing for space for chitosan to interact with chloroacetic acid. The mechanism for synthesizing *N*-CMC (Figure 2) begins with adding 15% NaOH at room temperature. At this stage, the base will attack the H^+ amine group in chitosan, thereby increasing the nucleophilicity of the chitosan anion formed [9]. Additionally, the newly produced chitosan anion will attack the methylene group on the positively charged monochloroacetic acid to create an alkaline carboxymethyl chitosan product [10].

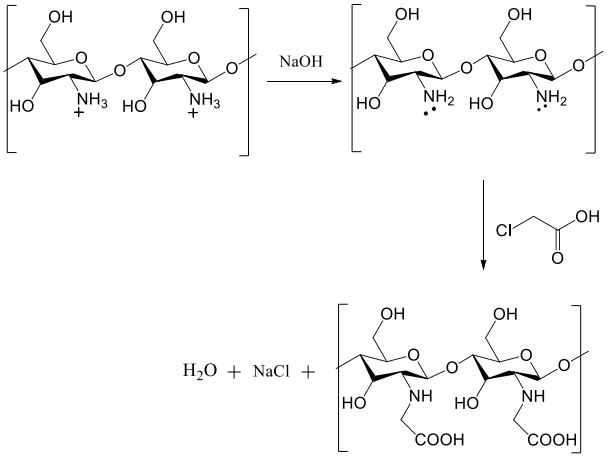

Figure 2. *N*-CMC synthesis mechanism

The purity test of the synthesis results was performed by dissolving *N*-CMC with water as a solvent. This demonstrates that the chloroacetic acid graft reaction against the amine groups of chitosan has been done successfully and that further integration with PVA and heparin graft reactions have been conducted. The success of *N*-CMC is analyzed from the FTIR results. The FTIR spectra of *N*-CMC are shown in Figure 3.

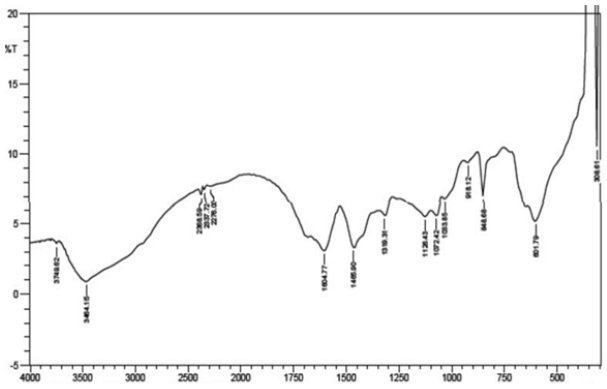

Figure 3. FTIR spectra of *N*-CMC

The FTIR spectra of *N*-CMC showed a broad absorption peak at 3438 cm^{-1}, which was identified as overlapping N-H and O-H stretching vibrations [11]. Weak absorptions also appear at 2944 cm^{-1} and 2876 cm^{-1}, which are assigned as asymmetric and symmetrical C-H sp^3 stretching vibrations, respectively [12]. In addition, absorption also appears at 1678 cm^{-1}, which is a stretching vibration of the C=O amide of chitosan that has not been deacetylated [13]. Strong absorption near ~1450 cm^{-1} is observed as bending deformation vibration (CH_2) [14]. Weak absorption also appears at 1126 cm^{-1}, identified as C-O stretching absorption [15]. The successful synthesis of *N*-CMC was indicated by the appearance of a peak at 1786 cm^{-1}, which was observed as the C=O vibration of the carboxyl group [16].

3.2. Integration and Determination of *N*-CMC/PVA Ratio

Optimum *N*-CMC was combined with PVA to improve the mechanical properties of CMC membranes. Since CMC is highly hydrophilic and dissolves in cold water, it is quite leaky and brittle when formed into a membrane. To increase the mechanical strength, integration with PVA is carried out to obtain the optimum value of flexibility. PVA is employed as a polymer because of its high elasticity and biodegradability [1]. Integration of *N*-CMC with PVA will result in the formation of hydrogen bonds between *N*-CMC and PVA molecules via intermolecular reactions (Figure 4).

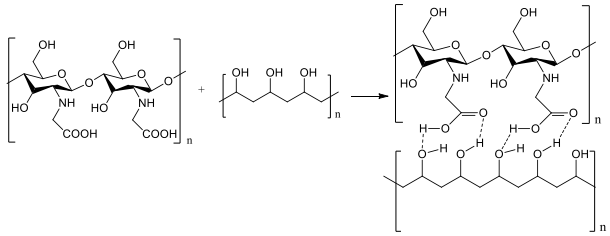

Figure 4. Hydrogen bonds between *N*-CMC and PVA

The optimum ratio of *N*-CMC to PVA was determined through a creatinine transport test at a concentration of 25 ppm for 6 hours. The integration of *N*-CMC and PVA increases the number of active groups

on the membrane, allowing it to attract creatinine molecules to the acceptor solution across the membrane. The results of the transport test showed that *N*-CMC/PVA2 was the best ratio that could transport 19.61% of the total creatinine used in the study. The optimum *N*-CMC/PVA membrane was then grafted using heparin.

3.3. The Grafting Reaction of *N*-CMC/PVA with Heparin

The grafting reaction was conducted by immersing the membrane in 100 IU heparin solution and 1% acetic acid catalyst. The graft reaction mechanism with heparin starts from the protonation of the amine group that does not bind to chloroacetic acid. The $-SO_3$ group in heparin will bind to the chitosan amine on the surface of the membrane [17]. Heparin can provide additional active sites on the surface of the membrane through the SO_3 group. Many oxygen atoms with high electronegativities can carry out hydrogen bonding reactions with creatinine [18]. It is hoped that an increase in the active site of the membrane can also increase the amount of membrane creatinine transport. It is necessary to characterize the functional groups using an FTIR spectrophotometer to determine the success of the heparin graft reaction.

After the graft reaction, the *N*-CMC/PVA spectra show a peak at wavenumber around 848 cm^{-1}, which was a $-SO_3$ group with low intensity (Figure 5). The $-SO_3$ group in heparin may be slightly grafted onto the surface of the membrane because most of the active groups of chitosan have been grafted with chloroacetic acid.

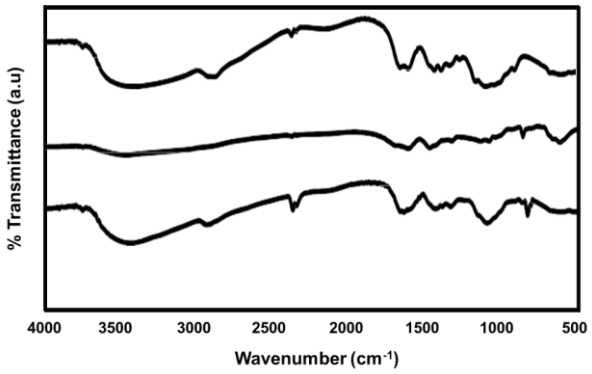

Figure 5. FTIR spectra of chitosan membrane, *N*-CMC, *N*-CMC/PVA.*g*.Hep

3.4. Membrane Characterization

3.4.1. Membrane Morphology

The surface morphologies of the *N*-CMC/PVA.*g*.Hep membrane before and after were determined using SEM analysis on the upper surface of the membrane. Figure 6 shows the surface morphology of the *N*-CMC/PVA.*g*.Hep membrane before transporting a mixture of creatinine and vitamin B12. The pores are shown to be evenly distributed throughout the membrane's surface. The pores in the membrane are formed due to the integration of *N*-CMC with PVA, which forms hydrogen bonds in the form of intermolecular reactions between *N*-CMC and PVA molecules, causing large distances and attractions.

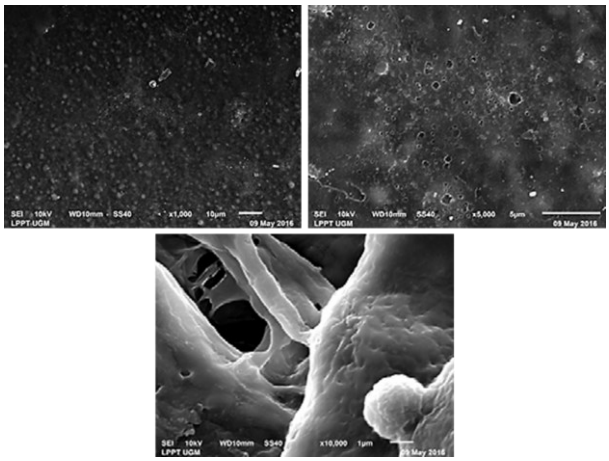

Figure 6. *N*-CMC/PVA.*g*.Hep before transporting a mixture of creatinine and vitamin B12

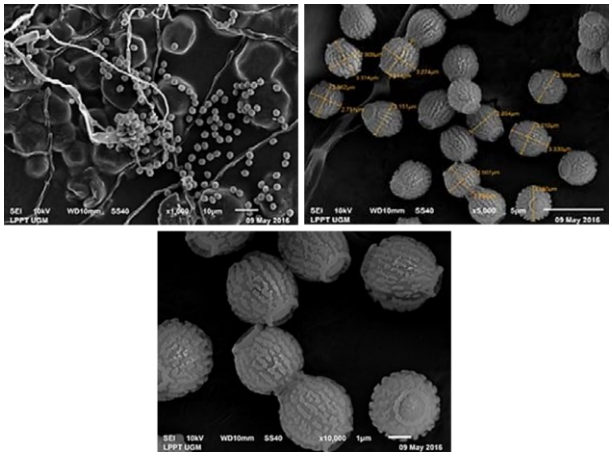

Figure 7. *N*-CMC/PVA.*g*.Hep after permeate transport (creatinine and vitamin B12)

Figure 7 shows a distinct difference between the membrane before and after it was employed to transport creatinine and vitamin B12. The surface of the membrane still has pores that are evenly distributed after being employed for transporting creatinine and vitamin B12; however, any molecules are attached to the surface of the membrane. The presence of a molecule attached to the surface is assumed to be a trapped vitamin B12 molecule since its size is insufficient to pass through the membrane pores and cover part of the membrane surface, causing reduced membrane function during transportation.

3.4.2. The Resistance of the Membrane to pH

The membrane for creatinine transport must be resistant to a wide range of pH. The pH resistance test was done by immersing the membrane in pH 3, 5, 7, 9, 11, and 13 solutions for 24 hours. The resistance to pH was assessed by measuring the weight loss before and after immersion. Based on Table 3, it can be seen that all membranes have decreased in weight. This demonstrates that the membrane is resistant to various pH conditions, including acidic, neutral, and alkaline pH; therefore, all membranes can be employed in various pH conditions.

Table 3. The results of membrane resistance to pH on various membranes

pH	Chitosan		*N*-CMC		*N*-CMC/PVA		*N*-CMC/PVA.*g*.Hep	
	Wo (g)	Wt (g)	Wo (g)	Wt (g)	Wo (g)	Wt (g)	Wo (g)	Wt (g)
3	0.0115	0.119	0.0256	0.0239	0.0472	0.0466	0.0334	0.0321
5	0.0158	0.0157	0.0221	0.0218	0.0510	0.0506	0.0579	0.0542
7	0.0255	0.0255	0.0076	0.0076	0.0657	0.0657	0.0398	0.0398
9	0.0268	0.0268	0.0133	0.0133	0.0566	0.0566	0.0345	0.0345
11	0.0228	0.0228	0.0738	0.0738	0.0445	0.0445	0.0337	0.0337
13	0.0240	0.0240	0.0163	0.0163	0.0528	0.0528	0.0261	0.0261

3.4.3. Water absorption test

The water absorption test aims to determine the ability of the membrane to absorb water and the hydrophilicity of the membrane. The water absorption of the membrane was determined by calculating the weight of the membrane before and after being immersed in water for 3 hours and 6 hours. All membranes calculated the percentage of water absorption. Based on Figure 8, there are differences in the percentage of absorption of water in various membranes. The chitosan membrane had the lowest water absorption value of 153.76%, *N*-CMC had a water absorption of 237.23%, *N*-CMC/PVA was 295.91%, while the *N*-CMC/PVA.*g*.Hep membrane decreased to 264.56 %. The highest percentage was achieved by the *N*-CMC/PVA membrane. The additional PVA increases the membrane's hydrophilicity because PVA contains several -OH groups that form hydrogen bonds with water molecules, allowing more water to diffuse across the membrane. The decrease in swelling in the *N*-CMC/PVA-g.Hep results from fewer hydroxyl groups that can bind to water, resulting in less water diffusing across the membrane.

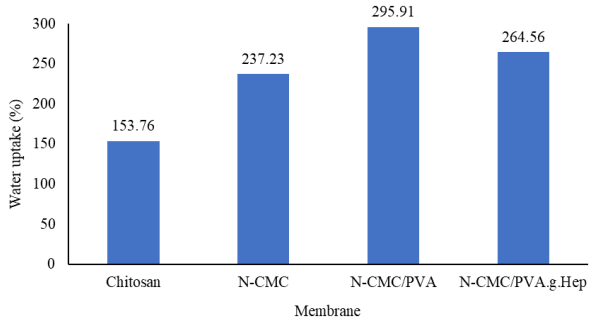

Figure 8. Graph of water absorption percentage on various membranes

3.4.4. Creatinine transport

The ability of the membrane to transport creatinine was carried out to determine the ability of the membranes to synthesize both chitosan, *N*-CMC/PVA, and *N*-CMC/PVA.*g*.Hep. Data from Figure 9 shows that the *N*-CMC/PVA.*g*.Hep membrane has the highest transport percentage of 24.81%, while the *N*-CMC/PVA membrane has a transport value of 19.61%. The significant increase showed that the graft reaction with chloroacetate combined with PVA and heparin grafting increased the active site of the membrane material so that it could transport greater creatinine into the acceptor phase.

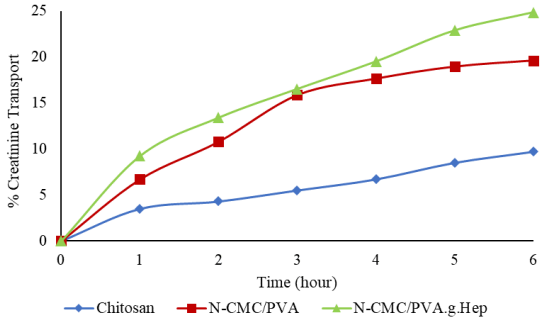

Figure 9. Graph of the percentage of creatinine transport

3.4.5. Effect of Vitamin B12 on Creatinine Transport

The membrane transport test of creatinine and vitamin B12 aims to determine the effect of another molecule (vitamin B12) on the ability of the membrane transport of creatinine. Vitamin B12 is a molecule found in the blood with creatinine. The molecular size of vitamin B12 is immense, with a molecular weight of 1355 g/mol (10 times the size of creatinine) [19], so it can block the process of creatinine transport.

Based on Figure 10 shows that the percentage of creatinine transport decreased in mixed transport. The percentage of creatinine transport became 16.53% from the previous 24.81%. The presence of vitamin B12 covering the membrane pores is one of the reasons for the decrease in the percentage of creatinine transport, with the large molecular size of vitamin B12 preventing creatinine from passing through the membrane pores. The existence of competition between the two molecules approaching the surface of the membrane results in collisions between the two molecules. The collision makes it difficult for creatinine to pass through the pore membrane, decreasing the transport process.

In mixed transport, there is no transport for vitamin B12, characterized by the absence of absorbance at a wavelength of 361 nm, which is the wavelength of vitamin B12. The size of vitamin B12 molecule that is too large cannot pass through the pore membrane, which is smaller compared to vitamin B12.

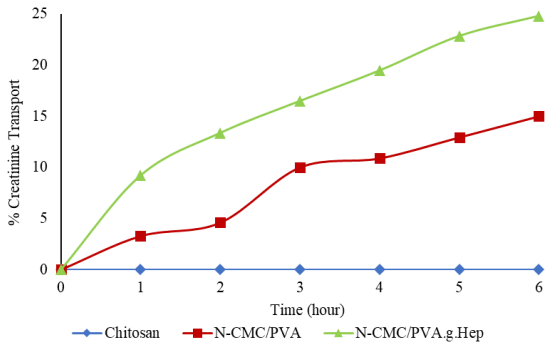

Figure 10. Graph of the percentage of vitamin B12 in creatinine transport

3.4.6. Viscosity test

The viscosity test aims to determine the molecular weight of the polymer synthesized by chitosan derivatives. The viscosity of the polymers *N*-CMC/PVA and *N*-CMC/PVA.*g*.Hep was measured using an Ostwald viscometer using five variations of polymer concentration. The results of the viscosity measurement can be seen in Table 4.

Table 4. Data on *N*-CMC and *N*-CMC/PVA viscosity measurements

Concentration (g/L)	t (s)	
	N-CMC	*N*-CMC/PVA
0	100.8	100.8
0.003	157.7	207.8
0.00375	180.2	245.1
0.005	200.6	280.7
0.0075	278.5	393.8
0.015	697.7	812.3

Based on Table 5, the *N*-CMC polymer has a molecular weight of Da (*N*-CMC/PVA). This shows that the number of polymer constituent components is directly proportional to the molecular weight value; the more polymer constituent components, the greater the molecular weight value.

Table 5. Molecular weight data of *N*-CMC and *N*-CMC/PVA polymers

Polymer	Molecular weight (Da)
N-CMC/PVA	448435.5146 Da
N-CMC	163066.816 Da

4. Conclusion

Chitosan derivative compounds were obtained through graft reactions with optimal chloroacetic acid and heparin. *N*-CMC/PVA.*g*.Hep membrane was obtained with a ratio of 1:1.5. Obtained information about the characteristics of the membrane, as follows: The membrane is resistant to various pH ratios. Integration of the membrane with PVA increases the tensile strength of the membrane. The ability to transport the membrane to creatinine was 24.81%.

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