# Modifying Surface Charge of Chitosan Membrane by *N,O*-Carboxymethyl chitosan Blended with Poly(vinylalcohol)

Retno Ariadi Lusiana, Dwi Siswanta, and Mudasir

***Abstract*---**Surface charge alteration were introduced to chitosan surfaces via carboxylation using monochloroacetic acid to produce N,O-carboxymethyl chitosan (N,O-CMC). The modified material should have carboxymethyl substituent on both the amine and hydroxyl groups, producing negatively charged carboxyl groups on the outer surface. Thus, it is also expected that protein adsorption on the membrane surface would be reduced due to electrical repulsion. Moreover, the ability of the resulted carboxyl group to form stronger hydrogen bonds should increase the membrane selectivity towards the target molecule. N,O-CMC was blended with PVA to improve the mechanical strength of the membranes, so that the membrane would have better permeability for low-molecular weight compounds. This novel membranes exhibited good permeability properties for small molecules as well as a decrease in protein adsorption on the membrane surface. The structure and the morphology of the resulting membranes were characterized by FTIR, NMR and Scanning Electron Microscopy (SEM).

***Keywords*--**Chitosan, Surface Charge, Carboxylation, Protein Adsorption, Permeability

## I. Introduction

PROTEIN adsorption on membrane's surface is a major problem when the membrane comes into contact with biological surroundings. It is impossible to remove protein adsorption completely but there are some strategies to decrease the adsorption on the surface of blood-contact membranes [7].

Chitosan have been suggested as materials for membrane due to their good solute permeability and mechanical strength. Chitosan has the ability to form thin film membranes with strong mechanical properties [2]. However, the existing amine and hydroxyl groups in chitosan are not strong enough for the interaction with target molecule. Furthermore, the use of unmodified chitosan can trigger protein adsorption on the membrane surface.

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This is because in acidic condition needed to prepare the chitosan membranes, the amine group on the chitosan backbone will be protonated to form positive charge on the surface, which in turn will lead to further interaction with the negative charge of the protein, hence the protein adsorption occurs. Heparin that has negative charges of sulfonate or sulfate groups, has been suggested as an effective way to reduce protein adsorption by chitosan [2,3].

Several routes can be used to change the surface of chitosan, the key purpose of which is to alter the chemical composition and the surface properties of chitosan to suit specific application [3,7]. For the purpose of this research, the modification of chitosan was conducted especially to prevent the interaction between protein and the membrane surface, so that the surface no longer exhibits positive charge. This was achieved by substituting amino groups of chitosan with carboxylic groups under homogeneous conditions [1,5]. Therefore, in our research, we attempted to prepare hemodialysis membrane using carboxylate substitution on amino groups of chitosan backbone to make *N,O*-CMC.

The material was prepared by carboxylation of the chitosan and the modified material should have carboxymethyl substituent on the amine, producing negatively charged carboxyl groups on the outer surface. Thus, it was also expected that protein adsorption on the membrane surface would be reduced due to electrical repulsion. Moreover, the ability of the resulted carboxyl group to form stronger hydrogen bonds should increase the membrane selectivity towards the target molecule [7].

The objective of the present work is to study the mechanism of small-molecule transport across N,O-CMC matrices and to determine the effects of surface modification through grafting reaction upon the permeation characteristics. In order to produce an appropriate proportion of hydrophobic and hydrophilic as well as mechanical properties, N,O-CMC was blended with compatible synthetic polymers poly(vinyl alcohol)(PVA) [3,10]. Urea, creatinine, vitamin B12 and albumin were used as the model compounds in the study. The structure, morphology, and mechanical properties of membranes were also investigated.

## II. Experimental

### A. Materials

Chitosan with deacetylation >85% was obtained from Seafresh Chitosan Co, Cirebon (Indonesia). PVA (weight-average molecular weight = 72,000 g/mol) provided by Aldrich Chemical Co. (St. Louis,MO) as the membrane matrix. Chloroacetic acid, sodium hydroxide (NaOH), acetic acid ($CH_3COOH$), isopropanol, ethanol provided by Merck Co. Urea was purchased from Sigma Chemical Co (USA).

### B. Synthesis of N,O-CMC

Carboxymethylation reaction of chitosan was done by adding NaOH using water/isopropyl alcohol as solvent. Purified chitosan (3 g) was dispersed in 65 mL of isopropanol. After 20 minutes of magnetic stirring at warm temperature, 20.4 g of aqueous NaOH and 14,4 g monochloroacetic acid/isopropanol solution were added to the suspension at 60-80 ºC for 20 h. The solid product were then filtered, suspended in 150 mL of ethanol and neutralized with glacial acetic acid. The product was then washed with 70% ethanol and dried at room temperature for 24 h.

### C. Preparation N,O-CMC/PVA film

The copolymer were dissolved in 3% aqeous acetic acid (100 mL) with continuous stirring for 20 h. 1.5 wt. % PVA solutions were prepared by dissolving PVA flakes in 80 ºC distilled water with stirring for 2-4 h. Then the mixture of copolymer and PVA solution were stirred for 24 h and poured into petri-dish at room temperature, heated at 50-60 ºC for 24 h. After heating treatment, the membranes were soaked in 2% NaOH(aq). The membrane was removed from plate and extensively washed using distilled water to remove residual sodium hydroxide.

### D. Analytical characterization

The physicochemical characterizations of *N,O*-CMC/PVA membranes were used by using FTIR spectrometry, NMR spectroscopy. The surface morphology of membrane was studied using SEM, (Hitachi U5800).

### E. Permeability test for the membranes

A dialysis chamber was used to control the permeability of membranes to various compounds, as a function of time at room temperature. The membrane was clamped between the chambers. One chamber was filled with 50 mL of phosphat buffer solution at pH 7.4, and the other was filled with a mixture of solutes containing urea (500 mg/L), creatinine (15 mg/L, Mw, 113), vitamin B12(20 mg/L, Mw 1113), albumin (1000 mg/L, Mw, 69,000) in 50 mL phosphate buffer at pH 7.4. The concentrations of solutes passing through the membrane were analyzed with a Shimadzu UV/VIS spectrophotometer at intervals of 0, 2, 4, 6, 8, 10 and 24 h, using p-dimethylaminobenzaldehyde reagent for urea, picric acid dissolved in alkaline solution for creatinine, and the Bradford method for Albumin. The percentages permeability were calculated using eq.

Percentage permeated at time t = Ct/C0 x 100

Where Ct is the concentration of the solute in the receiving cell diffused at time t; Co is the initial concentration of the solute in the feed cell and t is the time in minutes.

## III. Results And Discussion

### A. Synthesis of N,O-CMC

In this study, we have carried out the synthesis CMC by direct alkylation of chitosan with monochloroacetic acid at 40% NaOH and temperatures of arround 60 ºC. In highly alkaline medium however, both the amine and the hydroxyl groups are activated, hence *N,O*-CMC is formed. The reaction is in accordance with those published in literatures [1,5,9].

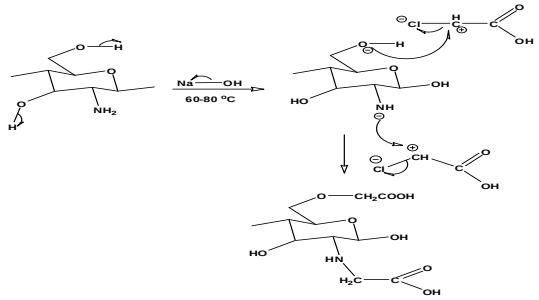


Fig. 1 Synthesis of carboxymethyl chitosan

### B. FTIR spectroscopy

The structural differences between chitosan and *N,O*-CMC was confirmated by FTIR spectroscopy (Fig.2). The basic characteristic peaks of chitosan were (1) strong and broad band due to the axial stretching of O-H and N-H bond at 3400 $cm^{-1}$ (2) splitting band at 1590-1650 $cm^{-1}$ corresponding to N-H primary bend (3) a band at 1378-1350 $cm^{-1}$ contribute to the stretching and bending vibrations of the C-N bond of the amide (3 band) (4) a band at 900 $cm^{-1}$ due to C-O stretch.

The spectrum of *N,O*-CMC can be shown in Fig.2, the broader band centered at 3400 $cm^{-1}$ shown the more hydrophilic character of CMC as compared to the chitosan. The strength band at 1635 $cm^{-1}$ indicating that the most of the primary amine had been changed to secondary amide. Indicating that amina gorup substituted by carboxymethyl groups. And the strength of the peak at 1411 $cm^{-1}$ related to the symmetrical vibration of –COO. The peak at 1310 $cm^{-1}$ indicated to the extension vibration of C-O is greatly increased. The strength peak at 1153-897 $cm^{-1}$ contributes the existence of a C-O-C and –C-O bond. The weak peak at 873 $cm^{-1}$ was demonstrating that the carboxymethylation happen at the amino group of chitosan. The absorption bands of N,O-CMC at 873 cm-1 suggests that a hydrogen atom of the $–NH_2$ group have been substituted by carboxymethyl groups. Strong peaks at 1071 and 1156 cm-1 were caused by the presence of a C-O-C bond, indicating that the hydroxyl groups of the chitosan were also substitued by the carboxymethyl group, *N,O*-CMC have been produced [9].

### *C. $H^1$ NMR*

The structural modifications induced by the carboxymethylation were observed by the H¹NMR spectrums of sample CMC (Figure 3). The H¹NMR spectrums at 400 MHz are reported for CMC in $D_2O/CD_3COOD$. The signals at 5.18 ppm were assigned to the hydrogen of C1 and substituted carboxymethyl-proton- (--OCH2COOD) of chitosan occured in the region of 4.60-4.80 ppm. The signals observed between 4.00 and 4.20 ppm corresponds to the hydrogen to the C3 glucosamine ring, while the signals between 3.50 and 3.80 ppm corresponds to hydrogen bonded to the carbon atoms C4,C5 of the glucopyranose that are overlaped. Signals observed between 3.00 and 3.25 ppm corresponds to carboxymehyl group substituted on –NH2. The signals in this region denoted occurence of N-carboxymethylation .

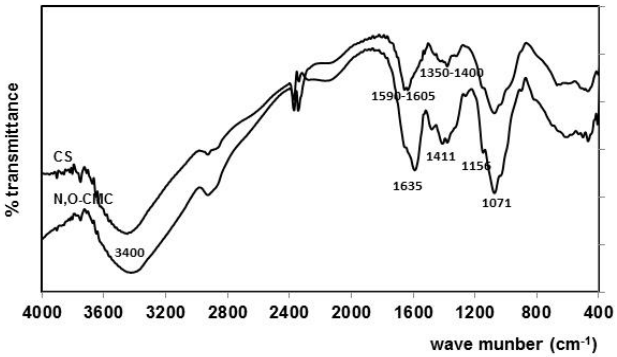


Fig. 2 FT-IR spectrum of membranes

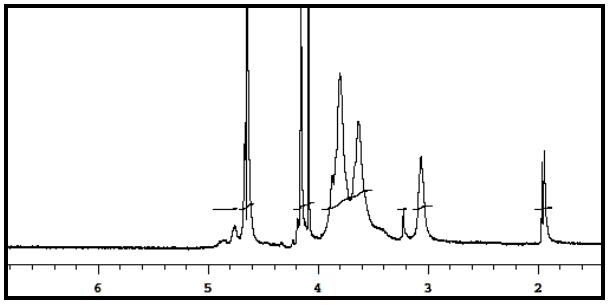


Fig. 3 H-NMR N,O-CMC in D2O/HCl

### *D. Water Uptake*

The hydrophilicity of the copolymers is measured by studying their water absorption capacity. It was informed that the chitosan is hydrophobic in nature and its swelling index at pH 7,4 is very low [4,6]. On grafting the chitosan with carboxyl groups was estimated to increase hydrophilicity, which is confirmed by our results. As shown in Fig. 4 water uptake CMC was the higher than chitosan. The higher capacity of CMC to adsorb water compared to the chitosan are indicated to the higher hydrophilicity of CMC. The presence of a large number of carboxyl groups in chitosan result in strong hydrogen bonding (may be both intermolecular and intramolecular types), which in turn affect the solubility of CMC in water. Water uptake of sample were decrease by addition of PVA.

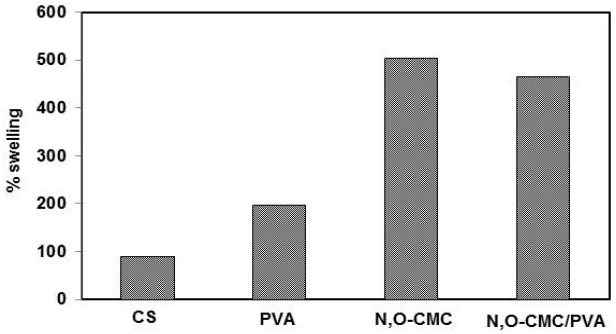


Fig. 4 Water uptake of various membranes

### *E.Permeation studies*

A normal hemodialysis proccess currently takes 5 hour for patients with kidney failure. Therefore, in this research, data collection was carried out for 1-24 h. The permeatibility of urea, creatinine, vitamin $B_{12}$ and albumin through *N,O*-CMC membrane were studied in 0,1 M phosphate buffer solution (pH 7.4) at room temperature. In this study, the concentrations of solutes passing through the membrane were analyzed with a Shimadzu UV/VIS spectrophotometer at intervals of 0, 2, 4, 6, 8, 10 and 24 hours.

The first study of this transport is testing the validity of the method that used. Figure 5 shows that the increasing urea and creatinine percentage transport in the acceptor phase in equivalent with the decrease of the percentage of transport in source phase.

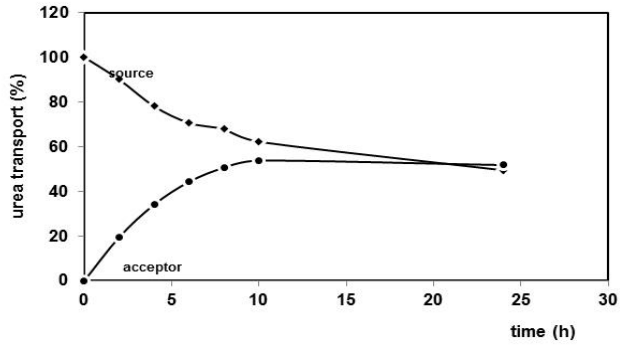


Fig. 5 Transport percentage data for concentration of urea in both source and acceptor phase of the membrane

The effect of vitamin $B_{12}$ and albumin in the transport of urea and creatinine in this research was studied by 3 different source phase content of compounds: 1) feed only contains urea or creatinine 2) feed containing urea, creatinine and vitamin $B_{12}$ 3) feed containing urea , creatinine, vitamin $B_{12,}$ and albumin. The transport was done for 24 hours using a membrane and the results can be seen in Figure 6.

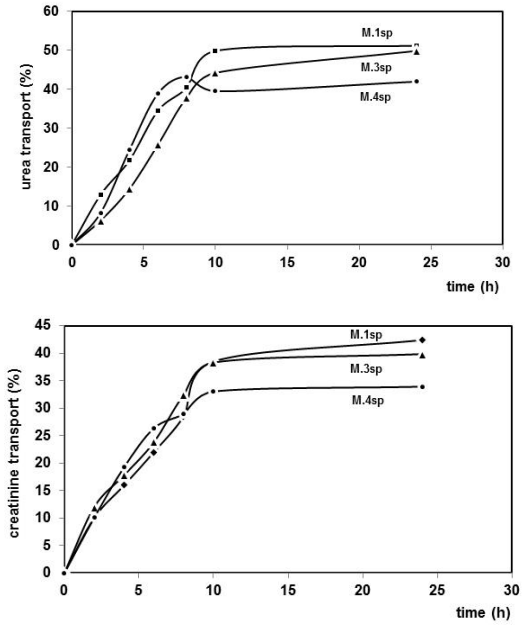


Fig. 6 Effect of vitamin $B_{12}$ and albumin in urea and creatinine transport of membrane

As seen as Fig. 6 the transport percentages of creatinine and urea transport for the three species mixtures (M.3sp) and four species mixtures (M.4sp) are lower than when urea and creatinine were transported individually (M.1sp). The transport of metabolites for four species look like lower thanthe others. The transport percentage of creatinine decreased from 42.39% to 39.83%, while transport percentage of urea decreased from 51.8% to 49.77% for three species micture in the sorce phase. And lower to 33.9% for creatinine percentage and 47.7% for urea percentage when four species mixture in source phase.

Vitamin $B_{12}$ and albumin are two of the molecules presents in blood together with creatinine and urea. Having a molecular weight of 1355.37 g/mol and 69.000 g/mol, the molecular size of vitamin $B_{12}$ and albumin are so large that it hinders the transport processes of creatinine and urea [10]. When mixed together, the much large molecular size of vitamin $B_{12}$ and albumin seems to have a little influence on the transport percentage of the other two metabolites. In addition, Figure 7 shows that there did not seem to be any transport of vitamin $B_{12}$ or albumin through the membrane, as pointed out by the almost zero absorbance of vitamin $B_{12}$ at 360 nm and albumin at 535 nm during UV-Vis analysis. It seems that the molecular size of vitamin $B_{12}$ and albumin are too large to pass through the membrane pores.

Figure 6 shows that the longer transport time, percentage of urea and creatinine transport is increasing. It is related to the contact time between the membrane with a solution of the compound. The longer the contact time between the membrane with a solution, the more target molecules that can diffuse and captured by the active group. Transport of metabolites appear to be fast since the beginning of the process up to 10 hours, then slowed down and is stable up to 24 hours. At 10-24 h the equilibrium state is reached, where the number of compounds not increased. Thus, it is estimated that the time for the transport membrane equilibrium is reached at the $10^{th}$ hour.

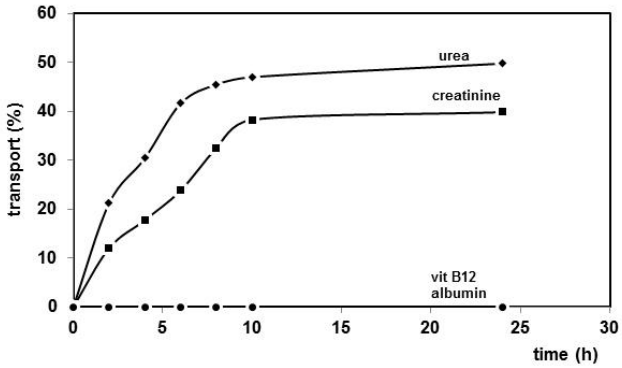


Fig. 7 Permeation of metabolites through N,O-CMC membranes.

Additionally, the presence of vitamin $B_{12}$ and albumin in the source phase reduces the percentage of creatinine and urea transport. With very large molecular size, albumin might hinder creatinine and urea to enter the membrane pores. The competition of the four metabolites to approach the membrane surface may also cause additional collision between the molecules, so that it is difficult for creatinine and urea to enter the membrane pores which lead to so longer transport time [11,12].

Albumin has a negative charge at pH 7.4. The negative charge will pull the positive charge of the amine group, which has not been completely substituted at CMC polymer. This causes the formation of albumin adsorption on the membrane surface [7]. Protein adsorption on the membrane is a major problem, because it causes a decrease in the speed of transport and membrane selectivity.

Urea, due to its lowest molecular weight, has the fastest dialysis rates than does any other metabolite trough all the membranes (Fig. 6) [8].

*F. Surface morphology of the film*

The SEM images at 400x magnificatian in Figure 8 show that the *N,O*-CMC films before used and after used in the transport process. It appears there a small part of the membrane surface covered by vitamin B12 and albumin. Fouling is probably due to interaction between the positively charge amine groups of chitosan with the negatively charged of protein. From these data it can be said that the charge changes from positive to negative charge of CMC makes only a litlle surface of membrane that covered by albumin.

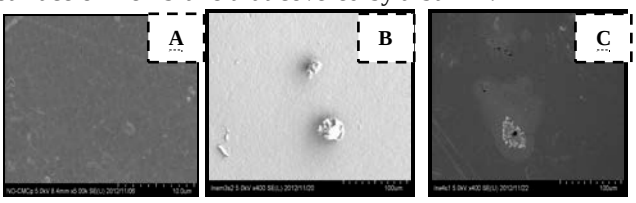


Fig. 8 SEM image of membrane mag. 400 x (A) before used (B) after used for 3 sp (C) after used for 4 sp

## IV. Conclusion

Biocompatible and mechanically stable membranes could be produced from CMC. The permeability of the membranes for the urea was found to be uuper than creatinine. The negative charge of carboxyl groups was expected to be able to reduce the adsorption of proteins on the membranes surface. In addition, the ability of the carboxyl group to form hydrogen bonds would increase the membranes selectivity towards the target molecule.

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