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PREPARATION AND CHARACTERIZATION OF ANTIBACTERIAL FILM BASED ON CARBOXYMETHYLCELLULOSE FROM GEBANG LEAF (*Coryphautan*), POLYVINYL ALCOHOL ANDCITRIC ACID

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ABSTRACT

Cellulose extracted from gebang (*Corypha utan*) leaves was characterized and used as a raw material for preparation of carboxymethyl cellulose which further used for preparing a film. Carboxymethyl Cellulose/polyvinyl alcohol films were prepared by the crosslinking method. The films were chemically crosslinked with citric acid for tuning their properties.The results indicate that the hydrogen bond interactions between carboxymethylcellulose/polyvinyl alcohol and citric acid can be formed. The films demonstrated excellent antibacterial effects against *Escherichiacoli* and *Staphylococcusaureus*. Increase the addition of citric acid can efficiently reduce the water absorption of the films. Moreover, the films present three-dimensional structure, porous networks and low toxicity. Therefore, the developed carboxymethyl cellulose/polyvinyl alcohol/citric acid film can be well-suited for biomedical application.

Keywords: Gebang,Carboxymethyl Cellulose, Polyvinyl Alcohol, Citric Acid, CrosslinkingMethod

INTRODUCTION

The largest and outermost organ covering the entire body that plays like a protective barrier against external insults is the skin[1].it is also concerned in immunological monitoring, vitamin D3 synthesis, protection of organisms against toxins and microorganisms, regulating body temperature, supporting blood vessels and nerves, and preventing dehydration of the body[3]. However, its structure and function performed by complex organs can be influenced by severe injuries[4].Ineffectivetreatment will facilitate bacterial contamination andposses difficulty to form as well as further trigger skin infections[5].In the early stages of the infection, the main involved organisms are gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*, while *Escherichia coli* and *Pseudomonas aeruginosa*as gram-negative bacteria are just obtained in the final phase during developed chronic wounds[8].

The most common types of infections are skin and soft tissue infections (SSTIs). In uncomplicated SSTIs, skin exhibits good self-regenerative[9]. However, Complicated SSTIs extending to subcutaneous tissue, fascia, or muscle[10] require complex treatment combining carefullyselective antimicrobial.In order to the rapid healing process, promising wound dressingmaterial whichis able tohinder both the penetration of bacteria and the growth of microorganisms is needed. Recently, researchers have developed modern polymer film-based biomaterials derived from various natural and synthetic biodegradable materials. The combination of both polymers loaded with antimicrobial drugs is often preferred.

Polyvinyl alcohol(PVA), the biodegradable synthetic polymer,has easy and excellent film-forming properties due to its good solubility, non-toxic and biocompatible properties[12].

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PVA has been applied in several advanced biomedical practices such as injectable bone substitute[2], biocomposite films[6], drug delivery,artificial organs and contact lenses[11].The polymeric material combined with PVA is to improve the mechanical and physicochemical properties of the material[12].

Carboxymethyl cellulose (CMC), a natural polyelectrolyte polymer,iscapable to absorb a certain amount of water and can retain moisture[13]. Due to its sensitivity to variations in pH and ionic strength, CMC is also called a "smart" cellulose derivative[13]. CMC has electrostatic charges connected to the network, thus having a double effect on swelling power capability[13]. In addition, CMC has many advantages such as water-soluble, easily degraded, a good degree of substitution (DS) and low viscosity[14]. CMC is one cellulose ether derivative with carboxymethyl (-CH_2COOH) substituents[15]. Substitution of carboxymethyl groups to hydroxyl groups is more dominant in the C-2 glucose position[16]. CMC has been widely used in the medical field because it is very good for wound dressing. In order to improve mechanical properties[17]and possess well-defined chemical structures, CMC is suitable to be combined with synthetic polymers like PVA.

CMC can be synthesized from various natural cellulose sources such as fibers, plant wastes, bacteria and various sources of plant cellulose. Gebang (*Coryphautan*) is a wild plant, has a high size, and lives in lowland areas[18]. Belonging to a family of *Arecaceae*, this palm tree,has a height of 30 m and age of 25-30 years old[18]. uniquely, gebang only blooms and produces fruit once in a lifetime[18]. The flowers produced are around 3-15 thousand flowers while the fruit produced is around 240,000-355,000 in each tree[18]. Therefore, the Gebang plant is also referred to as The Mount Everest of the Palm World[18]. Despite long growth, the distribution of gebang plants in Indonesia is still extensive and quite large[19]. Gebang has many benefits including Leaf parts as a traditional mat, traditional salak fruit packaging, traditional Indonesiandodol packaging, midrib as adhesive for building materials, gebang fruit as an anti-cancer agent[20], shipping materials reinforcement[21] and rubber filler and reinforcement[22].The obtained Cellulose content of gebang leaves is approximately 64%. The high cellulose content of gebang leaves is very promising to be used as a source of cellulose and its derivatives.

Preparation and mixing of CMC/PVAcanprovide high elasticity and good mechanical properties[23]. Comparison of the exact composition of both polymerscan produce mechanical properties of material similar to human tissue. To overcome long-term structural and mixed mechanical stability, several crosslinking methods have been made, namely physical, chemical and radiation[24]. The manufacture of the film has undergone new developments and techniques, but more chemically crosslinking methods have been chosen because they produce stable hydrogel structures and good swelling properties[25]. Frequently used crosslinker reagents such as glutaraldehyde, epichlorohydrin, boric acid and sodium trimethaphosphate cause health problems due to their toxicity[26].

Citric acid (CA) has been successfully used as a crosslinking agent for cellulose derivatives because it is non-toxic, easilydegraded, and good environmental[7,27]. CA is one of the poly carboxylicacids and as the primary organic acid in citrus fruits[28]. CA can be produced through glucose fermentation by *Aspergillusniger* and *Yarrowialipolytica* fungi[28]. CA has three carboxylic groups which facilitate tissue formation with cellulose and its derivatives, soCA is very well used as a promising crosslinking agent due to biocompatible and economical properties[29]. In addition, CA has powerful antimicrobial properties[30]. CA is the second most effective acid among several organic acids that can inhibit the growth of *S. aureus* in the health and food fields[31]. Combination of CA and natural polymers such as chitosan was able to significantly reduce the growth of negative *E. coli* bacteria[32]. Therefore, the addition of CA to CMC/PVA film can play a role as a crosslinking and antibacterial agent.

Synthesis CMC from cellulose pulp extracted from gebang leaf was the aim of the study. Then, Preparation CMC/PVA/CA film was carried out by the chemically crosslinking method. The structure, crosslinking degree and antibacterial properties of the film were also investigated.

EXPERIMENTAL

Material and Methods

Gebang leaves (*Coryphautan*) were obtained from North Padang Lawas, North Sumatera Province, Indonesia. Polyvinyl alcohol (PVA) with a 98% degree of hydrolysis, Sodium Monochloro acetic (NaMCA), monoanhydrate citric acid (CA) were purchased from Merck, Germany. Acetic acid, ethanol,

methanol, chloric acid, nitrate acid, acetone and other solvents used were of analytical grade and without further purification. Bacterial strains of *E. coli* and *S.aureus* were provided from microbiological laboratory at department biology, University of Sumatera Utara.

Isolation and Purification of Cellulose

Gebangleaves were washed with water and sun-dried for 4 days. its stick was removed from leaf and cut into small pieces about 1 cm. 75 g of product was put into beaker glass and soaked in 1 liter of 3,5% HNO_3 and 10 mg $NaNO_2$. The mixture was heated and stirred at 90 ^{0}C for 2 h. The obtained dark brown slurry was filtered and washed with water. The dark yellow product was cooked and stirred in 375 ml of 2% NaOH and 375 ml of 2% Na_2SO_3 at 50 ^{0}C for 60 minutes. The solid residue was filtrated and cleaned thoroughly with water. The crude cellulose was bleached with 500 ml of 10% H_2O_2 at 60 ^{0}C for 45 minutes. The filtrate was removed and the residue was rinsed with water. The bleached cellulose was dried at 60 ^{0}C. Dried cellulose was grounded with a blender to obtain its powder.

Synthesis of Carboxymethyl Cellulose (CMC) from Cellulose of Gebang Leaf

CMC was synthesized according to the optimized condition procedure performed by Golbaghi et al[33]. one gram of obtained cellulose powder was immediately immersedin 20 ml isopropanol. In order to form alkali cellulose, 4 ml of 28.4% NaOH solution was dropwisely offered to the solution and stirred for 1.5 h at 25 ^{0}C. Then, 1.14 g MCA was dissolved in 20 ml isopropanol and the solution was added gradually to the prior mixture under stirring for 15 min. The temperature of reaction slightly set at 57.85 ^{0}C for the reaction times of 4.01 h. Water from a constant temperature bath, with an approximate ±2 ^{0}C tolerance, was circulated through the jacket of the reaction vessel to hold the temperature constant during the carboxymethylation process. The mixture was filtered and the obtained CMC was poured into 40 ml absolute methanolunder stirring to dissolve byproduct. After a few minutes, pure acetic acid was provided to complete neutralization. Next, the mixture was refined and rinsed four times with 40 ml of 70% ethanol and continued washing with 40 ml absolute methanol for abolishingunwanted salts. Finally, the CMC was dried by exposing it to the blowing air followed by putting it in the oven at 60 ^{0}C for 12 h.

Preparation of CMC Film

A CMC hydrogel film was prepared by dissolving 1.8 g of CMC in 100 ml distilled water under magnetic stirring at room temperature for 30 minutes. Then 0.2 g PVA was added to CMC solution under stirring. After the mixture was stirred at 50 ^{0}C for 4 h and complete solubilization was occurred, the crosslinking agent CA was added under stirring at concentrations of10% (CMC/PVA/CA10) and 15% (CMC/PVA/CA15) m/m % of CMC+PVA polymer and homogenized for 20 minutes. Afterward, 10 ml of the solutions were cast in a petri dish (60 mm diameter) and allowed to dry at 40 ± 2 ^{0}C for 24 h to remove water. In the sequence, the samples were kept at 80 ± 2 ^{0}C for 24 h for the crosslinking reaction (slow evaporation method). As a reference, a sample without CA (CMC/CA0) was also prepared and dried following the same thermal treatment.

Yield of CMC production

In this research, determination of the yield CMC was performed the following equationRachtanapun*et al*[34]:

$$\text{Yield of CMC (\%)} = \frac{\text{weight of dry CMC}}{\text{Weight of dry cellulose}} \times 100 \quad (1)$$

The degree of Substitution (DS)

The DS of CMC was introduced by standard test method[51]. 4 g of CMC was treated with 75 ml of 95% ethanol under stirring for 5 min. In order to agitate, 5 ml of 2M HNO_3 was gained to the mixture. The obtained slurry was placed into hotplate for 2 min. Thereafter the solution was taken up and stirred for 10 min. Next, these were filtered and rinsed three times with 80% ethanol at room temperature. The obtained precipitate was washed with a 50 ml anhydrous methanol. After alcohol was completely removed, the filter was dried at 105 ^{0}C for 3 h and cooled in a desiccator for 0,5 h. The final dry CMC was added

with100 ml of water and 25 ml of 0.3 N NaOH solution under stirring. Then, once the solution was hot, it was placed into hotplate to boil for 15-20 min. The last phase, the mixture was treated by titration with 0.3 N HCl to a phenolphthalein end point.Finally, The DS of CMC haspresented the following formula:

$$DS = \frac{0.162A}{1-0.058A} \quad (2)$$

$$A = \frac{(BC-DE)}{F} \quad (3)$$

Where:
A: the obtained weight of alkali per gram of sample
B: the amount of NaOH solution (ml)
C: the normality of NaOH solution
D: the amount of HCl solution (ml)
E: the normality of HCl solution
F: the weight of the sample (gr)

Water Absorption of CMC Films

The films were divided into 5 × 5 mm^2 samples, dried at 40 ± 2 ˚C and weighted (W_1, initial mass). Then, the film (triplicates, n=3) were placed in 70 mL sample pots with 10 mL distilled water at RT. After 72 hours, the filmwas removed from the solution, gently wiped with filter paper to remove excess of liquid on the sample surface and weighted (W_2). Weight measurements obtained in each step of the process were used to calculate the absorption water of the films using Equations below:

$$\text{Water Absorption } (\%) = \frac{W2 - W1}{W1} \text{ x } 100\% \quad (4)$$

Degree of Crosslinking

To determine the degree of crosslinking quantitatively, the content of the filmwas explored. The gel content of the film was evaluated by extraction of the dried film (Wd) in hot distilled water at 100 ^{0}C for 36 h and dried at 50 ^{0}C for 4 h until they reached constant weight (We). The gel yield of the filmwas determined as follows :

$$\text{Gel } \% = (\frac{We}{Wd}) \text{ x } 100 \quad (5)$$

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectra of extracted cellulose, CMC, PVA and CMC film samples were recorded with Shimadzu IR Prestige-21 spectrophotometer. Pellets were made from each sample and KBr with a ratio of around 0.05 (g/g). Transmission levels were measured at wave number 500–4000 cm^{-1}.

X-Ray Diffractometry (XRD)

Diffraction diagrams of extracted cellulose, CMC, PVA and CMC film samples were analyzed using X-ray diffraction with a Shimadzu XRD-6100 Diffractometer operating at 40kV and 30 mA with Ni-filtered Cukα radiation. Diffraction patterns were recorded at a 2θ range of 7^0 - 70^0 with a scan rate of 2^0/min.

Scanning Electron Microscopy (SEM)

The surface morphologies of films were investigated using a scanning electron microscope CoxemBenchtop EM 30 AX Plus ,South Korea. The scanned surfaces of dried resin were coated with a thin gold layer to avoid charging under the electron beam. SEM photographs were taken at different magnification in the range of 50×, 100×, 250×, 500×, 1000× and 2000×.

Antibacterial Activity

Antibacterial nature of film having different amounts of CA was determined by test method AATCC 100-1998. The antibacterial activity was evaluated by a zone of inhibition and colony count methodagainst gram-positive bacteria, *S. aureus*and gram-negative bacteria, *E. coli.* In order to evaluate inhibition zone, the colonies of *S. aureus*(ATCC 25923) and *E. coli* (ATCC 35218) obtained from an overnight culture, were treated in Muller Hinton Broth (MHB) to form suspension and turbidity was set as 0.5 McFarland standards. 200 μL of the suspension was placed into Muller Hinton Agar (MHA) plates to produce a semi-confluent growth. The other membranes were located in inoculated medium and the plates were incubated for 24 h at 37 ^{0}C. finally, inhibition zones were observed the next day.

To determine the colony count method, *E. coli* (ATCC 11105) was for the testing bacterium. 105 CFU/ml was a Mother culture. In this procedure, the Luria/Nutrient broth solution was producedfrom bacteria inoculation with test and control samples in it in separate containers.

Sterilization was treated in an autoclave for 20 min at 121 ^{0}C at 15 lb pressure. Containers with nutrient broth, samples and bacteria culture were shaken at 200 rpm at 37 ^{0}C for 24 h. Agar plates were prepared by pouring the required quantity of a sterilized mixture of nutrient/luria agar and agar in the Petri dishes and allowing them to get solidified. After serial dilution, growth liquid was spread on the surface of solidified agar. All the Petri dishes were incubated thereafter at 37 ^{0}C for 24 h. The antimicrobial activity of the film was calculated.

RESULTS AND DISCUSSION

The degree of Substitution (DS) and Yield Percentageof CMC

The yield percentage of cellulose production from dried gebang leaf was found to be 64 %. Synthesis CMC was carried out by two stages. these were alkalization andcarboxymethylation using sodium monochloroacetate (NaMCA). The DS values were usually rangedfrom 0.4-1.3[36]. The solubility degree of CMC was based on substitution of a carboxymethylgroup in cellulose structure. According to the optimization of CMC production condition reported by Golbaghi et al, the obtained CMC from gebang leaf had the DS of 1.2 and the yield of 166%. Based onSjostrom, OH group of cellulose was substituted by carboxymethyl group in C2 position of cellulose structure due to higher acidity and dissociation value of OH group.

Degree of Crosslinking

The effect of the concentration of CA on the crosslinking percentage is increased. It was found that the percentage crosslinking of CMC/PVA/CA10% and CMC/PVA/CA15% is 9 % and 11%, respectively. With an increased concentration of CA, more monomer radicals may be available to interact with CMC macroradicals, which increases the percentage of crosslinking. Ester bonds, as a result of crosslinking reaction, was more potential formed in C6 position of cellulose chain due to higher reactivity of its OH group to esterification[38]. A possible reaction of film formation was shown in Fig.-1.

Water Absorption of CMC/PVA/CA Film

The effects of the concentration of CA on the water absorption of the film prepared from CMC/PVA are examined based on weight. The water absorption of the filmdecreases from 50% to 35%. The water absorption of film decreased with an increased concentration of CA. This was due to the increased cross-linked density with the increase in the concentration of CA. Therefore, the chain segments of the polymer chain shortened with increased cross-linking of the chain and reduced the water absorption values of the film because limited space was available for free water to enter into the vacant spaces of the cross-linked network[39].

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

According to figure 3, the frequency of absorption bands of cellulose and carboxymethyl cellulose were similar. It isindicated that both of them have similaritiesin functional groups.The broad peak at 3300-3450 cm^{-1} is presented by O-H stretching.Saturated aliphatic C-H group from cellulose chain appears at 2900-3000 cm^{-1}[40]. The band around 1060 cm^{-1} is due to C-O-C stretching[41]. In spectra of obtained cellulose, the band around 1635.65 cm^{-1} is related to O-H bond of cellulose structure. However, in spectra of CMC,

these peaks were completely removed. On the other hand, the new peak at 1604.77 cm^{-1} is assigned to stretching vibration of the carbonyl group from COO-. It is indicated that the O-H group of cellulose chain is successfully altered by carboxymethyl group[42]. The small peaks detected at 894.97 cm^{-1} were confirmed to β 1-4 glycoside bonds[43]. Absorptions differed from cellulose spectra at 1604.77 and 1419.61 cm^{-1} are related to C=O stretching and $–CH_2$, respectively. Based on Mario et al report, the existence of the carbonyl group and it salt that assigned to carboxymethyl group was observed at 1600-1640 cm^{-1} and 1400-1450 cm^{-1}. Therefore, this result confirmed that CMC could be synthesized from the cellulose of gebang leaf.

The FTIR spectrum of PVA also presented characteristic broad bands for hydroxyl groups at 3387 cm^{-1}, for the stretching vibration of C-H bonds at 2939 cm^{-1} and for stretching vibration of C-O bonds at 1419 and 1096 cm^{-1}. Other small peaks at 918.12 cm^{-1} and 848.68 cm^{-1} are related to the existence of –OH bonds dan –CH bending. These bands were previously reported by Silverstein et al[44].The decrease of the intensity of OH bands at 3400-3200 cm^{-1} that related to form hydrogen bond and the reference peaks at approximately 896 cm^{-1} of β 1-4 glycoside bonds were to confirm crosslinked cellulose. FT-IR Spectra of CMC/PVA/CA10% and CMC/PVA/CA15% films showed that hydroxyl groups of CMC possesseddepletion at approximately 3448.72 cm^{-1}during the crosslinking reaction. It was happened due to the chemical reaction with citric acid to form ester bonds.In addition, there were new peaks observed at 1720.50 cm^{-1} and 1205-1230 cm^{-1}.

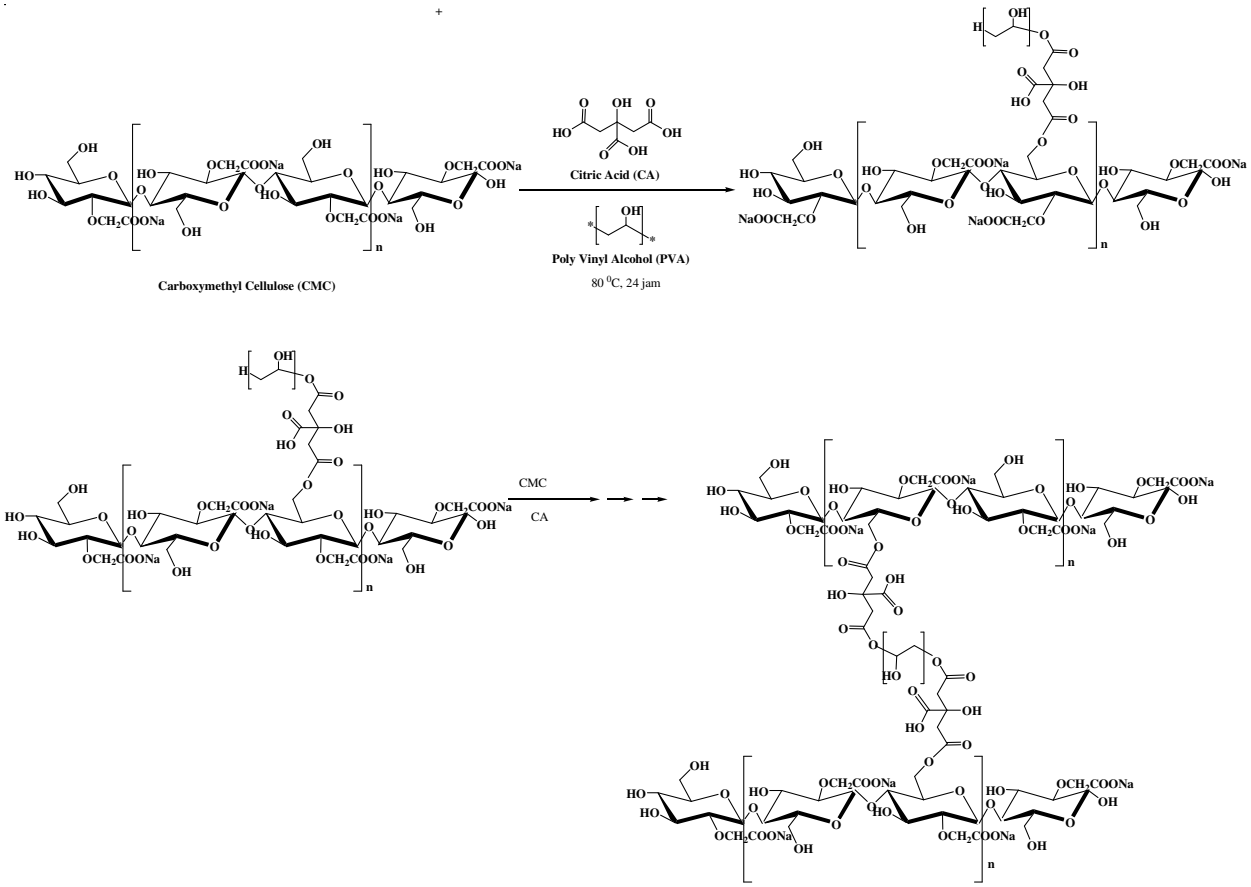


Fig.-1: Possible Reaction for the Formation of CMC/PVA/CA Film (B)

According to Azeredo et al[45], these were described to C=O and C-O of ester bonds formed as a product of the crosslinking reaction. However, the FTIR spectra werecomplicated to evaluatebecause of overlapping with carboxylic bands of CMC, forming different groups in the reaction and pH effect of the protonation of carboxymethyl groups of CMC by adding citric acid ($RCOO^-$/RCOOH). Therefore,thesewere not the main method to describe the crosslinking process. In other words, it was assumed there were other possible interactions overlapped in a similar area.

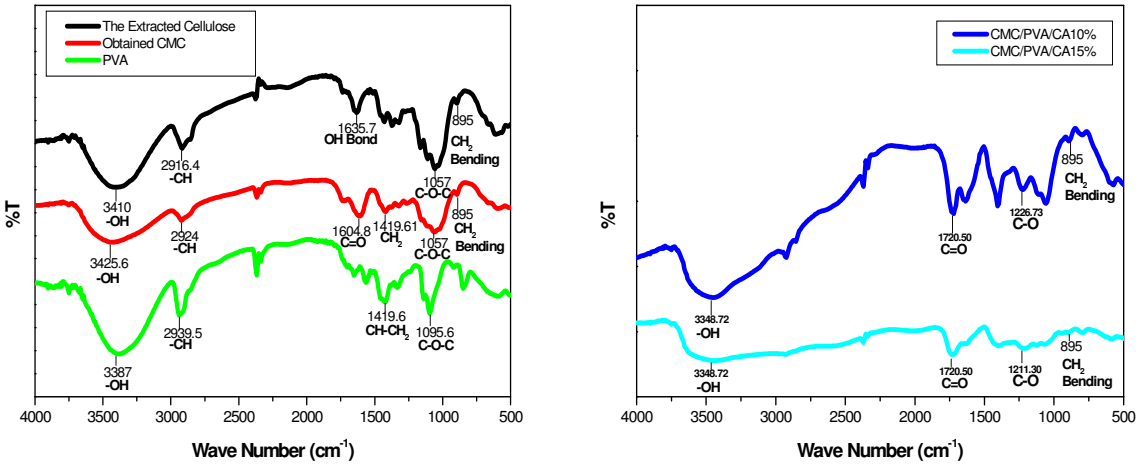


Fig.-2: FT-IR Spectra of Extracted Cellulose, Obtained CMC, PVA, and CMC/PVA/CA Films

X-Ray Diffraction (XRD) Analysis

Figure-3a shows the X-ray diffraction pattern, in which the typical peak of cellulose and carboxymethylcellulose are observed. The diffraction pattern of cellulose presented two broad and small peaks at 2θ values of $16.1^0$and $2\theta = 22.7^0$ that are related to the type of cellulose-1 structure. These peaks were also assigned to amorphous regions. The intense peaks observed around $2\theta = 43.97^0$and 64.34^0andthe small oneat $2\theta = 37.72^0$corresponded to the crystalline area of cellulose chain. After the carboxymethylation process , these reflection peaks were removed.

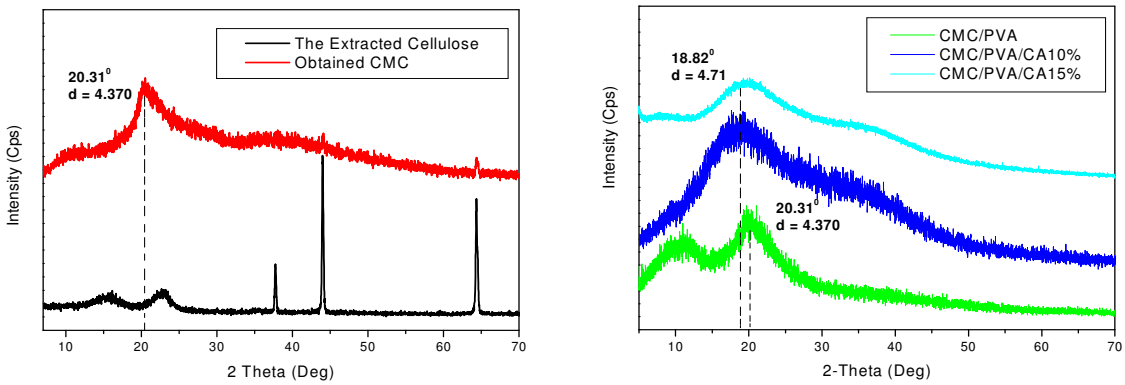


Fig.-3: X-Ray Diffraction Pattern of the Extracted Cellulose,Obtained CMC, CMC/PVA and CMC/PVA/CA Films

In the alkalization process, the addition of NaOH as swelling agent possessed the crystalline structure of cellulose in CMC spectra destructed. CMC has a maincharacteristic peak at $2\theta=20.31^0$ which described to less crystallin due to the cleavage of hydrogen bonds from NaOH[36]. In addition, the DS value of obtained CMC was of 1.2. DS value greater than 1 was decreased crystallinity of CMC as reported by Golbaghi et al. Low crystallinity properties were also found in CMC from durian rind[34]. Based on fig. 3, The diffractogram of the CMC/PVA film observed at $2\theta = 20.31^0$ is similar to the obtained CMC. It is possibly indicated that hydrogen bonding interaction was still not occurred. Therefore, It is proved that the chemical reaction in the CMC/PVA film was unavailable, but it was only a mixture[47]. While the citric acid (CA) is added the cross-linking occurs and the peak intensity of films have been moved to 18.82º. It could be explained that the crystallinity is decreased while cross-linking occurs between CMC and PVA because of hydrogen bonding interactions. In other words, the use of citric acid as crosslinking agent hindered the folding of polymer chains that resulted crystalline regions in the polymeric network.

Scanning Electron Microscopy (SEM) Analysis

In this study, uniformity and microstructural characterizations of films were assessedusing scanning electron microscopy (SEM). The SEM micrographs of CMC/PVA was given in Fig.-4. The figure of CMC/PVA film showed that morphological surface seemed to be less homogenous and smooth. It is possibly proved that both components were difficult to interact due to macromolecule of CMC. While the cross-linker CA content of film increased, crosslinking degree increased and film have more stiff structure. The SEM results showed that these structures have

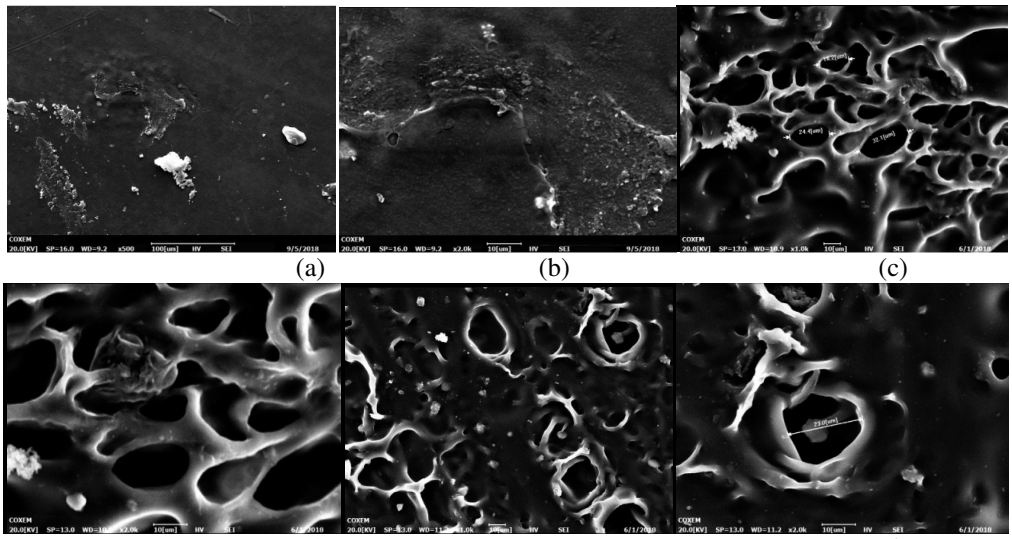


Fig.-4: SEM Micrograph of the Morphologies of the Samples (a) CMC/PVA at 500 x, (b) CMC/PVA at 2000 x, (c) CMC/PVA/CA10% at 500 x, (d) CMC/PVA/CA10% at 2000 x, (e) CMC/PVA/CA15% at 500 x and (f) CMC/PVA/CA15% at 2000 x

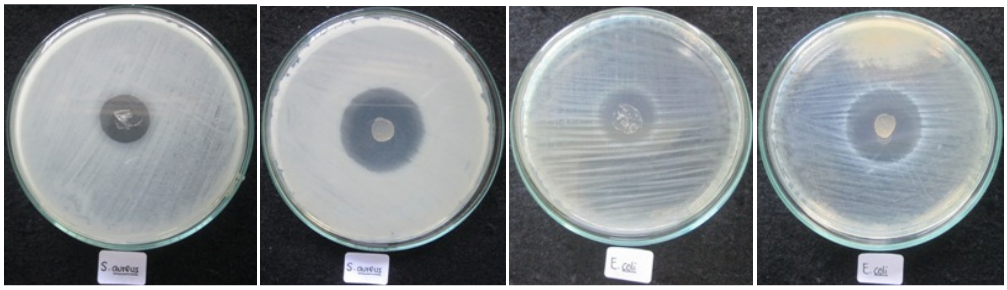
three-dimensional network structure.Moreover, CMC/PVA/CA10% film exhibits more porous,homogenousand good three-dimensional network along with interconnectivity between the poresdue to a less crosslinking percentage. Interconnected pore structure enables the diffusion of water molecules through the pore. Size pores of CMC/PVA/CA10% and CMC/PVA/CA15% film were 32 µm and 23 µm, respectively. While CMC/PVA/CA15% film showed less porous and denser structure. it indicated the large presence of ester-forming. Thus, the ability of film to absorb water was limited.

Antibacterial Activity

The antibacterial properties of the film were tested using the disk diffusion method. This method is carried out to determine the response of bacterial growth inhibition of compound by measuring the diameter of the clear zone using the calipers. The antimicrobial efficacy of CMC/PVA, CMC/PVA/CA10% and CMC/PVA/CA15% films was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli*. Figure 6(a)(b) was presented with the zones of inhibition. There is no zone of inhibition observed around the discs of CMC/PVA containing samples. Since CMC and PVA cannot be released out into the bacterial medium, they cannot observe a zone of inhibition. Antibacterial activity from the test showed that CMC/PVA/CA10% and CMC/PVA/CA15% films had the ability to effectively inhibit and kill the growth of both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. The inhibitory power of bacteria is getting better with increasing concentration of CA in the film. This can be seen from the results of the antimicrobial index zone of the two film samples in Table-1.

CMC/PVA/CA films have powerful antibacterial properties. This is due to the antimicrobial chemical properties of citric acid in the polymer network. According to the degree of crosslinking and FTIR spectra, CA, as crosslinker agent, did not fully react with both CMC and PVA. In other words, there is still COOH groups of CA remained in films. It possibly played a role as undissociated form in

antibacterial activity[48]. Thus, citric acid has an undissociated form which is a molecule that can penetrate the bacterial cell membrane, making it capable of donating hydrogen ions in the system[48].Citric acid can kill bacteria through the mechanism by which the undissociated molecules (COOH) are able to penetrate the cell membrane of microorganisms and ionize them[48]. To maintain intracellular pH, H^+ ions are released, resulting in a weak internal pH[48]. Acidic conditions in cells cause deformation and damage to enzymatic activity, protein and DNA structure, thus damaging the extracellular membrane[48]. In another mechanism, changes in cell membrane permeability inhibit substrate transport. In addition, changes in pH in cells suppress the oxidation of NADH which affects the electron transport system and causes the death of microorganisms[49].



(a) (b) (c) (d)

Fig.-5: Antimicrobial Activity of Sampels having (a) CMC/PVA, (b) CMC/PVA/CA10% (c) CMC/PVA/CA15% against *S. aureus* and (a) CMC/PVA, (b) CMC/PVA/CA10% (c) CMC/PVA/CA15% against *E. coli* by Zone Inhibition Method

Table-1: Antibacterial Inhibition Zone

Bacteria	CA Concentration	Sample Film Diameter (mm)	Clear Zone Diameter (mm)	Antimicrobial Index Zone
S. aureus	10%	7	16.9	1.41
	15%	7	29.6	3.22
E. coli	10%	7	18.1	1.58
	15%	7	25.9	2.70

CMC/PVA/CA15% films have a greater index of antimicrobial zones against gram-positive bacteria, namely *S. Aureus* compared to gram-negative bacteria *E. Coli*. This is because these microorganisms do not have an outer membrane which can facilitate antimicrobial agents to enter bacterial cells. Single-layer gram-positive bacterial cell wall structure with low lipid content (1-4%), whereas gram-negative bacteria have high lipid content (11-12%) and the outer membrane consists of 3 layers, namely lipopolysaccharide, lipoprotein, and pospolipid[50]. Thus, gram-positive bacteria such as *S. aureus* are more sensitive to antimicrobial agents.

CONCLUSION

The result of the characterization of structural analysis on CMC/PVA/CAfilm with FT-IR and XRD analysis indicated CMC/PVA was successfully crosslinked with CA. The percentage of crosslinking increased with the addition of CA was of 9% and 11%. The use of the crosslinkingreagents CA10% and CA15% on the CMC/PVA reduced its water absorption by 50% and 35%%, respectively. This resultwas due to the density of the crosslinking formation. The morphological analysis on film with SEM photographs showed that film created micropores and strong 3D polymeric networks. Antibacterial activity of films was examined on *E. choli* and *S. aureus* based on agar diffusion test. Films showed very good antibacterial activity against both gram-positive and gram-negative bacteria. Therefore, CMC/PVA/CA film could be considered to be a supporting material for different medical applications.

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