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EPICHLOROHYDRIN AS CROSSLINKING AGENT FOR SYNTHESIS OF CARBOXYMETHYL CELLULOSE SODIUM (Na-CMC) AS PHARMACEUTICAL EXCIPIENT FROM WATER HYACINTH (*EICHORRNIA CRASSIPES* L.)

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ABSTRACT

Water hyacinth is a plant, which contain high cellulose concentration that potential to be used as raw material for carboxymethylcellulose sodium. This study was conducted to observed the influence of crosslinker epichlorohydrin to the value of water holding capacity (WHC) and oil holding capacity (OHC) of carboxymethyl cellulose sodium synthesized from water hyacinth (Eichhornia crassipes (Mart.) Solm.). Research conducted through α-cellulose isolation, synthesis of Na-CMC, crosslinking Na-CMC, and characterization. The results showed α-cellulose yield of 26.334% and Na-CMC crosslink 150%. Physical quality evaluation of Na-CMC synthesized results and Na-CMC crosslink shows the most effective crosslink is in comparison 1:10. The value of WHC and OHC from Na-CMC, non crosslink Na-CMC and crosslink Na-CMC consecutively are 4.24 g/g and 4.39 g/g, 4.415 g/g and 3.03 g/g, 5.68 g/g and 2.87 g/g. Based on the study, epichlorohydrin can be used as a crosslinker in synthesis of Na-CMC from cellulose of water hyacinth for pharmaceutical application.

Key words: Carboxymethylcellulose sodium, Crosslink, Epichlorohydrin, α-Cellulose, Water hyacinth.

INTRODUCTION

Based on research conducted by Ismail et al.[1], water hyacinth is known to have

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64.5% of cellulose. This cellulose contain was high enough to be used as raw material for sodium carboxymethyl cellulose. Research conducted by Musfiroh et al.[2] have produced the Na-CMC from cellulose of water hyacinth, which meet pharmaceutical excipients parameter from USP NF 30 and Handbook of excipients but it still has shortcomings on the value of the degree of substitution (DS), water holding capacity (WHC), and oil holding capacity (OHC) compared with the standard. The degree of substitution of the analytical results for the Na-CMC derived from cellulose of hyacinth is at 0.7. While the value of the degree of substitution for Na-CMC in general is at 0.5-1.5[3]. WHC and OHC values for Na-CMC derived from cellulose of hyacinth was smaller than Na-CMC standard, the WHC value for synthesis is 2.3585 g/g and 5.2184 g/g for standard while OHC value is 1.64 g/g for synthesis and 2.38 g/g for standard. According to Basmal et al.[4], the value of the degree of substitution will affect the process of etherification of cellulose with sodium monochloroacetate, which in turn will affect the number of carboxymethyl groups generated. WHC and OHC value will affect the viscosity of the resulting Na-CMC.

One technique to improve the DS value, WHC and OHC is through the addition of crosslinker agent[5]. Crosslinker will improve the structure of Na-CMC polymer by forming bonds on the polymer so that it will be more stable and will produce Na-CMC with high viscosity. Crosslinker is a compound that can form chemical bond and linking the polymer chains with other polymer. Using crosslinker in the synthesis will change the straight chain into crosslinked structure so the resulting structure will have stronger structure and hard to retrodegraded[6]. Crosslinker will affect the physical properties of polymer such as reducing elasticity of the polymer, increasing the molecular weight, and improve the stability of the polymer. There are a wide variety of crosslinker that is often used in the manufacture of pharmaceutical preparations, such as methylene diphenylene diisocyanate (MDI), N, N'-methylene bisacrylamide (MBA) and epichlorohydrin[7-9]. Epichlorohydrin crosslinker is often used in cellulose[10,11]. These compounds form of a solution that is colorless and odorless, carcinogenic, slightly soluble in water but soluble in polar solvents[12]. Epichlorohydrin has a small molecular weight and is normally used in the manufacture of solvents and surfactants on pharmacists[13].

Based on that, this research is conducted to improve the characteristic of synthesized Na-CMC from water hyacinth by using epichlorohydrin as the crosslinker agent. The addition of crosslink is expected to improve the physical properties of Na-CMC synthesized from water hyacinth to obtain Na-CMC, which has similar properties to the standard. Research will be carried out by varying the concentration of crosslinker agent.

EXPERIMENTAL

Instrumentation that used in this study were digital scales (Mettler Toledo), grinding machines, magnetic stirrer (Yellow MAG HS7), 50 mesh sieve, oven (Heraeus), pH meter (Mettler Toledo), a water bath (memmert), electric furnace, Fourier Transform Infrared (FTIR) (Shimadzu, IR Prestige-21), and glass tools that commonly used in laboratories. The materials used in this study are the water hyacinth, distilled water, sodium hydroxide (NaOH) (Bratachem), sodium hypochlorite (NaOCl) (Bratachem), potassium dichromate ($K_2Cr_2O_7$), sulfuric acid (H_2SO_4) acid (Merck), an indicator Ferroin, ferrous ammonium sulfate [$(NH_4)_2Fe\ (SO_4)_2.6H_2O$], isopropyl alcohol (Bratachem), sodium monochloro acetate ($NaCH_2COOCl$) (Sigma Aldrich), methanol (Merck), ethanol (Bratachem), nitric acid (HNO_3), silver nitrate ($AgNO_3$), ammonium thiocyanate (Merck), acetic acid (CH_3COOH) glacial (Merck), alpha-naphthol (Merck), and epichlorohydrin (Merck).

The water hyacinth used were obtained from the Jatiroke, Jatinangor, West Java, Indonesia. The roots of the water hyacinth taken was discarded, then washed with water to remove mud attached and dried under the sun for 5-7 days. After it dried, the hyacinth is crushed into a coarse powder form, and dried again in the oven at 95^{o}C for 12 hrs, and sieved to 50 mesh[2].

Isolation of α-Cellulose from water hyacinth was conducted according to early research using NaOH 25%[2]. The water hyacinth grain was first boiled with water, and filtered. The insoluble part was boiled again with sodium hydroxide for one hour, and then filtered. The residue from this process was then washed with aquadestillata until the pH level reaching 6-7. The cleaned residue was then put into a plastic container, and the natrium hypochlorite (NaOCL) bleaching solution was further added to the residue. Both the residue and the solution were stirred evenly for 4 hrs in the closed container. The result was then filtered and again washed using aquadestillata until the chlorine scent was thoroughly undetectable. It was dehydrated in the oven at 50^{o}C. The final residue was called α-cellulose. The α-cellulose content then was analyzed by using titrimetry method in accordance with SNI 0444:2009 code[6] of conduct concerning alpha, beta and gamma cellulose in pulp examination method[14].

The Na-CMC synthesis of water hyacinth cellulose was done with the ratio as follows: isopropyl alcohol: NaOH: sodium monochloroacetate (1:1:0.25) according to early research[2]. Sodium carboxymethyl cellulose (Na-CMC) was synthesized in two-step reactions. In the first step, the cellulose was put in the suspension process by stirring it with isopropyl alcohol using a mechanical stirrer at room temperature; NaOH 40% was also added in the stirring process. This mixture was stirred for 90 min, and the result was alkali cellulose. The

stirring process was then continued by slowly adding sodium monochloroacetate ($ClCH_2COONa$) for 30 mins, and after the addition the stirring process was continued for 3.5 hrs at 55^oC. In the second step, 70% methanol was added to the reactor and the mixture was neutralized with 90% acetic acid. Na-CMC was thus obtained by washing and filtering the residue 6 times using ethanol. The obtained Na-CMC through ethanol cleaning was then washed again with pure methanol and dehydrated in the oven at 60%.

Crosslinking step of resulted Na-CMC was done in different variation of Na-CMC: Epichlorohydrin (1: 2.5; 1: 5; 1:10) 5 g of Na-CMC were mixed with 50 g of 17.5% NaOH and stirred at 50^oC for 20 min. Epichlorohydrin in 28% NH_4OH in the ratio 1: 2.5; 1: 5; 1:10 to Na-CMC were added. The mixture then put in the reflux apparatus for 4 hrs at 40^oC. Na-CMC that already crosslink then washed with ethanol and water, and then dried.

Characterization of crosslink Na-CMC was done according to SNI 06-3736-1995, The Joint FAO/WHO Expert Committee on Food Additives (JECFA)[15,16]. The characterization include :

(a) Organoleptic: Examining the shape, color, smell, and taste according to the Indonesian Pharmacopoeia 4th edition.

(b) Solubility: The sample is weighed three times as much as 0.1 g then dissolved in distilled water, ethanol, and ether.

(c) Water holding capacity (WHC) and oil holding capacity (OHC) determination: Distilled water and 25 mL of olive oil are added each to 1 g sample of crosslink Na-CMC, then stirred with a mechanical stirrer while incubated at 40°C for 1 hr. WHC and OHC values are calculated as a gram per gram sample[17].

(d) Determination of the degree of substitution (DS). The determination of the degree of substitution is performed by calculating the content of sodium in the Na-CMC and multiply with a factor.

Degree of substitution = 162 x % sodium/2300- (80 x % sodium).

(e) Characterization of crosslink Na-CMC with instrument method. The characterization of Na-CMC synthesized from water hyacinth cellulose was done by using fourier transform infrared (FTIR).

RESULTS AND DISCUSSION

The water hyacinth used were obtained from the Jatiroke, Jatinangor, West Java, Indonesia. The roots of the water hyacinth taken was discarded, then washed with water to

remove mud attached and dried under the sun for 5-7 days. Drying shrinkage of the materials then was determined, the results can be seen in Table 1.

Table 1: Drying shrinkage of water hyacinth results

Wet weight (Kg)	Dry weight (Kg)	Drying shrinkage (%)
5.19	0.492	90.52

After drying shrinkage determination, the water hyacinth sample was milled to form a coarse powder. Milling goal is to minimize the size of cellulosic materials and breaks down chemical bonds in long-chain molecules[3]. After that, the hyacinth is crushed into a coarse powder form, and dried again in the oven at 95^{o}C for 12 hrs, and sieved to 50 mesh[2].

The isolation of α-Cellulose from water hyacinth was conducted according to early research using NaOH 25%[2]. The water hyacinth grain was first boiled with water, and filtered. The insoluble part was boiled again with natrium hydroxide for one hour, and then filtered. The residue from this process was then washed with aquadestillata until the pH level reaching 6-7. This process was performed because this material will be use for pharmaceutical application so it should be in a neutral pH. The cleaned residue was then put into a plastic container, and the natrium hypochlorite (NaOCl) bleaching solution was further added to the residue. Both the residue and the solution were stirred evenly for 4 hrs in the closed container. The result was then filtered and again washed using aquadestillata until the chlorine scent was thoroughly undetectable. It was dehydrated in the oven at 50^{o}C. The final residue was called α-cellulose. The α-cellulose obtained from this procedure was 26,33%. This is not large enough because the washing process made a lot of loosing.

α-cellulose content analysis was performed according to SNI 0444: 2009 on how to test the levels of alpha, beta, and gamma cellulose in the pulp. The method used was back titration, where what counts is the content of β-γ-cellulose and cellulose. At this titration, we used boiling potassium dichromate ($K_2Cr_2O_7$) in an excess acid in order to oxidize the cellulose. Residual potassium dichromate, which has reacted with β-cellulose and cellulose-γ will be determined by titration using indicators ferroin as an indicator. The reaction was at follows :

$$6\ Fe^{2+} + Cr_2O_7^{2-} + 14\ H^{+} \longrightarrow 6\ Fe^{3+} + 2\ Cr^{3+} + 7\ H_2O$$

The content of α-cellulose is obtained from cellulose hyacinth at 89.0%. α-cellulose that obtained was higher than the value that have been investigated by Ismail et al.[1] that is equal to 64.51%. The content of α-cellulose is high because the isolation process performed

immersion in 25% NaOH solution, higher concentration of NaOH can affected to number of α-cellulose being isolated. In addition, the high content of α-cellulose is also caused by the bleaching process that can also increase the levels of α-cellulose contained in the sample.

The Na-CMC synthesis of water hyacinth α-cellulose was done with the ratio as follows: isopropyl alcohol: NaOH: sodium monochloroacetate (1:1:0.25) according to Musfiroh et al.[2] Sodium carboxymethyl cellulose (Na-CMC) was synthesized in two-step reactions. In the first step, the cellulose was put in the suspension process by stirring it with isopropyl alcohol using a mechanical stirrer at room temperature; NaOH 40% was also added in the stirring process. This mixture was stirred for 90 mins, and the result was alkali cellulose. The stirring process was then continued by slowly adding natrium monochloroacetate ($ClCH_2COONa$) for 30 min, and after the addition the stirring process was continued for 3.5 hrs at 55^oC. At this stage of reaction, in addition to the formation of Na-CMC occurs also occur formation of by products in the form of sodium glycolate and sodium chloride (NaCl). Thereafter, 70% methanol was added to the reactor. Then the mixture was neutralized with 90% acetic acid to remove the glycolic acid. Na-CMC was then obtained by filtering and washing the residue 6 times with ethanol. Na CMC that has been washed with ethanol then washed again with pure methanol and dried using an oven at 60°C[3]. At this stage, carboxymethyl cellulose into cleaner (seen from its physical appearance). This washing step causes the content of isopropyl alcohol in the Na-CMC decreased to 5%.

Crosslinking step of resulted Na-CMC was done in different variation of Na-CMC : Epichlorohydrin (1: 2.5; 1: 5; 1:10) 5 g of Na-CMC were mixed with 50 g of 17.5% NaOH and stirred at 50C for 20 min. This heating stage serves to accelerate the reaction between Na-CMC and 17.5% NaOH. Epichlorohydrin in 28% NH_4OH in the ratio 1: 2.5; 1: 5; 1:10 to Na-CMC were added. Epichlorohydrin serves as an agent that will form Na-CMC crosslinks. NH_4OH 25% made an alkaline condition in a crosslink process, besides NH_4OH also useful as long chain-forming bonds at crosslinking. The mixture then put in the reflux apparatus for 4 hours at 40^oC. This process is useful to break Na-CMC bonds and form a bond between the Na-CMC and epichlorohydrin. Na-CMC that already crosslink then washed with ethanol and water, and then dried.

The quality of crosslink Na-CMC were conducted by comparing their characteristics with the Na-CMC standard. Quality test include organoleptic examination, solubility, water holding capacity (WHC), and oil holding capacity (OHC). From organoleptic (physical appearance, color, smell, and taste) test, crosslink Na-CMC did not differ with Na-CMC standard. The results can be seen in Table 2.

Table 2: Quality test results of crosslink Na-CMC, non crosslink Na-CMC and Na-CMC standard

	Organoleptic	Solubility	Water holding capacity (WHC)	Oil holding capacity (OHC)
Na-CMC standard	Shape : Powder Color : Pale white Odor : Odorless	Disperse in water, not dissolve in ethanol and ether	4.415	3.03
Na-CMC non crosslink	Shape : Powder Color : Pale white Odor : Odorless	Disperse in water, not dissolve in ethanol and ether	4.24	4.39
Crosslink Na-CMC (1:10)	Shape : Powder Color : White Odor : Odorless	Disperse in water, not dissolve in ethanol and ether	5.68	2.87
Crosslink Na-CMC (1:5)	Shape : Powder Color : White Odor : Odorless	Disperse in water, not dissolve in ethanol and ether	4.97	2.66
Crosslink Na-CMC (1:2.5)	Shape : Powder Color : White Odor : Odorless	Disperse in water, not dissolve in ethanol and ether	4.49	1.84

Based on Na-CMC monograph that is written in the Indonesian Pharmacopoeia fourth edition. Na-CMC will form a colloidal solution with the addition of water, but insoluble by the addition of ether and ethanol. Crosslink Na-CMC showed the result of solubility that meet with the criteria. The solubility of Na-CMC is influenced by the degree of substitution (DS) value. Na-CMC with DS is less than or equal to 3 is soluble in alkaline solution, whereas the DS greater than 0.45 dissolved in water[18].

Water holding capacity (WHC) and oil holding capacity (OHC) is the ability of Na-CMC to bind either water or oil derived from Na-CMC itself or from outside[18]. From the test results, it can be seen that the WHC values for non crosslink Na-CMC is smaller compared to the Na-CMC standard, 4.24 g/g compare to 4.415 g/g consecutively. WHC values will affect the viscosity of the pharmaceutical dosage forms, that is why the epichlorohydrin was used to increase the WHC value. From the test results WHC on

crosslink Na-CMC, it can be seen that the higher the amount of crosslinker used, the higher the value of WHC. This is because more chemical bond can be formed by the crosslinker. OHC value of crosslink Na-CMC smaller than the standard, this is may be due to with the type of cellulose in the crosslink Na-CMC. Higher number of amorphous cellulose can absorb more water[4].

Degree of substitution also determines in this study according to SNI 06-3736-1995[15]. Results of the analysis of the degree of substitution (DS) for the Na-CMC standard is 0.17, while non crosslink Na-CMC is 0.285. Crosslink Na-CMC with composition 1: 2.5; 1: 5; 1:10, gives DS value 0.14, 0.063, and 0.035, respectively. DS value of crosslink Na-CMC was smaller than the standard and non crosslink, this is because crosslinking process will decreasing water solubility of a compound and degree of substitution. More crosslinker is added to the Na-CMC will further decrease the solubility and the degree of substitution[19].

Table 3: Sodium content and degree of substitution value of standard Na-CMC, non crosslink Na-CMC and crosslink Na-CMC

Sample	Sodium content	Degree of substitution (DS)
JECFA/FI 4/SNI 06-3736-1995	6.5 ≤ content ≤ 9.5	0.2 ≤ DS ≤ 9.5
Standard Na-CMC	2.23	0.17
Non crosslink Na-CMC	3.54	0.285
Crosslink Na-CMC 1:2.5	1.866	0.14
Crosslink Na-CMC 1:5	0.871	0.063
Crosslink Na-CMC 1:10	0.493	0.035

Analysis of functional groups on the Na-CMC standard, non crosslink Na-CMC and crosslink Na-CMC were done using FTIR instrument. According Latif et al.[3], carboxyl group as its salt has a wave number of about 1600 to 1640 cm^{-1} and 1400-1450 cm^{-1}. Infrared spectra of Na-CMC standard shows the absorption at wave number 1604.31 and 1425.41 cm^{-1}. The peak of the spectrum at a wavelength of 1604.31 cm^{-1} showed the presence of carbonyl groups and wavelength 1425.41 cm^{-1} showed the presence of methyl groups. This indicates the structure of carboxymethyl Na-CMC[20]. Some peaks were obtained from measurements using the FTIR results can be seen in the following Table 4.

Based on the results of the FTIR analysis, characteristic absorption peaks are at wave number 3415.48 to 3459.84 cm^{-1} (absorption peak of OH group), and the wave number 1047.36 to 1065.20 cm^{-1} (CO absorption peaks), which shows the glycoside bond in the structure of Na-CMC. Crosslink Na-CMC in different composition, has the same infra red spectrum as the standard.

Table 4: FTIR spectrum results of standard Na-CMC, non crosslink Na-CMC and crosslink Na-CMC

Sample	Functional groups and wavenumber (cm^{-1})				
	-OH Stretching	**-CH Stretching (CH_2 and CH_3)**	**-C=O (indication of Na-CMC)**	**-CH_2 Bending (Indication of Na-CMC)**	**-C-O symmetrical Stretching**
Na-CMC standard	3459.84	2913.50	1604.31	1425.41	1061.34
Non crosslink Na-CMC	3434.28	2900.96	1608.65	1420.10	1065.20
Crosslink Na-CMC 1:2.5	3415.48	2876.37	1660.12	1459.64	1056.52
Crosslink Na-CMC 1:5	3415.96	2912.05	1650.60	1462.05	1047.36
Crosslink Na-CMC 1:10	3416.44	2835.96	1643.86	1456.27	1049.28

Based on the research that has been done crosslinking can improve the value of WHC and OHC of Na-CMC and reduce the degree of substitution of Na-CMC. Comparison of effective crosslinker used in the Na-CMC was 1:10 against the amount of Na-CMC.

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