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LOW MOLECULAR WEIGHT OF HYDROXYETHYL CELLULOSE TO EXTRACT PROTEINS IN NATURAL RUBBER LATEX

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Abstract. Natural rubber latex (NRL) with "low protein content" is regarded as less allergenic latex for medical gloves, medical products, condoms, etc. Therefore, this study was conducted to strengthen the fundamental approach of making "low protein NRL" via oligosaccharide hydroxyl ethyl cellulose (oligoHEC) treatment. OligoHEC (Mw ~10,000 g/mol) is a decomposition product of HEC (Mw ~90,000 g/mol) by treatment with 1.5 % H_2O_2 combined with hydrothermal processing at a temperature of 121 °C , a pressure of 0.38 mPa for 30 minutes in an autoclave. OligoHECs were employed to form electrical bonds with proteins. The objective of this study is to obtain the protein content in NRL ≤ 50 µg/g of rubber and to evaluate the effect of oligoHEC treatment on the soluble protein content in NRL. The results show that oligoHEC at low concentration (0.25 %) effectively extracted protein molecules. Interestingly, the allergenic protein content in NRL decreased proportionally with the increase in oligoHEC concentration. These results suggest a potential approach to produce NRL products without allergenic proteins.

Keyword: HEC, natural rubber latex (NRL), proteins content.

Classification numbers: 1.3.1, 2.9.3.

1. INTRODUCTION

Natural rubber latex (NRL) is one of the materials with many outstanding properties such as water resistance, insulation, flexibility, elasticity, and environmental friendliness. It has been widely used to fabricate medical equipment, household appliances, and industrial equipment. After centrifugation, the protein content of NRL products is about 0.3 - 0.45 % [1]. Studies have proven that the protein component in NRL is the cause of allergies for users when using products such as medical gloves, medical products, condoms, mattresses [2]. In the world, there are many methods for deproteinization of NRL, applied for production and processing, including treating the pre-centrifugation latex, such as thickening process [3], removing proteins by urea

[4, 5], combining with sodium dodecyl sulfate (SDS) surfactants [4, 6], using solvents (ethanol, propanol, acetone) [7], $CaCl_2$ [8], and proteolytic enzyme [9]. Another method is to use water-soluble synthetic polymers such as polyvinyl pyrrolidone (PVP) [10], polyethylene glycol (PEG) 6000 [3, 11] to obtain the skimmed NRL with a protein content of 0,67 mg/g of rubber. In Vietnam, protein-free natural rubber latex has been prepared by incubation with urea in SDS surfactant solution [4, 12] or SDS combining with magnetic coated alumina ($Fe_3O_4@Al_2O_3$) nanoparticle [13] or only $Fe_3O_4@Al_2O_3$ nanoparticle [13]. Low protein NRL was also prepared using synthetic polymers such as PVA with molecular weight (Mw) ~22,500 and 90,000 g/mol, PVP with Mw 40,000 and 360,000 g/mol at a concentration of 3 phr (part per hundred of rubber) [14]. Research on using natural polymers or oligomers to extract proteins from NRL has not been published much. The positively charged low molecular weight HEC combined with the negatively charged protein of NRL, producing soluble proteins that have been removed by centrifugation. HEC has been used as a green technology solution. In this study, we present the results of using hydroxyl ethyl cellulose (HEC) with Mw ~10,000 g/mol at different concentrations to extract soluble proteins from NRL by centrifugation process to obtain low protein NRL with contents less than 50 µg per gram of rubber.

2. MATERIALS AND METHODS

2.1. Materials

Natural rubber latex was extracted directly from rubber trees of Binh Long Rubber Company, Binh Phuoc province, Viet Nam. Hydroxyl ethyl cellulose (HEC) with Mw ~ 90,000 g/mol was supplied by Aldrich Sigma (Singapore). Chemicals such as 30 % H_2O_2, Dimethylformamide (DMF), NaOH, HCl, NH_3, and $(NH_4)_2HPO_4$ were from Merck (Germany). Alcohol (C_2H_5OH) was supplied by Duc Giang Chemical Company (Viet Nam).

2.2. Methods

2.2.1. Adjusting molecular weight of HEC by hydrothermal treatment with H_2O_2

50 g of HEC was dissolved in 700 mL of DMF solvent, then 30% H_2O_2 was added to obtain HEC solutions with a concentration of H_2O_2 of 0.5 to 1.5 %. The HEC/H_2O_2 solution mixture was hydrothermally decomposed in an autoclave at 121 ^oC for 30 minutes. The obtained HEC was then precipitated in 99 degrees absolute alcohol at an alcohol/HEC ratio of about 1/6. After precipitation, the HEC was dried at 60 °C for 6 hours.

2.2.2. Removing protein by oligoHEC in production of HA natural rubber latex

After being havested, the latex was stored in a tank, then $(NH_4)_2HPO_4$, ammonium lauryl sulfate (10 %) and oligoHEC (0.25 - 0.75 %) were added to the latex, which was then incubated for 36 hours for $(NH_4)_2HPO_4$ to react with Ca^{2+}, Mg^{2+} ions to form phosphate salts of Ca, Mg. During the incubation, ionic bonds between oligoHEC and proteins in latex were formed, causing the proteins to be separated. After that, the lower part of the tank containing Ca^{2+}, Mg^{2+} ions was removed through a blow-down valve, the rubber latex in the upper part of the tank was continuously fed into a centrifuge to separate the water and to collect the NRL as a product. Protein content after centrifugation was determined by Lowry method according to

ASTM D5712-10 [15]. Finally, natural rubber latex is supplemented with NH_4OH to enhance mechanical strength.

2.2.3. Measurement

Gel permeation chromatography (GPC) was used to determine the molecular weight of HEC. The GPC measurement was performed on 1260 Infinity GPC (Agilent PL-GPC 50, Agilent PL-GPC 220), using an RI detector and a 5 µm MIXED-C PLgel column (7.5 × 300 mm) at the Institute of Applied Materials Science. The mobile phase consisted of *N*, *N*-dimethylformamide (DMF) containing 0.1M LiBr at 50 °C and a flow rate of 1 mL/min with the PEO/PEG standards.

The Fourier-transform infrared (FT-IR) spectra of HEC samples were performed with a Model FT–IR 8400S spectrometer (Shimadzu, Japan). The test specimens were prepared by the KBr disk method.

The X-ray diffraction (XRD - D8 Advance Bruker, Germany) measurement was used to study the structure of HEC by applying CuKα radiation (λ = 1.5405 Å) under a constant current and a voltage of 30 mA and 40 kV, respectively, and the diffraction scan angle (2θ) ranged from 5^o to 80°.

The amount of protein in the latex was determined by the modified Lowry test according to ASTM D 5712-10. The latex proteins were first solubilized in 1 % SDS and 50 mM sodium phosphate buffer (final concentration). 300 µL of the redissolved latex protein solution, an ovalbumin protein standard, or the blank reagent (0.2 M NaOH) were added to a 625 µL volume of alkaline copper tartrate. The solution obtained was well mixed and allowed to stand for 15 minutes at room temperature. 75 µL of 2-Folin-Ciocalteu Phenol reagent was added to the solution which was thoroughly mixed immediately and then allowed to stand for 30 minutes at room temperature. The absorbance of the resulting solution was measured at 750 nm using a UV visible spectrophotometer, then a calibration curve was calculated and the protein concentration in the test specimens was determined [14, 15].

3. RESULTS AND DISCUSSION

3.1. Adjusting molecular weight of HEC

In this study, we carefully adjusted the molecular weight of HEC by H_2O_2 that in common is a capable oxidizing agent at lower concentrations typically combined with hydrothermal treatment. In carbohydrate degradation process, if elevated concentrations H_2O_2 could due to the assured destruction of glucose rings, physicochemical and structural characteristics of polysaccharides would instantly change. The specific objective of this academic study correctly was to carefully prepare HEC (Mw ~10,000 g/mol) for practical use for the successful extraction of proteins from NRL.

The results in Figure 1 show that the molecular weight of HEC decreased from 90,000 g/mol to 6,900 g/mol as the concentration of H_2O_2 increased from 0 to 1.5 % after hydrothermal process. Hydrogen peroxide is substantially more acidic than water, with a pKa of 11.6. So, the perhydroxyl anion is instable [16]. The reduced stability of H_2O_2 is due to the instability of HOO^+. Temperature and base will also increase H_2O_2 decomposition. Perhydroxyl negative ions react with H_2O_2 to form activated hydroxyl radicals ($HO^{\bullet}$) [7]. As reported by Quin *et al*. (2002), H_2O_2 was dissociated in water according to the following equation [16]:

$$H_2O_2 \rightarrow H^+ + HOO^-$$

The instability of HOO^- anions causes the decomposition of H_2O_2 as they reacted with H_2O_2 to create $OH^\bullet$ free radicals with strong oxidizing activity.

$$HOO^- \rightarrow OH^- + (O)$$

$$H_2O_2 + HOO^- \rightarrow {}^\bullet OH + O_2{}^\bullet - + H_2O$$

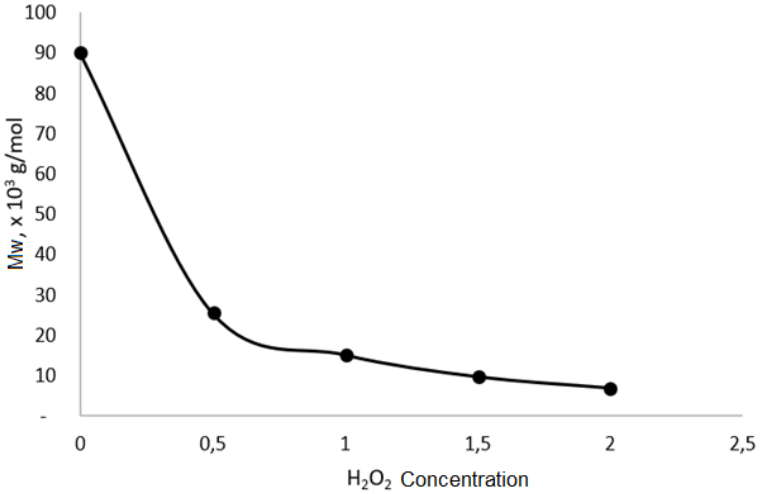

Figure 1. Molecular weight reduction of HEC corresponding to various H_2O_2 concentrations.

Studies show that hydroxyl radicals react extremely quickly with carbohydrates, forming a new atom to break H-C bonds according to the general equation: $RH + HO^\bullet \rightarrow R^\bullet + H_2O$

According to Von Sontag *et al*. (1980) [17], $^\bullet OH$ radicals rapidly attack the carbohydrate structure in a non-selective random manner by hydrogen atoms of CH groups on the glucose ring, forming macromolecular free radicals. Then the process of cutting the glycosidic linkage will occur due to the effect of this free radical: $P^\bullet$ (polymer) $\rightarrow P^\bullet{}_1 + P_2$

Table 1. The dependence of molecular weight (Mw, Mn) and polydispersity index (PI = Mw/Mn) of HEC on the concentration of H_2O_2.

H_2O_2conc., %	**0**	**0.5**	**1.0**	**1.5**
Mw, g/mol	90,000	25,500	15,100	9,700
Mn, g/mol	40,000	10,800	7,900	5,700
PI (Mw/Mn)	2.25	2.36	1.91	1.70

The results in Table 1 showed that when the concentration of H_2O_2 increased, the polydispersity index (PI) value decreased from 2.36 to 1.70, indicating that the low molecular weight HEC has a uniform distribution. The molecular mass of HEC decreased rapidly as the concentration of H_2O_2 increased from 0.5 % to 1.0 % and then decreased slowly with increasing H_2O_2 concentration up to 1.5 %. Therefore, the optimum concentration of H_2O_2 in the range of 1.0 - 1.5 % is used for the molecular weight decomposition of HEC in combination with hydrothermal treatment.

FTIR spectra of pure HEC and oligoHEC are presented in Figure 2. Figure 2a shows that for the pure HEC sample, the broadband at the 3379 cm^{-1} position characterizes the elongated fluctuation of the hydrogen in O–H bonds, at the 2880 cm^{-1} position represents prolonged oscillation of the hydrogen in C–H bonds, at the 1739 cm^{-1} position represents the double bonding oscillation of the carbonyl group, at positions 1353 cm^{-1} and 1016 cm^{-1} characterizes fluctuations in carboxyl salts, stretching vibration of ether C–O and hydrogen bending in O–H bonds. Figures 2b, c, d show that for the oligoHEC samples, the broadband at the positions of wavenumbers from 3340 to 3475 cm^{-1} is characteristic for the OH-stretching vibration, the band from 2896 to 2981 cm^{-1} represents the C–H stretching vibration. The absorption band from 1640 to 1660 cm^{-1} is assigned to absorb H_2O, the absorption band from 990 to 1059 cm^{-1} represents the C–O vibration, and the absorption band from 1411 to 1458 cm^{-1} represents the CH_2. Besides, the absorption band at 850-897 cm^{-1} represents the C–O–C stretching at β-(1 → 4)-glycosidic linkages. The results showed that after the degradation, the oligoHEC did not change functional groups compared with the original HEC.

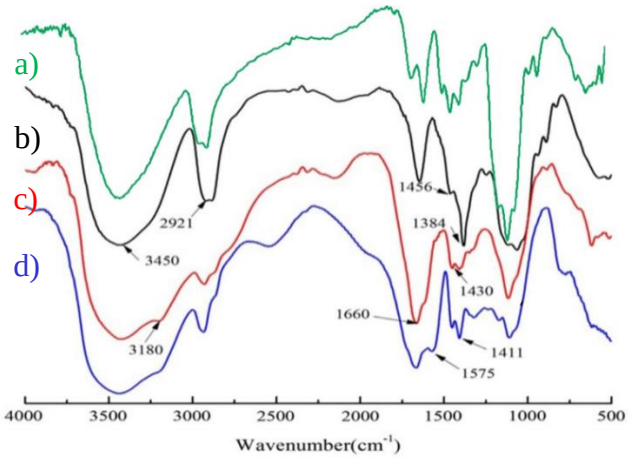

Figure 2. FT-IR spectra of HECs: pure HEC (a); HEC Mw~25,500 g/mol (b); HEC Mw~15,100 g/mol (c); HEC Mw~9,700 g/mol (d)

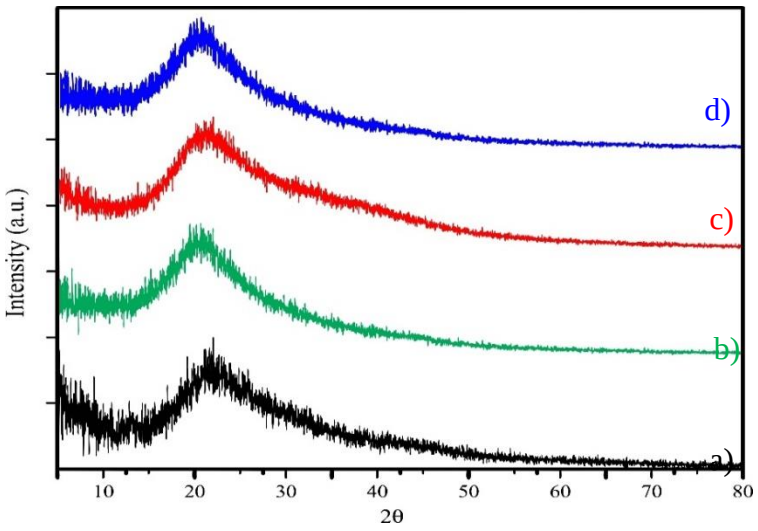

Figure3. The XRD pattern of HECs: pure HEC (a); HEC Mw~25.500 g/mol (b); HEC Mw~15.100 g/mol (c); HEC Mw~9.700 g/mol (d)

Figure 3 shows the X-ray diffraction (XRD) pattern of HECs in the range of 2θ from 5 to 80°. Peaks are observed for the initial HEC at a 2θ of approximately 20°. This suggests that the HEC has an amorphous structure because it is a cellulose derivative with the presence of hydrogen bonds between the polymer chains. The results in the XRD pattern show that the molecular weight degradation of the HEC does not alter the structure of HEC. The diffraction peak of oligoHECs was observed at a diffraction angle of 2θ larger than the diffraction angle of the original HEC, which suggests that their structure is also more amorphous than that of the original HEC.

3.2. Removing proteins in natural rubber latex with oligoHEC

OligoHEC (Mw ~ 9,700 g/mol) was added to the NRL, which was then incubated with $(NH_4)_2HPO_4$ to study the effect of oligoHEC concentration on protein removal from the NRL. The results are presented in Table 2.

Table 2. The dependence of protein content of NRL after centrifugation on oligoHEC concentration.

Sample	OligoHECconcentration, %	Protein content, µg/g of rubber
NL01-HEC	0	26.400
NL02-HEC	0.25	47
NL03-HEC	0.50	30
NL04-HEC	0.75	21

After centrifugation, the protein content of NRL was determined according to ASTM D5712-10. As observed by Mekonnen *et al*. (2019) [18], negatively charged proteins in the layer covering the rubber particles bind to positively charged oligoHEC when the pH of rubber latex is higher than the isoelectric point of the rubber. Once this bond is formed, the proteins can be easily separated from the NRL through centrifugation. Thus, oligoHEC is effective in extracting proteins of NRL. The results given in Table 2 showed that when the oligoHEC concentration increased, the remaining protein content in the NRL decreased after centrifugation. Specifically, when the NRL was treated with 0.25% oligoHEC before centrifugation, the protein content in the NRL was measured as 47 µg/g of rubber, which is lower than the target value of this study.

As recommended by the US Food and Drug Administration, the protein content of latex products must be less than 200 µg/g of rubber. Extracted protein (EP) content < 200 µg/g of rubber is the current recommendation to reach the safe limit. The allergenicity of rubber products will be reduced when the protein content in latex rubber is low and preferably free-extractable proteins [19]. Low protein (< 50 µg/g of rubber) NRL materials are essential for high-tech products such as medical gloves, condoms, household appliances, etc.

4. CONCLUSION

OligoHEC (Mw 9,700 g/mol) was obtained from the degradation of the HEC molecular weight (Mw 90,000 g/mol) at an initial HEC concentration of 5 % by using 1.5 % H_2O_2 as an oxidizing agent combined with hydrothermal treatment at a temperature of 121°C and a pressure

of 0.38 mPa for 30 minutes. This method is easy to implement, with high degradation efficiency and simple equipment. OligoHEC with a concentration of 0.25 % incubated with NRL for 36 hours before centrifugation is suitable for the manufacture of NRL with low protein contents (< 50 µg/g of rubber). According to calculations, 1 kg of oligoHEC can be used for 1,000 liters of latex with a rubber content of ~ 40 %.

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CRediT authorship contribution statement. L.T.K.D: Designing the simulations and experiments; Performing the experiments. B.D.D: Supervising, Writing manuscript. L.N.A.T: Performing the simulations; Analyzing data; Writing manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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