



Antibacterial Activity of Hydrogels Produced from Nanocellulose Derivatives for Controlled Drug Delivery

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ABSTRACT

Hydrogels are a 3D polymer network capable of absorbing large amounts of water or biological fluids without dissolving, due to chemical or physical crosslinking. They are made from cellulose derivatives and are a promising research area for developing smart drug carriers in modern drug delivery systems. The present research aimed to synthesize hydrogels based on nanocellulose derivatives and evaluate their antibacterial activity. A crosslinker termed epichlorohydrin (ECH) was incorporated into Sengon plant cellulose to produce cellulose derivatives, specifically abietic nanocellulose (CAB). Hydrogels were synthesized from the nanocellulose derivatives. The hydrogels were characterized using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis, and scanning electron microscopy (SEM). The degree of substitution, release, and swelling tests, as well as antibacterial activity tests, were conducted. The results of the FTIR spectra revealed absorption between 1400 and 1600 cm^{-1} , indicating a crosslinking process on the hydrogels. Furthermore, SEM cross-sectional observations demonstrated the formation of pores of various sizes. The hydrogel characterization results showed the impact of the preparation conditions, specifically the ECH concentration. The nanocellulose derivatives (NCAB): ECH concentration ratio varied between 1:0.5, 1:1, and 1:2. Based on the research results, the highest hydrogel swelling ratio was found in the CAB: ECH reaction conditions, which was 1:0.5. The findings of the present study demonstrated that modification by rosin esterification can result in hydrogels with desirable properties that can be employed for controlled drug delivery. Hydrogel from NCAB can inhibit the growth of *E. coli* bacteria in the weak category, but is moderate against *S. aureus*.

Keywords: Drug-delivery, Hydrogels, Nanocellulose, Nanocellulose-derivative.

Introduction

Hydrogels are a major candidate for various biomedical applications, including drug delivery, due to their many qualities. These qualities include biocompatibility, biodegradability, tunable mechanical properties, aversion to different upgrades, the ability to embody different remedial specialists, and the ability to regulate the delivery of drugs.¹ One of the most abundant regular polymers on earth is probably cellulose, which is made up of a straight chain of several hundred to ten thousand linked D-glucose units. It makes up a sizeable portion of the vital mass of the cells of green plants.² Until now, cellulose ethers have been acknowledged as an essential component of the pharmaceutical industry. In the formulation of drug devices, their main function is to be considered excipients.³ Hydrogels based on cellulose derivatives are an interesting and challenging area of research to study for the development of smart drug carriers in modern drug delivery systems.

Cellulose can be extracted from Sengon wood (*Paraserianthes falcataria*) waste, and the cellulose obtained is hydrolyzed to produce nanocellulose, which is cellulose with a smaller size. The physical and chemical properties of Sengon wood nanocellulose have been reported in previous research.⁴ Nanocellulose is modified with rosin through an esterification reaction to produce nanocellulose ester.^{5,6} A commodity that is common in Indonesia is rosin, which contains abietic acid.^{7,8} The chemical structure of abietic acid consists of three cyclic compounds that are linked together and have a carboxylic functional group in one of the rings.⁹

The present study was conducted to synthesize hydrogels from nanocellulose from Sengon wood (*Paraserianthes falcataria*) whose surface structure was modified by rosin plant abietic acid to obtain nanocellulose esters. The antibacterial activity of the hydrogels was also investigated.

Materials and Methods

Materials used

The material used in this study included cellulose from Sengon, rosin (technical grade), ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), chloroform (CH_2Cl_2 ; 99% purity from Merck, Germany), sodium hydroxide (NaOH; 99% purity from Merck, Germany), urea ($(\text{NH}_2)_2\text{CO}$; 99% purity from Merck, Germany), epichlorohydrin ($\text{C}_3\text{H}_5\text{ClO}$; 99% purity from Merck, Germany), curcumin extract, Mueller Hinton Agar (MHA; 99% purity from Merck, Germany), nutrient broth (99% purity from Merck, Germany), n-hexane (C_6H_{14} ; 99% purity from Merck, Germany), cotton, sterile cotton swab, sterile gauze, and distilled water (H_2O). *Escherichia coli* and *Staphylococcus aureus* were the bacterial isolates used for the study.

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Preparation of cellulose derivatives

The modification of cellulose was carried out by the esterification method. A saturated solution of cellulose in chloroform was prepared at room temperature. Cellulose was added to the solution. The suspension was stirred at 300 rpm at 100°C for 6 hours. After the reaction was completed, the suspension was filtered, and the solid was washed with ethanol and then ethyl acetate. The solid was dried at room temperature to a constant weight.

Preparation of hydrogels

The hydrogels were prepared using the chemical crosslinking technique. A solution of NaOH:urea:quadedest (0.7:1.2:8.1) was prepared (Table 1). The mixture was stirred until the solid was completely dissolved. A modified nanocellulose polymer was added to the mixture. The mixture was stirred again until completely dissolved to form a cloudy liquid. The solution was cooled to -4°C for 15 hours, and the frozen solution was allowed to thaw. Then, epichlorohydrin was added as a crosslinker. The solution was again stirred at 600 rpm for 30 minutes. Curcumin extract was added and then stirred again. The slurry was printed and dried in an oven at 60°C for 5 hours.

Characterization of the hydrogels

a) Characterization using Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) analysis was used to examine the functional groups in hydrogel and modified cellulose. The procedure was performed by combining 5 mg of the sample with 95 mg of potassium bromide. After being crushed, the mixture was formed into a thin pellet. The dainty plate that had been made was placed in a cut, and the bar was passed through to establish the FTIR spectrum.¹⁰ The FTIR spectra on all product sheets were between 500 and 4500 cm⁻¹. In addition, the FTIR spectrum was also used to determine the degree of substitution by comparing the absorbance of the carbonyl (about 1700 cm⁻¹) and hydroxyl (about 3300 cm⁻¹) groups of modified cellulose.

b) Characterization using X-ray diffraction analysis

There are crystalline and amorphous phases in cellulose. The crystallinity index, which is calculated using the Segal equation, can be used to determine the crystalline phase.¹¹ Each sample was measured with a range of 2θ = 10-90° and a scan rate of 2°/min.

c) Characterization using scanning electron microscopy

Scanning electron microscopy (SEM) was used to observe the surface morphology of cellulose and cellulose acetate. The materials were analyzed using a Field Emission Scanning Electron Microscope (FEI SEM) Inspect-S50 after being placed in a specimen holder.¹²

Determination of the degree of substitution

The degree of substitution (DS) is the average number of glucose substituted by other groups. In this case, the group in concern is oleate. Based on the FTIR results, the degree of substitution was ascertained by comparing the absorbance of the carbonyl group with the absorbance of the hydroxyl group. The DS was calculated using the following equation:¹³

$$DS = 0.76 \times \frac{\text{Abs C=O}}{\text{Abs-OH}}$$

DS: Degree of substitution; Abs C=O: Absorbance of the carbonyl group; Abs-OH: Absorbance of the hydroxyl group.

Release and swelling tests

The release ability of the curcumin extract contained in the hydrogels was tested to determine the ability of the extract to be released from the hydrogel pores. During the 24-hour release test, a sample was taken every two hours and subjected to UV-Vis spectrophotometer measurements at a wavelength of 231 nm. The wavelength of 231 nm is the λ_{max} of the ethanol extract of turmeric rhizome.

Evaluation of the antibacterial activity of the hydrogels

The antibacterial activity of the hydrogels was evaluated by the disc diffusion method. The test bacteria used were the Gram-negative bacterium, *E. coli*, and the Gram-positive bacterium, *S. aureus*.

Table 1: Concentration of hydrogel constituents

Sample code	Nanocellulose (g)	Urea (g)	Sodium hydroxide (g)	Epichlorohydrin (mL)	Curcumin extract (g)
H-NCAB2	0.4	1.2	0.7	0.2	0.02
H-NCAB4	0.4	1.2	0.7	0.4	0.02
H-NCAB6	0.4	1.2	0.7	0.6	0.02

H-NCAB2: Nanocellulose derivatives with 2% of cross-linker; H-NCAB4: Nanocellulose derivatives with 4% of cross-linker; H-NCAB6: Nanocellulose derivatives with 6% cross-linker.

Results and Discussion

Nanocellulose derivatives

Nanocellulose was modified with abietic acid from rosin to produce nanocellulose derivatives. The reason for the modification process was to protect the hydroxyl groups to obtain a more nonpolar compound so that this compound could slowly release curcumin extract from inside the hydrogel. The modified reaction of cellulose with abietic acid is shown in Figure 1. An ester compound will be created when the hydroxyl group of cellulose and the carboxylic group of abietic acid react. The esterification reaction on nanocellulose is most likely to occur at C6 because there is a large space to bind to the abietyl group at this location.⁶ Thus, it can weaken the steric effect that occurs.

Characteristics of nanocellulose and nanocellulose derivatives according to FTIR analysis

The changes in the functional groups of the samples were examined by measuring the FTIR spectra of nanocellulose and its derivative (Table 2). It was observed that there was a new peak in the area of 1745 cm⁻¹ that appeared after the esterification reaction. This peak correlates with the appearance of the C=O ester bond, which occurred due to the formation of a bond between cellulose and abietic acid.

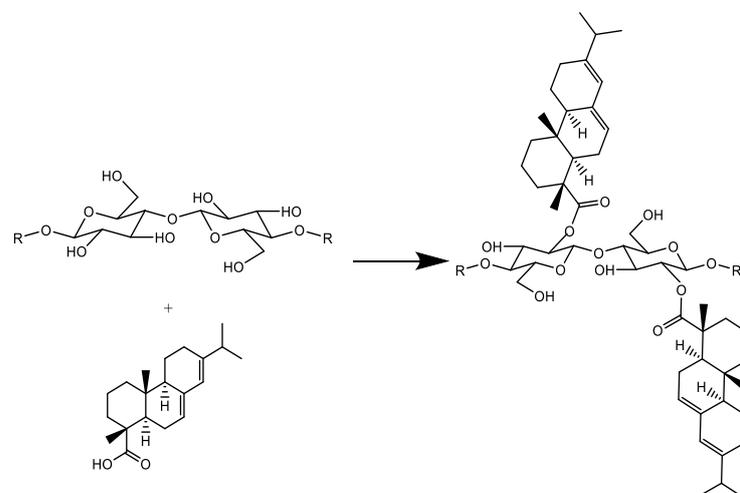


Figure 1: Esterification reaction of cellulose with abietic acid of rosin.

Furthermore, the intensity of the peak in the area of 2800 cm^{-1} increased. This may be influenced by the addition of the amount of C-H from abiatic acid.²⁰ Based on the FTIR spectrum in Figure 2, the DS value was determined for abiatic nanocellulose (CAB). The computation results indicated that the DS value was 0.602. Theoretically, nanocellulose has a DS value between 0 and 3. A DS value of 0 indicates pure nanocellulose, while a DS value of 3 shows all the hydroxyl groups of nanocellulose substituted by other functional groups.²¹ In the present study, the CAB had a DS value of 0.602, suggesting that 20% of the hydroxyl groups of nanocellulose were substituted by abietyl groups.

The nanocellulose derivative (NCAB) crystallinity analysis was conducted by XRD analysis, and the results of the diffractogram of nanocellulose before and after the esterification reactions are presented in Figure 2. Based on the diffractogram obtained, there was a significant difference between them. The nanocellulose diffractogram had two peaks at 16° and 22° . Meanwhile, after the esterification reaction, the peak in the 22° region disappeared and was replaced by a single 16° region peak. This indicated that the crystalline region of the sample had changed into an amorphous one.^{3,4,11,18,22} However, it is necessary to further study the purification process from NCAB samples because the machine product had a low DS value according to the FTIR results. A low DS value indicates that a few crystalline areas turn amorphous.^{23,24}

Morphology of nanocellulose derivatives according to scanning electron microscopy

Figure 3 shows the morphology of the modified nanocellulose according to SEM. The reaction slightly transformed into a targeting product, as indicated by the amorphous phase observed.²⁵ The results also showed increased agglomeration and aggregation due to more surface hydroxyl group exposure, resulting in enhanced intra- and intermolecular hydrogen bonding.²⁵ The SEM image of the cross-section of the NCAB hydrogel is shown in Figure 6. The outcomes showed well-defined, interconnected, three-layered permeable designs with critical macropores. These pores served as regions of water permeation, allowing water to easily diffuse into them. Also, the honeycomb-like macroporous structure of hydrogel has significant functions for biomedical applications due to its capacity to facilitate drug loading and release.²⁶ The space in the network of hydrogels is large due to the electrostatic repulsion by the large anion, abietyl.²⁷ Based on the results of the analysis with Image J software, the hydrogel pores ranged from 2.2 to 4.4 micrometers.

Release and swelling properties of the hydrogels

The swelling test was conducted to assess the ability of the hydrogels to absorb water within 24 hours. Table 3 shows the results of the swelling test of the hydrogel, indicating that the swelling ability increased with the amount of crosslinker supplied. This is because when epichlorohydrin concentration rises, so does hydrogel cross-linking, resulting in the formation of an increasing number of hydrogel network structures. Consequently, there is also an increased capacity to absorb water.²⁸ To determine how quickly the curcumin extract was released from the hydrogels, a release test was conducted on the hydrogels. The release test was carried out on all synthesized hydrogels, namely H-NCAB2, H-NCAB4, and H-NCAB6. Figure 7 displays the release test chart for each hydrogel. Using a UV-Vis spectrophotometer, the

concentration of curcumin extract was measured on the y-axis, while the x-axis displays the testing time. The hydrogels exhibited a high release of extract during the first eight hours, followed by a steady release for up to twenty-four hours, according to the obtained graph. The total maximum concentration of curcumin released for 24 hours was 36%. In contrast to the hydrogel derived from unmodified nanocellulose, this release test revealed superior outcomes, as evidenced by the higher concentration of curcumin retained within the hydrogel. This finding aligns with its enhanced swelling capabilities compared to nanocellulose-based hydrogels.¹⁴

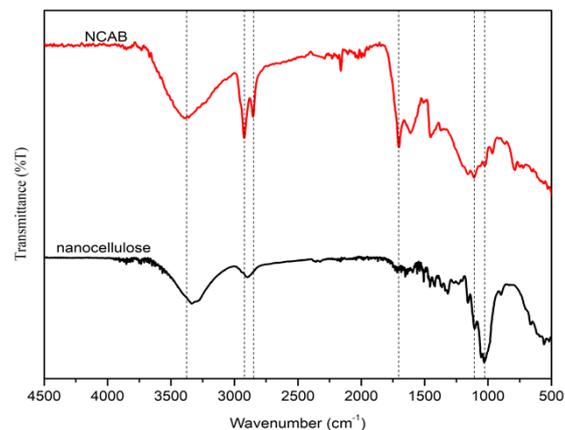


Figure 2: Fourier transform infrared spectroscopy (FTIR) spectra of nanocellulose and nanocellulose derivatives.

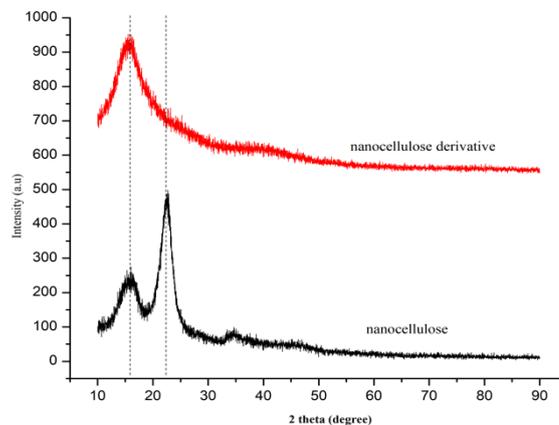


Figure 3: X-ray diffraction (XRD) diffractograms of nanocellulose and nanocellulose derivatives.

Table 2: Fourier transform infrared spectroscopy (FTIR) spectra interpretation of nanocellulose and nanocellulose derivatives

Wavenumber (cm^{-1})		Functional group	References
Nanocellulose	Nanocellulose derivative (NCAB)		
3333	3387	-OH stretching	[14]–[16]
-	2904	C-H cellulose	[14]–[16]
2895	2880	C-H cellulose	[14]–[17]
-	1745	C=O ester	[4], [11], [18]
1005	1105	C-O-C Pyranose ring	[3], [4], [11], [15], [17], [19]

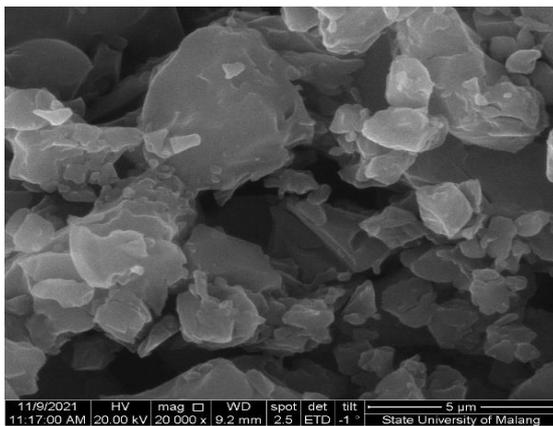
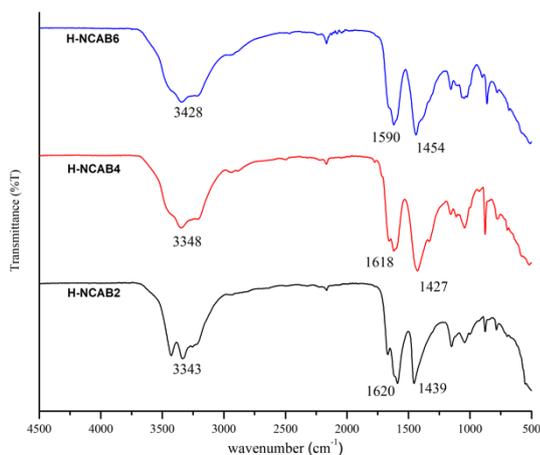
Antibacterial activity of the hydrogel

The results of the hydrogel antibacterial test demonstrated that the hydrogels did not affect the curcumin extract's capacity to inhibit the test bacteria (Figure 8). The NCAB hydrogel was observed with two repeats against the bacteria *S. aureus* and *E. coli*. After a 24-hour incubation period, the clear zone for *E. coli* measured 3.02 mm and for *S. aureus*, 5.51 mm. Theoretically, antibacterial activity is categorized as weak if the diameter of the inhibition zone is <5 mm, moderate if it is 5-10 mm, strong if it is 19-20 mm, and very strong if the diameter of the inhibition zone is >20 mm.²⁹⁻³² As a result, the hydrogels had moderate antibacterial activity against *S. aureus* and weak antibacterial activity against *E. coli*. This outcome resulted from the hydrogels effectively trapping the curcumin extract. The potency of the hydrogel's antibacterial properties is diminished compared to unmodified nanocellulose-based hydrogels due to limited curcumin release into the bacterial growth medium.¹⁴

Table 3: Percentage swelling ratio of hydrogels

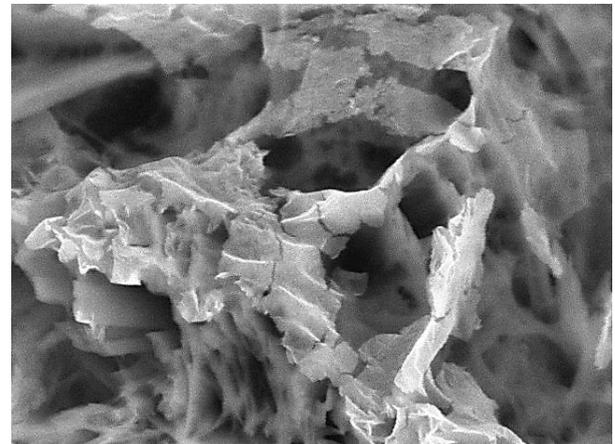
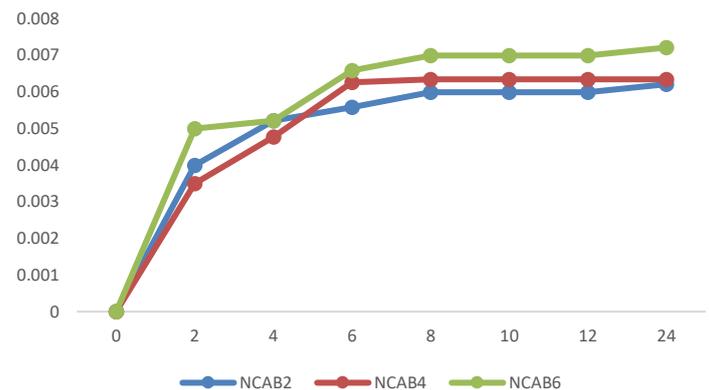
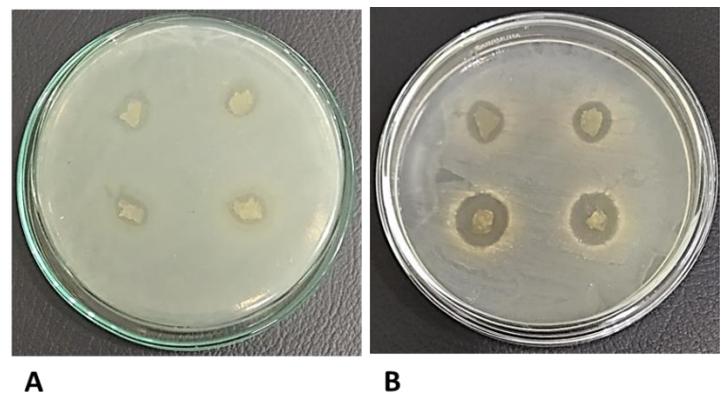
Sample	% swelling
H-NCAB2	159.52
H-NCAB4	174.40
H-NCAB6	185.24

H-NCAB2: Nanocellulose derivatives with 2% of cross-linker; H-NCAB4: Nanocellulose derivatives with 4% of cross-linker; H-NCAB6: Nanocellulose derivatives with 6% cross-linker.

**Figure 4:** Electron micrograph of nanocellulose derivatives.**Figure 5:** Fourier transform infrared spectroscopy (FTIR) spectra of hydrogels-based nanocellulose derivatives (NCAB) with different concentrations of epichlorohydrin (ECH).

Conclusion

The findings of the present study revealed that nanocellulose could be modified through an esterification reaction with rosin. Chemical crosslinking can be used for producing modified nanocellulose hydrogels, and the crosslinker was epichlorohydrin. The release and swelling tests were not significantly impacted by the crosslinker concentration; the higher the crosslinker concentration, the lower the swelling ratio.

**Figure 6:** Cross-sectional of hydrogels from nanocellulose derivatives with 2% of cross-linker (H-NCAB2).**Figure 7:** Release properties of curcumin extract from hydrogels.**Figure 8:** Antibacterial activity of hydrogels from nanocellulose derivatives.

A: *Escherichia coli*; B: *Staphylococcus aureus*

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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