

ISSN 2181-8622

Manufacturing technology problems



Scientific and Technical Journal Namangan Institute of Engineering and Technology

INDEX  COPERNICUS
INTERNATIONAL

**Volume 10
Issue 4
2025**



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OXIDATION OF VARIOUS CELLULOSE CONTAINING MATERIALS USING THE $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ SYSTEM

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Abstract: The oxidation of cellulose-containing materials is an important approach for producing functional derivatives with biomedical applications. In this study, the oxidation behavior of cotton cellulose, medical gauze, and viscose fabric was systematically investigated using an $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ oxidizing system. The effects of oxidizing mixture composition, sodium nitrite concentration, reaction temperature, and reaction duration on the degree of oxidation (DO), oxidation rate, and product yield were evaluated. The results demonstrated that increasing the content of orthophosphoric acid significantly enhanced the DO of the obtained products due to improved fiber swelling and increased accessibility of reactive hydroxyl groups. An optimal NaNO_2 concentration of 1.4% was identified, providing maximum oxidation efficiency through increased formation of nitrogen dioxide in the reaction medium. The oxidation rate was highest during the initial stages of the reaction (1–6 h) and gradually decreased with time as the concentration of oxidizing species diminished. Elevated temperatures and prolonged reaction times led to higher DO values but caused a reduction in product yield due to polymer chain degradation.

Comparative analysis revealed that viscose fabric exhibited the highest reactivity, while cotton cellulose showed the lowest oxidation rate under identical conditions, which was attributed to differences in crystalline structure and amorphous content. Overall, the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ system proved to be an efficient and selective method for obtaining monocarboxylated cellulose with high degrees of oxidation, provided that the reaction parameters are carefully optimized.

Keywords: cellulose oxidation, monocarboxyl cellulose, degree of oxidation, cellulose based materials, oxidized viscose, nitrogen dioxide, carboxyl groups.

Introduction. One of the most practically significant derivatives of cellulose is oxidized cellulose, particularly monocarboxylated cellulose. The growing interest in this material is primarily associated with its medical applications, as oxidized cellulose exhibits hemostatic and biodegradable properties that are characteristic of only a limited number of natural biopolymers. A key advantage of oxidized cellulose over many conventional hemostatic agents is its excellent biocompatibility: it does not induce allergic reactions in the human body and, after implantation, is gradually resorbed and completely degraded into carbon dioxide and water.

The properties of oxidized cellulose strongly depend on the nature of the polysaccharide precursor used in its preparation. Specifically, the type and origin of cellulose directly influence the morphology, physicochemical characteristics, and pharmacological performance of the resulting product. Consequently, careful selection of the cellulose source is a critical factor in tailoring oxidized cellulose for biomedical applications.

The hemostatic activity of monocarboxylated cellulose is closely related to its degree of oxidation (DO). Numerous studies have demonstrated that an increase in the content

of carboxyl functional groups leads to a corresponding enhancement of hemostatic efficiency [1]. This effect is attributed to the ability of carboxyl groups to participate in both specific and nonspecific interactions with amino groups of blood proteins, particularly serotonin-containing components present in blood plasma. These interactions promote local coagulation processes and ultimately result in the manifestation of hemostatic activity.

Despite the high medical importance of oxidized cellulose, its production process remains highly complex and requires carefully controlled conditions. During cellulose oxidation, various chemical systems have been employed, and their selectivity, efficiency, and practical applicability have been extensively discussed in the literature. One of the classical approaches is the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ system, which has been widely used for the preparation of oxidized cellulose. Kumar and Yang reported that oxidation of cellulose in the presence of the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ system results in carboxyl group contents of approximately 14–18% with product yields of 75–81%; however, the process is relatively time-consuming and the strongly oxidizing conditions may lead to partial degradation of the polymer chains [2]. Nevertheless, this system has been extensively applied in industrial practice and continues to play an important role in the production of hemostatic materials. The principal advantage of this system lies in the preferential oxidation of primary hydroxyl groups at the C6 position, which ensures the formation of monocarboxylated cellulose.

Consistent with these findings, Xu et al. reported that oxidation of bamboo pulp using the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ system achieved efficient carboxyl group introduction within short reaction times (15–60 min), accompanied by notable changes in fiber morphology and structural properties [3].

In contrast to nitric-acid-based methods, TEMPO-mediated oxidation represents a modern and highly selective approach for cellulose modification. Seminal studies by Saito and Isogai revealed that the TEMPO/NaBr/NaClO system selectively oxidizes the C6 primary hydroxyl groups to carboxyl groups while largely preserving the crystalline structure of cellulose [4]. Subsequent investigations further demonstrated that TEMPO-oxidized cellulose can be readily disintegrated into individualized nanofibrils through mild mechanical treatment, yielding highly dispersed cellulose nanofiber suspensions with superior functional properties [5].

Nevertheless, several drawbacks associated with TEMPO-mediated oxidation have been identified. The use of NaClO and NaBr necessitates strict pH control and raises concerns regarding reagent consumption, halide residues, and environmental impact. Recent comprehensive reviews have emphasized the need for optimization strategies aimed at reducing oxidant dosage and improving the sustainability of the TEMPO process while maintaining its high selectivity toward C6 oxidation [6].

Alternative TEMPO-assisted systems employing sodium chlorite (NaClO_2) as the primary oxidant have also been explored. Hirota et al. reported that cellulose can be efficiently oxidized under acidic to neutral conditions using NaClO_2 in the presence of TEMPO and NaClO, resulting in selective formation of carboxyl groups without severe

degradation of the cellulose backbone [7]. Such approaches offer additional flexibility in reaction conditions and may mitigate some of the limitations associated with conventional TEMPO/NaClO systems.

More recently, nitric-acid-based “nitro-oxidation processes” (NOPs) have been proposed as emerging alternatives for the functionalization of lignocellulosic materials. These methods typically involve combined liquid- and gas-phase treatments using nitric acid and nitrite species, enabling efficient carboxylation while reducing water and energy consumption. Chen et al. demonstrated that NOP techniques can produce highly functionalized cellulose-based materials with promising adsorption and application potential [8]. Although such methods appear environmentally and technologically attractive compared to traditional $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ systems, further optimization and standardization of processing parameters are required before large-scale implementation can be realized.

Although numerous studies have been conducted on the synthesis of monocarboxylated cellulose, there is a lack of systematic investigations on the oxidation reactions of various cellulose-containing materials that differ in composition and crystalline structure. Therefore, it is of particular interest to comparatively examine the oxidation behavior of structurally distinct cellulose-containing substrates, such as cotton cellulose, medical gauze, and viscose fabric, in order to identify the most suitable material for obtaining monocarboxylated cellulose with a high degree of oxidation.

Methods. Reagents and Materials. The following reagents and materials were used in this study: cotton cellulose (DP = 1400, MW = 225 kDa), viscose (Ss 8871-84), medical gauze (Ts 25060463-004:2021), nitric acid, phosphoric acid, sodium nitrite, isopropyl alcohol and acetone. All reagents were used as received without further purification.

Oxidation of Cellulose Containing Materials Using $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$

A mixture of 70% nitric acid and 85% phosphoric acid in a 1:1 volumetric ratio was prepared. To 70 mL of this acid mixture, 5 g of the cellulose-containing sample (cotton cellulose, viscose, or medical gauze) was added. Once the sample was completely immersed in the solution, 1 g of sodium nitrite was introduced. The flask was immediately sealed when the evolution of a reddish-brown gas was observed. The reaction mixture was stirred periodically throughout the process.

The oxidation reactions were carried out at temperatures ranging from 10 to 40°C for 6–72 hours. Upon completion of the reaction, the mixture was filtered, and the solid residue was washed with water until the pH reached 4–4.5. The residue was then sequentially washed with acetone and subsequently with isopropyl alcohol, followed by drying to obtain the oxidized cellulose products.

Determination of Carboxyl Group Content

The content of carboxyl groups in the cellulose-containing oxidized materials was determined using the calcium acetate method [9]. For this purpose, 1 g of the sample (previously dried to constant weight) was placed in a 250 mL beaker, and 60 mL of 0.25 M calcium acetate solution along with 100 mL of distilled water was added. The mixture was stirred continuously for 6 hours to ensure complete interaction. Subsequently, a 25

mL aliquot of the resulting solution was titrated with 0.01 M NaOH solution. Bromothymol blue was used as an indicator to determine the endpoint of the titration.

The carboxyl group content was determined following the guidelines of the United States Pharmacopoeia. Initially, a 2% calcium acetate solution and a 0.1 M NaOH solution were prepared. A 0.5 g sample of oxidized cellulose immersed in 50 mL of the 2% calcium acetate solution for 0.5 hours. After thorough soaking, the resulting mixture was titrated with 0.1 M NaOH using phenolphthalein as an indicator. The titration volume was corrected by subtracting the blank value. The carboxyl content was then calculated using the following formula:

$$-COOH (\%) = \frac{N * V * MW(COOH)}{m} * 100$$

where N is the normality of 0.1 M NaOH solution, V is the consumed volume of NaOH which is corrected for the blank, $MW(COOH)$ is the molecular weight of carboxyl group and m is the weight of the sample.

In our study, an oxidizing mixture composed of $HNO_3/H_3PO_4-NaNO_2$ was employed for cellulose oxidation. This choice was motivated by the relatively high content of nitrogen oxides present in this mixture, which facilitates efficient oxidation of the cellulose substrate. It is known that the oxidation process of cellulose begins with the removal of one hydrogen atom from the cellulose molecule by unpaired electrons in the NO_2 molecule and the formation of $Cel-C(\bullet)H-OH$. The release of $NO\bullet$ and HNO_2 from the intermediate products formed as a result of the subsequent attack of $NO_2\bullet$ leads to the formation of $Cel-CH(OH)_2$ and $Cel-CHO$. In the next stage, the elimination of HNO_2 and the subsequent attack of $NO_2\bullet$ leads to the formation of $Cel-COOH$ from $Cel-CH(OH)_2$. In the case of $Cel-CHO$, the intermediate product formed as a result of hydrogen evolution and $NO_2\bullet$ attack is first hydrolyzed and $Cel-COOH$ is formed [2]. The oxidation reaction of cellulose is illustrated in Figure 1.

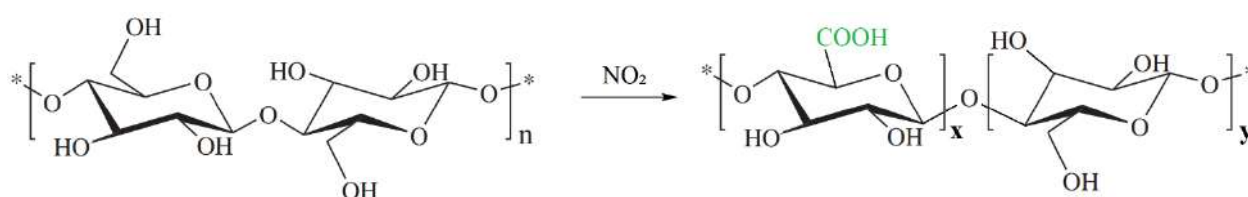


Figure 1. Oxidation of cellulose using nitrogen dioxide (NO_2)

In our study, the influence of various factors on the oxidation reaction was investigated to determine its optimal conditions. Initially, with the temperature and reaction time kept constant ($t = 25^\circ C$, $\tau = 24$ h), the effect of the phosphoric acid content in the oxidizing mixture on the progress of the reaction was examined. It was observed that increasing the amount of phosphoric acid led to higher degrees of oxidation (DO) in the obtained products. The results of this investigation are presented in Figure 2.

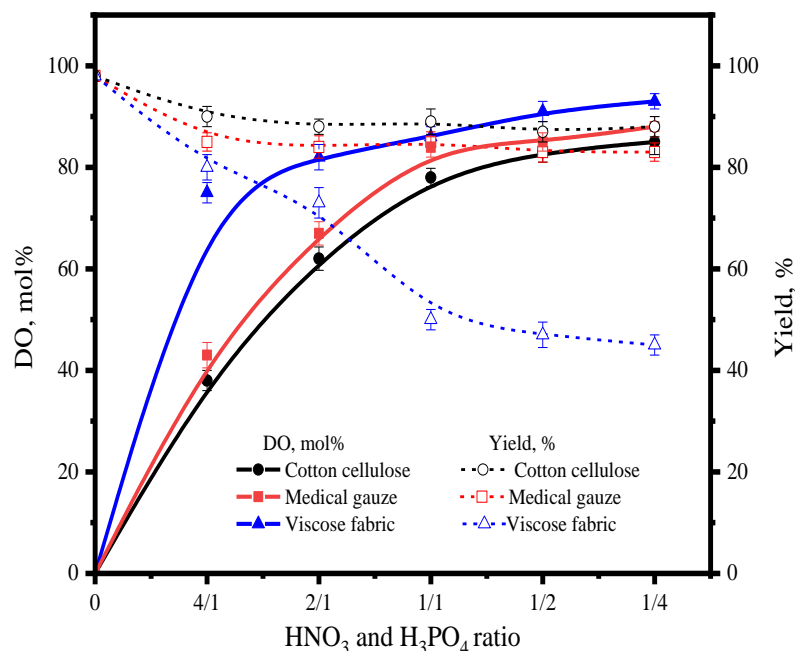


Figure 2. Dependence of the degree of oxidation (DO) and yield of the obtained products on the acid ratio in the oxidizing mixture

As shown in Figure 2, when the volumetric ratio of nitric acid to orthophosphoric acid was varied from 4:1 to 1:4, the degree of oxidation of the obtained products increased significantly: from 38 to 85 mol% for cotton cellulose, from 54 to 88 mol% for samples based on medical gauze, and from 75 to 93 mol% for viscose fabric. This clearly indicates the critical role of H₃PO₄ in the oxidation process.

We suggest that orthophosphoric acid acts partially as a swelling agent during cellulose oxidation. Its presence promotes the swelling of cellulose fibers and induces changes in fiber morphology, leading to an increase in the amorphous regions of the macromolecule. Consequently, oxidizing molecules can move more freely along the polymer chains, facilitating the oxidation reactions.

Our results demonstrated that, regardless of the ratio of nitric to phosphoric acid in the oxidizing mixture, the oxidation reactions proceeded with high efficiency. However, in the case of viscose fabric, unlike cotton cellulose and medical gauze, an increase in the phosphoric acid content resulted in a pronounced decrease in product yield. This behavior can be attributed to the structural features of viscose: its macromolecules contain fewer crystalline regions and relatively weaker hydrogen bonding, making them more susceptible to hydrolysis and degradation under the influence of phosphoric acid.

Our investigations revealed that with an increasing content of orthophosphoric acid in the oxidizing mixture, the fibrous characteristics of the products gradually disappeared, and the material became progressively more rigid. We attribute this phenomenon to the partial dissolution of cellulose samples during the oxidation process, which occurs as the H₃PO₄ content in the oxidizing mixture increases, leading to alterations in fiber morphology.

Additionally, the effect of NaNO_2 concentration in the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ oxidizing mixture on the progress of cellulose oxidation was studied (Figure 3). It was observed that an increase in NaNO_2 content in the reaction mixture led to a higher degree of oxidation of the products. This behavior can be explained by the interaction of NaNO_2 with the acids in the mixture to form HNO_2 , which subsequently decomposes rapidly to produce NO_2 . As a result, the concentration of NO_2 in the oxidizing mixture increases, enhancing the oxidative capacity of the system.

Our results showed that a NaNO_2 concentration of 1.4% provided the maximum degree of oxidation of the products. Further increases in NaNO_2 content did not significantly affect the DO values.

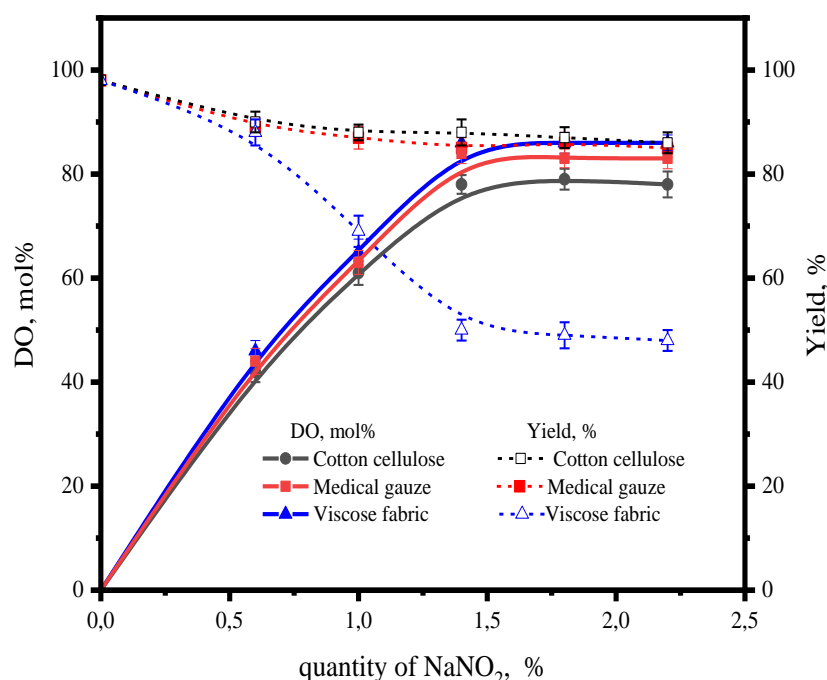


Figure 3. Dependence of the degree of oxidation (DO) and yield of the obtained products on the NaNO_2 content in the oxidizing mixture (Reaction conditions: $t = 25^\circ\text{C}$, $\tau = 24$ h)

In subsequent experiments, the effect of temperature on the progress of the oxidation reaction was investigated (Figure 4). In these experiments, the composition of the oxidizing mixture was kept constant (volumetric ratio of $\text{HNO}_3:\text{H}_3\text{PO}_4 = 1:1$, $\text{NaNO}_2 = 1.4\%$). It was observed that increasing the reaction temperature led to higher degrees of oxidation in the products. Specifically, raising the temperature from 10°C to 40°C resulted in DO values increasing from 21 to 92 mol% for cotton cellulose, from 24 to 93 mol% for samples based on medical gauze, and from 30 to 96 mol% for viscose fabric.

However, a significant decrease in product yield was noted with increasing temperature (Figure 4). This behavior is attributed to the enhanced reactivity of the acids at higher temperatures, which promotes degradation of the polymer chains, thereby reducing the overall yield.

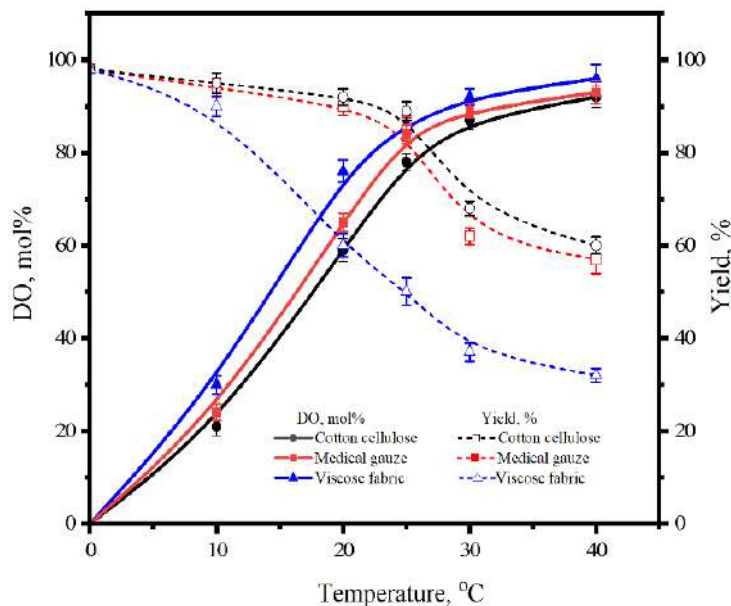


Figure 4. Dependence of the degree of oxidation (DO) and yield of the obtained products on the reaction temperature

In the subsequent experiments, the effect of reaction duration on the oxidation process was investigated (Figure 5). In these experiments, the composition of the oxidizing mixture was kept constant (volumetric ratio of $\text{HNO}_3:\text{H}_3\text{PO}_4 = 1:1$, $\text{NaNO}_2 = 1.4\%$). It was observed that extending the reaction time led to an increase in the degree of oxidation (DO) of the products. Specifically, increasing the reaction duration from 6 to 72 hours resulted in DO values rising from 25 to 90 mol% for cotton cellulose, from 30 to 93 mol% for medical gauze, and from 76 to 98 mol% for viscose fabric (Figure 5).

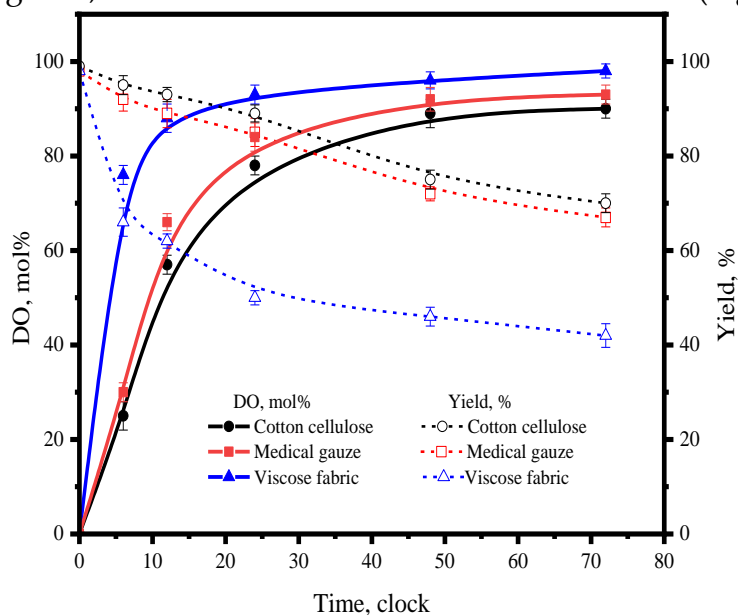


Figure 5. Dependence of the degree of oxidation (DO) and yield of the obtained products on the reaction duration

Our investigations revealed that each cellulose-containing sample entered the oxidation reaction at a different rate. Specifically, under constant conditions of the oxidizing mixture composition and temperature (volumetric ratio $\text{HNO}_3:\text{H}_3\text{PO}_4 = 1:1$, $\text{NaNO}_2 = 1.4\%$, $T = 25^\circ\text{C}$), the average oxidation rate of the products varied differently with increasing reaction time (Figure 6).

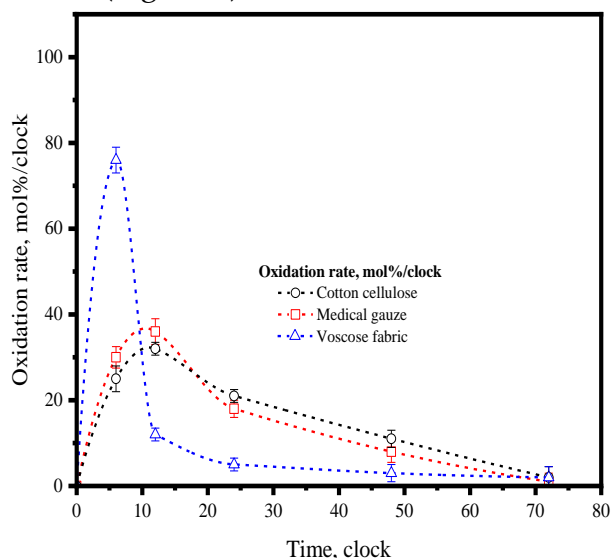


Figure 6. Variation of the oxidation rate of the samples during the oxidation process in the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ oxidizing mixture

As illustrated in Figure 6, during the initial 6 hours of the reaction, the oxidation rates were 4.17 mol\%/h for cotton cellulose, 5.06 mol\%/h for samples based on medical gauze, and 12.67 mol\%/h for viscose fabric. In the subsequent 6-hour period (6–12 h), the rates were 5.33 mol\%/h for cotton cellulose, 6.08 mol\%/h for medical gauze, and 2.07 mol\%/h for viscose fabric. Over the following 12 hours (12–24 h), the reaction rates decreased to 1.75 mol\%/h , 1.50 mol\%/h , and 0.42 mol\%/h for cotton cellulose, medical gauze, and viscose fabric, respectively. During the next 24 hours (24–48 h), the rates further declined to 0.46 mol\%/h , 0.33 mol\%/h , and 0.125 mol\%/h , and in the final 24-hour interval (48–72 h), the oxidation rates reached 0.08 mol\%/h for cotton cellulose, 0.04 mol\%/h for medical gauze, and 0.08 mol\%/h for viscose fabric.

The results indicate that the oxidation rate is highest during the first 1–6 hours of the reaction. This can be attributed to the high concentration of the oxidizing reagent at the initial stage and the abundance of free hydroxyl groups in the polymer macromolecule. As the reaction progresses, the amount of oxidizing reagent decreases, leading to a gradual decline in the oxidation rate.

Among the samples studied, cotton cellulose exhibited relatively low reactivity, whereas viscose fabric showed high reactivity. The differences in reactivity in the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ oxidizing mixture are associated with variations in their crystalline structure and physicochemical properties. The relatively lower reactivity of cotton cellulose can be explained by its macromolecular structure, which hinders the diffusion of reagents to hydroxyl groups and involves these hydroxyl groups in

intermolecular hydrogen bonding [10]. Figure 7 shows the structural arrangement of the cellulose macromolecule.

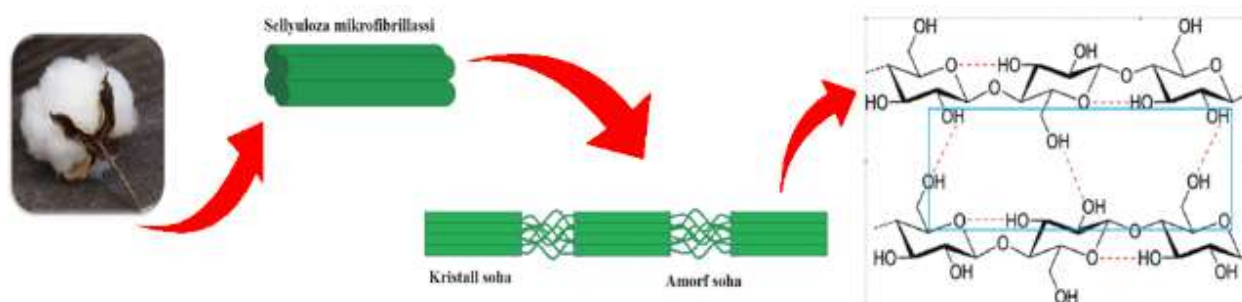


Figure 7. Structural representation of the cellulose macromolecule

Cellulose with a higher content of amorphous regions reacts more readily than crystalline cellulose, as the disordered domains in its macromolecule allow easier penetration and interaction of reactive species [11].

Conclusion. In this study, the oxidation behavior of various cellulose-containing materials, including cotton cellulose, medical gauze, and viscose fabric, was systematically investigated using the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ oxidizing system. The results demonstrated that the composition of the oxidizing mixture, as well as reaction temperature and duration, play a decisive role in determining the degree of oxidation (DO), reaction rate, and product yield.

An increase in the orthophosphoric acid content in the oxidizing mixture led to a significant enhancement of the DO values for all studied materials, indicating the crucial role of H_3PO_4 in promoting fiber swelling, partial dissolution, and increased accessibility of reactive sites. Similarly, increasing the NaNO_2 concentration resulted in higher oxidation efficiency due to the elevated formation of nitrogen dioxide in the reaction medium, with an optimal NaNO_2 content of 1.4%, beyond which no substantial improvement in DO was observed.

The oxidation rate was found to be highest during the initial stages of the reaction (1–6 h), which can be attributed to the high concentration of oxidizing species and the abundance of accessible hydroxyl groups. Prolonged reaction times and elevated temperatures further increased the DO; however, these conditions also caused a notable decrease in product yield due to enhanced acid-induced degradation of the polymer chains.

Comparative analysis revealed that viscose fabric exhibited the highest reactivity, while cotton cellulose showed the lowest oxidation rate under identical conditions. These differences were attributed to variations in crystalline structure, hydrogen bonding, and amorphous content of the materials. Overall, the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NaNO}_2$ system proved to be an effective and selective approach for producing monocarboxylated cellulose with high degrees of oxidation, provided that the reaction parameters are carefully optimized to balance oxidation efficiency and product yield.

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