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PREPARATION, CHARACTERIZATION, AND HEMOSTATIC PROPERTIES OF TRICARBOXYCELLULOSE

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ABSTRACT

In this study, tricarboxycellulose samples were synthesized through the sequential oxidation of cellulose, woven gauze, and medical cotton using $NaClO$, $NaIO_4$, and H_2O_2. The resulting materials were thoroughly characterized in terms of their physicochemical and sorption properties. The oxidation process led to a substantial increase in the carboxyl group content, reaching 53% in cellulose, 46% in woven gauze, and 42% in medical cotton. Infrared (IR) spectroscopy confirmed structural modifications in cellulose, indicating the formation of new functional carboxyl groups. The modified tricarboxycellulose samples exhibited markedly improved sorption capacities-up to 830% for cellulose, 670% for woven gauze, and 520% for medical cotton. Owing to their enhanced sorption performance, biodegradability, and hemostatic properties, tricarboxycellulose-based materials show strong potential for application in medical settings as effective hemostatic agents.

Introduction

Cellulose is a biodegradable, non-toxic, and hygroscopic material that is also insoluble in water and common organic solvents. Issues related to its dissolution have been reported in the literature [1]. The insolubility of cellulose is attributed to its chemical structure as well as a number of contributing factors. Firstly, cellulose is a macromolecule with a high degree of polymerization, and its dissolution is hindered by the presence of strong hydrogen bonds, which results in a reduced entropic gain for macromolecules in solution compared to small molecules [2–3]. In addition, cellulose contains a large number of hydroxyl groups, and the five oxygen atoms of these hydroxyl groups, in addition to the ring and bridging oxygens, form complex hydrogen-bonding networks [4]. The reactive functional groups of cellulose are hydroxyl groups, which are prone to various chemical modifications; therefore, it is possible to obtain a wide range of cellulose derivatives [5].

In particular, the sequential oxidation of cellulose with various oxidizing agents results in the formation of oxidized celluloses containing multiple functional groups. In this process, mono-carboxycellulose containing up to 1–25% –COOH groups was obtained [6].

Oxidation of cellulose can be carried out using various oxidizing systems. These include oxidizing agents such as nitrogen oxides, nitrates, and nitrites [7], peroxides [8], sodium chlorite, and permanganates [9].

In selective oxidation, the process is influenced by several factors, including the nature and type of the oxidizing agent, the pH of the reaction medium, temperature, solvent, and other parameters. In this case, the final products differ not only in the nature of the newly introduced functional groups but also in the specific positions at which they are attached to the cellulose molecule [10].

The type of oxidation depends on the position of the hydroxyl group and proceeds as follows: primary hydroxyl groups (at the C6 carbon atom) are converted into aldehyde or carboxylic acid groups [11], while secondary hydroxyl groups (at C2 and C3) are transformed into ketone and aldehyde groups. In this process, cleavage of the C2–C3 bond leads to the opening of the glucose ring [12].

The oxidizing agents most widely used in the selective oxidation of cellulose include nitroxyl radicals and sodium periodate [13]. Sodium periodate is considered a highly effective oxidizing agent in the selective oxidation of cellulose, as it acts on the vicinal secondary hydroxyl groups located at the C2 and C3 positions of the anhydroglucose unit, cleaving the C2–C3 bond and generating adjacent aldehyde groups [14]. Oxidation with $NaIO_4$ results in the formation of dialdehyde cellulose (DAC) [15]. The oxidation process is carried out under mild conditions: at pH 3–3.5 (in an acidic medium), at room temperature (20°C), protected from light, and lasting from 24 to 250 hours [16]. Under these conditions, the mechanism of selective oxidation of cellulose in the presence of sodium periodate is shown in Figure 1.

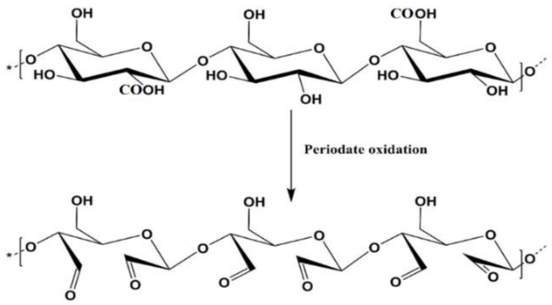


Figure 1. *Mechanism of selective oxidation of cellulose with sodium periodate under mild conditions* [17]

One of the advantages of this method is its high yield and the ability to readily produce dispersible cellulose nanofibrils. Furthermore, the resulting aldehyde groups can be converted into carboxylic acid groups [18] or into imines (Schiff bases) through reactions with amines [19]. At the initial stage of the reaction, the periodate ion acts on the C2-C3 bonds in the crystalline regions, leading to the disruption of the highly ordered macromolecular structure [20]. Even at comparatively low oxidation levels, the intermolecular hemiacetal linkages generated in the highly oxidized regions promoted the formation of more compact and densely organized supramolecular structures [21].

To reduce the reaction time, the experiment was conducted at an elevated temperature (85°C)-above the decomposition temperature of periodate (55°C)-in the presence of metal salts (LiCl, $ZnCl_2$, $CaCl_2$) that activate cellulose. Under such conditions, it has been shown that dialdehyde cellulose (DAC) with a high content of aldehyde groups can be obtained even at relatively low periodate concentrations.

In studies conducted by Martinsson and colleagues [22], it was reported that oxidation of wood cellulose in an acidic medium in the presence of hydrogen peroxide (H_2O_2) resulted in an increase in the formation of carbonyl groups. In some reports, it has been demonstrated that the use of widely employed oxidants, including H_2O_2, as oxidizing agents enables the introduction of carbonyl groups into the cellulose structure. Furthermore, it has been noted that the large-scale production of cellulose with enhanced moisture resistance can be achieved even without the use of additional additives. The reaction mechanism of H_2O_2 under alkaline pH conditions has been studied by researchers [23].

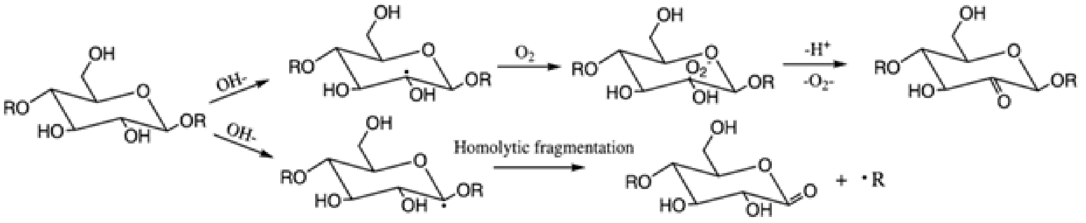


Figure 2. *Mechanism of H_2O_2-mediated oxidation of carboxyl groups to ketones under alkaline conditions*

During the oxidation process under alkaline pH conditions, the main effect is attributed to the hydroperoxide anion generated from the decomposition of hydrogen peroxide, resulting in a pH range of 10-12. At low pH values, hydrogen peroxide undergoes decomposition either via the Fenton reaction with metals or through interaction with organic substances, such as cellulose, leading to the formation of hydroxyl radicals [24]. Hydroxyl radicals can react with several atoms of the carbohydrate chains, with the primary sites being the oxidation of secondary hydroxyl groups or the anomeric carbon atom. The oxidation process is initiated by the abstraction of a hydrogen radical from the carbon atom associated with a secondary hydroxyl group, ultimately leading to the incorporation of oxygen and the formation of a ketone functional group [25].

However, according to the analysis conducted by scientists, this reaction under alkaline conditions is considered to lead to high energy costs.

On the other hand, during the ozonation process under acidic pH conditions, it has been shown that the formation of carbonyl groups primarily occurs through radical-mediated mechanisms. Hydroxyl radicals also oxidize the anomeric carbon atom (C1). The abstraction of a hydrogen radical at the C1 position leads to homolytic cleavage, resulting in chain scission and the formation of carbonyl groups [26].

The environmental impact of peroxide-based oxidation, particularly when carried out with H_2O_2, is considered environmentally benign compared to other chemical oxidants. Upon decomposition, hydrogen peroxide yields water and oxygen, which are harmless to the environment. Nevertheless, this process may generate free radicals and perhydroxyl anions, which can lead to the degradation of cellulose and, in some cases, the formation of harmful by-products.

The aim of this investigation is to explore the possibility of tricarboxycellulose preparation and to study its physicochemical, toxicological and hemostatic properties.

Materials and methods

Materials

Cellulose $(C_6H_{10}O_5)_n$, ≥98%, CAS No.: 9004-34-6; Woven Gauze (Medical Cotton Bandage, ≥95% cellulose, CAS No.: 9004-34-6); Medical Absorbent Cotton (≥98% cellulose, CAS No.: 9004-34-6); Sodium Hypochlorite (NaClO, 10-15%, CAS No.: 7681-52-9); Sodium Periodate ($NaIO_4$, ≥99%, CAS No.: 7790-28-5); Hydrogen Peroxide (H_2O_2, 30–35%, CAS No.: 7722-84-1); Hydrogen Chloride (HCl, 99-100%, CAS No.: 7647-01-0); Sulfuric acid (H_2SO_4, 96–98%, CAS No.: 7664-93-9); sodium hydroxide (NaOH, 98.0%, CAS No.: 1310-73-2); Distilled Water (CAS № 7732-18-5). All the other chemicals were of analytical grade.

Method for the Preparation of Tricarboxycellulose

The oxidation reaction was carried out through the stepwise oxidation of cellulose for its application as a hemostatic agent. In the first stage, 5 g of cellulose was treated with a 14 g/L NaClO solution at a 1:10 liquor ratio for 3-4 hours at 60°C. At the end of the process, the oxidized cellulose was filtered, washed 4-6 times with distilled water until neutral, and lyophilized for 10-12 hours at 80°C. To increase the content of carboxyl groups in the sodium hypochlorite-oxidized cellulose sample, sodium periodate ($NaIO_4$) solution was used to cleave the C2 and C3 carbon atoms. In the subsequent step, the reaction was carried out with a 1.5-2.5% $NaIO_4$ solution for 10-15 minutes at a temperature of 30-35°C. The oxidized cellulose sample was washed 10-12 times with distilled water until neutral and lyophilized for 6-8 hours at 80°C. In the final stage, 3 g of oxidized cellulose was treated with H_2O_2 at a 1:10 ratio by mass, with a concentration of 4-10%, for 1-3 hours at 70-80°C. The resulting reaction mixture was filtered, washed until neutral, and dried to a moisture content of 4 ± 0.5% [27].

Determination of the swelling degree of tricarboxycellulose

The water retention value (WRV) method [28] is considered one of the most optimal approaches for measuring the swelling capacity of oxidized or chemically modified fibers. Initially, 0.2-0.5 g of dry tricarboxycellulose was accurately weighed. The sample was allowed to swell in distilled water for 30 minutes at 25-30°C. Subsequently, the sample was filtered through membranes with a pore size of 50-200 nm or filters of 50-100 μm, and the excess water was removed using a dry filter paper. The sample was centrifuged at 8000 rpm for 10 minutes, and the wet weight was determined.

The following formula was used to calculate the swelling degree of tricarboxycellulose.

$\text{WRV (g/g)} = (m_2 - m_1) / m_1$

Where:

m_1 - initial dry mass of tricarboxycellulose, g;

m_2 – mass of tricarboxycellulose, g, filtered and dried to constant weight.

Method for Determining the Sorption Properties of Tricarboxycellulose

Determination of the sorption properties of oxidized cellulose toward liquids (e.g., blood, water, physiological solution) or ions (e.g., metal cations) [29]. A 1 g sample of tricarboxycellulose was dried at 105°C for 24 hours. The fully dried tricarboxycellulose sample was immersed in distilled water or physiological solution at 25°C, using a 1:10 liquor ratio, for 24 hours. After 24 hours, the sample was blotted with filter paper to remove surface liquid and then weighed.

Calculation of the degree of sorption (SD):

$\text{SD} = W_1 - W_0 / W_0 \times 100\%$

Where:

W_0 –initial dry mass of tricarboxycellulose, g;

W_1 – mass of tricarboxycellulose, g, filtered and dried to constant weight.

Determination of molecular weight and polydispersities of TCC

To determine the molecular weight and polydispersities of TCC samples, Gel-chromatography method employed using an Agilent 1260 Infinity high-performance liquid chromatograph. A refractometric detector utilized for analysis. The polymer solution concentration was set at 2 mg/ml, and the dispenser volume was 20 μl. The eluent flow rate was maintained at 0.8 ml/min. The chromatographic column, made of cylindrical stainless steel with dimensions of 25 cm length and 0.8 cm inner diameter, was packed with TSK GM PW XL sorbent from Toya Soda, Japan. The assay results calculated using Windows Chemstation 7 software.

Infrared spectroscopic analysis method

The structure of the samples was analyzed using an 'Inventio-S' FTIR spectrophotometer (Bruker, Germany) in the wavelength range of 500-4000 cm^{-1}.

Determination of Carboxyl Groups

The content of carboxyl groups (mmol/g) in tricarboxycellulose samples was determined by titration using a Mettler Toledo conductometer (Mettler Toledo, Switzerland). In this procedure, 0.2 g of tricarboxycellulose was added to 20 mL of 0.1 mol L^{-1} HCl solution and stirred for 15 minutes, after which the resulting suspension was titrated with 0.5 N NaOH [30]. The content of carboxyl groups in the tricarboxycellulose sample was determined using the following formula:

$\text{Carboxyl content (mmol/g)} = V \times N / W \times 1000$

Where:

V – volume of NaOH consumed (mL)

N – normality of NaOH (typically 0.1)

W – mass of the sample (g)

Determination of the pH value

The pH values were determined using a FiveEasy Plus FP20 pH/mV meter (Mettler Toledo, Switzerland), which measures the proton (H^+) concentration in the solution.

Determination of the aldehyde group content in DAC samples

0.5 g of DAC was dissolved in 25 mL of water, and 4-5 mL of 1 M NaOH solution was added dropwise until the pH reached 5. Subsequently, 20 mL of a 0.05 g/mL hydroxylamine hydrochloride ($NH_2OH\cdot HCl$) solution was added to the obtained solution and stirred at 40°C for 4 hours. The resulting solution was titrated with 1 M NaOH solution until the pH reached 5. The number of aldehyde groups in the resulting product was calculated using the following formula:

$$\boldsymbol{AG}\% = \frac{(V_a - V_b)\cdot C_M}{\frac{m}{M_r}} \quad (1)$$

Where:

AG – aldehyde group content, %.
CM – molar concentration of the consumed alkali, mol/L.
Va – volume of alkali consumed for DAC, mL;
m – mass of the dry sample, g;
Vb – volume of alkali consumed for cellulose, mL;
Mr – molecular weight of dialdehyde cellulose, g/mol.

Medico-biological investigations

The methodologies for determining the hemostatic and toxic properties of powdered hemostatic agents are presented. In this study, powdered tricarboxycellulose was used as the research object. The preparation was administered to animals via various routes:

orally (via an intragastric tube);

local application (to the wound surface or subcutaneously) and others.

The hemostatic efficacy of tricarboxycellulose was evaluated in laboratory animals following local application (to the wound surface or subcutaneously). Healthy male rats (Wistar strain) with an average body weight of 198 g were used in the experimental studies. The animals were provided with standard food and water and were included in the experiment after a quarantine and adaptation period. All procedures were carried out in accordance with international bioethical guidelines and the standards of the OECD (Organization for Economic Co-operation and Development) [31].

Hemorrhage models

To evaluate the hemostatic efficacy of the powdered hemostatic agent, experimental studies were conducted using the following model. The right major lobe of the liver was designated as the injury site during the surgical procedureThis procedure was performed on rats under anesthesia via laparotomy. A standardized injury (5 × 5 mm, 2 mm depth) was inflicted on the liver parenchyma. The preparation was applied to the injured area in an amount of 0.05-0.1 g. The primary evaluation parameters were bleeding time and blood loss, which were measured using a stopwatch [32].

Assessment of Acute Toxicity

To determine acute toxicity, the preparation was administered once at various doses. The doses were determined based on the principle of dose escalation. The animals were observed for 14 days. General condition, locomotor activity, food and water intake, body weight, respiratory rate, and signs of pathology were recorded. Several doses were administered according to the dose escalation principle to determine the LD_{50} (the dose causing 50% mortality) and the maximum tolerated dose [33].

Results and discussion

In the first stage of tricarboxycellulose production, the optimal conditions for obtaining oxidized cellulose (OC) were determined by oxidizing cellulose, woven gauze, and medical cotton with a NaClO solution [34]. In this process, the effects of various factors on oxidation, including oxidant concentration, reaction duration, and temperature, were investigated. Under various conditions, the content of carboxyl groups in the samples and the product yields were determined, and the obtained results are presented in Table 1.

Table 1

The oxidation of cellulose, woven gauze, and medical cotton with NaClO solution

Samples	**NaClO concentration, %**	**pH of the medium**	**Reaction duration, hours**	**Temperature, °C**	**Carboxyl group content, %**	**Yield, %**
Cellulose	14	9-9.5	3	40	6.0	68
			4	40	7.5	64
			3	50	8.4	58
			4	50	9.0	53
			3	60	10.5	48
			4	**60**	**14.0**	**44**
Cotton-based gauze	14	9-9.5	3	40	5.2	65
			4	40	6.5	59
			3	50	6.8	55
			4	50	8.0	50
			3	60	8.2	45
			4	**60**	**10.8**	**40**
Medical cotton	14	9-9.5	3	40	4.5	78
			4	40	5.8	70
			3	50	6.6	66
			4	50	7.2	60
			3	60	7.8	56
			4	**60**	**9.5**	**50**

NaClO is a strong oxidizing agent that enables the oxidation of hydroxyl groups in cellulose to carboxyl groups. A 14% solution exhibits high oxidizing activity and significantly accelerates the modification process.

During the oxidation process, the reaction efficiency is significantly dependent on the pH of the medium. NaClO is relatively stable under alkaline conditions and exhibits higher activity as an oxidizing agent. The experimental results indicated that the optimal pH range is 9.0-9.5; at pH < 7, NaClO rapidly decomposes, leading to reduced oxidation activity. If the pH of the medium exceeds 11, cellulose degradation occurs, compromising the structural integrity of the samples.

The reaction duration directly affects the carboxyl group content and the product yield.

During the oxidation process, conducting the reaction for 2-3 hours does not produce a sufficient amount of carboxyl groups. Within a 5-6 hour period, excessive exposure to the oxidizing agent leads to the degradation of cellulose molecules. The optimal condition is a 4-hour reaction period, during which the product quality reaches its maximum.

Considering the above analyses, it was observed that with increasing reaction duration, the carboxyl group content increased to 14% in cellulose, 10.8% in woven gauze, and 9.5% in medical cotton; however, the product yield decreased by 40%.

The FTIR spectra of OC samples obtained by oxidizing cellulose, woven gauze, and medical cotton with NaClO solution are presented in Figure 3.

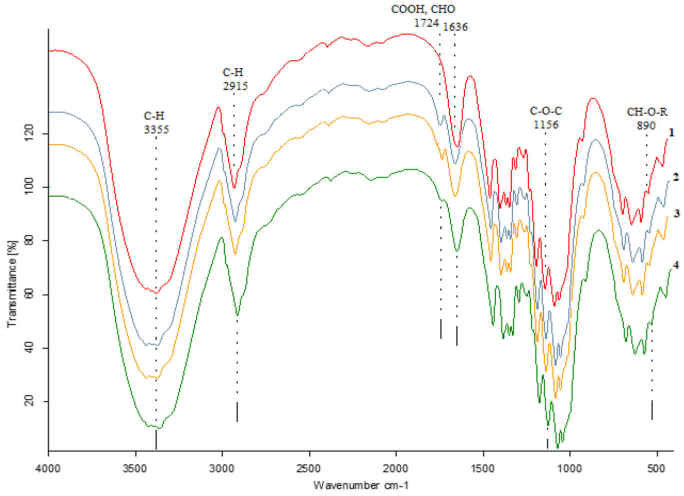
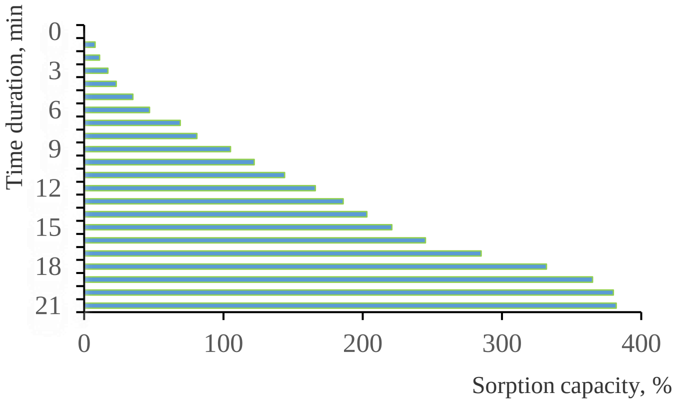


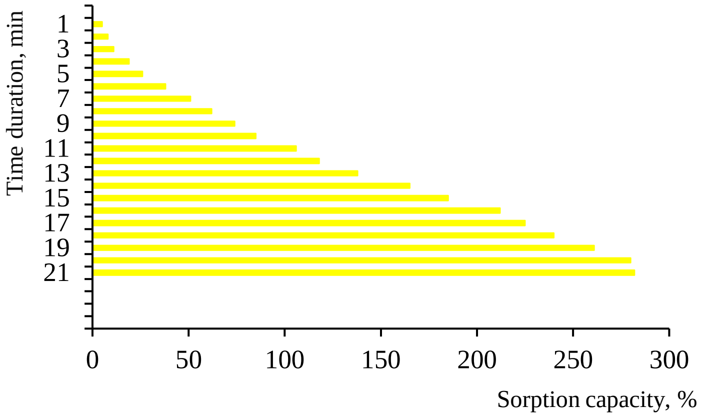
***Figure 3.** FTIR spectroscopic analysis results of oxidized cellulose samples **1.** Cellulose; **2.** Oxidized cellulose (OC) obtained from cellulose; **3.** OC obtained by oxidizing woven gauze, **4.** OC obtained by oxidizing medical cotton*

As shown in Fig. 3, changes in the functional groups of cellulose and samples of cellulose, woven gauze, and medical cotton oxidized with a 14% NaClO solution were studied using infrared (IR) spectroscopy. This peak is typically characteristic of aldehydes and carboxylic acids, confirming the formation of carboxyl groups in cellulose through oxidation with NaClO. The peak at 1600–1635 cm^{-1} corresponds to the vibrational frequencies of carboxylate ions ($-COO^{-}$). This peak indicates the formation of the ionized form of acidic groups. The peaks at 1420 cm^{-1} and 1375 cm^{-1} correspond to the vibrations of $-CH_2-$ and -CH- groups in the cellulose backbone, indicating that the main structure is preserved. The peak at 1030–1050 cm^{-1} corresponds to the vibrations of C–O–C ether bonds and glycosidic linkages. This is one of the main peaks indicating that the polysaccharide nature of cellulose is preserved. The peak at 890 cm^{-1} is characteristic of β-glycosidic bonds, indicating the preservation of β-1.4-linked glucosidic bonds in cellulose.

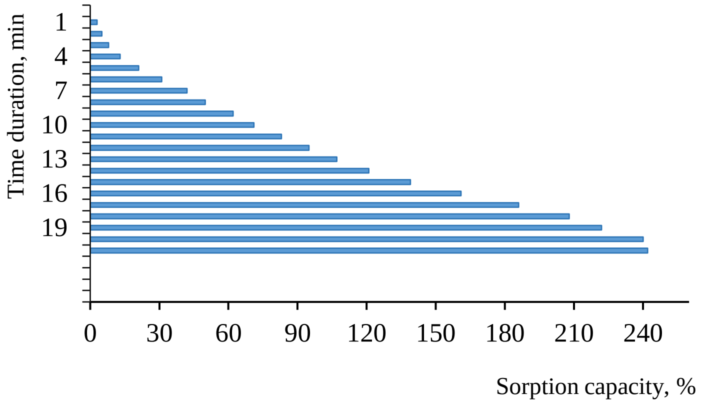
Studies were conducted to investigate the sorption properties of oxidized cellulose. The sorption properties of the oxycellulose sample obtained through oxidation with a 14% NaClO solution were analyzed. During the modification process, the hydroxyl groups in cellulose were partially converted into carboxyl and aldehyde groups, resulting in significant changes in its physicochemical properties.



Sorption properties of the OC sample obtained by oxidizing cellulose



Sorption properties of the OC sample obtained by oxidizing woven gauze



Sorption properties of the OC sample obtained by oxidizing medical cotton

Figure 4. *Sorption properties of OC samples obtained by oxidizing cellulose, woven gauze, and medical cotton with NaClO solution*

According to the experimental results, oxidized cellulose possesses the ability to absorb liquid media such as water, blood, and physiological saline. The increase in this property is associated with the rise in the number of polar (hydrophilic) functional groups in the cellulose structure, namely carboxyl (-COOH) and carbonyl (-C=O) groups. In this case, the sorption capacity increases from 300% to 800% depending on the degree of oxidation, indicating that the modified cellulose possesses a high water retention capability. According to the results of the study, the sorption capacity of the OC sample obtained by oxidizing cellulose increased by 380%, that of the OC sample obtained from oxidized woven gauze increased by 280%, and that of the OC sample obtained from oxidized medical cotton increased up to 240%; these increases can be attributed to the rise in the content of carbonyl and carboxyl groups. This, in turn, enhances its potential applications in biomedical fields, particularly as a hemostatic agent or a biosorbent.

When cellulose is first oxidized with NaClO and subsequently re-oxidized with $NaIO_4$, $NaIO_4$ oxidizes the vicinal diols in the cellulose chain (i.e., two adjacent hydroxyl groups) to aldehyde groups. In this process, the hydroxyl groups at the C2-C3 carbon atoms cleave the C-C bond, resulting in the formation of dialdehyde cellulose (DAC). This modification enhances the reactivity and multifunctional properties of cellulose.

The obtained oxycellulose sample was oxidized with $NaIO_4$ (10-25 minutes, 35℃, 2.5% concentration), resulting in the cleavage of the C2-C3 diol and the formation of 2.3-dialdehyde cellulose, and the aldehyde group content was determined. The primary purpose of re-oxidizing oxycellulose (OC) samples, obtained by the primary oxidation of cellulose with 14% NaClO, with $NaIO_4$ is to convert the vicinal diol groups located at the C2 and C3 carbon atoms into aldehyde groups.

Table 2

Oxidation of the aldehyde groups at the C2 and C3 carbon atoms in the OC sample obtained by oxidizing cellulose with NaClO using $NaIO_4$

Samples	**$NaIO_4$, %**	**pH**	**Reaction duration, hours**	**Temperature, °C**	**Aldehyde group content, mmol/g**	**Yield, %**
OC sample obtained from cellulose.	2.5	3.5	10	35	4.8	72
			15		5.7	61
			20		**7.2**	**55**
			25		7.4	48
OC sample obtained from woven gauze			10		3.7	66
			15		4.9	58
			20		**6.2**	**49**
			25		6.4	43
OC sample obtained from medical cotton			10		3.2	75
			15		4.3	66
			20		**5.0**	**56**
			25		5.3	47

As can be seen from Table 2, the oxidation of OC was carried out using $NaIO_4$ at a concentration of 2.5%, under controlled conditions of pH, at a temperature of 35°C, and for a duration of 10-25 minutes. With increasing concentration, the reaction rate accelerated, and a corresponding increase in the amount of aldehyde groups was observed. At this temperature, $NaIO_4$ exhibits high activity, and the selective oxidation is considered effective; however, with increasing temperature, the decomposition of $NaIO_4$ intensifies, which negatively affects the reaction efficiency. When the reaction temperature exceeds 35°C, the activity of periodate decreases, resulting in a reduced material yield. The oxidation process with $NaIO_4$ was carried out in an acidic medium at a pH of around 3.5, under which conditions $NaIO_4$ exhibits maximum activity, selectively converting the C2–C3 diol groups in the cellulose structure into aldehyde groups.

Considering the above, the optimal conditions were determined as pH 3.5, a reaction duration of 20 minutes, and a temperature of 35°C. Under these conditions, the aldehyde group content was found to be 7.2 mmol/g in the OC sample obtained from cellulose, 6.2 mmol/g in the OC sample obtained from woven gauze, and 5.0 mmol/g in the OC sample obtained from medical cotton. These values enhance the activity of dialdehyde cellulose and increase its potential for further modifications. The yield of the process was found to be 49-56%. The yield was found to depend on the reaction conditions; if pH, temperature, or reaction time increased beyond the optimal range, a significant decrease in yield was observed.

The infrared (FTIR) spectra of the samples obtained by re-oxidizing the oxidized OC samples with $NaIO_4$ are presented in Figure 5.

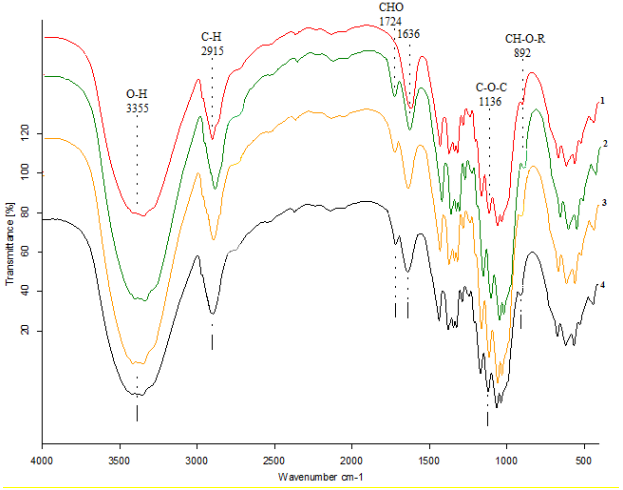
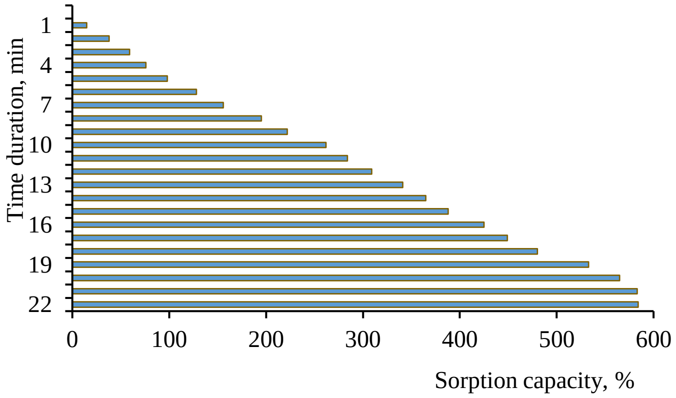


Figure 5. Results of FTIR spectroscopic analysis of oxidized cellulose samples.
1. *Cellulose,* ***2.*** *Dialdehyde cellulose obtained by the oxidation of cellulose,* ***3.*** *Dialdehyde cellulose obtained by the oxidation of woven gauze,* ***4.*** *Dialdehyde cellulose obtained by the oxidation of medical cotton*

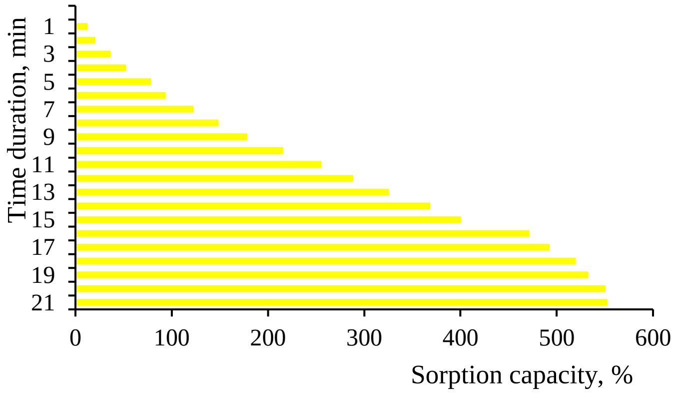
The sequential oxidation of cellulose with NaClO and $NaIO_4$ leads to the conversion of hydroxyl groups (-OH) in its molecular structure into aldehyde (-CHO) and carboxyl (-COOH) groups. In the region of 3400–3300 cm^{-1}, the broad absorption band corresponding to hydroxyl group vibrations is observed, and its decreased intensity after oxidation indicates a reduction in the number of -OH groups due to the formation of aldehyde and carboxyl groups. The absorption band in the region of 1730–1740 cm^{-1}, corresponding to aldehyde and carboxyl groups, confirms the cleavage of the bond between the C2 and C3 carbon atoms under the action of $NaIO_4$, resulting in the formation of aldehyde groups (-CHO). During the reaction process, the oxidation of some aldehyde groups also leads to the formation of carboxyl groups. In this case, the C=O stretching vibrations result in high intensity. In the region of 1615 cm^{-1}, the presence of COO^- (carboxylate anion) vibrations is observed. The absorption band at 1136 cm^{-1} indicates the preservation of C-O bonds or the formation of hemiacetals. These spectral changes confirm the oxidation of aldehyde groups within the cellulose structure during the modification process.

The sorption properties of dialdehyde cellulose, obtained by oxidation of NaClO-treated OC samples with $NaIO_4$, were investigated.

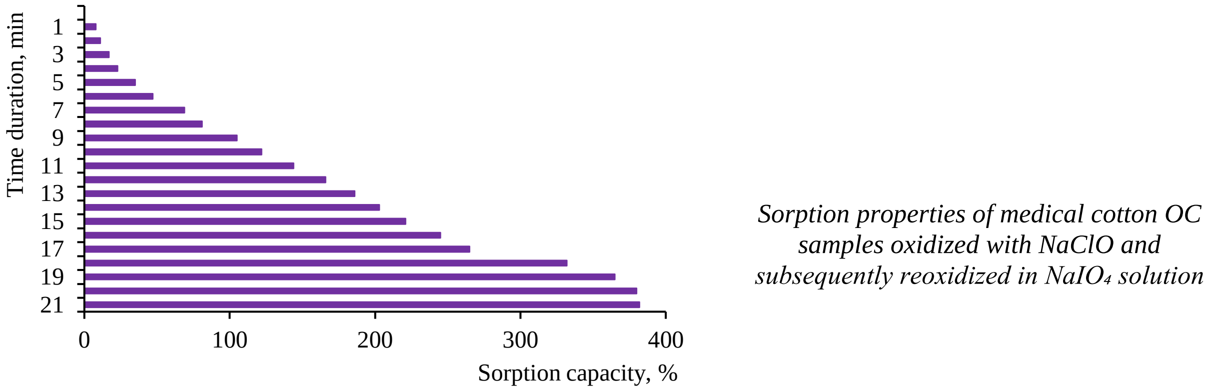
Figure 6 presents the graphs of the sorption properties of oxidized cellulose (OC) samples obtained by NaClO oxidation of cellulose, woven gauze, and medical cotton, followed by re-oxidation with $NaIO_4$ solution.



Sorption properties of oxidized cellulose (OC) samples obtained by NaClO oxidation of cellulose and re-oxidized with $NaIO_4$ solution



Sorption properties of woven gauze OC samples oxidized with NaClO and subsequently reoxidized in $NaIO_4$ solution



Sorption properties of medical cotton OC samples oxidized with NaClO and subsequently reoxidized in $NaIO_4$ solution

Figure 6. *Sorption properties of cellulose, woven gauze, and medical cotton OC samples oxidized with NaClO and subsequently reoxidized in $NaIO_4$ solution*

As can be seen from Figure 6, the results indicate that the sorption capacity of OC samples of cellulose oxidized with NaClO and subsequently re-oxidized in $NaIO_4$ solution increased by up to 600%, that of woven gauze samples by up to 550%, and that of medical cotton samples by up to 380%. This enhancement can be explained by the increased content of aldehyde and carboxyl groups. Accordingly, oxidation with NaClO leads to the formation of carboxyl and aldehyde groups in cellulose. Subsequent oxidation with $NaIO_4$ leads to the formation of additional aldehyde groups in the cellulose chains, which enhances its hydrophilicity and ability to bind water molecules. The high sorption properties of such modified cellulose samples can be explained by the conversion of hydroxyl groups into carboxyl and aldehyde groups, resulting in an increased surface charge and an enhanced ability to form a greater number of hydrogen bonds with water molecules.

The oxidation of cellulose using H_2O_2 as an oxidizing agent through a catalytic chemical reaction is considered one of the most important modification processes. This process proceeds via a radical chain reaction mechanism: H_2O_2 reacts with cellulose to generate highly reactive radical intermediates. These radicals interact with cellulose to form new radicals, which subsequently react with H_2O_2 to produce additional intermediate species. This reaction proceeds until the radicals undergo mutual interaction or react with the catalyst to yield stable products.

Factors such as temperature, pH conditions, and the type of catalyst during the oxidation process have a significant influence on the products of cellulose oxidation with peroxide. H_2O_2 is considered an important oxidizing agent from both industrial and environmental perspectives.

The modification of cellulose is carried out to improve its physicochemical properties and to enrich it with functional groups.

At this stage, reactions were carried out to obtain tricarboxycellulose, characterized by a high content of carboxyl groups as well as enhanced hydrophilic and reactive properties, through the stepwise oxidation of cellulose, woven gauze, and medical cotton. In this process, the oxidation of the C6, C2, and C3 carbon atoms is carried out using an H_2O_2 solution, whereby the cellulose chain simultaneously acquires three carboxyl groups, resulting in strong hydrophilicity and high ion-exchange capacity.

The obtained DAC sample was oxidized (2-3 h, 70-80℃, 6-8 % concentration) to oxidize the C6 and C2-C3 carbon atoms, yielding tricarboxycellulose. The results are presented in Table 3.

Table 3

The variation in the content of carboxyl groups depends on the duration of the modification process and the concentration of the oxidizing agent

№	H_2O_2 concentration, %.	Time, min	Carboxyl group content, %	Yield, %
Cellulose + NaClO+ $NaIO_4$ + H_2O_2	6	2	38	47

Woven gauze + NaClO + $NaIO_4$ + H_2O_2	6	2	32	50
Medical cotton + NaClO+ $NaIO_4$ + H_2O_2	6	2	28	52
Cellulose + NaClO+ $NaIO_4$ + H_2O_2	8	3	53	35
Woven gauze + NaClO + $NaIO_4$ + H_2O_2	8	3	46	38
Medical cotton + NaClO+ $NaIO_4$ + H_2O_2	8	3	42	41

As shown in the 3 tables of the obtained results, an increase in the concentration of the oxidizing agent and the reaction time during the oxidation process led to a decrease in product yield. Meanwhile, the content of carboxyl groups in the samples increased, reaching 42% in tricarboxycellulose obtained from oxidized medical cotton, 46% in tricarboxycellulose obtained from oxidized woven gauze, and up to 53% in tricarboxycellulose obtained from oxidized cellulose. This demonstrates the possibility of obtaining tricarboxycellulose compounds highly enriched with carboxyl groups through the oxidation of the C6, C2, and C3 elementary units. The yield was found to decrease by up to 35%. This indicates that the observed material loss resulted from the cleavage of the main polymer chains, fragmentation of the crystalline regions, and the formation of amorphous domains during the oxidation reactions. Therefore, although the yield was relatively low, the high extent of modification during the process was confirmed.

The FTIR spectral analysis of the tricarboxycelluloses, sequentially oxidized from cellulose, woven gauze, and medical cotton using NaClO, $NaIO_4$, and H_2O_2, is presented in Figure 8.

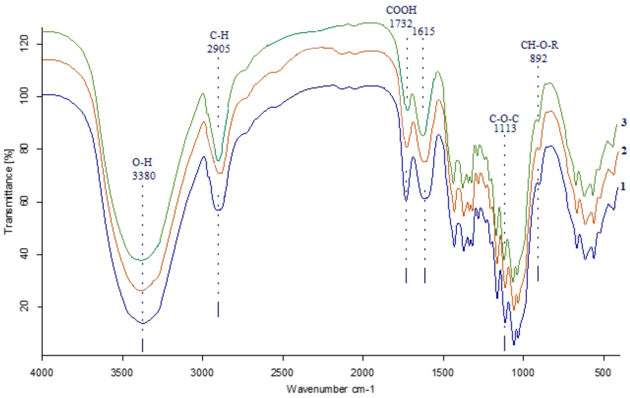
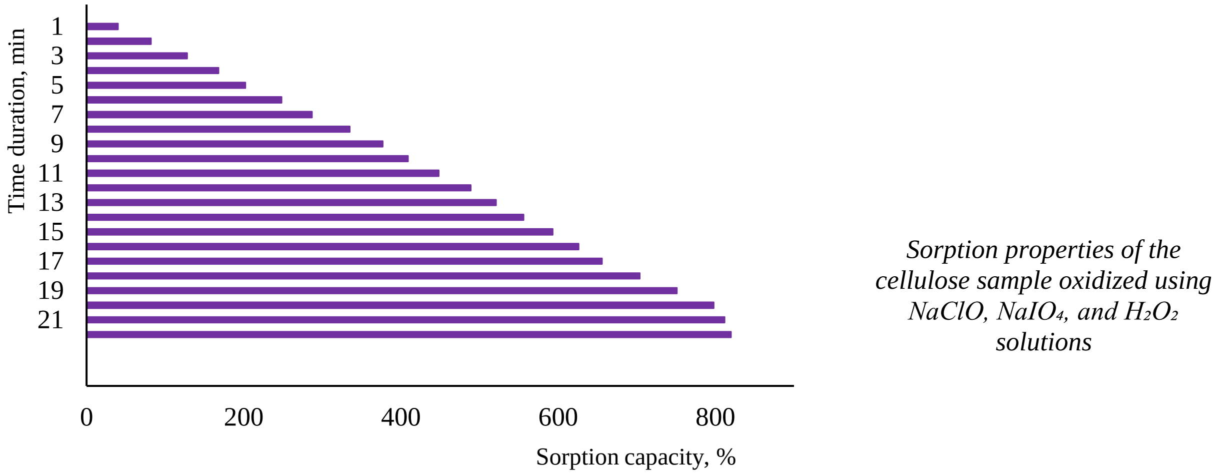


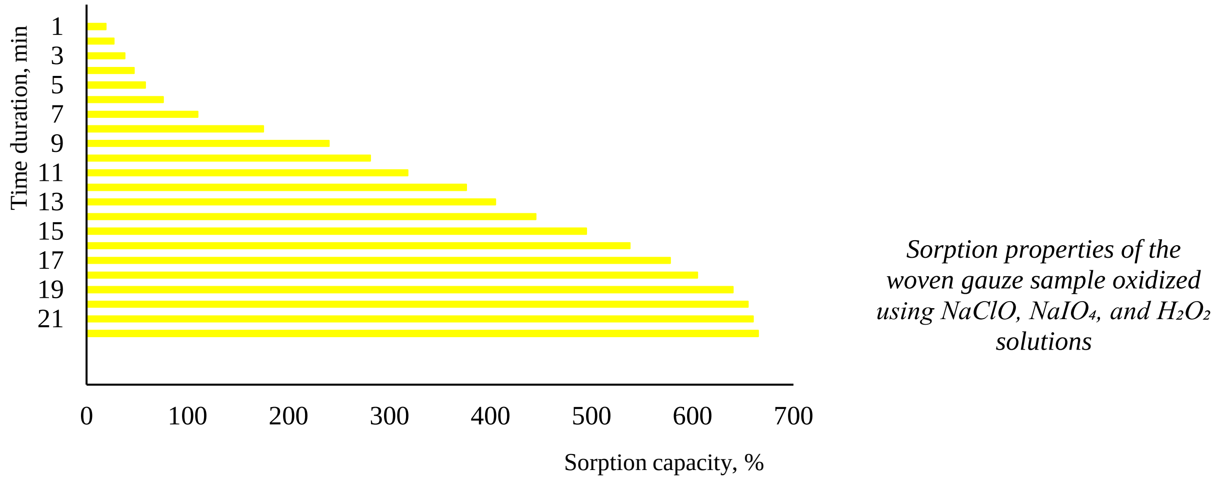
Figure 7. *Fourier-transform infrared (FTIR) spectroscopic analysis of the oxidized cellulose samples*
1. Tricarboxycellulose obtained from oxidized cellulose; 2. Tricarboxycellulose obtained from oxidized woven gauze; 3. Tricarboxycellulose obtained from medical cotton

As shown in Figure 7 of the obtained results, during the three-step oxidation of cellulose, various functional groups (aldehyde, carboxyl, and hydroxyl) were selectively introduced, leading to significant changes in its molecular structure. The vibrations observed in the 3300–3400 cm^{-1} region correspond to the hydroxyl groups of cellulose, associated through hydrogen bonding within its natural polysaccharide structure. After the oxidation steps, the intensity of this signal slightly decreased, indicating that some hydroxyl groups were oxidized and converted into carboxyl groups. In the final stage of oxidation, a distinct peak around 1732 cm^{-1} was observed in the tricarboxycellulose samples, indicating the presence of carboxyl groups. The increased intensity of this peak confirms the formation of carboxyl groups at the C6, C2, and C3 carbon atoms within the tricarboxycellulose structure. The vibrations observed around 1615 cm^{-1} in almost all oxidized samples indicate the presence of ionized carboxyl groups, specifically carboxylate anions ($-COO^-$). This indicates that the carboxyl groups are partially dissociated, which also affects their solubility in water. The vibrations of the peaks observed around 1113 cm^{-1} indicate the preservation of the glycosidic bonds in the cellulose chain. The vibrations of the peak at 890 cm^{-1} indicate the preservation of the β-1.4-glycosidic bonds in the main cellulose chain, confirming that complete degradation did not occur.

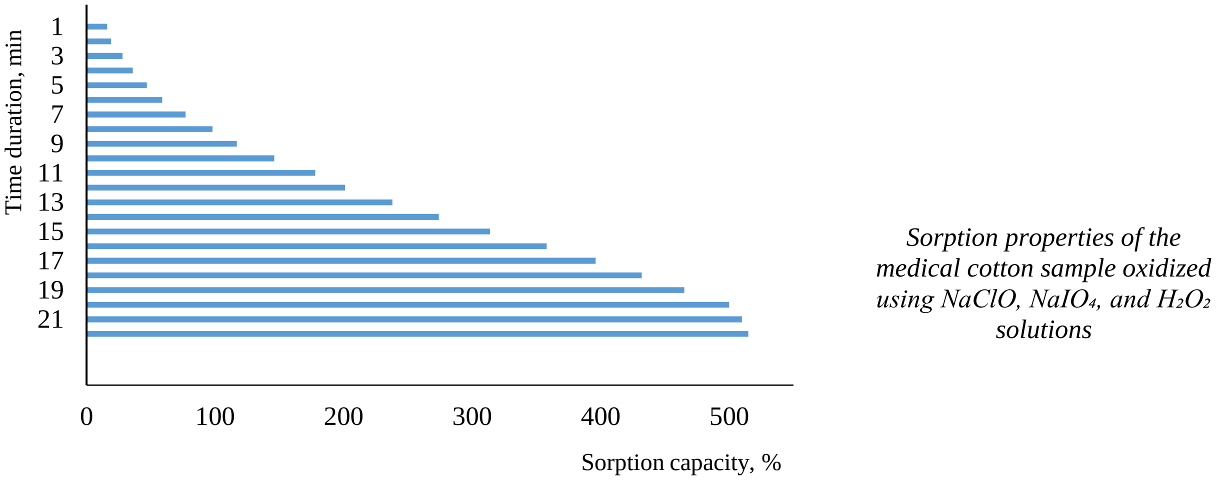
The sorption (liquid uptake) properties of cellulose-based materials depend on their chemical composition, structural amorphousness, and degree of modification. Functionalization of cellulose through oxidation, particularly by enriching it with carboxyl groups, enhances its interaction with water. In this study, the sorption properties of tricarboxycelluloses obtained from various sources (cellulose, woven gauze, and medical cotton) were analyzed.



Sorption properties of the cellulose sample oxidized using NaClO, NaIO$_4$, and H$_2$O$_2$ solutions



Sorption properties of the woven gauze sample oxidized using NaClO, NaIO$_4$, and H$_2$O$_2$ solutions



Sorption properties of the medical cotton sample oxidized using NaClO, NaIO$_4$, and H$_2$O$_2$ solutions

Figure 8. Graph of the sorption properties of tricarboxycelluloses obtained from cellulose, woven gauze, and medical cotton samples through consecutive oxidation using NaClO, NaIO$_4$, and H$_2$O$_2$

As shown in Figure 8, the results indicate that cellulose, gauze, and medical cotton samples, subjected to stepwise oxidation using NaClO, NaIO$_4$, and H$_2$O$_2$ solutions, yielded tricarboxycelluloses with high sorption capacity and enriched functional groups. In this case, the cellulose-based samples were found to exhibit liquid sorption capacities of up to 830%, woven gauze up to 670%, and medical cotton up to 520%. This can be explained by the fact that each step of the oxidation process modifies the structure of cellulose, increasing the number of hydrophilic groups on its surface and enhancing its polarity. Furthermore, this indicates that the modified biopolymer materials could have expanded potential for applications in medicine and biotechnology, where high sorption activity is required.

Taking the above data into account, in the first stage of the oxidation process, cellulose, woven gauze, and medical cotton samples were treated with a 14% NaClO solution for 3-4 hours at a temperature range of 40-60℃. Based on the results of the conducted studies, the sample exhibiting a high content of carboxyl groups, obtained with an oxidizing agent concentration of 14%, a reaction time of 4 hours, and a temperature of 60℃, was selected as the optimal sample.

In the subsequent stage of the oxidation process, the samples selected as optimal were further oxidized using $NaIO_4$ solution to convert the vicinal diol groups located at the C2 and C3 carbon atoms into aldehyde groups. In this process, samples oxidized with 2.5% $NaIO_4$ at pH 3.5, a temperature of 35℃, and a reaction time of 20 minutes, exhibiting a high content of aldehyde groups, were selected as the optimal samples.

To achieve complete oxidation of the C6, C2, and C3 carbon atoms, tricarboxycellulose samples were treated with 8% H_2O_2 for 3 hours at 60℃; the samples exhibiting a high content of carboxyl groups were selected as the optimal samples and used for subsequent studies.

In the next phase of the study, the sterilization feasibility of tricarboxycellulose and the physicochemical properties of the samples after sterilization were investigated, and the obtained results are presented in Table 4.

One of the primary requirements for pharmaceutical preparations intended for medical use is their sterility.

The sterilization of the hemostatic preparation was carried out using the gas sterilization method with ethylene oxide, and it was found that its physicochemical properties remained almost unchanged before and after the sterilization process.

Table 4

Physicochemical and hemostatic properties of the tricarboxycellulose sample before and after sterilization

Samples of tricarboxycellulose	**Before sterilization**			**After sterilization**		
	Content of carboxyl groups, %.	**MM, kDa**	**Bleeding time, sec**	**Content of carboxyl groups, %.**	**MM, kDa**	**Bleeding time, sec**
Cellulose	53	127.6	123 ±1	53	127.4	121 ±3
Woven gauze	46	109.7	137±4	46	109.3	135±6
Medical cotton	42	98.5	148±8	42	98.2	145±4
SURGICEL® Powder (ORC-powder)	24	123.2	134 ±3	24	123	130 ±4

According to the data in Table 4, the content of carboxyl groups, molecular weight (MW), and hemostatic properties of tricarboxycellulose samples synthesized stepwise from cellulose, gauze, and medical cotton using $NaClO–NaIO_4–H_2O_2$ solutions, as well as the SURGICEL® Powder (ORC-powder) sample, were analyzed before and after sterilization.

As can be seen from the table, the content of carboxyl groups in the samples remained unchanged during sterilization, which can be explained by the chemical stability of their structure. A slight decrease in the molecular weight of the samples was observed, indicating that partial disintegration may occur during sterilization; however, this does not affect the subsequent processing of the obtained samples. The hemostatic properties remained almost unchanged, indicating that their hemostatic efficacy was preserved.

Assessment of acute toxicity of tricarboxycellulose by intraperitoneal administration.

The acute toxicity of tricarboxycellulose was investigated in specialized laboratory rats by administering various doses of the compound orally. The experimental animals were divided into five groups, with six rats in each group. According to the results of the experimental study, when the compound was administered orally to rats at single doses of 5000, 5500, 6000, 6500, and 7000 mg/kg, effects on the central nervous system were observed after 24 hours, along with signs of refusal of food and water in the experimental animals.

No signs of intoxication were observed in the animals of Group 1 that received a dose of 5000 mg/kg. By the end of the second day, in Group 2 (dose 5500 mg/kg), one out of six rats had died; in Groups 3 and 4 (doses 6000 and 6500 mg/kg, respectively), two rats in each group died; and in Group 5 (dose 7000 mg/kg), four rats died.

The LD_{50} of tricarboxycellulose, determined for acute toxicity after intraperitoneal administration, was 6654.0 mg/kg (range: 6005.8-7302.2 mg/kg). Thus, based on the results of acute toxicity following intraperitoneal administration, the LD_{50} of tricarboxycellulose was determined to be 6654.0 mg/kg. This places the compound in toxicity class 4, corresponding to slightly toxic substances, indicating that the compound is practically non-toxic.

Conclusions

In the initial stage of stepwise oxidation of cellulose, woven gauze, and medical cotton, oxidation with NaClO followed by re-oxidation with $NaIO_4$-aimed at exposing the C2 and C3 carbon atoms to introduce multiple functional groups-and subsequent complete oxidation with H_2O_2 were carried out. This procedure was found to result in partial cleavage of the glucosidic chains and formation of carboxyl groups. Accordingly, the content of carboxyl groups in the tricarboxycellulose samples was found to be the highest among all samples, amounting to 53%, 46%, and 42% in cellulose, woven gauze, and medical cotton samples, respectively.

The study revealed that the water absorption capacities of cellulose, woven gauze, and medical cotton samples containing carboxyl groups increased significantly, reaching 830%, 660%, and 520%, respectively. According to the results of the study, the hemostatic activity, measured as clotting time, of tricarboxycellulose samples was determined to be 69 ± 2 s for the cellulose-based sample, 78 ± 2 s for the sample obtained from woven gauze, and 121 ± 2 s for the sample obtained from medical cotton.

Tricarboxycellulose samples with a high content of carboxyl groups can be applied as polymer-based hemostatic agents in medical practice.

CRediT authorship contribution statement

Xolturayev B.J.: Investigation, Writing-Original Draft & Methodology; Yunusov Khaydar E: Writing – review & editing, Investigation, Methodology, Project administration, Conceptualization; Sarymsakov Abdushkur A: Supervision & Visualization.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declaration of interests

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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