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Analysis of oxycellulose obtained by partial oxidation with different reagents

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The object of the present work was to study the possibilities for activating of dressings of cellulose and synthetic fibre by a partial oxidation with sodium hypochlorite, hydrogen peroxide and sodium periodate and to investigate the effect of the reaction parameters on the quantitative content of aldehyde groups in the obtained product.

Textile dressings of cellulose and synthetic fiber were treated with oxidizing solutions of sodium hypochlorite, hydrogen peroxide and sodium periodate with an aim to modify the material and form aldehyde groups in the cellulose molecule. The highest values of the copper number and content of aldehyde groups indexes were obtained for pure cellulose treated with sodium periodate solutions. The obtained textile materials could be used as carriers for immobilization of proteolytic enzymes to achieve a biologically active dressing.

Key words: cellulose, copper number, aldehyde groups, oxidation, dressings.

INTRODUCTION

Cellulose, probably the most abundant biopolymer in the world, is present in all higher plants and exists in its purest form in cotton fiber. The materials of natural cellulose fibre still have a major application in medicine as conventional linters and textile dressings that provide for a passive protection of wounds and have a number of advantages: mechanical strength, high absorption capacity, accessibility with respect to sources and price, good physiological endurance and others. The disadvantages that are known: adhering to the wound surface and injuring of the newly formed epithelium when changing the dressings, lack of therapeutic effect for stimulation of the wound healing process, lack of hemostatic and antibacterial action, can be eliminated by structural or chemical modification of the cellulose materials.

The modification of the cellulose materials allows the obtaining of new products from natural cellulose materials. An example of a structural modification is the microcrystalline cellulose widely applied in the food and pharmaceutical industries. Variety of products with different properties and application can be obtained by chemical modification of the cellulose – nitro derivatives, cellulose esters, acetates, xanthogenates and others. The diethylaminoethylcellulose (DEAE-cellulose) and the carboxymethylcellulose (CMC) are widely used in the laboratory practice as ion-exchange substances.

In the case of dressings, a certain percentage of functional groups can be introduced by chemical modification, without disruption of the fibre structure and with preservation of the basic structural-mechanical properties and characteristics of the material. The activated textile materials are fit to be used as carriers of different biologically active components - enzymes, stimulators of the regeneration and epithelization or antibacterial agents [3, 11]. In such a way, the conventional dressings acquire new properties and as a result of this they effectively improve the wound microenvironment and create favourable healing conditions [12]. The immobilization of proteolytic enzymes, (trypsin, chymotrypsin, collagenases, lysozyme, plasmin, papain, bromeline, microbial proteases and others) on the dressing material has a favourable effect for debridement of the wounds [2, 4]. There are different methods for immobilization of the enzymes on cellulose or its derivatives: adsorption on cellulose derivatives with ion-exchange properties (*e.g.* DEAE-cellulose); formation of chelate complexes with cellulose or cellulose derivatives, activated by metal ions; covalent connection [1]. The activating of the carrier prior to the covalent bonding immobilization can be realized in different ways: interaction of the cellulose with cyanogen bromide, through glutaraldehyde and aminoethylcellulose; by using of triazine method; by azide method with carboxymethylcellulose, and others.

In view of its chemical structure the cellulose represents β-1,4-D-glucan. The native cellulose is a typical linear homopolysaccharide consisting β-(1→4) linked D-glucose units *n* (Fig. 1). In the

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native fibres *n* is from 10 000 to 14 000 and in the purified cellulose it is from 1000 to 3000. The polyanhydroglucose chain is sensitive to certain influences and it is partially interrupted in the process of purification. A multitude of hydrogen bond connections between the separate polymer chains in the cellulose fibrils determines the high stability, tensile strength and low solubility of the cellulose.

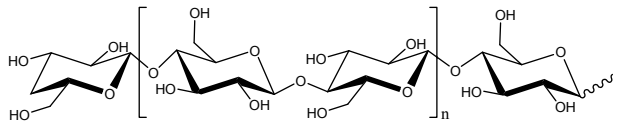

Fig. 1. Chemical structure of cellulose.

The cellulose content in the cotton fibres is 90−98%. After purifying, defatting, bleaching and deviling the raw material a product with all the basic properties of cellulose is obtained, the most important property, with an application in medicine, being its great hygroscopic capacity. The cotton can absorb water and liquids exceeding several times its own weight that justifies its usage as a dressing material and styptic for small wounds. The cotton-based dressings drain the wound, absorb toxins, protect the wound surface from infection and prevent the unnecessary loss of moisture, and after chemical modification and activation, they can serve as carriers of biologically active substances. The choice of the method of activation depends on the structure and the texture of the material, thickness and number of the filament doublings, textile density and other factors.

The partial oxidation of the hydroxyl groups is one of the most widely used methods for activation of cellulose materials. In the process of oxidation aldehyde, ketone and carboxyl groups can be formed, depending on the nature of the oxidizer and the parameters of the process. The sodium hypochlorite, for example, is a non-specific oxidizer and the relative content of the obtained aldehyde, ketone and carboxyl groups depends on pH of the reaction medium [6]. The chloric acid oxidizes the aldehyde groups to carboxyl, with no impact on the hydroxyl groups, while the selective oxidation using nitrogen (IV) oxyde gives carboxyl group at C6. The oxidizing effect of the hydrogen peroxide on cellulose is comparatively weaker, but still induces a transformation of part of the cellulose molecules into oxycellulose [10]. The alkaline salts of the periodic acid oxidize specifically the cellulose through a disruption of the glucopyranose ring at C2-C3 position forming two aldehyde groups per monomer unit [2]. A highly reactive product – dialdehyde cellulose is obtained. However, the introducing of a higher percentage of aldehyde groups in C2-C3 position increases the propensity to hydrolysis in an alkaline medium and decreases the degree of polymerization of the cellulose molecules (Fig. 2).

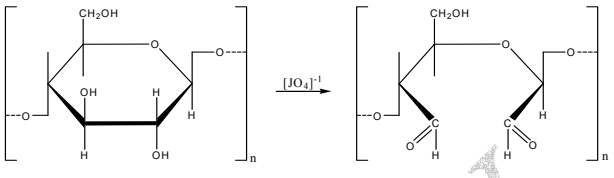

Fig. 2. Obtaining of dialdehyde cellulose.

Activated textile and other cellulose materials, containing aldehyde groups, have been used as carriers for covalent immobilization of biologically active components, incl. enzymes [3]. It has been assumed that the forming of covalent bond occurs between the aldehyde groups and the amino groups of the polypeptide chain.

The object of the present work was to study the possibilities for activating of dressings of cellulose and synthetic fibre, by oxidation with sodium hypochlorite, hydrogen peroxide or sodium periodate and to investigate the effect of the reaction parameters on the content of reactive aldehyde groups in the obtained product.

EXPERIMENTAL

Materials

Experiments with three types of textiles were carried out: gauze compresses and knitted bandage, containing pure cotton fibres and unwoven textile of viscose/polyester. All reagents and chemicals used were of p.a. quality (Merck).

Activation of the textile carriers

The textile materials were preliminary treated with a 14% NaOH solution. Sodium periodate, sodium hypochlorite in slightly acidic or alkaline solutions, and hydrogen peroxyde (Table 1) were used as activating agents.

Analyses of the activated textile

Spectrophotometric determination of copper number. Method according to Chai X. et al. [5]. Copper number is commonly defined as the number of grams of metallic copper (as Cu_2O) resulting from the reduction of $CuSO_4$ by 100 g of the cellulose fibres. The method is based on the reaction between reducing groups (mainly CHO) in the cellulose molecule and copper ions (Cu^{2+}).

Table 1. Options of treatment of textile materials.

No	Activating agent	Parameters			
		Concentration	pH	Temperature, °C	Time, h
I	Sodium hypochlorite solution	2 mg Cl/ml	9.0	20	24
	Sodium hypochlorite solution	5 mg Cl/ml	9.0	20	24
	Sodium hypochlorite solution	11 mg Cl/ml	9.0	20	24
II	Sodium hypochlorite solution	2 mg Cl/ml	5.5	20	24
	Sodium hypochlorite solution	3 mg Cl/ml	5.5	20	24
	Sodium hypochlorite solution	5 mg Cl/ml	5.5	20	24
III	Hydrogen peroxyde solution	4 .0%	9.5	90	24
	Hydrogen peroxyde solution	8.0%	9.5	90	24
	Hydrogen peroxyde solution	12.0%	9.5	90	24
IV	Sodium periodate solution	0.5%	3.0	20	7
	Sodium periodate solution	0.5%	3.0	20	24
	Sodium periodate solution	0.5%	3.0	20	42

Content of aldehyde groups in the oxidized cellulose – Iodometric method (according to TAPPI, T430). The method is based on the oxidation of aldehyde groups to carboxyl groups by iodine in alkaline media. In order to achieve a specificity of the reaction it is necessary to use a buffer solution with pH 9.3 to 9.5.

RESULTS AND DISCUSSION

The preliminary treatment of the textile materials with 14% sodium hydroxide solution results in hydrating and swelling of the cellulose fibres, which helps the oxidizing interaction of the material with the activating agent. Solutions of sodium hypochlorite, hydrogen peroxide and sodium periodate were used as activated agents. The technological parameters of the process and the oxidizer concentrations were varied in 4 sample series (Table 1).

The rate of oxidation of the experimental series activated textile and the content of the introduced aldehyde groups were analyzed in two ways: through spectrophotometric determination of copper number and by iodometric titration of the free aldehyde groups.

There are different methods for determining of carbonyl groups and parts of them are based on chemical reactions: oxidation, reduction and condensation. The oxidation by solutions of chlorous acid and iodine allows determining only the aldehyde groups in oxycellulose. The ketone groups do not react under these conditions. Under the reaction conditions mentioned in the Experimental section, the iodometric method is specific for aldehyde groups and it can be used for quantitative determination in oxycellose products.

In Tables 2–5 the experimentally established data of copper number and aldehyde groups content are shown for each separate type of textile material, after treating by the respective oxidizer.

It is known, that the copper number for the purified cotton is below 0.4−0.5. From the results in Tables 2 and 3 it is seen, that after a partial oxidation by sodium hypochlorite in acidic media, this index increases up to 18.93. The applied oxidizers (sodium hypochlorite and hydrogen peroxide) transform part of the hydroxyl groups in the cellulose fibres into carbonyl groups (aldehydes or ketones). In contrast to the sodium hypochlorite and the hydrogen peroxide, the sodium periodate is a strongly specific oxidizer for the cellulose that disrupts the glucopyranose ring at C2-C3 position and forms two aldehyde groups per monomer unit (Fig. 2). Because of this reason, the values for copper number in this series are higher and reach up to 24.74. The reaction intensity and the degree of oxidation depend on the type of the reagent and the parameters of the process.

Higher values of copper number are accounted for gauze compresses and knitted bandages, which are made of cellulose fibres. The results, obtained for unwoven textile, which contains a mixture of regenerated cellulose (viscose) and synthetic fibres, are much lower. In spite of the identical chemical composition, there are differences in the physical structure (degree of polymerization, location and orientation of the macromolecules) and in the physical-mechanical properties of the textile fibres from natural and regenerated cellulose. These differences appear also on the electronic microscope photos (Fig. 4).

In unwoven textile, the percentage of the cellulose component is much lower than that in the other investigated textile materials. These are the reasons that can explain the considerably weaker interaction of this material with all applied oxidizers.

The results in Table 2 and Table 3 show that the reaction of cellulose with sodium hypochlorite is strongly influenced by the pH. At the same con-

centration of free chlorine – 5.0 mg/ml, the copper number of the samples of cotton gauze increases from 3.53 (pH 9.0) to 18.93 (pH 5.5), *i.e*. the content of reducing groups in oxycellulose, obtained by oxidation in slightly acidic medium, is higher.

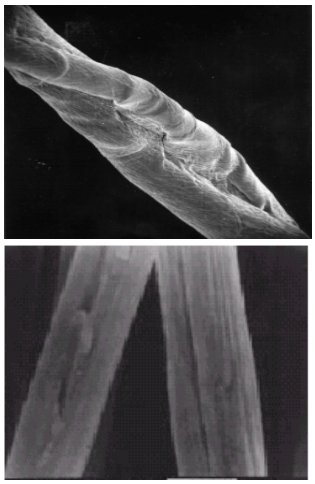

Fig. 4. Structure of textile fibers SEM: a - cotton; b - viscose.

Similar conclusions have been reached by Nevell and Singh who have also established that the reaction of hypochlorite oxidation of cellulose is influenced to the greatest extent by pH, than by the duration of the process [8].

The results from the analysis of the samples, treated by solutions of hydrogen peroxide in alkaline media are presented in Table 4. The copper number is much lower compared to the samples oxidized by sodium hypochlorite. The hydrogen peroxide is a non-specific oxidizer that attacks a lot of organic compounds and it is widely applied in the textile industry as a bleaching agent. Levin and Ettinger have studied the reaction between H_2O_2 and purified cotton fibres [7]. They have observed the action of the hydrogen peroxide in relation to pH and have established that in alkaline solutions a perhydroxyl radical is formed that oxidizes part of the hydroxyl groups at position C2 or C3. The obtained oxycellulose contains aldehyde and ketone groups, which are in β-position with respect to the glycoside bond.

Table 2. Copper number (g/100 g) and aldehyde groups (mmol/100 g) with different kinds of textile materials – oxidizer sodium hypochlorite, pH 9.0.

NaClO (pH 9.0) concentration	Gauze compress		Knitted bandage		Unwoven textile	
	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g
2 mg Cl /ml	2.78 ± 0.24	2.83 ± 0.07	0.47 ± 0.034	1.44 ± 0.09	0.209 ± 0.019	0.05 ± 0
5 mg Cl/ml	3.53 ± 0.46	3.62 ± 0.12	1.44 ± 0.072	2.10 ± 0.26	0.83 ± 0.027	0.11 ± 0
11 mg Cl /ml	5.67 ± 0.53	5.86 ± 0.18	4.14 ± 0.065	4.24 ± 0.21	1.09 ± 0.033	0.12 ± 0.01

Table 3. Copper number (g/100 g) and aldehyde groups (mmol/100 g) with different kinds of textile materials – oxidizer sodium hypochlorite, pH 5.5.

NaClO (pH 5.5) concentration	Gauze compress		Knitted bandage		Unwoven textile	
	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100g
2 mg Cl /ml	3.309 ± 0.53	4.52 ± 0.08	3.020 ± 0.032	3.97 ± 0.11	0.170 ± 0.02	0.06 ± 0.01
3 mg Cl/ml	11.910 ± 0.72	8.97 ± 0.22	9.980 ± 0.045	8.48 ± 0.18	0.960 ± 0.012	0.09 ± 0.01
5 mg Cl /ml	18.930 ± 0.36	16.14 ± 0.13	17.050 ± 0.48	15.14 ± 0.12	1.20 ± 0.042	0.17 ± 0.01

Table 4. Copper number (g/100 g) and aldehyde groups (mmol/100 g) with different kinds of textile materials – oxidizer hydrogen peroxyde, pH 9.5.

H_2O_2 (pH 9.5) concentration	Gauze compress		Knitted bandage		Unwoven textile	
	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g
4.0%	0.779 ± 0.046	1.0 ± 0.07	0.810 ± 0.067	0.86 ± 0.03	0.091 ± 0.03	0
8.0%	1.588 ± 0.019	1.28 ± 0.03	1.705 ± 0.057	1.24 ± 0.06	0.611 ± 0.019	0
12.0%	1.068 ± 0.038	1.66 ± 0.17	0.98 ± 0.028	1.62 ± 0.07	0.207 ± 0.024	0.14 ± 0.01

Table 5. Copper number (g/100 g) and aldehyde groups (mmol/100 g) with different kinds of textile materials – oxidizer sodium periodate.

$NaIO_4$ (pH 3.0) time, h	Gauze compress		Knitted bandage		Unwoven textile	
	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g	Cu number g/100 g	CHO mmol/100 g
7	10.11 ± 0.30	36.20 ± 1.07	9.20 ± 0.23	33.82 ± 0.9	0.53 ± 0.021	1.16 ± 0.01
24	16.54 ± 0.11	43.82 ± 0.44	15.41 ± 0.29	40.39 ± 0.3	0.96 ± 0.015	1.45 ± 0.02
42	24.74 ± 0.30	51.31 ± 0.18	23.84 ± 0.51	49.17 ± 1.24	1.7 ± 0.031	2.34 ± 0.01

In the process of oxidation, the content of the ketone groups increases more rapidly, compared to the aldehyde and carboxyl groups, and the product of cotton oxidation at pH 9.5 is ketocellulose. According to Lewin and Ettinger the forming of ketone groups occurs predominantly at C3 atom of the anhydroglucoside monomer. The rate of oxidative interaction between the cellulose and the hydrogen peroxide is lower than 10%, while for the sodium hypochlorite it reaches up to 40%.

The weaker interaction, as well as the forming of predominantly ketone groups, explains the lower values for copper number in the third group of samples.

The copper number is highest for cellulose oxidized by sodium periodate (Table 5), which can be explained by the mechanism of the oxidation reaction and forming of two aldehyde groups per monomer unit (Fig. 3).

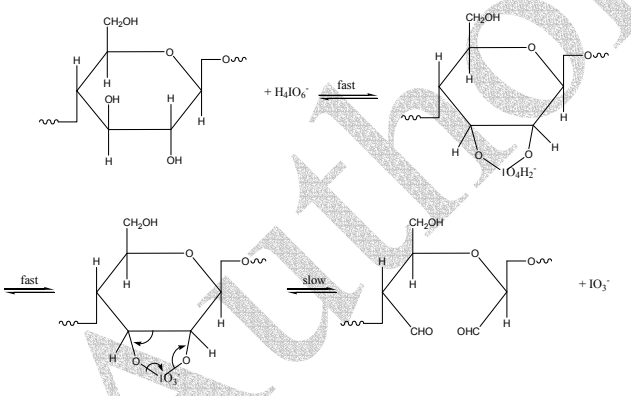

Fig. 3. Mechanism of the reaction of cellulose with sodium periodate.

It is assumed that concentrations of sodium periodate above 0.1 M lead to a much higher degree of cellulose oxidation, to a decrease of the stability of the glycoside connections and respectively to a deterioration of the structural and sorption characteristics of the tissue. For that reason we used sodium periodate with concentration 0.025 M with the purpose to achieve a partial oxidation of cellulose and to form reactive aldehyde groups without destructive changes. The copper number increases upon increasing the duration of the process at one and the same concentration of sodium periodate and pH.

The results, obtained for the content of aldehyde groups in the samples, correlate with the values for copper number. The aldehyde groups introduced into cellulose molecule have a capacity to interact with free groups of enzymes and other biologically active substances, thus achieving an immobilization of the biological component on the textile carrier.

According to literature data, the presence of reactive aldehyde groups with content from 0.06 to 3.1 mg/g textile allows the achievement of a covalent immobilization of collagenase on cellulose carrier. Immobilization of trypsin or alkaline protease has been carried out by similar methods [2–4].

CONCLUSION

Textile materials of cellulose and synthetic fibres can be activated by oxidation with solutions of sodium hypochlorite, hydrogen peroxide and sodium periodate, thus introducing reactive aldehyde groups into them. From the investigated textile materials, highest content of aldehyde groups was established for the samples, containing pure cellulose and those treated by sodium periodate. The obtained activated textile materials are appropriate as carriers for immobilization of proteolytic enzymes with the aim to achieve a biologically active wound dressing.

REFERENCES

1. S. Afaq, J. Iqbal, *Elect. J. Biotech*., **4**, 1 (2001).
2. A. A. Belov, N. F. Kazanskaya, V. N. Filatov, Y. N. Belova, *Vestn. MGU Ser. 2: Khimiya*, **47**, 87 (2006).
3. BG Patent 51438 (A3), 5/1993.
4. N. D. Burkhanova, S. M. Yugai, M. Y. Yunusov, G. V. Nikonovich, T. I. Usmanov, *Chem. Nat. Compd.*, **33**, 488 (1997).
5. X. Chai, D. Zhang, Q. Hou, S. Yoon, *J. Ind. Eng. Chem*., **13**, 597 (2007).
6. M. Lewin, A. Ettinger, *J. Polym. Sci*., 58, 1023 (1962).
7. M. Lewin, A. Ettinger, *Cellul. Chem. Tech.,* **3**, 9 (1969).
8. T. P. Nevell, O. P. Singh, *Text. Res. J*., **56**; 270 (1986).

9. TAPPI Official Test Method T430, TAPPI Press, Atlanta, revised 1994, p. 1.
10. T. Topalovic, V. A. Nierstrasz, L. Bautista, D. Jocic, M. Warmoeskerken, A. Navarro, *Cellulose*, **14**, 385 (2007).
11. US Patent 5098417.
12. T. N. Yudanova, I. V. Reshetov, *Pharm. Chem. J.*, **40**, 430 (2006).

ОХАРАКТЕРИЗИРАНЕ НА ОКСИЦЕЛУЛОЗА ПОЛУЧЕНА СЛЕД ЧАСТИЧНО ОКИСЛЕНИЕ С РАЗЛИЧНИ РЕАГЕНТИ

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(Резюме)

Целта на настоящата работа бе да се проучат възможностите за активиране на превързочни материали от целулозни и синтетични влакна, чрез частично окисление с натриев хипохлорит, водороден пероксид и натриев перйодат и да се изследва влиянието на параметрите на реакцията върху количественото съдържание на реакционноспособни алдехидни групи в получения продукт.

Текстилни превързочни материали от целулозни и синтетични влакна бяха третирани с разтвори на окислители: натриев хипохлорит, водороден пероксид и натриев перйодат, с цел модифициране на материала и формиране реакционноспособни алдехидни групи в целулозната молекула. Най-високи стойности на показателите медно число и съдържание на алдехидни групи бе отчетено при пробите, съдържащи чиста целулоза и обработени с разтвори на натриев перйодат. Получените активирани текстилни материали биха могли да се използват като носители за имобилизация на протеолитични ензими с цел получаване на биологично активно превързочно средство.