Carboxymethyl Cellulose Oxidation to Form Aldehyde Groups

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Oxidation of carboxymethyl cellulose (CMC) is a method of its modification, which allows to improve the functionality of this compound by increasing its reactivity. Presented work describes two methods of oxidation of carboxymethyl cellulose. The first is the conversion of CMC to its dialdehyde derivatives (DCMC) by sodium periodate. In the second method, hydrogen peroxide in the presence of iron tetrasulfophthalocyanine (FePcS) as catalyst was applied. Oxidation degree of CMC in different process parameters and time intervals was estimated using hydroxylamine hydrochloride. Modified CMC will be used in nanoparticles preparation in medical diagnostics.

Keywords and phrases: oxidation, carboxymethyl cellulose, sodium periodate, hydrogen peroxide, iron tetrasulfophthalocyanine.

Introduction

Carboxymethyl cellulose (CMC) is one of the main derivatives of cellulose, which is a natural polysaccharide and one of the best renewable resources available for humanity. CMC occurs in the sodium salt form (Fig. 1) and is produced in reaction of alkali cellulose with sodium monochloroacetate under strictly controlled conditions. Because of its properties such as biodegradability, non-toxicity and biocompatibility, CMC has found various application in many sectors of industry including pharmaceutics, cosmetics, food, etc. [1].

The most innovative applications of CMC are in the area of medicine. CMC solutions are used to form gels that are used in heart, thoracic and cornea surgery. In thorax operations, the lungs are stapled and then covered with a solution of CMC to prevent air leaks and fluid ingress. In the field of orthopedics, CMC solutions are used in lubricating the joints of the bones, most often in the wrists, knees and hips. The fluid is injected into these joints to prevent erosion, swelling and possible destruction of the cartilage attached to bones.

In the pharmaceutical industry, it is used to coat tablets with high degrees of purity and low viscosity [2]. CMC is insoluble in the acidic environment of the stomach but soluble in the basic medium of the intestine. It is also used for forming gels, transporting drugs, disintegrating tablets and as a stabilizer for suspensions, emulsions, sprays and bioadhesive tablets which attach internally to the mucus of a body part. What is more CMC is used as a 0.25–0.5% eye drop solution which helps keep the eye moist and protect the eye from injury and infection, and decrease symptoms of dry eyes such as burning or itching.

In the cosmetics industry, it is used in dental impression materials, toothpastes and gels. This water soluble substance serves as a thickener, stabilizer, suspending agent and former of films in creams, lotions, or shampoos. Additionally CMC is widely used in hair care products [3].

Oxidative modification of carboxymethyl cellulose is carried out in order to improve the functionality of the compound by increasing its reactivity. Traditional oxidation involves the use of stoichiometric amounts of inorganic oxidants such as NaOCl, N_2O_4 to introduce carboxyl groups or $NaIO_4$ to obtain C-C bond cleavage and aldehyde functions. Nevertheless, NaOCl and N_2O_4 are rather expensive or toxic and produce a large amount of waste such as chlorinated products or nitrate salts. Several catalytic approaches, such as 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in combination with NaOCl/ NaBr, have been proposed for improved oxidation. However, this method does not eliminate necessity of use of NaOCl [4].

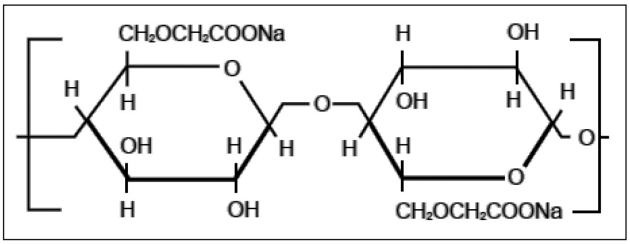

Fig. 1. Unit structure of NaCMC.

Oxidative modification of CMC, present in this paper, is the first step for nanoparticles' preparation for medical diagnostics and treatment. Further medical use of CMC, imposes selection method in which used reagents have the lowest toxicity or can be easily removed from post-reaction mixture. Moreover, CMC application in nanoparticles synthesis in future studies, makes it necessary to control amount of introduced aldehyde groups and to obtain a result in accordance with the theoretically assumed amount in repeatable manner.

Nanoparticles are structures with size in the 1–100 nm range. The reason why these particles are attractive for medical purposes is based on their important and unique features, such as their surface to mass ratio that is much larger than that of other particles. A great advantage is also their ability to bind, adsorb and carry other compounds like drugs, proteins, probes, vaccines, nucleic acids, genes etc. [5]. As drug delivery system, nanoparticles can entrap drugs or biomolecules into their interior structures or/and absorb drugs or biomolecules onto their exterior surfaces. Nanoparticle drug delivery systems have great advantages such as ability to pass through the smallest capillary vessels because of their ultra-tiny volume and to avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged. They can also penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lung, spinal cord and lymph. Additionally, they can improve the utility of drugs and reduce toxic side effects [6]. Nanoparticles are very promising materials to be used as anticancer drug carriers. The application of known drugs but encapsulated in nanoparticle delivery system can improve efficiency and decrease side effects of anticancer therapy by increasing drug concentration in cancer tissue. With the ability to investigate the fate of nanoparticles in the cell using fluorescent markers, there can be created nanoparticles that efficiently and selectively attack cancer cells.

Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible and biodegradable, what makes carboxymethyl cellulose a material which is suitable for this purpose. In addition CMC is polysaccharide which can be easily chemically and biochemically modified, resulting in many kinds of derivatives. From both economic and medical point of view, we decided to choose two presented methods for oxidative modification of CMC. The first of them, is the oxidation of CMC by sodium periodate, and the second one is the oxidation of CMC by hydrogen peroxide in the presence of iron tetrasulfophthalocyanine (FePcS) catalyst. In the present study, we investigated the oxidation of CMC by the determination of the aldehyde content. It was based on the reaction between hydroxylamine hydrochloride (0,5 N, water solution) and the aldehyde groups in DCMC chain. It is important to introduce aldehyde groups into the CMC chain, in order to bind them to the active molecules, such as drugs or fluorescent dyes. They are also necessary to create nanoparticles. However, literature research shows the cytotoxic activity of aldehyde groups on the human body. For these reasons, the number of introduced aldehyde groups is very important for further studies. The number of these groups determines the size of the obtained nanoparticles. Therefore it has to be repeatable and consistent with the established theoretical amount. The resulting nanoparticles are to be used in medicine, so a very important parameter is the non-toxicity of used reagents. This is why we examine these two methods.

Experimental

Materials

Sodium periodate, trisodium salt of 4-sulfophthalic acid, ammonium chloride, ammonium molybdate and iron sulfate heptahydrate were purchased from Sigma–Aldrich®. Carboxymethyl cellulose sodium salt (CMC) was kindly donated by HERCULES. Hydrogen peroxide, urea and N,N-dimethylformamide were purchased from POCh S.A. Hydroxylamine hydrochloride was purchased from Fluka.

Method 1

The first method is sodium periodate oxidation of CMC. It is based on the specific cleavage of the C2–C3 bond of glucose units. The result is the formation of two aldehyde groups per one glucose unit [1], what is shown in Fig. 2. With the introduction of aldehyde groups, CMC can be converted to primary alcohols, carboxylic acids or imines with primary amines [7].

To find the best conditions of CMC oxidation we prepared a series of experiments. Various parameters of the process are given in Table 1, where modes a, b, c refer to the different reaction conditions.

Oxidation of CMC using $NaIO_4$

Dialdehyde carboxymethyl cellulose (DCMC) was prepared as follows: CMC was dissolved in 25 mL distilled water in the flask which was immersed in a DF-101S temperature controlled water bath with a magnetic stirrer. Then, sodium periodate was added to the CMC solution under stirring. The pH was adjusted with 1 M sulfuric acid solution. After the mixture was stirred in the dark at desig-

Table 1. Choice of parameters for method 1.

Factors	Method 1		
	Modes		
	a	b	c
Reaction time	4 h	24 h	24 h
Temperature	35°C	35°C	22°C
pH	3.0	7.0	7.0

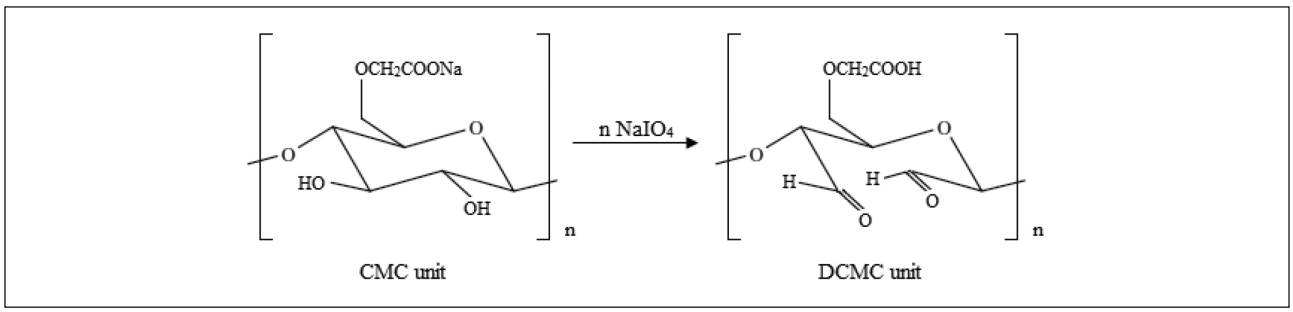

Fig. 2. Periodate oxidization of CMC to dialdehyde carboxymethyl cellulose (DCMC).

Table 2. The amount of reagents used in the oxidation of CMC with NaIO4.

Degree of oxidation	CMC [g]	$NaIO_4$ [g]
30%	1	0,57
80%	1	1,51

nated reaction temperature for a predetermined time, the oxidized product, referred to dialdehyde carboxymethyl cellulose was extensively dialyzed against deionized water at 22°C for 3 days (water was changed every 24 hours). The product was dried at 40°C to constant weight for the subsequent use [8, 9]. The reaction was carried out in following conditions: a) 4 h in 35°C with reduced pH = 3 b) 24 h in 35°C with pH = 7 and c) 24 h in 22°C with pH = 7.

Method 2

The second method is the oxidation of CMC by hydrogen peroxide in the presence of iron tetrasulfophthalocyanine (FePcS) catalyst. It is characterized by cleavage of the C2–C3 bond of vicinal diols of glycoside units [4]. This leads to the formation of carbonyl and carboxyl functions along the polysaccharide (Fig. 3).

To find the best conditions of CMC oxidation we prepared a series of experiments. Various parameters of the process are given in Table 3, where modes d, e refer to the different reaction conditions.

Preparation of iron tetrasulfophthalocyanine (FePcS)

The sodium salt of iron tetrasulfophthalocyanine was prepared according to a modified method developed by Weber and Bush [10]. Trisodium salt of 4-sulfophthalic acid (6.4 mmol), urea (38.6 mmol), ammonium chloride (3.5 mmol), ammonium molybdate (0.02 mmol), and iron sulfate heptahydrate (1.2 mmol) were ground together in a mortar until a homogenous powder is obtained. Then the mixture was heated to 280°C for 2 h.

Oxidation of CMC using H_2O_2 in the presence of FePcS, in the aquatic environment

FePcS was mixed with CMC in 25 mL of distilled water until complete solubilization, and then an aqueous solution containing H_2O_2 was added. Resulting mixture was stirred and allowed to react at designated reaction temperature during 18 h. After this time, the reaction was stopped and oxidized product was dialyzed against deionized water at 22°C for 3 days (water was changed every 24

Table 3. Choice of parameters for method 2.

Factors	Method 2	
	Modes	
	d	e
Reaction time	18 h	18 h
Temperature	35°C	50°C
pH	7.0	7.0

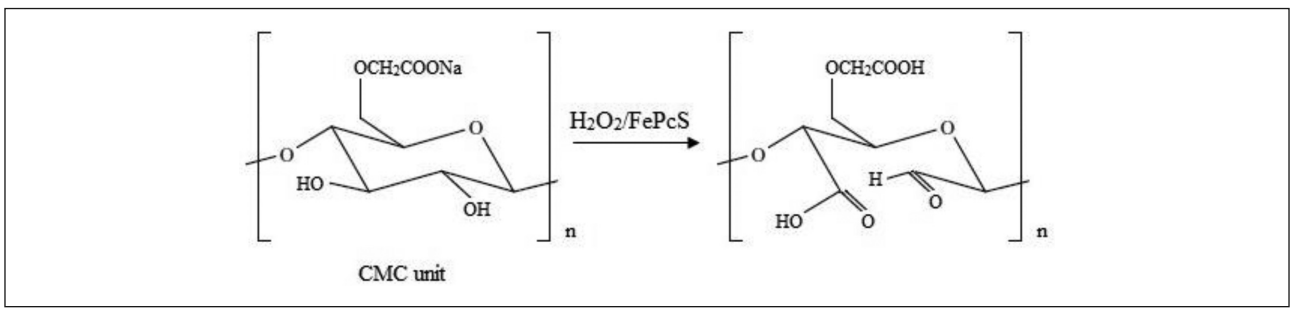

Fig. 3. Oxidation of CMC by hydrogen peroxide in the presence of iron tetrasulfophthalocyanine (FePcS) catalyst.

Table 4. The amount of reagents used in the oxidation of CMC with H2O2/FePcS.

Degree of oxidation	CMC [g]	FePcS [mg]	H_2O_2 [µl]
30%	1	1.4	24
80%	1	3	65

hours). The product was dried at 40°C to constant weight for the subsequent use. The selected temperatures for carrying the reaction were d) 35°C and e) 50°C.

Preparation of 0,5N hydroxylamine hydrochloride solution

17,5 g hydroxylamine hydrochloride was dissolved in 150 mL distilled water to which was added 6 mL of methyl orange reagent (0,05%). The solution was diluted to a volume of 0,5 liter and the pH adjusted to 4 [11].

Determination of aldehyde content

Aldehyde content determination was carried out to assess the degree of oxidation of CMC. The procedure was as follows [11]: DCMC, dried to constant weight with sample sizes ca. 0,1 g, was dissolved in 12,5 mL distilled water and the pH was adjusted to 7 with 0,1 N sodium hydroxide (NaOH). Then 12,5 mL of 0,5 N hydroxylamine hydrochloride — methyl orange solution ($H_2NOHHCl$) was added into the DCMC solution. The mixture was stirred and allowed to react for 2 h, and was then titrated with 0,1 N sodium hydroxide solution until the red-to-yellow end point was achieved by matching the color of the sample solution with that of a blank one. The analytical reaction is as follows:

$$CMC-(CHO)_n + H_2N-OH\cdot HCl \rightarrow CMC-(CH=N-OH)_n + H_2O + HCl \quad (1)$$

$$HCl + NaOH \rightarrow NaCl + H_2O \quad (2)$$

As a blank was used the same concentration of CMC solution at pH 7.

The number of aldehyde groups per 100 anhydroglucose units were determined using the following formula:

$$AC = N_{NaOH}\cdot V_{NaOH}\cdot\frac{MW}{m_{DCMC}}\cdot\frac{100}{S}\cdot 10^{-3} \quad (3)$$

where AC is aldehyde content; N_{NaOH} is the concentration of standardized sodium hydroxide solution [N]; V_{NaOH} is the volume of NaOH [mL]; MW is the molecular weight of CMC [Da]; m_{DCMC} is the weight of DCMC sample [g]; S is the number of anhydroglucose units in the chain of carboxymethyl cellulose with a MW.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra of CMC and DCMC were obtained from discs containing ~ 2.0 mg dry sample [1]. This analysis was done to compare the spectra of compounds obtained after the modification to the spectrum of unmodified CMC. For this purpose we used the Thermo Scientific Nicolet ™ 6700 spectrometer with Omnic 8 software which is used for computer analysis of spectra.

The study of solubility in water and organic solvents

Two samples of DCMC with sizes ca. 0,1 g were weighed. To the first one was added 10 mL distilled water, and 10 mL of N,N-dimethylformamide was added to the second one. Both samples were stirred for 2 h.

Results and Discussion

In our studies the presence of aldehyde groups in the obtained DCMC was determined using a hydroxylamine hydrochloride method. The number of aldehyde substitution per 100 glucose units was estimated from the amount of standardized 0.1 N sodium hydroxide used in the titration, using the equation (1). Table 5 shows the results of the determination of alehyde content. It follows that the number of aldehyde groups obtained in most cases is lower than results from theoretical calculations. For the degree of oxidation of 30% there should be created 60 aldehyde groups per 100 glucose units (one glucose formed two aldehyde groups). Degree of oxidation, after reaction was calculated by subtracting the total degree of oxidation of unmodified CMC from the total degree of oxidation of the compounds after modification. One can say that the best oxidation conditions were found when the reaction was conducted at the temperature of 22°C for 24 h in case of method 1, and at the temperature of 50°C for 18 h in case of method 2.

DCMC synthesis was verified by FT-IR spectroscopic studies. The resulting FT–IR spectra were compared with the spectrum of unmodified CMC. Fig. 4 and Fig. 5 show the FTIR spectra of CMC before and after the oxidation with different aldehyde content. Fig. 4 relates to the sodium periodate oxidation (method 1). There are two characteristic IR bands at ~1736 cm^{-1} and ~895 cm^{-1} regions, which confirm the oxidation of CMC. Fig. 5 is about the oxidation of CMC by H_2O_2/FePcS (method 2). Characteristic IR bands appear at ~1737 cm^{-1} and ~891 cm^{-1} regions. In general, the absorbance at about 880 cm^{-1} is assigned to the formation of hemiacetal bonds between the aldehyde groups and neighbor hydroxyl groups, while the band around 1740 cm^{-1} is characteristic of aldehydic carbonyl groups [8]. It is not easy to detect the existence of aldehyde groups because they readily combine with water or neighboring groups to produce hydroxyl group absorbance in the IR spectrum. Second derivative spectroscopy could reproducibly identify aldehyde bands in oxidized CMC only if the samples were dried at 110°C for 24 h; the aldehyde band appeared at 1732–1734 cm^{-1} [12].

Characteristic peaks for lower oxidation degree (30%), are masked by the strong results peaks of carboxyl groups preasent in CMC. This result are very similar to those presented in literature [13]. In addition the peaks were highest for 80% of oxidation, which confirms the higher number of aldehyde group in CMC chain. To decide which reaction conditions are the most effective, that is, in which case the oxidation is highest, we compared spectrum obtained for various parameters. Fig. 6 shows a comparison of FR-IR spectra for the 30% degree of oxidation, obtained in different reaction conditions a), b) and c). Considering the height of the peak, we can conclude that the best oxidation occurred when the reaction was carried out at 22°C for 24 h, with pH = 7. A similar analysis was also performed for method 2.

In Fig. 5 there is an interesting area in the range 1800–2500 cm^{-1}. It is based on Ruff degradation. In this reaction the final aldehyde group of glucose is oxidized to glycolic acid, and then during the subsequent oxidation undergoes decarboxylation (disappears CO_2 group and the chain is reduced by one carbon atom), and previous group of carbon and OH group, are oxidized to aldehyde. In our case there is only decarboxylation resulting with disappearance of the COOH group from the carboxymethyl group, and remaining CH_2OH group. This is why the intensity derived from the carbonyl bands around 1730 cm^{-1} is reduced. There can also appear band, derived from the OH group of about 3500 cm^{-1}.

The results of the analysis of the FTIR spectra show that more efficient way to modify the CMC is method 1. The spectra derived from compounds oxidized with this method, have higher peaks characteristic for the presence of aldehyde groups, which means that there are more of these groups. This is confirmed by the results of determination of the content of aldehyde groups in unmodified CMC and in oxidized CMC using both methods. In method 1, most of aldehyde groups were introduced into the CMC chain, when the reaction proceeded at 22°C for 4 h with pH = 7. For the desired degree of oxidation of 30% was achieved higher oxidation, which was 36%. However, after taking into account the degree of oxidation of the unmodified CMC, this result is consistent with the application. For the given degree of oxidation of 80%, was also observed the highest oxidation under these conditions, but the determination of the content of aldehyde groups suggests that the reaction had not gone fully.

In the method 2, the oxidation process went more efficiently at a higher temperature of 50°C. It was observed for both the desired degrees of oxidation of 30% and 80%. It has been shown that for the lower oxidation degree (30%), the obtained result is consistent with the established, but after subtraction of the degree of oxidation of the unmodified CMC, we found that the reaction had not gone to the end. For the oxidation of 80% is also not obtained the desired degree of oxidation.

In the preparation of the assumed degree of oxidation, it is important to determine at the beginning the number

Table 5. The number of aldehyde groups in DCMC per 100 anhydroglucose units.

Method	Conditions of the reaction	Expected degree of oxidation	Number of aldehyde groups per 100 anhydroglucose units	Total degree of oxidation	Degree of oxidation, after reaction
0	-	CMC	10,2	5%	0
1	T = 35°C, t = 4h, pH = 3.0	30%	38,2	19%	14%
	T = 35°C, t = 24h, pH = 7.0	30%	59,8	30%	25%
	T = 22°C, t = 24h, pH = 7.0	30%	72,5	36%	31%
	T = 35°C, t = 4h, pH = 3.0	80%	69,3	35%	30%
	T = 35°C, t = 24h, pH = 7.0	80%	85,9	43%	38%
	T = 22°C, t = 24h, pH = 7.0	80%	91,4	46%	41%
2	T = 35°C, t = 18h, pH = 7.0	30%	35,8	18%	13%
	T = 50°C, t = 18h, pH = 7.0	30%	59,2	30%	25%
	T = 35°C, t = 18h, pH = 7.0	80%	67,8	34%	29%
	T = 50°C, t = 18h, pH = 7.0	80%	87,4	44%	39%

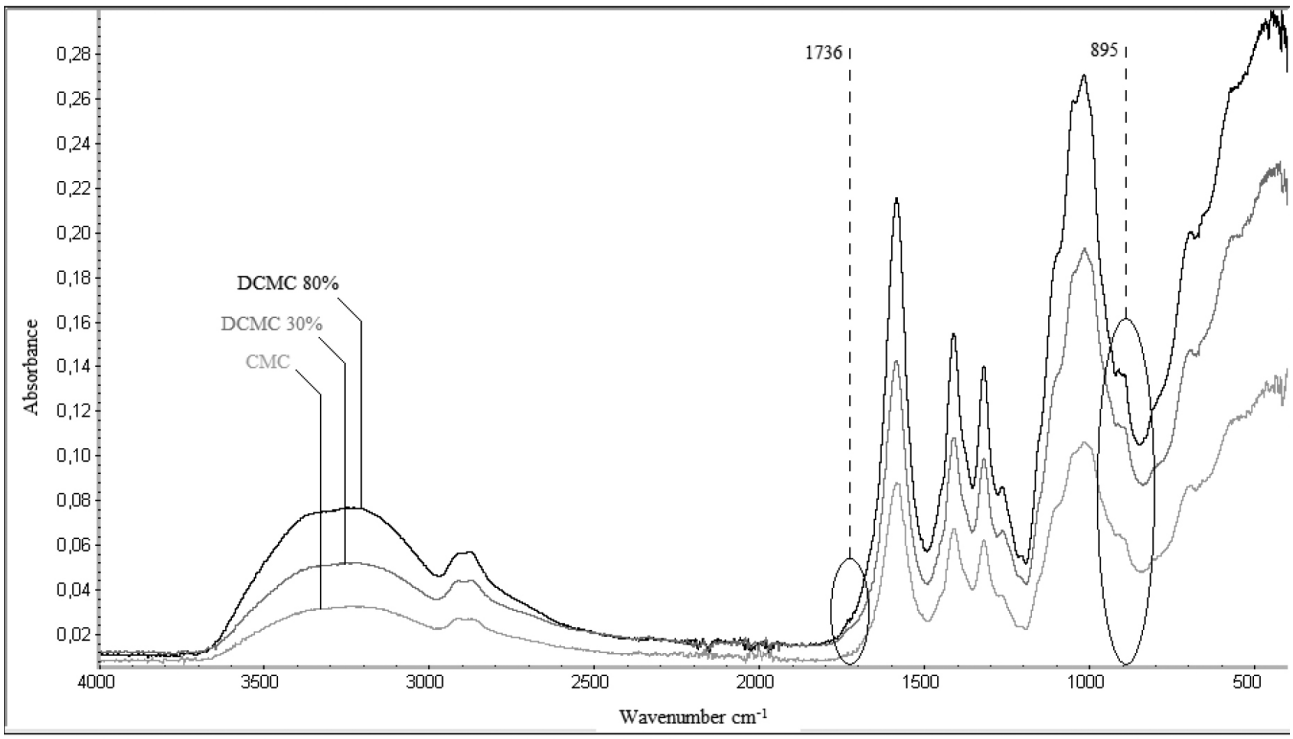

Fig. 4. FTIR spectra of CMC before and after the sodium periodate oxidation. CMC — unmodified carboxymethyl cellulose; DCMC 30% — oxidized carboxymethyl cellulose with 30% degree of oxidation; DCMC 80% — oxidized carboxymethyl cellulose with 80% degree of oxidation.

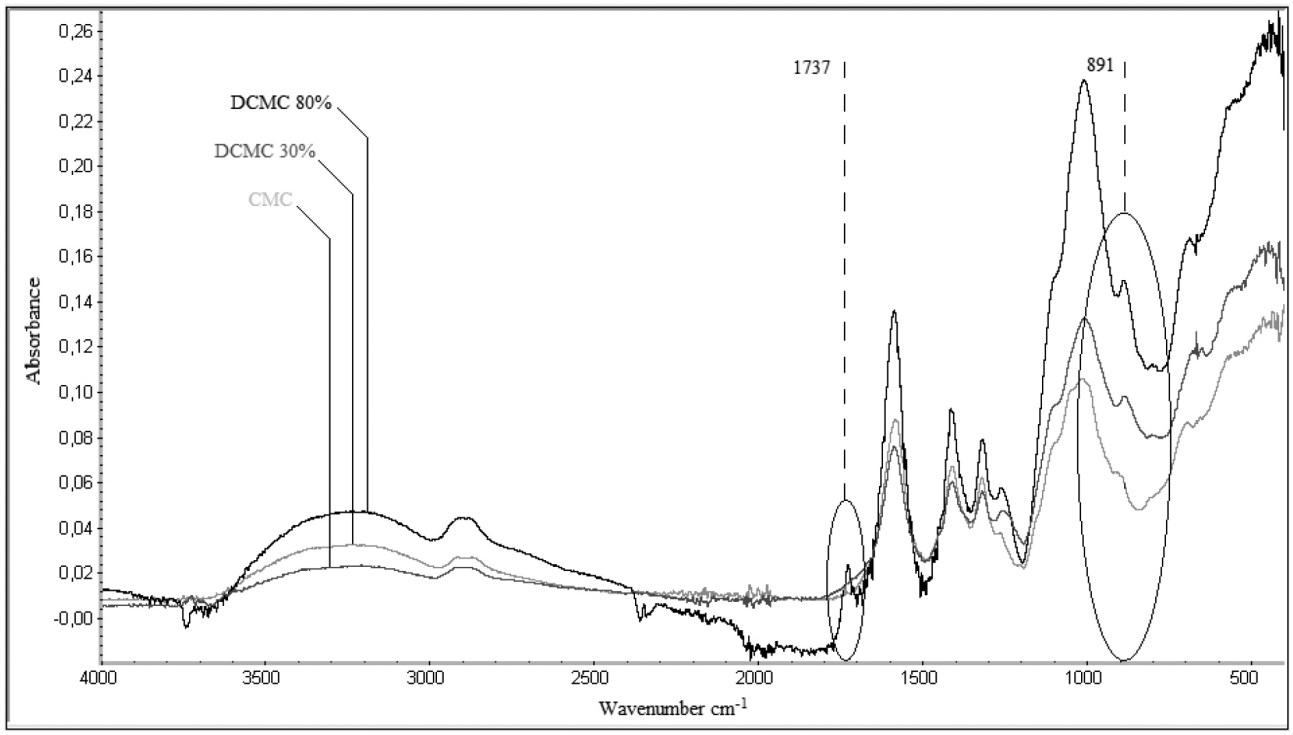

Fig. 5. FTIR spectra of CMC before and after the oxidation by H_2O_2/FePcS. CMC — unmodified carboxymethyl cellulose; DCMC 30% — oxidized carboxymethyl cellulose with 30% degree of oxidation; DCMC 80% — oxidized carboxymethyl cellulose with 80% degree of oxidation.

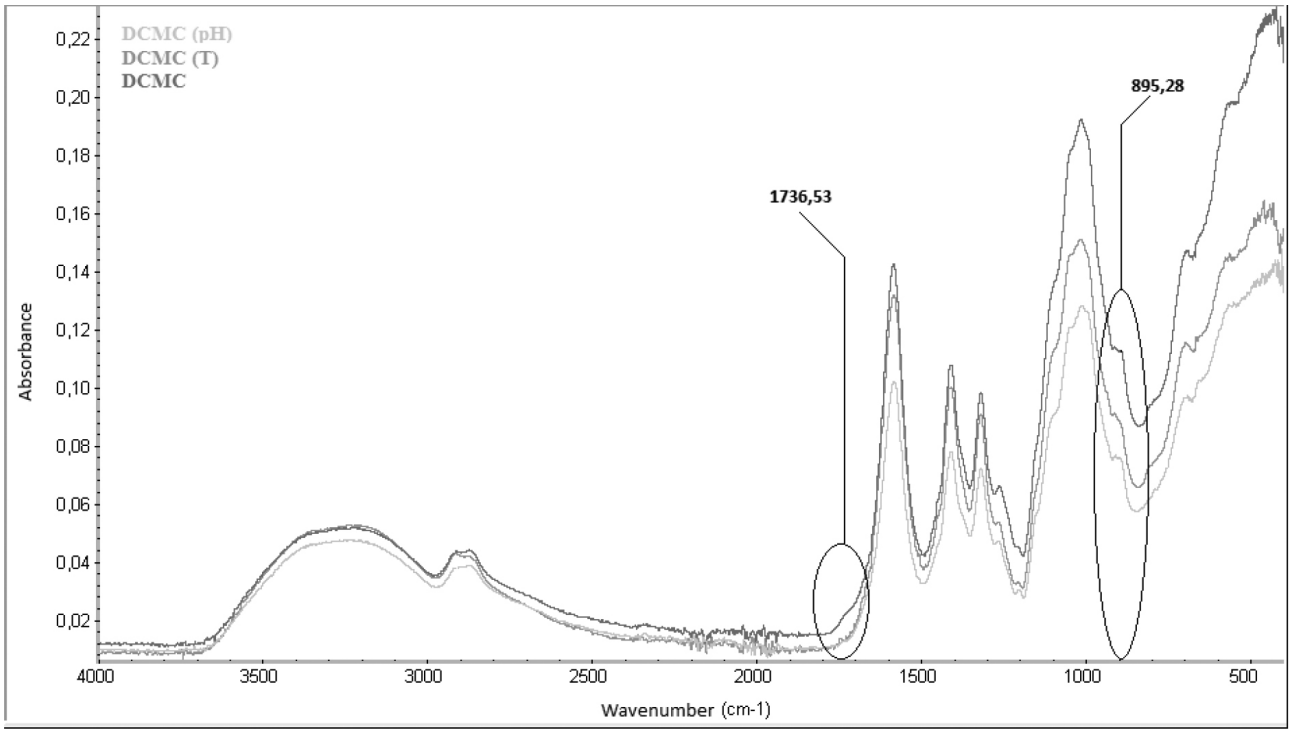

Fig. 6. FTIR spectra of CMC after the sodium periodate oxidation (30%): a) DCMC (pH) — 4 h in 35°C with reduced pH = 3 b) DCMC (T) — 24 h in 35°C with pH = 7 c) DCDM — 24 h in 22°C with pH = 7.

of aldehyde groups in unoxidized CMC. This must be taken into account when calculating the quantities of reagents needed for the reaction of oxidation. They should be correspondingly lower, so as to obtain oxidation, without the need to diminish it by oxidation of unmodified CMC. This will allow control of the process, rather than getting random results.

Conclusion

The aim of this study was the modification of carboxymethyl cellulose by its oxidation. It was accomplished using two methods: in the first (1) we used sodium periodate and in the second (2) hydrogen peroxide in the presence of iron tetrasulfophthalocyanine as catalyst. Taking into account the mentioned earlier parameters, such as the number of aldehyde groups introduced into the CMC chain, as well as the non-toxicity of used reagents, we indicate that the method 1 is the most efficient. Analysis of results shows that both methods allow to introduce aldehyde groups, however, the results that we obtained in the method 1 are the most similar to the theoretical calculations. We received the assumed degree of oxidation, when the reaction was carried out at 22°C for 24 h with pH = 7. This is confirmed by FTIR spectrum, which shows the presence of characteristic IR bands at ~1736 cm^{-1} and ~895 cm^{-1} regions. Advantage of the method 1 is also the fact that the catalyst can be purchased, and in the method 2, the catalyst must be prepared, which causes a longer research time. In addition there is no need to purify the product from the catalyst. Correspondence between the experimental and theoretical results and their repeatability, qualifies method 1 as the most promising for the further research.

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