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One-shot carboxylation of microcrystalline cellulose in the presence of nitroxyl radicals and sodium periodate

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Abstract

Water soluble cellulose derivatives are highly required products for many practical purposes, expanding the limited applications of pure cellulose, caused by highly ordered hydrogen bond network and high crystallinity. In this connection, this paper, presents a new approach to obtain water soluble carboxyl-functionalized cellulosic materials, combining two of the most common selective oxidation protocols for cellulose, i.e. the nitroxyl mediated and periodate, in one-shot reaction. It was found that, under specific reaction conditions, fully oxidized, 2,3,6-tricarboxy cellulose can be obtained in high amounts. The other valuable oxidized fractions were found to possess large amounts of carboxylic groups, as determined by potentiometric titration. ^{13}C-NMR evidenced the presence of three distinctive carboxylic groups in the fully oxidized product, whereas for the partially oxidized samples, ^{13}C CP-MAS solid-state NMR did not detect any carbonyl signals. The oxidized products were characterized by means of FTIR and X-ray

photoelectron spectroscopy (XPS). Moreover, the changes on the degree of polymerization occurred after oxidative treatments were viscometrically determined.

Keywords: Cellulose, TEMPO oxidation, NHPI oxidation, sodium periodate, carboxylic groups.

1. Introduction

Cellulose, among the most versatile and widely prevalent biopolymers in nature, has been used for millennia for human basic needs, e.g. as building material, for clothing fabrication and as energy source, but founded today, through its derivatives, new and *exotic* applications in food industry, medicine, cosmetic, flexible display panels, electronic devices and many others.[1-3] Chemical modification of polysaccharides represent the most important route to design new materials with new structures and properties.[4] Cellulose, having three reactive hydroxyl groups (one primary and another two, secondary) in its repeating unit, can be easily modified, following typical alcohol group chemistry, most important transformations referring to esterification,[5] etherification,[5] and oxidation.[6-9] Particularly, the oxidation reaction aroused many researcher groups around the world, due to the large variety of the products which could be obtained, depending on the reactive site, and employed reagents, offering a broad spectrum of cellulose derivatives for industrial applications.[1,2,4] Many attempts has been made in order to improve the reaction selectivity of the cellulose oxidation, to found benign and cheap reagents, and even to found new paths, able to supply new and highly value-added products. There are hitherto, two main approaches for the cellulose selective oxidation: i) nitroxyl radical-mediated oxidation of the primary OH groups, and ii) periodate oxidation of the two secondary OH groups, see Fig.1. These two protocols are considered the most selective processes, in this kind of transformations.

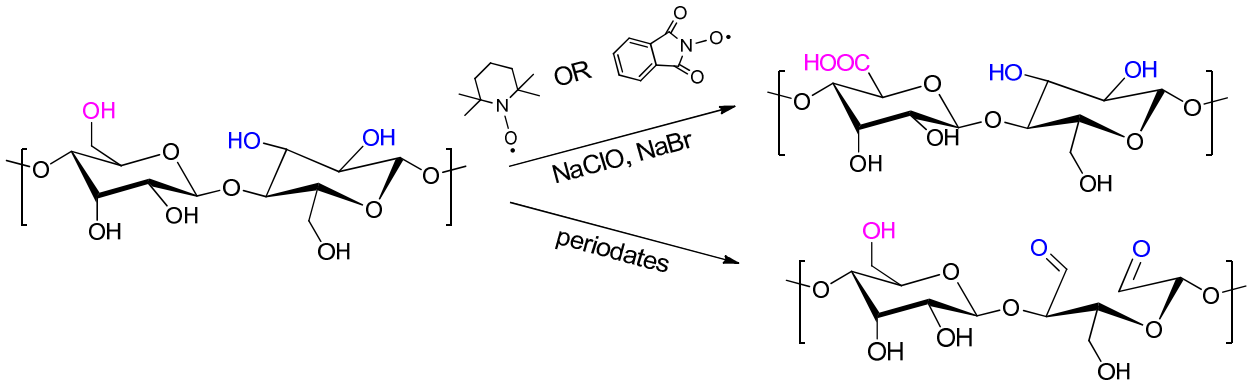

Fig. 1 Possible oxidation routes for the cellulose selective oxidation, in presence of nitroxyl radicals or periodates.

The introduction of 2,2,6,6-tetramethyl-piperidine-1-oxyl radical (TEMPO), for the selective oxidation of primary hydroxyl groups in cellulose, has been an important step forward in the field of cellulose oxidation, Fig. 1.[10,11] The stable nitroxyl radical TEMPO acts as a mediator in the presence of sodium bromide and sodium hypochlorite, to selectively oxidize the primary hydroxyl groups in cellulose. Recently, an alternative to this protocol has been reported,[7-9] which implies the use of a non-persistent nitroxyl radical, i.e. phthalimide-*N*-oxyl (PINO) which is generated *in situ* from its parent hydroxyl amine, *N*-hydroxyphthalimide (NHPI) and an adequate cocatalyst. The use of NHPI for the selective oxidation of cellulose fibers was another evidence of the exceptional catalytic activity of this catalyst proved previously on a wide range of organic compounds,[12-18] envisaging the possibility to implement NHPI homogeneous catalysis for industrial applications.[19,20] The NHPI/NaBr/NaClO system oxidize also the primary hydroxyl groups in cellulose, see Fig. 1, the reported values of the carboxylic groups formed during oxidation being however smaller than in the case of using TEMPO.[10] Nevertheless, the use of NHPI is preferred when products having higher degrees of polymerization are targeted.[8]

The second protocol for the cellulose selective oxidation, presented in Fig. 1, uses periodates, an oxidant able to oxidize the vicinal hydroxyl groups, resulting two aldehyde groups with a simultaneously breaking of the C_2-C_3 linkage.[21,22] This protocol creates (masked) aldehyde groups which further might serves as anchoring points, suitable for modifying and functionalizing cellulose. Moreover, the dialdehyde cellulose is biodegradable and biocompatible. Also, the aldehyde groups can be supplementary oxidized to carboxylic groups,[23] or to introduce an imine bonds between cellulose and amine groups, through a Schiff base

reaction with an amine.[24] However, the difference in the reactivity of hydroxyl groups in cellulose, as well as accessibility and regioselective control, could offer the circumstances to prepare cellulose products with new and specific features for various applications. Among the huge variety of the cellulosic products, water-soluble derivatives are significant for a large number of applications, as film forming, emulsion stabilizers, lubricating, gelling agents, in fields ranging from agriculture to food, cosmetics, oil industry, textile, pharmaceutical, etc.[25] Most water soluble cellulose derivatives correspond to cellulose ethers, some of them (carboxymethyl, hydroxyethyl, and hydroxypropil cellulose) being produced industrially in large quantities. Oxidation reaction of cellulose, could be used as an efficient tool to prepare water soluble cellulosic products. However, for this purpose, carefully reaction conditions, suitable reagents and the raw cellulose source are to be considered. 6-Carboxy cellulose, or β-1,4-linked glucuronic acid ("cellouronic acid") has been prepared as a water-soluble fraction during TEMPO-mediated oxidation of cellulose samples pretreated with ammonia.[26] The yield of the soluble fraction was only 17% in the case of using Avicel, and the degree of polymerization was found to be 75. Recently two-steps oxidations were applied to microcrystalline cellulose to obtain 6-carboxy cellulose.[27] The authors found that the water soluble product had weigh-average DP value of only 38, as against 220, the DP of the starting material. To prepare cellouronic acids by TEMPO-mediated oxidation, pretreatments of the native cellulose to convert cellulose I to III with lower crystallinity has been also proposed.[28]

Another useful oxidizing product which could be prepared by cellulose oxidation is 2,3,6-tricarboxy cellulose (TCC), named also mesotartaric acid/monohydrated glyoxilic acid alternating co-polyacetal. TCC was initially synthetized in a three-step reaction of cellulose with N_2O_4, $NaIO_4$, and $HClO_2$.[29] A two-step process to prepare TCC has been later reported, using N_2O_4 and $NaIO_4$ for the oxidation of cellulose.[30] Besides their multi-step character, these methods uses harsh reaction conditions and harmful reagents, like N_2O_4. Very recently, a one-step preparation of TCC was published, which is using 2-azaadamantane *N*-oxyl (AZADO) in combination with sodium bromide and high excess of sodium hypochlorite to oxidize regenerated cellulose.[31] Although, this is a one-step process, the use of very expensive AZADO, hinders its potentially industrial implementation. Moreover, the large excess of sodium hypochlorite used, has serious consequences on the depolymerization of the cellulose chain,

which dramatically decreased from 1270 in the initial cellulose sample to only 26 in the fully oxidized product ![31]

Taking into account the serious impediments aforementioned, we envisaged that a reconsideration of the oxidizing protocol could be beneficial. Therefore, in this work, we combined the two of the most selective protocols for cellulose oxidation in one shot reaction, to achieve the oxidation of all three hydroxyl groups in anhydroglucose unit of cellulose. In this way, we report the production of a series of partially oxidized products, and for the specific reaction conditions, even pure 2,3,6 - tricarboxy cellulose has been isolated. Besides the "*single reaction*" character of the proposed protocol, we avoided the use of very expensive reagents (TEMPO is 250 times cheaper than previously reported AZADO) or larger excess of NaClO as previously used.[31] A special attention was paid to the depolymerization phenomena, which drastically decreased the molecular weight of the oxidized products. The oxidation reactions were performed at room temperature, in the presence of either TEMPO and periodate, or NHPI and periodate. Two separate control experiments were carried out, in the absence of any nitroxyl radicals. All the oxidized products were rigorously characterized by means of FTIR, NMR (for water soluble compounds) and solid state NMR for the water insoluble products. X-ray photoelectron spectroscopy (XPS) has been further used for the characterization of the oxidized products, whereas the content of the carboxylic (aldehyde) groups were determined by using titration methods. The changes occurred on the degree of the polymerization of the oxidized products were determined through viscometric measurements.

2. Materials and methods

2.1. Materials

Avicel® PH 101 microcrystalline cellulose purified, partially depolymerized α-cellulose, with a mean degree of polymerization (DP) of 140, was purchased from Sigma-Aldrich. TEMPO, NHPI, sodium periodate, sodium bromide, 9% (wt) sodium hypochlorite and other chemicals and solvents were of pure grade (Sigma Aldrich), and used without further purification.

2.2. Oxidation reaction protocols

TEMPO-mediated oxidation of cellulose, using TEMPO/periodate/NaBr/NaClO system

TEMPO (0.4 g, 2.5 mmol), sodium periodate (2.7 g, 12.5 mmol) and NaBr (4 g, 40 mmol) were dissolved in 600 mL distilled water under vigorous stirring. Microcrystalline cellulose (5 g) was then suspended in the reaction mixture. The reaction vessel was covered with aluminum foil to prevent the photo-induced decomposition of periodate. The 9% NaClO solution (2.97 g, 40 mmol) was added to the cellulose slurry under continuous stirring and the resulting suspension was stirred for certain time: 4 h and 24 h respectively, at room temperature. The pH of the suspension was carefully maintained at about 10.5 by adding 2 M NaOH solution. After the designed time, the oxidation reaction was stopped by adding 5 mL of ethanol and the oxidized cellulose was filtered and washed several times with deionized water and 0.5 M HCl solution. The obtained water-insoluble fraction was dried by lyophilization, followed by vacuum-drying at 40°C for 48 h, and weight to measure the mass recovery ratios. The water-soluble fraction was precipitated with ethanol, the formed precipitate being collected by centrifugation. After centrifugation, the solid fraction was re-dissolved in water, desalted, oligomers being removed by diafiltration through a Millipore ultrafiltration membrane from polyethersulfone (cut-off: 10,000 g cm^{-1}) in Amicon cell equipped with a tank filled with pure water (conductivity lower than 3 µS m^{-1}). The diafiltration was stopped when the filtrate conductivity was lower than 10 µS m^{-1} and the oxidized cellulose was recovered by freeze-drying.

NHPI-mediated oxidation of cellulose, using NHPI/periodate/NaBr/NaClO system

The oxidation protocol is similar with the procedure presented for the TEMPO-mediated oxidation of microcrystalline cellulose using TEMPO/periodate/NaBr/NaClO, except the disperse medium, which in this case was a mixture of water (500 mL) and acetonitrile (100 mL), to ensure a better solubility of NHPI. All of the other parameters were maintained, including the reaction times: 4h and 24 hours. respectively.

Periodate-mediated oxidation of cellulose

Microcrystalline cellulose (5 g) was immersed for 4 h and 24 h respectively, in water (600 mL) containing sodium periodate (2.7 g, 12.5 mmol). The mixtures, were gently stirred at room temperature, in the dark. The pH of the suspension was maintained at about 4 by adjusting the pH with 2 M NaOH or 0.5 M HCl solutions. The oxidation was stopped by adding 5 mL glycerin

and the oxidized cellulose was filtered and washed several times with deionized water and 0.5 M HCl, and then dried by lyophilization followed by vacuum-drying at 40^0C for 48 h.

2.3. Characterization methods

UV Vis Measurements

The electronic absorption spectra were recorded using a SPECORD 200 Analytik Jena spectrometer.

FT-IR

Approximately 1 mg of dry cellulose sample was pressed into a pellet with 200 mg of potassium bromide and Fourier transform infrared (FT-IR) spectrum was recorded by Bruker Vertex 70 with accumulation of 32 scans and a resolution of 2 cm^{-1}, from 4000 to 500 cm^{-1}.

Determination of the aldehyde groups content

The dialdehyde group content was determined using a titrimetric method.[32] According to this method, each -CHO group reacts with hydroxylamine hydrochloride to form an oxime, while one proton is released. A certain amount of dialdehyde sample was suspended in water, and the pH was adjusted to 3.5 using HCl, then 25 mL hydroxylamine hydrochloride solution (5 % w/w) was added to the suspension. The pH of the suspension was carefully maintained around 3.5 by adding 0.1 mol/L NaOH, until no change of pH was observed. The cellulose sample was washed several times with water and collected by filtration. The weight of each sample was measured after drying, and the aldehyde content was determined by the consumption of the NaOH solution.

Determination of the carboxylic group contents

To a dried sample, equivalent to 0.5-1.0 g was added 100 cm^3 of 0.5 M NaCl. 10 cm^3 of 0.1 M HCl in 0.5 M NaCl was added to the suspension, prior to titration. The titration was carried out by adding 0.1 M NaOH in 0.5 M NaCl from a precision burette. The solution was stirred with a glass propeller and kept in an airtight titration vessel, during titration. All experiments were carried out under thermostatically controlled conditions at 25 °C. An inert atmosphere was maintained by continuous flow of argon. After each addition, the potential was recorded automatically with a Mettler Toledo 70 titrator. It usually took 2.5 h until a stable potential was attained. The stability criterion was a drift of less than 0.5 mV/min. All presented values are the mean values of three parallel measurements, the standard deviation of measurements being within 4%.

Degree of polymerization (DPv)

The degree of polymerization (DP) was determined viscometrically after dissolving the cellulose samples in cuoxam according to Klemm.[33] The viscosity measurements were performed in a modified Ubbelohde viscometer (capillary length 78 mm, capillary bore width 0.75 mm, volume of the bulb between the marks 7 cm^3). The cellulose solution in the viscometer was employed for one measurement only to avoid possible degradation of the sample. The viscosity (η) was calculated from the efflux time of the cellulose solution (t), the blank cuoxam solution (t_0), and from the concentration of cellulose in solution (c). The DP was calculated from the specific viscosity:

$$\eta_{spec} = \frac{\eta - \eta_0}{\eta_0} \qquad (1)$$

according to:

$$DP = \frac{2000 \cdot \eta_{spec}}{c \cdot (1 + 0{,}29 \cdot \eta_{spec})} \qquad (2)$$

Where:

c – cellulose sample concentration in g/L

^{13}C NMR analyses of water-soluble fractions

^{1}H-NMR and ^{13}C-NMR spectra were obtained by Bruker-Avance DRX 400 MHz Spectrometer, equipped with a 5 mm QNP direct detection probe and z-gradients, from solutions in D_2O, using tetramethylsilane TMS (δ=0.0 ppm) as the internal standard.

^{13}C CP-MAS solid-state NMR

^{13}C CP-MAS NMR spectra were recorded with a Bruker-Avance III AQS 400 SB instrument, operating at 9.4 T, fitted with a double air-bearing two-channel probe head. Samples were packed uniformly in a 4 mm zirconium oxide rotor. All measurements were performed at 296 (± 1) K. The MAS rate was 10 kHz. Acquisition was performed with a CP pulse sequence using a 2.95 microseconds proton 90 degree pulse, an 800 microseconds ramped (100 - 50%) falling contact pulse and a 2.5 seconds delay between repetitions. A SPINAL64 pulse sequence was used for ^{1}H decoupling. Hartman-Hahn matching procedure was performed on glycine and the chemical shift scale was calibrated to TMS ($(CH_3)_4Si$)

by assigning the data point of maximum intensity in alfa-glycine carbonyl signal a chemical shift of 176.03 ppm.

X-ray photoelectron spectroscopy (XPS)

The compositional analysis of the studied samples was carried out by X-ray photoelectron spectroscopy (XPS) using a PHI-5000 VersaProbe photoelectron spectrometer (*Φ* ULVAC-PHI, INC.) with a hemispherical energy analyzer (0.85 eV binding energy resolution for organic materials). The shape of the samples was "tablet" of dried fibers. A monochromatic Al Kα X-ray radiation (hν = 1486.7 eV) was used as excitation source. The standard take-off angle used for analysis was 45^{o}, producing a maximum analysis depth in the range of 3-5 nm. Spectra were recorded from at least three different locations on each sample, with a 1 mm x 1 mm area of analysis. Low-resolution survey spectra were recorded in 0.5eV steps with 117.4 eV analyzer pass energy. In addition, high-resolution carbon (1s) spectra were recorded in 0.1 eV steps with 58.7 eV analyzer pass energy. The XPS data were acquired using the PHI SUMMIT XPS for VersaProbe software.

3. Results and Discussion

Nitroxyl radicals, such as stable TEMPO, or non-persistent PINO, are known to be efficient mediators for the C_6 oxidation in cellulose, the other two hydroxyl groups being unafected. On the other hand, sodium periodate is often used to oxidize C_2 and C_3 atoms in anhydroglucose unit of cellulose, to form dialdehydes, whereas the C_6 atom remains unoxidized. Based on these findings, we can allege that these two processes are very selective routes to perform cellulose oxidation. However, no information on how the cellulose oxidation will be influenced by the simultaneously presence of the two reagents (nitroxyl radicals and sodium periodate) are reported. The selectivity of each reagent will exist, or other some synergistic effects are to be prevalent? To answering of these questions, in our present work, the oxidation of cellulose was accomplished by using simultaneously, both oxidizing agents: nitroxyl radicals (either TEMPO, or PINO) and sodium periodate in one-shot reaction, in the presence of sodium hypochlorite and sodium bromide. Following this procedure, a total oxidation of the three available hydroxyl groups in cellulose becomes feasible. In one parallel run, the oxidation was performed in the absence of nitroxyl radicals, only periodate being employed, a *classic* periodate oxidation of

cellulose. Two series of experiments were carried out, varying the reaction time from 4h to 24h respectively.

Until few years ago, TEMPO and its derivatives, were the only nitroxyl radicals able to perform the selective oxidation of C_6 in cellulose. Recently, NHPI was implemented as an efficient mediator for the selective conversion of the primary OH groups in cellulose into carboxylic moieties. However, in this case, the presence of another compound is mandatory, to achieve the activation of NHPI, by its conversion into PINO radical. Several compounds are used as co-catalysts for the homolytic bond cleavage between O and H atoms in NHPI.[12-16] This requirement seems to be a drawback, since compounds as lead tetraacetate, or cerium ammonium nitrate, co-catalysts for the PINO *in situ* generation, are themselves interfering with the cellulosic substrate, causing side reactions, especially by degrading the macromolecular backbone. In order to avoid this, we tested the ability of sodium periodate, as a possible agent for the *in situ* formation of PINO radical. Fortunately, periodate could act as very efficient co-catalyst for the PINO radical formation, as UV-vis spectra highlights, Figure 2.

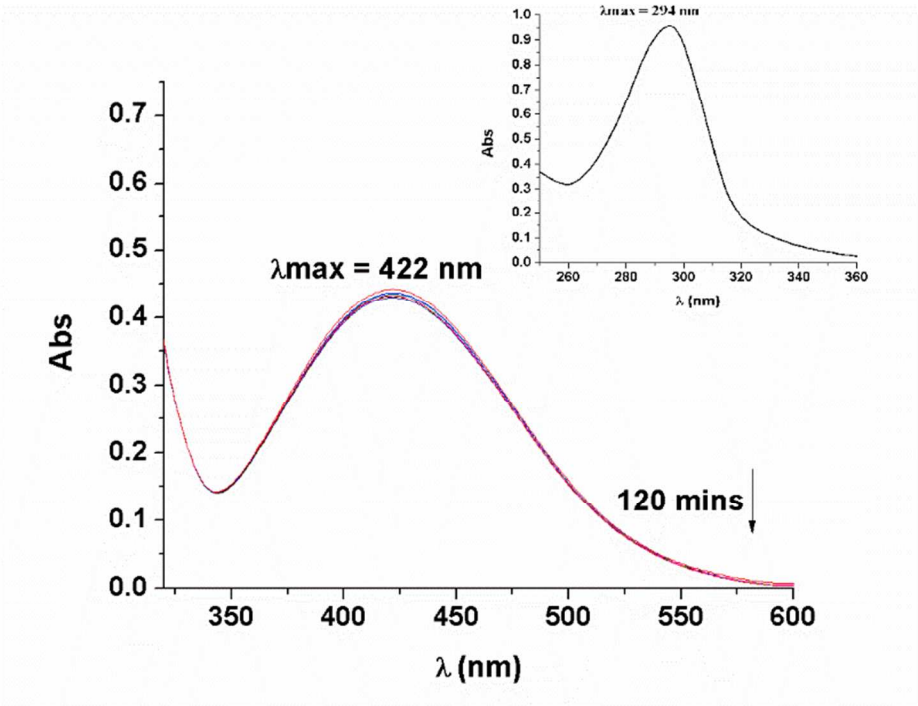

Fig. 2 UV-vis spectra of a mixture of NHPI (1 mM) and sodium periodate (1 mM) in acetonitrile-water (1:1 volume %); Inset: UV-vis spectrum of 1mM NHPI solution in acetonitrile.

The maximum UV-vis adsorption of neat NHPI in acetonitrile, at λ=294 nm, shifts instantaneously to λ=422 nm when an equimolar amount of periodate aqueous solution is added. This is a clear evidence of the PINO radical formation, which, further can act as a mediator for the cellulose oxidation. The simultaneous presence of TEMPO (or NHPI) and perioadate will fulfill the conditions for the oxidation of all the three hydroxyl groups in anhydroglucose unit, as is suggested in Fig. 3.

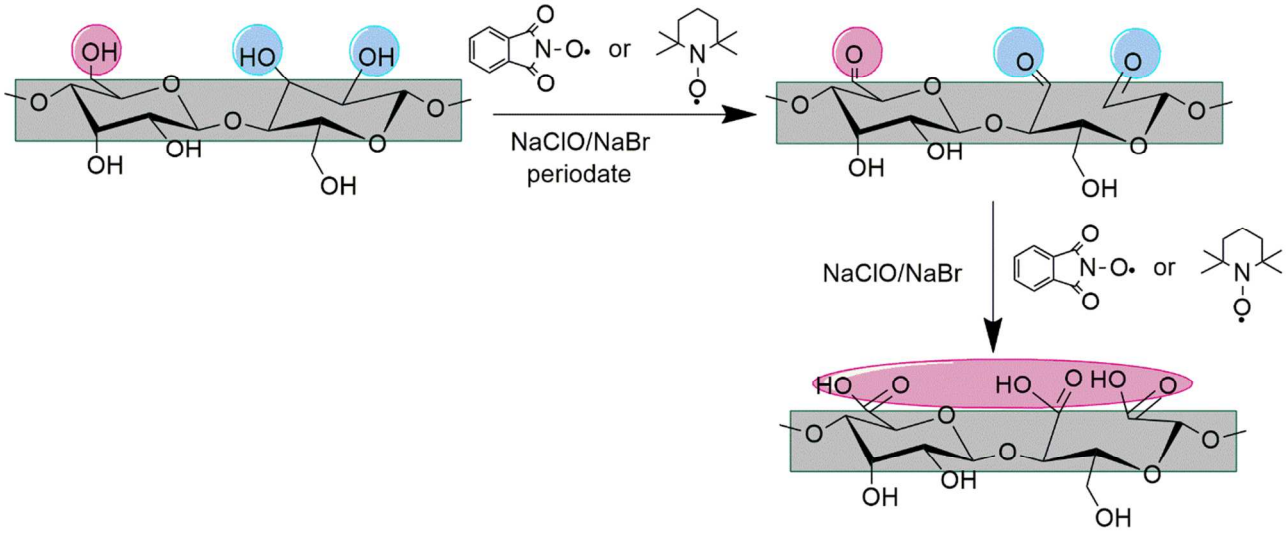

Fig. 3 Illustration scheme of the full oxidation of cellulose in the presence of both nitroxyl radical (TEMPO or PINO) and periodate.

In the first stage of the reaction, the three hydroxyl groups, are converted to aldehydes, as follow: the accessible C_6-OH groups on the crystalline surface are converted due to the presence of the nitroxyl radicals (TEMPO or NHPI) in the presence of sodium hypochlorite, whereas the two secondary OH groups, at C_2 and C_3, are being converted into 2,3-dialdehyde cellulose, in a *classic* periodate oxidation mechanism, concomitantly with the cleavage of the cellulose's glucopyranose rings between C_2-C_3 bond. In the next step, due to the presence of nitroxyl radicals and sodium hypochlorite, the aldehyde groups are further oxidized, to form the final oxidation product: the carboxylic groups.

Table 1 presents the carboxylic (aldehyde) groups contents, yield and degree of polymerization of the oxidized samples. The samples were labeled by letters O, N, T, and P. Sample O is the untreated (original) microcrystalline cellulose, whereas N denotes samples simultaneously oxidized by NHPI and periodate, T those simultaneously oxidized by TEMPO and periodate, and P means the samples oxidized only by periodate. Each label contain also a number (4 or 24)

which describes the reaction time (in hours) used to prepare the respective sample. The water soluble samples are supplementary identified by a lowercase s, added after the corresponding number.

Table 1. The content of carboxylic (aldehyde) groups, and degree of polymerization of cellulose samples, oxidized at room temperature, in the presence of 2.5 mM periodate / g cellulose.

Sample	Reaction time (h)	NHPI (mM/g cellulose)	TEMPO (mM/g cellulose)	Content of carboxylic groups (mM/Kg)	Content of aldehyde groups (mM/Kg)	Yield (%)	Degree of Polymerization (DP)
O	-	-	-	51	-		140
N4	4	0.5	-	456	-	82	86
N4s	4	0.5	-	-	-	< 1	n.d
N24	24	0.5	-	553	-	79	65
N24s	24	0.5	-	-	-	< 3	n.d
T4	4	-	0.5	1760	-	52	69
T4s	4	-	0.5	3110	-	31	35
T24	24	-	0.5	1790	-	48	55
T24s	24	-	0.5	3128	-	34	28
P4	4	-	-	-	315	95	107
P24	24	-	-	-	1325	88	75

Once the conversion of the hydroxyl groups in the anhydroglucoside unit, to carboxylic ones, reach a certain level, the fully oxidized product, tend to become water soluble: see samples N4s, N24s, T4s, and T24s, in Table 1. The amount of the carboxylic groups formed upon oxidation for 4h, is almost four times higher in the case of using TEMPO/periodate, than in the case of using NHPI/periodate (samples T4 and N4, Table 1). When solely periodate was used for oxidation, no evidences of the carboxylic groups formation were found, the only hydroxyl groups converted being those on the positions 2 and 3 on anhydroglucose unit, to form dialdehydes. Increasing the oxidation time from 4h to 24h, no further improvement of the amount of the carboxylic groups

formation was noticed, samples N24, and T24. However, the prolongation of the reaction, cause supplemental degradation of the cellulose chain, from a degree of polymerization of 86 to 65 for the samples oxidized with NHPI/periodate, and from a degree of polymerization of 69 to 55, for the samples oxidized with TEMPO/periodate. Periodate oxidation of cellulose for longer time (24h) instead, lead to an impressive increase on the amount of 2,3-dialdehyde groups formation as compared as with the 4h time reaction, see samples P4, and P24. Moreover, the degradation processes in the case of periodate oxidate are less pronounced as compared with the case of using nitroxyl radicals-mediated oxidations. Sample T24s has the highest amount of the carboxylic groups, more than 3000 mM/Kg cellulose. This remarkable amount, induced the solubility of this sample in water, as well as a highly negative value of the ξ-potential: -58 ± 3.85 mV. It has to be pointed out, that the water soluble fractions are exclusively obtained when employing the TEMPO/periodate protocol, in the case of the NHPI/periodate system, only small amounts of the water-soluble product could be obtained.

FTIR

The FTIR technique can be used as a straightforward method to evaluate the structural changes occurred in cellulose after oxidation. However, for the periodate oxidized samples, the aldehyde groups estimation appears to be ticklish, since these groups are existing partially or even totally hydrated, and the resulted hemiacetal or hemialdol structures do not exhibit the classical adsorption in FTIR of the carbonyl group. In these conditions, the aldehyde group originates from periodate oxidation appears hardly to be detected and not quantitatively by FTIR.[34] Moreover, all the possible oxidized functionalities appeared after oxidation reaction (aldehyde, keto and carbonyl) absorb in a very narrow region of the spectrum, between 1700 and 1750 cm^{-1}. Figure 4 shows the FTIR spectra of original and 24h oxidized samples.

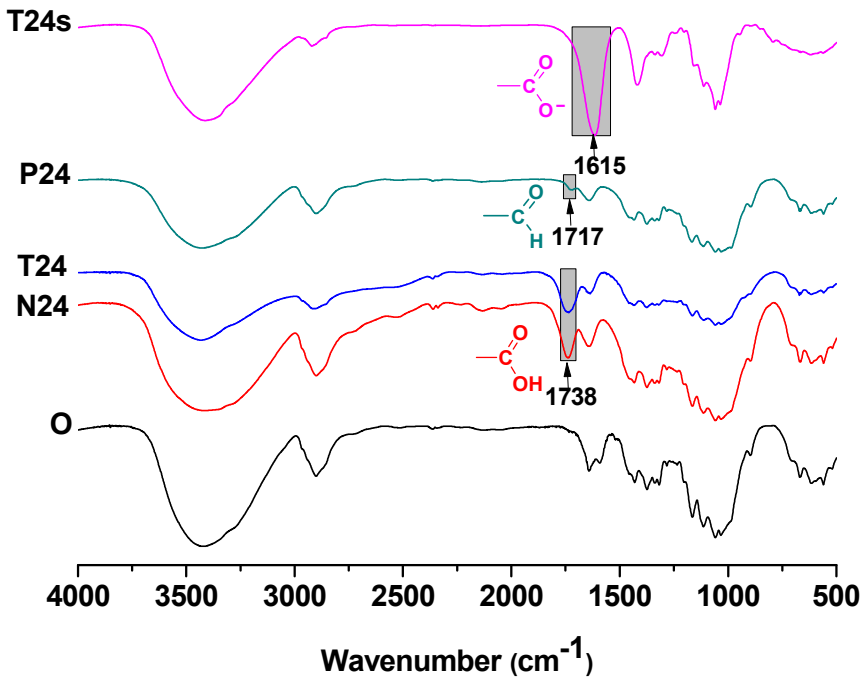

Fig. 4 FTIR spectra of untreated and 24h oxidized cellulose samples.

The characteristic cellulose peaks for hydrogen bonded O-H stretching, centered at 3420 cm^{-1} and for sp^3 hybridized C-H stretching at 2900 cm^{-1} are presented in all the samples. However, the later peak, drastically decrease after oxidation (see samples T24 and T24s), due to the oxidation at secondary hydroxyl groups (OH-2 or OH-3 positions). After oxidation, the presence of another peaks can be denoted, thus for the samples N24 and T24 a sharp absorption at 1738 cm^{-1} is attributed to carbonyl groups in the free COOH group, whereas the large and sharp absorption at 1615 cm^{-1} in sample T24s is due to carboxylate groups in their sodium salt forms. The absence of these absorption bands in P24 sample denote that no carboxylic groups are formed during periodate oxidation (in the absence of any nitroxyl radicals), an absorption peak being yet localized around 1717 cm^{-1}, which is assigned to C=O stretching vibration of aldehyde groups. The other characteristic peaks of unoxidized cellulose, such as –OH inplane bending, at 1201 cm^{-1}, C-H deformation stretching vibration, at 1112 cm^{-1} and asymmetry stretch vibration, around 1165 cm^{-1}, C-O-C stretching vibration of pyranose ring skeleton at 1059 cm^{-1}, tend to became weaker after oxidation, in all samples, indicating the possible decomposition processes during oxidation.[35,36]

XPS

X-ray photoelectron spectroscopy (XPS) technique was intensively used in the last decade to study structural changes which occur in polysaccharides backbone during various physical or chemical treatments.[37-39] A low resolution scan of the untreated and 24h cellulose oxidized samples was performed. These scans were used to determine the O/C ratio, Table 2 and Fig. 5. As expected, the only elements found, were carbon and oxygen, except T24s sample, when Na was supplementary detected. This is due to the fact that carboxylic groups in this sample, are in their sodium carboxylate form, see also the FTIR spectrum.

Table 2. The elemental surface composition from XPS survey spectra for original and oxidized cellulose samples.

Sample	C1s (%)	O1s (%)	O/C
Avicel	61.30	38.70	0.63
N24	59.46	40.56	0.68
T24	58.74	41.26	0.70
T24s	57.02	42.98	0.75
P24	60.09	39.91	0.66

As Table 2 highlights, the atomic ratio O/C, gradually increase from 0.63 in unoxidized sample, to 0.66 in P24 sample, and reached the highest value, of 0.75 in T24s sample, which denote the highest percent of the introduced oxygen in the cellulose units.

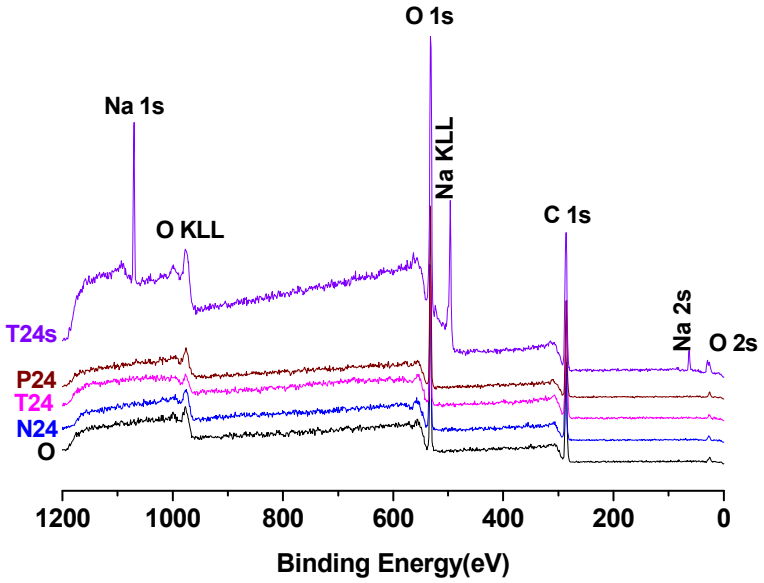

Fig. 5 XPS survey spectra of original and oxidized cellulose samples.

To examine the types and relative amounts of the different C-O bonds present on the surface of cellulose samples, the high resolution C1s region was performed. High resolution C1s XPS spectra, reveal chemical shifts that could be classified as follows:[40] unoxidized carbon (C-C, binding energy: 285 eV), carbon with one oxygen bond (C-O- or C-OH, binding energy: 286.5 eV), carbon with two oxygen bonds (O-C-O or C=O, binding energy: 288 eV), and carbon with three oxygen bonds (O=C-O, binding energy: 289.4 eV), Table 3.

Table 3. Relative amounts of differently bound carbons as determined from high resolution carbon C1s determined by XPS.

Sample	Relative concentration (%) and Binding energy (eV)				
	C-C/C-H C1 285	C-O-/C-OH C2 286.5	O-C-O/C=O C3 288	O-C=O C4 289.4	C4/C2
O	16.96	64.15	17.39	1.50	0.023
N24	17.77	56.53	21.50	4.20	0.074
T24	27.37	47.08	19.93	5.62	0.120
T24s	23.32	43.51	25.59	7.58	0.170
P14	15.73	59.61	21.24	3.42	0.057

From Table 3 one can conclude that after oxidation, the C2 peak area decrease substantially, due to the conversion of OH groups into CHO or COOH groups. Another reason of the decrease could be related with the degradation of the D-glucose ring during oxidation. Conversely, the C3 and C4 peaks, consisting of O-C-O/C=O and O=C-O linkage of carbon respectively, significantly increased after cellulose oxidation, which denote a high amount of carbonylic/carboxylic groups introduced into anhydroglucose unit. As a measure of these changes, we used the ratio of C4/C2 area peaks, to evaluate the unoxidized and oxidized cellulose samples. This ratio increased from 0.023 in pure cellulose, to 0.074 in N24 sample, and reached the highest value, of 0.170 in T24s sample. The ratio C4/C2, differ due to the changes occurred on both carbon types contributions. On the one hand, C2 peak area became smaller after oxidation (due to the OH groups disappearance), but on another hand, the C4 peak area largely increased after cellulose oxidation, due to the introduction of high amount of carboxylic groups.

^{13}C-NMR of water soluble fractions

Due to the high content of carboxylic groups incorporated, T4s and T24s samples becomes highly water soluble, allowing therefore the acquisition of the ^{13}C NMR spectra in deuterated water. Fig. 6 shows the typical spectrum of such compounds. Since for the original (unoxidized) cellulose sample is not possible to record the ^{13}C NMR in deuterated water, its spectrum can be seen later as a solid state NMR. The T4s sample exhibit in ^{13}C NMR spectra a quite intense peaks signal, between 176.87 and 178.35 ppm, which are characteristic for carbons originating from carboxylic groups. The presence of three peaks (see the inset of Fig. 6) prove that there are three distinctive COOH groups inside the anhydro glucose unit.

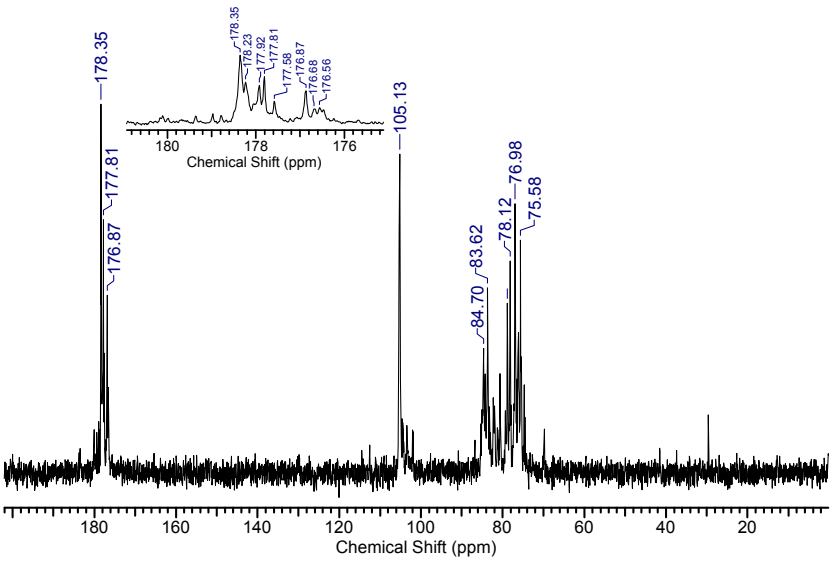

Fig. 6 ^{13}C-NMR spectrum in D_2O of T4s sample. *Inset*: magnified peaks of "carboxylic" signals.

In the case of preparation of other water soluble cellulose derivative, i.e. 6-carboxylcellulose, ^{13}C NMR spectrum, there is only one carbon peak around 175 ppm, (corresponding only to one carboxylic group type formed) according with C_6 oxidation performed with nitroxyl radical and sodium hypochlorite (no periodate was present).[41]

^{13}C CP-MAS solid-state NMR

Partially oxidized cellulose products, those which were not water soluble, were analyzed by means of ^{13}C CP-MAS solid-state NMR**.** The spectrum of the unoxidized cellulose, Fig. 7, displays the typical cellulose signals in the range of 110 – 60 ppm. A multiplet around 105 ppm is assigned to the C_1 carbon of cellulose, two signals at 90 and 84 ppm are assigned to the C4 carbons in crystalline and non-crystalline regions, whereas crystalline and non-crystalline C_6 signals are located at 64.4 and 62.5 ppm respectively. Between 76.6 and 70.7 ppm there is a region of overlapping signals, originating from the C_2, C_3, and C_5 carbons. After oxidation some spectral changes occurred. The most noticeable is a new signal peak at 170.5 ppm in N24 sample, which increased in intensity in T24 sample. This new signal, confirms the introduction of the COOH groups in these samples, as previously evidenced by FTIR. Also, there are changes of the C6 non-crystalline signals, which decrease in intensity from N24 to T24 samples, correlating with the expected conversion of CH_2-OH groups to COOH groups. Chemical modification of a fibril surface, can indirectly alter signal intensities at neighboring chemically unmodified surfaces. Also, significant changes occur in the C4 non-crystalline region as the result of the chemical modifications. Further, loss of signal intensity at a particular signal

position in CP/MAS ^{13}C-NMR spectra as the result of chemical modifications can be the result of shifting signal intensity to another position or loss of signal intensity (discrimination) due to changes in polymer mobility. For all oxidized samples, the signals originating from the $C_{2,3,5}$ atoms are lower in intensity than in the original, unoxidized samples, correlating with the conversion of the OH groups linked to C_2 and C_3 to carboxyl (carbonyl) groups. An interesting feature of the sample P24 in ^{13}C NMR, is the lack of any resonance in the carbonyl region (210 – 180 ppm) expected due to the presence of the two carbonyl moieties formed during periodate oxidation. This absence is due to the fast recombination reactions of carbonyl groups to form hemiacetals structures with remaining hydroxyl groups.[32,42]

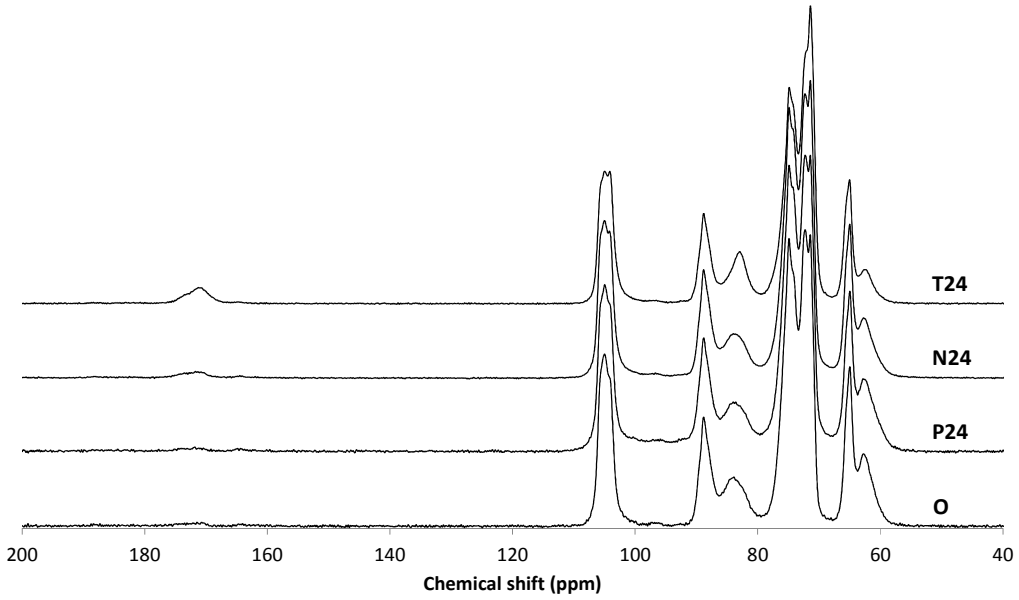

Fig. 7 ^{13}C CP-MAS solid-state NMR spectra of unoxidized and oxidized cellulose samples.

4. Conclusions

The highly water-soluble 2,3,6-tricarboxy cellulose has been prepared by combining the synergic actions of the two cellulose selective oxidants: nitroxyl radicals (TEMPO) and sodium periodate, in one-shot reaction, at room temperature and pH 10.5, within 4 h. The carboxylic groups content as found by potentiometric titration was as high as 3110 mM/kg. No further significant increase of this amount by prolonging the reaction time to 24h, was observed. In the case of combining another nitroxyl radical (PINO, obtained *in situ* from its parent hydroxyl amide) with periodate,

rather modest amounts of water soluble derivative were formed, as well as the overall content of carboxylic groups formed was scarce. Notably, the polymerization degree of the synthetized 2,3,6-tricarboxy cellulose was found to be only 4 times lower than that of the starting material, indicating a better preservation of the macromolecular chain than in the case of other reported oxidizing protocol, when the polymerization degree was, impressively 500 times lower as compared with the original cellulose sample.[31] When periodate alone, was employed, no carboxylic groups formation were detected, 2,3-dialdehyde cellulose products were revealed instead.

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