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# Oxidation of cellulose in pressurized carbon dioxide

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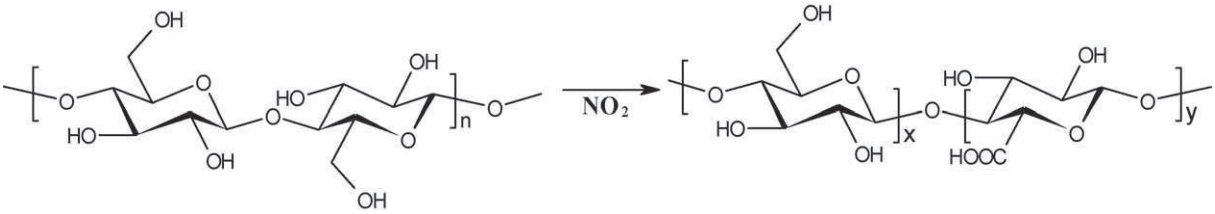
A B S T R A C T

This work presents first results upon oxidation of type II cellulose by nitrogen dioxide dissolved in carbon dioxide at high pressure. This reaction leads to oxidized cellulose, a natural-based bioresorbable fabric used for biomedical applications. The oxidation reaction takes place in a heterogeneous fluid–solid system. Kinetics of oxidation is presented here and effects of operating conditions such as pressure, temperature and initial moisture content of cellulose are investigated. Results are presented in terms of degree of oxidation of cellulose and quality of the final oxidized cellulose, which has been characterized using liquid-state and solid-state $^{13}C$ NMR. The experimental results show the existence of possible secondary reactions which may lead to oxidized cellulose with insufficient mechanical strength. An attempt is made to evidence and understand the role of $CO_2$ as a solvent in this system. Indeed, although supercritical $CO_2$ appears to be a suitable candidate as a solvent for oxidation reactions, some inhibiting effect on nitrogen dioxide activity are observed in this case.

## 1. Introduction

Oxidized cellulose is a very interesting material for biomedical applications, because it is degradable in human body [1] and also exhibits haemostatic and antibacterial properties. Oxidized cellulose is thus used as a raw material for medical devices such as adhesion barriers, sutures, absorbable haemostats or scaffolds for tissue engineering. To obtain this product, nitrogen dioxide ($NO_2$) is the most suitable oxidant because, in this case, oxidation does not affect secondary hydroxyl groups of the cellulose, and thus secondary reactions are avoided. In this case oxidized cellulose maintains acceptable mechanical properties, allowing its use as a biomedical device. The global reaction is illustrated by the following scheme:




The first industrial process for oxidation of cellulose was based on the use of gaseous nitrogen dioxide, where the gas was circulated across layers of fabrics [2]. But this process was abandoned because of the difficulty of handling a gas (especially when its atmospheric condensation point is 21 °C, as it is the case for $NO_2$), as well as because mastering the exothermicity of the reaction in a gaseous system at industrial conditions is uneasy. Consequently, solvent based processes were proposed. In this case, nitrogen dioxide was dissolved in an organic solvent, and thus the oxidation took place in the liquid phase [3]. Nevertheless, the oxidant could cause some damage to the solvent, and solvent degradation products were often present. Therefore, the biomedical company Johnson and Johnson proposed the use of inert halogenated solvents, and patented the use of perfluorocarbons as solvents for this reaction [4].

Recently the use of supercritical carbon dioxide as a suitable inert solvent to perform oxidation of cellulose was proposed and patented [5]. Because of its peculiar physical properties and its specific "green" character, supercritical $CO_2$ is now growing interest as a reaction medium for many chemical syntheses, such as hydrogenation, hydroformylation, oxidation or polymerization [6]

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**Nomenclature**

| | |
|---|---|
| AC | carboxylic acid content of the cloth (%w/w) |
| DO | degree of oxidation of cellulose |
| $V_a$ | HCl solution volume used to neutralise basic cellulose solution (L) |
| $V_b$ | HCl solution volume used to neutralise basic solution without cellulose (L) |
| $C$ | HCl solution concentration (mol $L^{-1}$) |
| $M$ | molar weight of COOH group = 45 g $mol^{-1}$ |
| $m$ | weight of the cellulose sample (g) |

for instance. Especially, as it is the result of complete oxidation of carbon, $CO_2$ is a solvent of choice for oxidation reactions considering the absence of by-products possibly issued from degradation of the solvent by the oxidant. In the view of biomedical applications for oxidized cellulose, using $CO_2$ has the major advantage to yield processed fabrics as "solvent residue free". More widely, carbon dioxide is considered as a green non-expensive solvent, and, in this case, compares very favourably with perfluorocarbons.

This work focuses on the main aspects of oxidation of cellulose in high-pressure $CO_2$ and using nitrogen dioxide as the oxidant.

## 2. Generalities on cellulose oxidation by nitrogen dioxide

Nitrogen dioxide does not exist as pure molecular specie, but is in equilibrium with its dimer, nitrogen tetroxide, as follows:

$$N_2O_4 \leftrightarrows 2NO_2$$

When temperature is less than 262.15 K, nitrogen dioxide is completely dimerized giving nitrogen tetroxide ($N_2O_4$) and this dimer dissociates into 2 mol of $NO_2$ as temperature increases, proportion of each compound depending on conditions of temperature and pressure. Under atmospheric pressure, the mixture boils at 294.25 K, the liquid being yellowish brown and the vapour reddish brown. If moisture is present, $N_2O_4$ reacts with water forming nitric and nitrous acids which are thus involved in complex equilibria mechanisms [7]. This decomposition phenomenon makes it very aggressive to numerous metals. Acute toxicity of this reactant makes it quite delicate to handle, so individual protective equipments must be used.

Physical properties of the mixture and equilibrium constant value depend on temperature and have been the subject of several studies. For example, Reamer and Sage [8] have done density measurements in the liquid–vapour coexistence region. Values of equilibrium constants in the gas phase as a function of temperature can be found in works of Verhoek and Daniels [9] or Chao et al. [10]. James and Marshall [11] have determined equilibrium data for the liquid mixture and Redmond and Wayland [12] have been interested in equilibrium constant data for the nitrogen dioxide dissolved in organic solvents.

In order to run the reaction in a homogeneous mixture of oxidant and solvent, fluid phase equilibria involved in the reactional mixture have been previously experimentally investigated [13]. These measurements give us information upon solubility of $NO_2$ in high-pressure $CO_2$, and about interactions which exist between $NO_2$ and $CO_2$. Such interactions may have a dramatic effect on the reaction of oxidation of cellulose, as mentioned by some authors who studied this reaction in organic solvents [14]. Indeed, the $NO_2$ specie is suspected to be the only active oxidant specie [15,16] and its concentration depends upon the dimerization equilibrium. In their investigations upon the oxidation of cellulose by nitrogen dioxide dissolved in various solvents, it was shown that the degree of dissociation of $N_2O_4$ increases in non-polar solvents, leading to an increase in the degree of oxidation of cellulose. Considering that supercritical $CO_2$ is considered as a non-polar solvent, this observation seems a favourable clue for its use for the reaction of oxidation.

Mechanisms of oxidation of cellulose with $NO_2/N_2O_4$ in gas phase or in solvent phase have been the subject of different studies that have been summarized by Stilwell et al. [1] where it appears that formation of cellulose nitrites as intermediates is necessary to achieve oxidation, which occurs then through physical and chemical adsorption of the $NO_2$ onto the cellulose. Cellulose must contain initially between 5.5 and 11% (w/w) of moisture to ensure adsorption of the reactant. The proposed stoichiometry of the oxidation reaction is then:

$$RCH_2OH + NO_2 \rightarrow RCOOH + H_2O + (1/2)N_2$$

Considering nitric acid formation by the reaction of $NO_2$ with $H_2O$, Stilwell et al. [1] proposed the overall stoichiometry of the reaction to be:

$$RCH_2OH + 2N_2O_4 \leftrightarrows RCOOH + 2HNO_3 + NO + (1/2)N_2$$

Because of the by-products of this reaction, an important step of the production process lies in the cleaning of the oxidized cellulose at the end of the reaction, because the remaining acidic by-products, as well as the remaining adsorbed $NO_2$ are responsible of degradation of the product during storage.

## 3. Materials and methods

### 3.1. Cellulose

Regenerated cellulose, i.e., man-made cellulose fibres produced by the cuprammonium process [17] from Bemberg company, was used as the starting raw material for all this study. This regenerated cellulose, usually termed rayon, consists in fibres that are spun in such a way to form a continuous filament. Such continuous filaments can be knitted together to form simple braid or meshes.

### 3.2. Chemicals

$NO_2/N_2O_4$ was supplied by Air Liquide (water content max 0.5%wt) and $CO_2$ TP by Linde Gas.

### 3.3. Oxidation experimental set-up

Experiments were performed using the set-up presented in Fig. 1. The reactor (250 mL), from Parr Instrument Company is thermo-regulated and comprises two sapphire windows allowing visual control of the reactional mixture. Homogeneity of the mixture is ensured due to a magnetic stirrer. A Teflon® made supporting grid is used to maintain the piece of cellulose to be oxidized in the upper part of the reactor volume. Pressure and temperature sensors are used to control operating conditions and are connected to a digital recorder (Memo-graph RSG10, Endress + Hauser). Liquid $CO_2$ is pumped due to an air operated reciprocating piston pump (High-pressure pump ref. 0902 10000, Top Industrie). A Coriolis type mass flow meter (Micro-Motion) allows the control of the amount of $CO_2$ introduced in the reactor.

Reactant $NO_2$ (note that we refer here to the $NO_2/N_2O_4$ mixture) is introduced into the reactor by using a 50 mL high-pressure air operated syringe injector (Injector 1644 1000, Top Industrie). This part of the set-up is equipped for nitrogen circulation in order to dry and inert the system, and this is necessary to avoid nitric acid formation during $NO_2$ introduction.

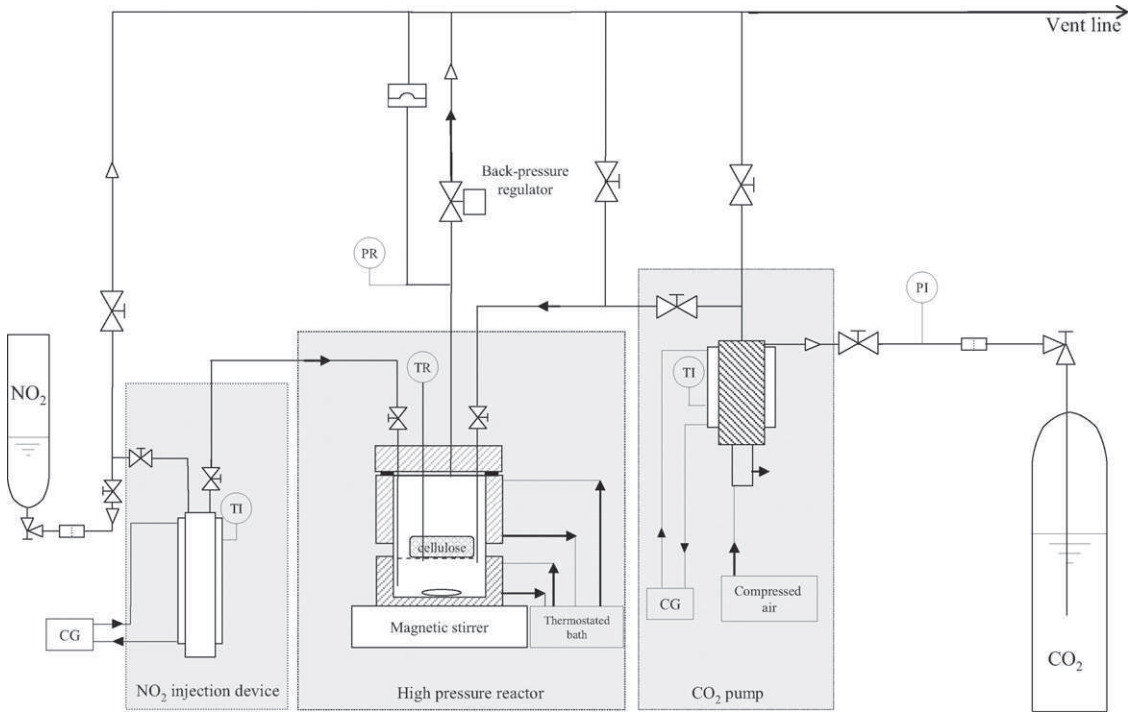


**Fig. 1.** Scheme of the high-pressure oxidation experimental set-up.

At the outlet of the reactor, the closing valve is followed by a heated back-pressure regulator (Tescom). This system is used to maintain a constant reactor pressure, especially during the $CO_2$ washing procedure at the end of the reaction (washing by dilution, see next paragraph).

All parts of the experimental set-up in contact with $NO_2$ are made of 316L stainless steel, and gaskets are made of Teflon. Because of the toxicity of $NO_2$, in case of accidental leakage, operators are protected using individual protecting equipment.

### 3.4. Operating procedure

The reactor is firstly heated to the desired temperature (around 313 K) and the cellulose fabric sample is placed on the Teflon grid. After closing the reactor, sweeping with gaseous $CO_2$ is done before filling with $CO_2$ until the desired pressure is reached. From measurement of the course of the injector piston, a given amount of reactant $NO_2$ is then introduced into the reactor and oxidation starts.

Post-oxidation washing of oxidized samples is fastidious step of the process. Indeed, if a simple depressurization of the reactor is carried out, residual $NO_2$ may condense onto cellulose and could cause irreversible damage to the product. As a consequence, a washing by dilution with $CO_2$ under pressure is carried out in order to maintain the reacting mixture as a homogeneous phase and avoid condensation of $NO_2$ onto cellulose. This washing is done by pumping $CO_2$ into the reactor, while the diluted reacting mixture is released through the back-pressure regulator.

Then, a second washing step is necessary to remove acidic by-product of the oxidation and residual adsorbed $NO_2$. In accordance with industrial oxidation processes ([2,3]), this washing is done at atmospheric pressure outside the reactor with an isopropyl alcohol–ethanol mixture (50/50 vol.) until pH is neutral. Residual water is then removed by washing three times with anhydrous alcohol (isopropyl alcohol or ethanol). Finally, the sample is dried in an oven at 333 K during 24 h.

### 3.5. Titration by colorimetric method

The degree of oxidation is directly related to the carboxylic acid content of the oxidized cellulose samples. This latter is determined by a back-titration method: a small amount of dried oxidized sample (about 200 mg) is dissolved in 2 mL of 0.5N solution of sodium hydroxide diluted with 8 mL of distilled water. The solution is then titrated with standard 0.05N HCl to a phenolphthalein end point. A blank is also run using only the NaOH solution without the cloth. The titration is repeated at least three times. This titration allows determination of the carboxylic acid content of the cloth (AC), 100% of oxidation corresponding to 25.5% carboxylic acid content, and a degree of oxidation equal to unity. The degree of oxidation (DO) represents the number of oxidized hydroxyl groups per anhydroglucose unit of the cellulose sample, following the relations:

$$\mathrm{AC} = \frac{(V_b - V_a) \times C \times M}{m} \times 100 \quad (1)$$

$$\mathrm{DO} = \frac{M_{\mathrm{anhydroglucose}} \times (V_b - V_a) \times C}{m - 14 \times (V_b - V_a) \times C} \quad (2)$$

In relation (2), $M_{\mathrm{anhydroglucose}}$ is the molar mass of one anhydroglucose unit of the cellulose (162.15 g mol$^{-1}$), and 14 is the difference between the molecular weight of a glucuronic acid unit and an anhydroglucose unit.

### 3.6. Characterization of the cellulose by solid-state NMR

A Brucker MSL spectrometer was used to obtain solid-state $^{13}C$ NMR spectra, operating at 50 MHz $^{13}C$ frequency.

The solid-state NMR spectrum of unprocessed rayon is presented in Fig. 2. Peaks corresponding to each carbon group of the sample can be observed, as referred on the anhydroglucose group above it. Characteristic peak of "–$CH_2OH$" group is observable at 62.3 ppm, peaks of "–CH" groups at positions 2, 3 and 5 of the cycle are between 68 and 80 ppm, and carbons at position 4 and 1 are characterized by peaks between 80 and 90 ppm and 105 ppm,

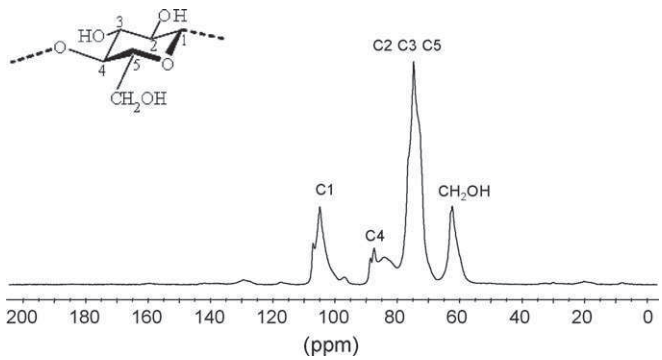


**Fig. 2.** Solid-state $^{13}C$ NMR spectrum of original rayon.

respectively. Shoulders which appear on C1 and C4 peaks evidence existence of crystalline parts.

### 3.7. *Characterization of the cellulose by liquid-state NMR*

$^{13}C$ NMR spectra of oxidized samples are obtained on a Brucker AC 300 spectrometer (operating frequency of 75.47 MHz). Samples are studied after dissolution in NaOD solution (60 mg in 1 mL of NaOD 2 M, followed by neutralisation (pD ~ 7) with DCl) at 298 K in 5 mm o.d. tubes. The methyl signal of acetone was used as internal standard ($^{13}C(CH_3)$ at 31.5 ppm relative to $Me_4Si$). $^{13}C$ spectra were recorded using 30° pulses, 7500 Hz spectral width, 0.5 s acquisition time, 0.5 s relaxation delay and 50,000 scans were accumulated.

## 4. Results and discussion

### 4.1. *Influence of the pressure*

When considering phase equilibria of the $CO_2$–$NO_2/N_2O_4$ mixture at 313 K issued from [13] and presented in Fig. 3 we can deduce that, if reaction is run above 8.5 MPa, it takes place in a homogeneous reacting mixture, whatever its composition. Indeed, the biphasic zone where liquid and vapour are in equilibrium, has to be avoided in order to insure that the reaction is run in a homogeneous phase to obtain homogeneously oxidized samples. Under 8.5 MPa, depending on the composition, the reaction may take place either in a "dense gaseous phase" ($CO_2$ rich mixtures) or in an "expanded liquid" ($NO_2/N_2O_4$ rich mixtures).

Oxidation of a sample of rayon (3.3 g) has been studied under conditions where the reacting mixture is a monophasic at 313 K for two values of the oxidant concentration in the reactor, i.e., 132 g $L^{-1}$ and 12 g $L^{-1}$ of $NO_2$ (note that we arbitrarily refer to "concentration of $NO_2$" as if the mixture $NO_2$–$N_2O_4$ was completely dissociated). Considering the overall stoichiometry of the reaction, oxidation with $[NO_2] = 132$ g $L^{-1}$ is conducted with a large excess of reactant (stoichiometric ratio [cellulose:$NO_2$] is equal to [1:35]), and $[NO_2] = 12$ g $L^{-1}$ corresponds to a slight default of oxidant (stoichiometric ratio [cellulose:$NO_2$] is equal to [1:3]). From preliminary kinetics experiments, the reaction was performed during 4 h, since most of the oxidation occurs during this time.

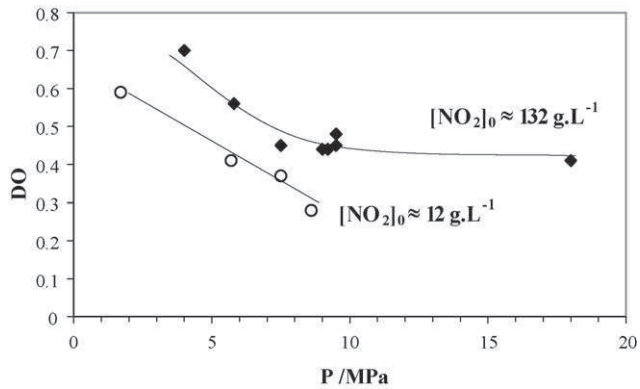


**Fig. 4.** Influence of the pressure on oxidation of rayon with $NO_2$ in $CO_2$ at 313 K during 4 h.

Because the oxidation reaction is run under constant volume condition, at a given $NO_2$ concentration, an increase in the $CO_2$ amount in the mixture leads to an increase of the total pressure of the reaction. At low pressure, i.e., low $CO_2$ and high $NO_2$ mass fractions, oxidation is actually performed in a dense medium that can be regarded as an "expanded" liquid $NO_2$-phase. Conversely, reactions performed at high pressure, i.e., low $NO_2$ mass fraction, correspond to dense gaseous $NO_2/CO_2$ mixtures. Evolution of the degree of oxidation of rayon samples with the pressure is presented in Fig. 4.

At constant $NO_2$ concentration, we clearly observe that when the pressure, i.e., the amount of $CO_2$ in the system, is increasing, the degree of oxidation of rayon globally decreases, whatever the initial $NO_2$ concentration. This behaviour is quasi-linear for pressure below 9 MPa, but seems to level off above this value. Same results, expressed in terms of mass fraction of $CO_2$ inside the reactor, as done in Fig. 5, are more explicit because they clearly reflect effect of the amount of $CO_2$ in the system. Indeed, above the critical pressure of $CO_2$, an increase of the amount of $CO_2$ has a drastic effect on the pressure because of the high density of $CO_2$ as com-

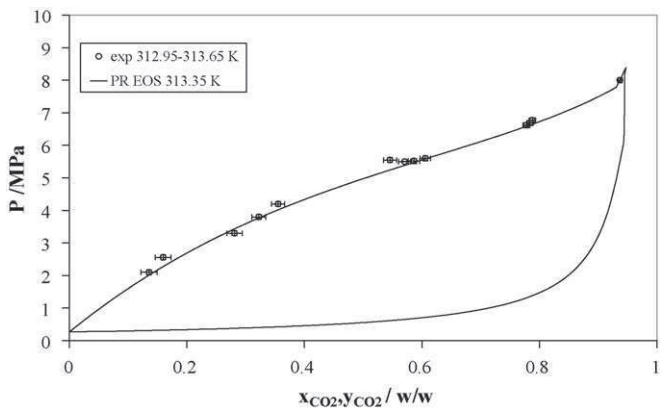


**Fig. 3.** *P–x, y* data of $CO_2$–$NO_2$ mixture around 313 K. Experimental (○) and calculated with Peng–Robinson equation of state with $k_{ij} = 0$ (—) (from [13]).

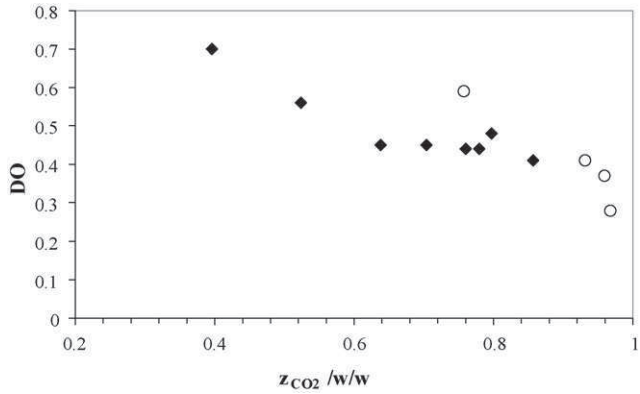


**Fig. 5.** Influence of mass fraction of $CO_2$ ($z_{CO_2}$) on the degree of oxidation of rayon in $CO_2/NO_2$ mixtures at 313 K during 4 h with $[NO_2] = 12$ g $L^{-1}$ (♦) and $[NO_2] = 132$ g $L^{-1}$ (○).

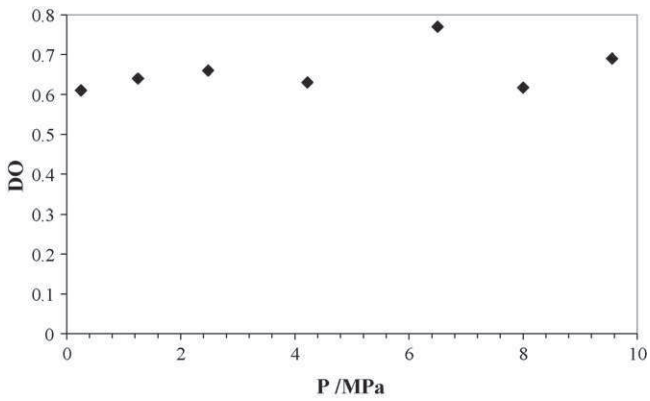


**Fig. 6.** Influence of pressure of $N_2$ on the degree of oxidation of rayon in $NO_2/N_2$ mixtures at 313 K, 4 h and $[NO_2] = 12\,g\,L^{-1}$.

pared to its gaseous density. Anyway, the more $CO_2$ is introduced in the system, the less effective is the oxidation.

In Figs. 4 and 5, we observe that a large increase in the $NO_2$ mass concentration does not lead to an important improvement of oxidation. Oxidation is mostly dependant on the fraction of $NO_2$ in the $NO_2/CO_2$ mixture, i.e., of the amount of $CO_2$. However, this behaviour is not reproduced if $CO_2$ is replaced by $N_2$ for example, as it can be seen in Fig. 6. In the case of $N_2$, it is observed that the degree of oxidation is not significantly influenced by the total pressure. At 313 K and $[NO_2] = 12\,g\,L^{-1}$, 4 h oxidation leads to DO values around 0.6, whatever the amount of $N_2$ in the system. We can conclude that in the case of $N_2$, oxidation of rayon does not depend on the quantity of $N_2$ introduced into the system.

Fig. 7 shows solid-state $^{13}C$ NMR spectra of non-oxidized and oxidized samples both in $N_2$ and $CO_2$ ($[NO_2] = 12\,g\,L^{-1}$ in both cases). Spectra of oxidized samples show a decrease of the peak corresponding to $-CH_2OH$ group and present the subsequent appearance of the peak corresponding to the carboxylic acid function, around 170 ppm. Moreover, we can deduce from these spectra that cellulose seems to maintain its partial crystallinity during oxidation in $CO_2/NO_2$ mixtures, while it looses it during oxidation in $N_2/NO_2$ mixtures. Indeed, with $CO_2$, no important modification in size or shapes of C1 and C4 peaks is observed, and, especially, shoulders are still present on oxidized samples. This is not the case for

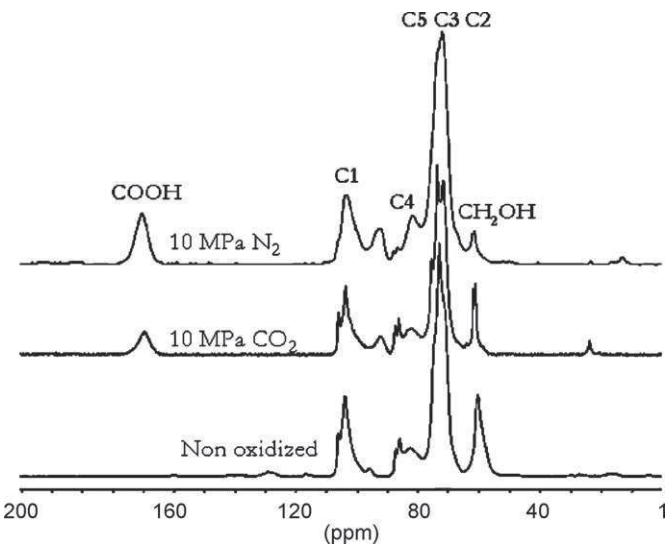


**Fig. 7.** $^{13}C$ NMR spectra of non-oxidized and oxidized samples of rayon obtained in $N_2$ and $CO_2$ at 313 K, 4 h and $[NO_2] = 12\,g\,L^{-1}$.

samples that have been oxidized in $N_2$ media. Another observation concerns the increase of a peak comprised between C1 and C4 peaks (around 90 ppm). A hypothesis could be a possible breakage of cellulose chains during oxidation, leading to degradation of cellulose, and/or to secondary reactions of hydroxyl groups (C2 and C3) leading to formation of hemiacetal groups. This peak is particularly important for samples that have been oxidized in $N_2/NO_2$ mixtures. Thus, these samples are both more oxidized and more degraded. Finally, these spectra are consistent with values of DO obtained by colorimetric titration, since they show that samples are oxidized but degraded also.

As a conclusion, from these results of oxidation in $CO_2$ and $N_2$, it appears that $CO_2$ seems to have an "inhibiting" effect on oxidation mechanism. Several hypotheses may be proposed concerning the role of $CO_2$ in this system.

First of all, although chemically inert towards oxidant, $CO_2$ may interact with $NO_2$ in such a way that reactivity may be reduced, i.e., through the modification of the $NO_2/N_2O_4$ equilibrium towards $N_2O_4$. $CO_2$ is considered as non-polar, especially as compared to polar solvents which were shown to have a dramatic negative effect on oxidation [14]. Nevertheless, the $CO_2$ molecule has a significant quadratic dipolar moment, and recent studies suggest that supercritical $CO_2$ can participate in conventional or non-conventional hydrogen-bonding interactions [19]. As a consequence, specific interactions may exist between $CO_2$ and $NO_2/N_2O_4$ or between $CO_2$ and cellulose. In this latter case, $CO_2$ may compete with adsorption of $NO_2$ onto cellulose.

A conventional approach would consider that carbon dioxide, as well as nitrogen, acts as a diluting agent in the system. The mass action law, applied to the dimerization equilibrium, indicates that, in the case of addition of a diluting agent at constant temperature and volume (thus inducing a pressure increase), the equilibrium reaction is not affected, if the mixture can be considered as a mixture of perfect gases. This is not the case for real gases for which the equilibrium constant must be expressed in terms of fugacities of the components in the mixture, instead than in terms of partial pressures. Modification of fugacities of $NO_2$ and $N_2O_4$ may occur with the addition of $CO_2$, under high pressure, depending upon interactions between the components. A possible explanation to our results, which have shown a decrease of oxidation when $CO_2$ is added to the system, could be a displacement of the $NO_2/N_2O_4$ chemical equilibrium in favour of the presumed inactive oxidant compound, $N_2O_4$. This is consistent with experimental results obtained in the case of $N_2$, where no effect of $N_2$ pressure has been detected. Indeed, it is well known that the thermodynamic behaviour of $N_2$ at high pressure, which is far from its critical temperature, is not very different from the behaviour of a perfect gas.

Another possible explanation of the negative effect of $CO_2$ could be its role as a drying agent for cellulose. Stilwell et al. [1] reported an optimum of moisture content for cellulose oxidation, in the range 5.5–11% (w/w), this optimum being related to an effective adsorption of nitrogen dioxide onto cellulose. Several hypotheses are reported upon physisorption and/or chemisorption of nitrogen dioxide onto cellulose, but moisture content is clearly evidenced to be a key parameter for oxidation. To interpret this result, the supermolecular structure of cellulose must be considered. Indeed, most of the reactions on cellulose fibres can be considered as non-homogeneous because the solid is indeed a kind of two-phase solid system, composed of less-ordered (amorphous) zones (mainly located as the surface of the fibrils or in the interlinking regions between crystallites) and well-ordered elementary crystallites. As a consequence, any reaction starts in the less-ordered regions (firstly at the surface and then in the regions interlinking elementary crystallites), and then proceeds into the crystallites [17]. However, a quasi-homogeneous oxidation can only be achieved when cellu-

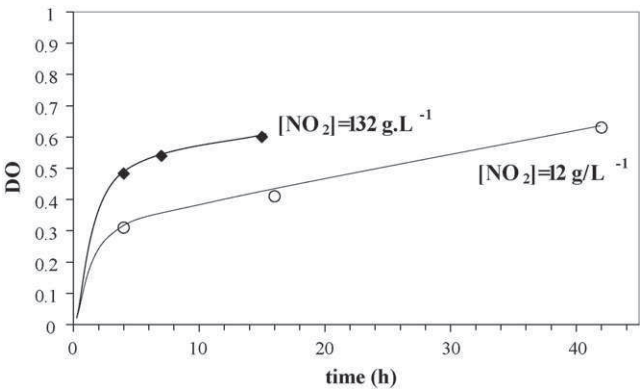


**Fig. 8.** Kinetics of oxidation of rayon in $CO_2/NO_2$ mixtures at 10 MPa and 313 K.

lose looses its crystalline structure, due for instance to a swelling pre-treatment. Water can be regarded as a swelling agent because it inserts between cellulose chains in amorphous zones, breaking hydrogen bonds between hydroxyl groups of cellulose, forming its own hydrogen bonds. This insertion induces an inter-crystalline swelling of the textile in amorphous zones, and can facilitate penetration of reactants in these zones, but not in crystalline zones of the cellulose [18]. Water alone is not sufficient to induce a complete swelling, including an intra-crystalline swelling of the cellulose. However, the primordial role of water had been already underlined for the treatment of cellulose with supercritical $CO_2$ in the domain of textile dyeing [20]. In this case, the drying effect of $CO_2$ is taken into account through controlled pre-addition of water in the system. In the context of cellulose oxidation, we may expect an analogous role for water, which allows the oxidant penetrating amorphous zones of cellulose and adsorbing onto the textile to react. Indeed, because solubility of water in $scCO_2$ is significant (around 1 g/L of $CO_2$ at 10 MPa and 313 K), a part of the water initially present onto cellulose dissolves in the supercritical phase. Influence of initial water content will be discussed later.

### 4.2. Kinetics of oxidation

Kinetics of oxidation in high-pressure $CO_2/NO_2$ mixtures has been studied, and, as previously, oxidations have been run at two reactant concentrations. Temperature of oxidation was set at 313 K and pressure was chosen to be 10 MPa.

Observation of Fig. 8 shows a two-step kinetics of oxidation, which appears to be rapid until around 4 h, and then undergoes a strong slow-down, whatever the initial oxidant concentration. Indeed, with a large excess of $NO_2$ in the reactor, oxidation degree is greater, but we always observe a levelling off after about 4 h of reaction. The second step is a slow reaction, and we observe that the maximum DO value of 1 was never reached in our experiments, even for high $[NO_2]$ or long reaction time.

Once again, in the case of oxidation in $CO_2/NO_2$ mixtures, we can observe from solid-state $^{13}C$ NMR analysis, that cellulose maintains its crystalline structure during oxidation. Fig. 9 shows spectra of oxidized celluloses obtained from experiments in $CO_2/NO_2$ mixtures, with $[NO_2] = 132\,g\,L^{-1}$. Indeed, no modification in size or shapes of C1 and C4 peaks is observed. Main modifications concern the decrease of the size of $CH_2OH$ peak and the subsequent appearing and increasing, as far as the reaction proceeds, of the COOH peak, so as the increase of the peak of secondary reactions (around 90 ppm).

Additional observations can be extracted from $^{13}C$ NMR spectra obtained by dissolving oxidized sample in NaOD solution. Spectra of dissolved oxidized samples (experiments with $[NO_2] = 132\,g\,L^{-1}$), shown in Fig. 10, present some slight signal displacement of C2, C3,

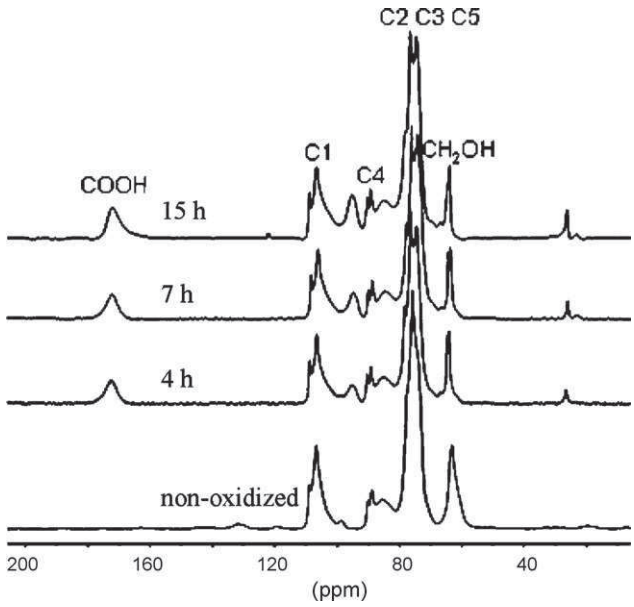


**Fig. 9.** $^{13}C$ NMR spectra of non-oxidized and oxidized samples of rayon obtained in $CO_2/NO_2$ mixtures.

C4 and C5 between carbons from anhydroglucose units and carbon from oxidized units. As long as the reaction proceeds, we observe an increase in the number of signals around 92 ppm, corresponding to a possible secondary reaction on cellulose chains. Peaks of residual iso-propanol from the washing step are also present in the spectra.

As mentioned above, in $CO_2/NO_2$ mixtures at 10 MPa and 313 K (see Fig. 8) the reaction proceeds as a two-step phenomenon. The first step corresponds presumably to oxidation of easily reachable zones of cellulose fibrils, and the second step to zones where primarily hydroxyl groups are less easily reachable. However, as observed on solid-state NMR spectra, these two types of zones do not correspond to amorphous and crystalline zones since these latter do not seems to be oxidized. As a conclusion, under these operating conditions, $NO_2$ dissolved in $CO_2$ seems to be unable to sufficiently swell the cellulose in order to destroy its crystalline structure to yield effective oxidation (cf. Fig. 9).

The influence of $CO_2$ as the solvent has been investigated once again by operating the reaction without any solvent. In this case only liquid $NO_2$ is injected in the air filled reactor at 313 K, and the quantity of injected $NO_2$ is adjusted in order to ensure that $NO_2$ vaporizes completely. This phenomenon can be observed experimentally because the recorded pressure inside the reactor increases all along the injection. After a given value of the injected quantity, the pressure inside the reactor is no longer increasing and stabilises, indicating the presence of a liquid–vapour equilibrium for $NO_2$. In this case, the total pressure remains constant because, whatever the quantity of $NO_2$, its partial pressure is equal to its vapour pressure for the experimental temperature. This limiting value could be predicted by simple calculations using the perfect gas law, but the computed theoretical value is indeed smaller (around $7\,g\,L^{-1}$) than the experimental observed one. This may be because a significant part of gaseous $NO_2$ adsorbs onto the cellulose sample present in the reactor and do not participate to the partial pressure of $NO_2$. Consequently the oxidation was done in low pressure gaseous $N_2/NO_2$ mixture at a $NO_2$ concentration of $12\,g\,L^{-1}$, corresponding to the observed maximum gaseous concentration which can be obtained at 313 K. We also performed oxidation in pressurized $N_2/NO_2$ mixtures at 7 MPa, 313 K and $12\,g\,L^{-1}$ of $NO_2$. For these two kinds of experiments, the same amount of cellulose is placed inside the reactor.

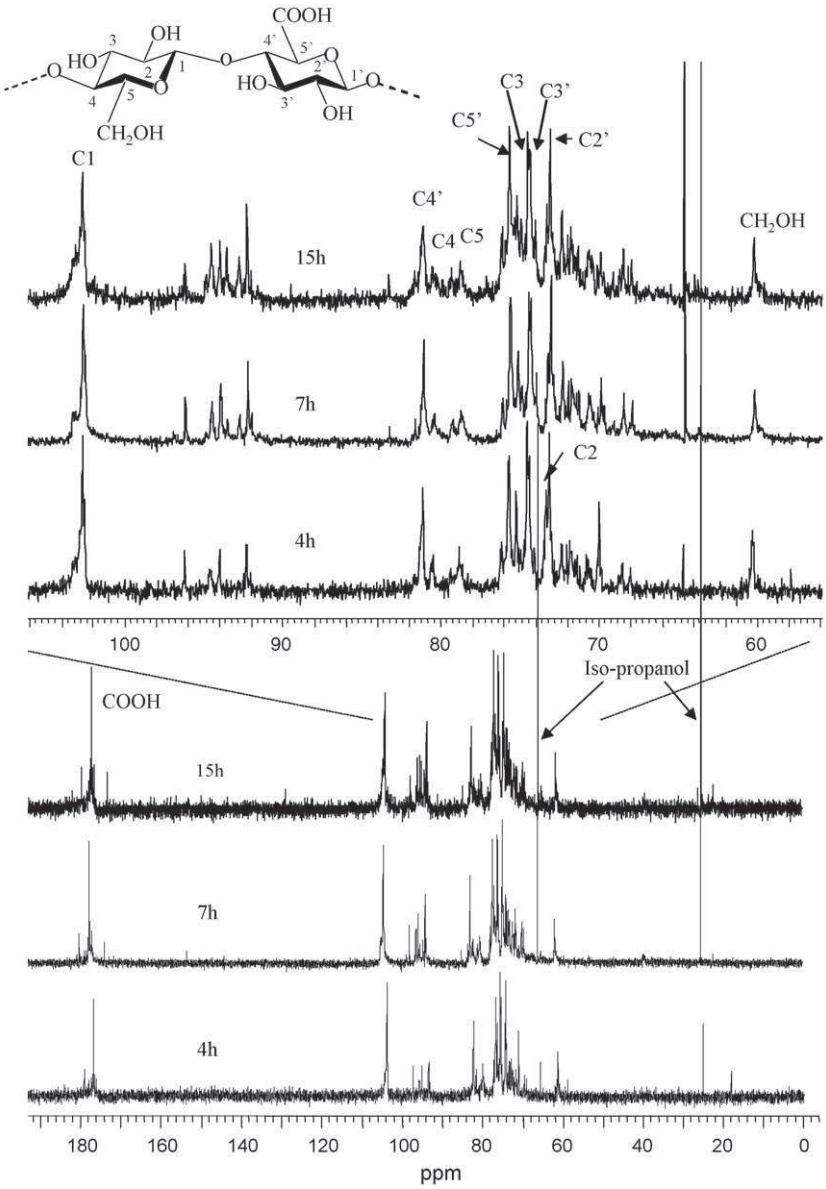


**Fig. 10.** $^{13}C$ NMR spectra of oxidized samples of rayon dissolved in $NaOD/D_2O$ solution.

Kinetic curves are presented in Fig. 11. These curves represent kinetics of oxidation ($[NO_2] = 12\,g\,L^{-1}$ at 313 K) in different reacting media. An obvious conclusion from these curves is that the presence and nature of the 'solvent' has a drastic influence on reaction of oxidation. Indeed, when oxidation is performed in low pressure gaseous $N_2/NO_2$ mixture, a 0.85 value of the degree of oxidation is reached after 4 h of oxidation. This is a higher value than in $CO_2/NO_2$ mixture or pressurized $N_2/NO_2$ mixture. However, operation in pressurized $N_2/NO_2$ mixture seems to have a lower impact on oxidation as compared to operation in pressurized $CO_2/NO_2$ mixture. Nevertheless, in all cases, we observe that reaction is more rapid during the four first hours of reaction. On $^{13}C$ NMR spectra of oxidized samples obtained in low pressure gaseous $N_2/NO_2$ mixture (Fig. 12), we notice the disappearance of shoulders on C1 and C4 peaks, (that we previously attributed to crystalline zones of cellulose). This tends to show that structure of cellulose is rapidly loosened in that case. Increase of "COOH" peak and decrease of "$CH_2OH$" one, evidence oxidation of primary hydroxyl groups of cellulose. Collateral reaction can be detected from the increase of the peak around 90 ppm. This peak is quite large for highly oxidized sample, such as the one obtained after 250 min, and thus such samples could not be used for application of biomedical device, because cellulose would not have satisfactory mechanical properties. Indeed, oxidation in low pressure gaseous $N_2/NO_2$ mixture allowed obtaining oxidized cellulose with higher DO (even completely oxidized at DO = 1) as compared to oxidation in $N_2/NO_2$ mixture. In low pressure gaseous $N_2/NO_2$ mixture, oxidation seems to be a quasi-homogeneous phenomenon, probably because of a rapid swelling and loosening of cellulose crystallinity, allowing oxidation of all zones of cellulose.

### 4.3. *Influence of initial moisture content of the cellulose*

As mentioned above, initial moisture content of cellulose is suspected to influence the oxidation mechanism. So an attempt has been made to evaluate its importance on oxidation results. Thermogravimetric analysis of raw cellulose has been done due to differential scanning calorimetry and to thermogravimetric analysis apparatus (DSC–TGA, TA Instruments Q600 SDT) in order to evaluate the initial moisture content of cellulose under normal

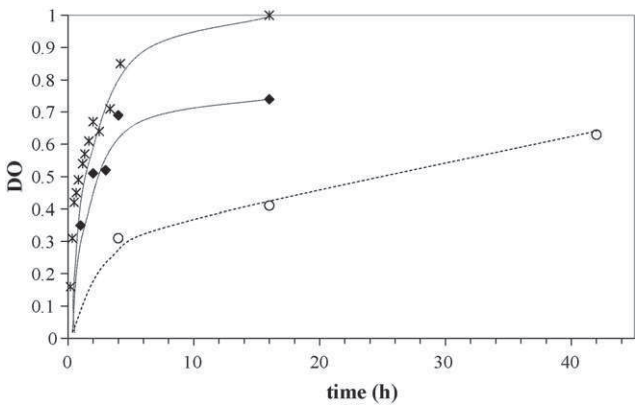


**Fig. 11.** Kinetics of oxidation at 313 K in low pressure gaseous $N_2/NO_2$ mixture (✱), $N_2/NO_2$ mixture at 10 MPa (♦), in $CO_2/NO_2$ mixture at 10 MPa (○).

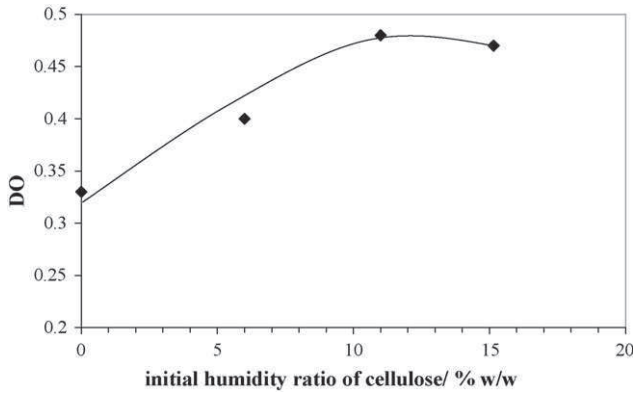


**Fig. 13.** Influence of initial moisture content of cellulose upon the degree of oxidation in $CO_2/NO_2$ mixture at 313 K, 10 MPa, 4 h and $[NO_2] = 132\,g\,L^{-1}$.

atmospheric conditions. This reveals that rayon at ambient naturally contains around 11% (w/w) of water.

To evaluate the importance of initial moisture of cellulose on oxidation with $NO_2$, we have modified the initial moisture content of the samples. Firstly, this was done by simply drying the cellulose during one night in an oven at 333 K before oxidation. Two other samples have been equilibrated with saturated salt solutions. In that case, non-oxidized cellulose samples have been maintained inside a closed vessel during several days in the atmosphere equilibrated with of a solution saturated with a given salt. A first solution has been saturated with NaOH, providing a water activity equal to 0.0698 at 293.15 K, and the second one has been saturated with NaCl, providing a water activity of equal 0.7542 at 293.15 K. In all cases, moisture content of cellulose before oxidation is evaluated by gravimetric method.

Oxidation of these equilibrated cellulose samples has been done in $CO_2/NO_2$ mixture at 10 MPa, 313.15 K, at $[NO_2] = 132\,g\,L^{-1}$ during 4 h, and results of oxidation are presented in Fig. 13. On this figure, we reported also degree of oxidation of cellulose obtained without further modification of initial humidity (initial moisture content equal to 11%).

Results presented in Fig. 13 clearly show that the degree of oxidation of cellulose is, in a certain range, directly related to its initial moisture content. This result is consistent with previously reported results indicating an optimum moisture content range for cellulose [14] between 5.5 and 11% (w/w) for oxidation, an excess of moisture in the cellulose leading to a predominant nitration phenomenon. As a matter of fact, water seems to have an important role in the mechanism of oxidation. This has been confirmed by solid-state $^{13}C$ NMR analysis of oxidized samples obtained from non-equilibrated cellulose, and from previously dried sample (in the oven), showing that the pre-dried sample is less oxidized and less-damaged than the non-equilibrated one, and as a consequence of the low degrees of oxidation, crystalline structure seems to be still present in both samples.

Moreover, oxidation having been realized in $CO_2$ at high pressure, it has to be considered that water could be stripped out because of the significant solubility of water in the $CO_2/NO_2$ mixture (solubility of water is around $0.74\,g\,L^{-1}$ in pure $CO_2$ at 313 K and 10 MPa). The lowering of the water content of cellulose, due to the presence of $CO_2$, is then likely to influence the reaction of oxidation. Surprisingly, no effect was observed when preliminary addition of water in the system is done, in a similar way which we mentioned for dyeing experiments.

Finally, from these results, we observe that optimum degree of oxidation was actually obtained from cellulose samples where no modification regarding water content had been done. Consequently, these results confirm the importance of water in the system, but they do not evidence the suspected negative effect of $CO_2$ on the distribution of water.

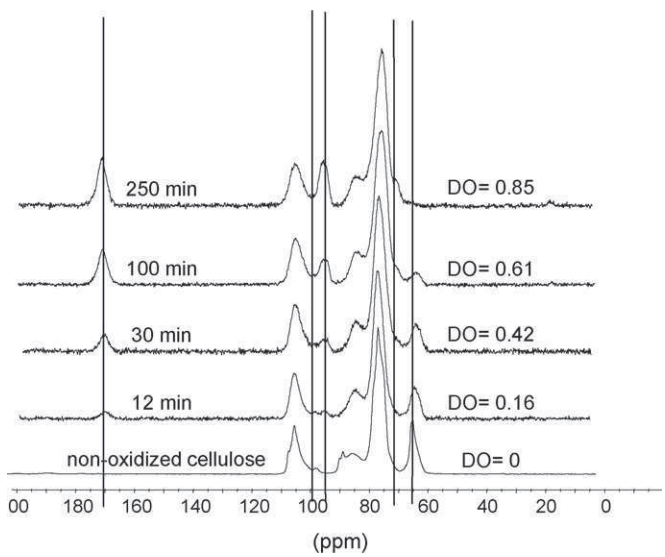


**Fig. 12.** $^{13}C$ NMR spectra of non-oxidized and oxidized samples of rayon obtained in low pressure gaseous $N_2/NO_2$ mixture.

### 4.4. Influence of the temperature

Temperature can influence several parameters of oxidation like reaction rate and selectivity, chemical equilibrium between $NO_2$ and $N_2O_4$, thermodynamic behaviour of the reacting mixture and adsorption of $NO_2$ onto cellulose.

Although literature upon oxidation of cellulose by nitrogen dioxide is very scarce and not recent, Ermolenko et al. [21] early observed that oxidation rate was surprisingly slower when the temperature was increased, in contradiction with a simple approach based on the conventional Arrhenius law. These authors attributed this phenomenon to the detrimental effect of the temperature on the necessary adsorption of $N_2O_4$ onto cellulose. Note that, according to these authors, the active oxidant specie was $N_2O_4$. Moreover, they argued that, considering $NO_2/N_2O_4$ equilibrium, an increase of temperature favoured dissociation into $NO_2$, which was in their sense detrimental for oxidation. However, the same research team later called this hypothesis into question by showing that the reactive specie was actually $NO_2$. This last result arose from the observation that an increase in the degree of oxidation of cellulose was obtained when non-polar solvents were used for oxidation with $NO_2$ [1], assuming that these non-polar sol-

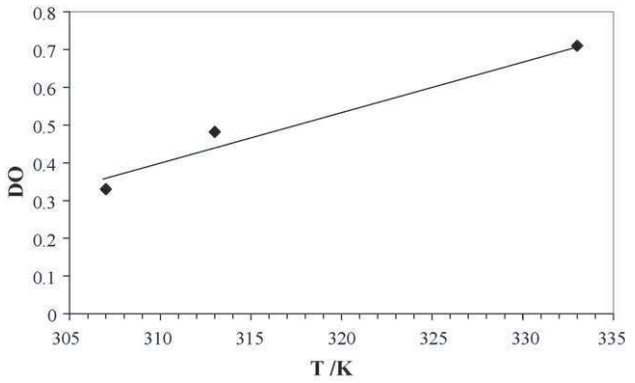


**Fig. 14.** Influence of the temperature on the degree of oxidation in $CO_2/NO_2$ mixture at 10 MPa, 4 h and $[NO_2] = 132\,g\,L^{-1}$.

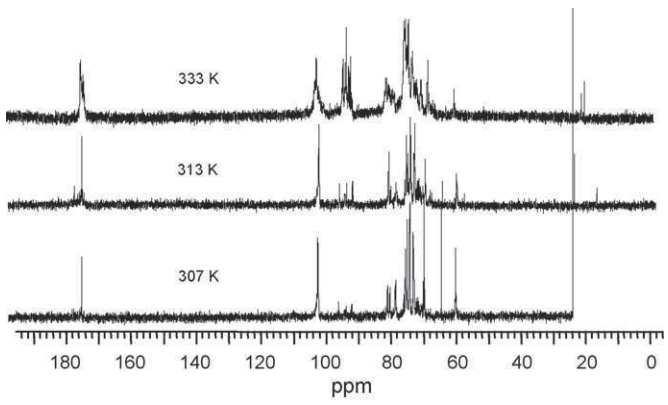


**Fig. 15.** $^{13}C$ NMR Spectra of oxidized samples of cellulose dissolved in $NaOD/D_2O$ solution. Oxidation in $CO_2/NO_2$ mixture at various temperatures at 10 MPa, during 4 h and $[NO_2] = 132\,g\,L^{-1}$.

vent favoured an increase in the degree of dissociation of $N_2O_4$ into $NO_2$.

In our case, we performed oxidation with temperature varying between 307 and 333 K at 10 MPa in $CO_2/NO_2$ mixture during 4 h and $[NO_2] = 132\,g\,L^{-1}$. This range of temperature has been chosen in such way that the reaction is run in a single-phase mixture. Results obtained in these conditions are presented in Fig. 14.

Our results clearly show an increase in the degree of oxidation of cellulose when temperature is increased, and, in this case, these results are consistent considering the Arrhenius law. Moreover, these results tend to show that $NO_2$ is the active oxidant since an increase of temperature is favourable to $NO_2$.

A more complete characterization of the oxidized samples, due to solid and liquid $^{13}C$ NMR spectroscopy, has revealed that increasing the temperature had lead to strongly damaged cellulose. Liquid-state $^{13}C$ NMR spectra of cellulose samples are shown in Fig. 15. At 333 K, we observe that the peak corresponding to –COOH group (around 178 ppm) is not only important but "multimodal" too, and this could be due to occurrence of a carboxylation reaction on carbon atoms other than C6. In addition, peaks attributed to breakage of cellulose chains (between 90 and 100 ppm) are important. These analyses are confirmed by tactile analysis where oxidized samples were found fragile and brittle. As a conclusion, temperature of oxidation has to be kept as low as possible in order to limit degradation of the product mechanical properties.

## 5. Conclusion

Results upon oxidation of cellulose with nitrogen dioxide in high-pressure $CO_2$ medium are reported here for the first time. Kinetics of oxidation, influence of pressure, temperature and initial moisture content of cellulose were investigated. Although high-pressure $CO_2$ matches all the requirements to be a green solvent for the reaction of oxidation with nitrogen dioxide, it indeed appears that its role is not as neutral as expected and the degree of oxidation of cellulose depends on the amount of $CO_2$ introduced in the system. Our first attempts to understand the role of $CO_2$ have not allowed clarifying completely its action on cellulose or/and the oxidant but it can be suspected that $CO_2$ interacts with $NO_2$, inhibiting the reactivity of this latter towards cellulose. The nature and the intensity of the interactions between $NO_2$ and $CO_2$ have still to be investigated, for example through spectroscopy measurements in parallel with *ab initio* calculations.

Nevertheless, in the specific process of production of oxidized cellulose for biomedical application, $CO_2$, although not the most efficient solvent for this system, can be regarded as an efficient "moderator" agent, allowing soft and homogeneous oxidation, which could be easily controlled by varying the pressure of the system. Furthermore, if we remind that the post-oxidative washing procedure is a very important step of the process, use of high-pressure $CO_2$ is a very good way to improve and simplify the whole process. At last, one of the major advantages of such a system is also related to the green aspect of using $CO_2$ as a solvent, especially for biomedical applications where biocompatibility of the product must be strictly ensured.

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