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## New insights into the mechanism of photodegradation of chitosan

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1 **New insights into the mechanism of photodegradation of chitosan**

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6  
7  
8 **Abstract**

9  
10 (150 words)

11 Chitosan films were subjected to accelerated artificial weathering at  $\lambda > 300$  nm and 60°C in  
12 the presence of O<sub>2</sub>. The resulting variations in the chemical structure were characterized by IR  
13 spectroscopy and UV-visible spectroscopy, and a photooxidation mechanism was proposed  
14 based on the identified oxidation photoproducts. The formation of gluconolactone derivatives  
15 leading to chain scissions was shown. In addition, low molecular weight photoproducts,  
16 which accounted for chitosan deacetylation, were detected. Furthermore, crosslinking  
17 reactions occurred, as revealed by gel fraction characterization. Variations in the mechanical  
18 and surface properties were characterized by AFM, and the reduction in macroscopic  
19 properties was correlated with the structural changes observed at the molecular scale by a  
20 multiscale approach.

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25 **Keywords:** chitosan; photodegradation; oxidation; mechanism

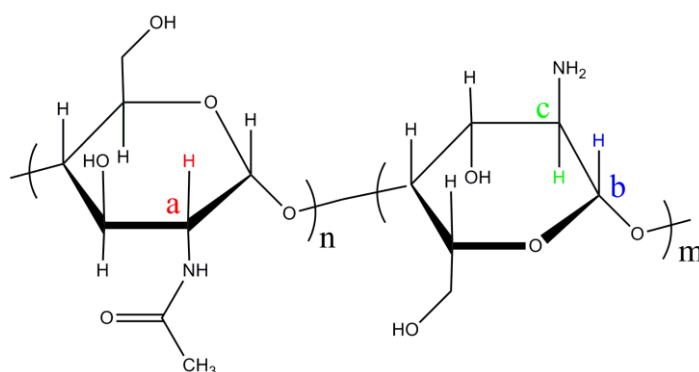
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32 **1. Introduction**

33 Chitosan is a natural polysaccharide composed of N-acetyl glucosamine and glucosamine  
34 units, two derivatives of glucose, that are linked together by glycosidic bonds (Synytsya,  
35 Grafova, Slepicka, Gedeon & Synytsya, 2012) (Scheme 1). The presence of hydroxyl and  
36 amino groups promotes H bonds between macromolecular chains. Thus, chitosan can  
37 crystallize into a hydrated form and an anhydrous form (Ogawa, Yui & Okuyama, 2004). Due  
38 to its solubility in water, biodegradability, biocompatibility and non-toxicity, many studies  
39 have focused on the use of chitosan for medical purposes such as soft tissue engineering  
40 (Tchemtchoua et al., 2011), bone tissue engineering (Nazeer, Yilgor, Yilgor, 2017) and drug  
41 release (Lien et al., 2012). Due to the same “green” characteristics, chitosan was studied to be  
42 employed as a scaffold for water treatment (Moczek & Nowakowska, 2007) and as packaging  
43 material in the food industry (Aider, 2010).

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Scheme 1: Chemical structure of chitosan

50 The dependence of the thermal degradation of chitosan on the degree of deacetylation was  
51 studied (Gamiz-Gonzalez et al., 2017), (Villar-Chavero, Dominguez, Alonso, Oliet &  
52 Rodriguez, 2018). Changes in the degree of deacetylation of chitosan were reported during  
53 thermal treatment above 200°C (Zawadzki & Kaczmarek, 2010). The effect of the degree of  
54 deacetylation on the radiation-induced degradation of chitosan (Taskn, Cansag, Sen, 2014)  
55 was also investigated and revealed that deacetylation is an important factor for controlling the  
56 degradation rate of chitosan. Degradation of chitosan under different stresses (microwave  
57 (Wasikiewicz & Yeates, 2013) and cavitation (Huang, Wu, Huang, Yang & Ren, 2013) has  
58 also been reported. Regarding photodegradation, a few papers report the use of UV irradiation  
59 as a sterilizing agent at a wavelength of 254 nm: chitosan (Sionkowska, Planecka,

60 Lewandowska, Kaczmarek & Szarszewska, 2013) (Andrady, Torikai, Kobatake, 1996)  
61 (Mucha & Pawlak, 2002); blends of chitosan/poly(vinyl pyrrolidone) (Sionkowska,  
62 Wisniewski, Skopinska, Vicini & Marsano, 2005) and of chitosan/poly(ethylene oxide)  
63 (Kowalonek, 2017); and chitosan modified with keratin (Sionkowska, Skopinska-  
64 Wisniewska, Planecka & Kozłowska, 2010), with silk fibroin (Sionkowska, Planecka,  
65 Lewandowska & Michalska, 2014) or with tannic acid (Sionkowska, Kaczmarek, Gnatowska  
66 & Kowalonek, 2015). The effect of UV degradation was mainly characterized on the thermal  
67 or mechanical properties but also on the surface of chitosan films (Sionkowska, 2006). UV  
68 irradiation at  $\lambda = 254$  nm was shown to provoke a decrease in molecular weight as a result of  
69 chain scissions. Changes in IR spectra were observed, such as the formation of bands  
70 attributed to carbonyl groups at  $1730\text{ cm}^{-1}$  and an increase of bands attributed to amide at  
71  $1655\text{ cm}^{-1}$  (C=O) and  $1560\text{ cm}^{-1}$  (N-H) accompanied with a decrease of the IR band at  $1590$   
72  $\text{cm}^{-1}$  corresponding to the amine group (Sionkowska et al; 2013) (Mucha & Pawlak, 2002).  
73 One study reports the  $\text{TiO}_2$  photocatalytic-oxidation of solid-state chitosan under fluorescent  
74 irradiation (Nawi, Jawad, Sabar & Wan Ngah, 2011); more recently, the effect of UV  
75 irradiation on the physicochemical characteristics of chitosan modified with nanoparticles of  
76 silver vanadate (Abdelghany, Ayaad & Aboelkheir, 2019) was reported. However, weathering  
77 of chitosan films under UV-light irradiation at long wavelengths ( $\lambda > 300$  nm) under  
78 accelerated conditions of natural outdoor weathering has received little attention  
79 (Sionkowska, Planecka, Kozłowska, Skopinska-Wisniewska, Los, 2011), and no mechanism  
80 of photooxidation has been proposed.

81 The aim of this work is to propose a photodegradation mechanism of chitosan in  
82 photooxidative conditions at  $\lambda > 300$  nm under accelerated artificial conditions (at  $60^\circ\text{C}$  in the  
83 presence of  $\text{O}_2$ ), which can explain the variations in material properties. The main objective of  
84 the paper is to provide evidence of the consequences of photochemical reactions on the  
85 macroscopic properties of chitosan and to qualitatively and quantitatively confirm the direct  
86 relationship between variations in the chemical structure and variations in the mechanical  
87 properties due to a multiscale approach of the degradation.

88

## 89 **2. Experimental**

### 90 **2.1. Materials**

91

92 Chitosan was purchased from Polysciences Inc. as a purified powder with an average  
93 molecular weight of 15 000 g.mol<sup>-1</sup> and a minimum degree of deacetylation of 85%. –Thus,  
94 the R group in Scheme 1 is defined as –H for 85 % of monomer and as acetamide group for  
95 15 % of monomer. The deacetylation degree of the chitosan film (after Soxhlet treatment) was  
96 monitored using IR spectra by following the formula given by Kasaai (Kasaai, 2008) for the  
97 ratio of absorbance at 1655 cm<sup>-1</sup> and 3450 cm<sup>-1</sup>, at 1320 cm<sup>-1</sup> and 1420 cm<sup>-1</sup>, and at 1560 cm<sup>-1</sup>  
98 and 2875 cm<sup>-1</sup>. The degree of deacetylation was then estimated to be 82 ± 4 %, and the NH<sub>2</sub>  
99 group predominated the acetamide group in this chitosan sample.

100 To prepare the films, chitosan powder was dissolved in acidified water (acetic acid 1%  
101 v/v, pH = 5) at a concentration of 10-20 mg.mL<sup>-1</sup> and stirred for 3 h at 60°C. Solutions were  
102 then deposited in Petri dishes, and self-standing films were obtained after drying in ambient  
103 air for a few hours. Films of thickness in the range 25 – 50 µm or 150 µm were obtained for  
104 the different subsequent analyses. Purification of the films was performed by Soxhlet  
105 extraction in MeOH for 24 h. The films were then dried at 60°C under vacuum for 14 hours.

106

## 107 **2.2. Irradiation**

108 UV-visible light irradiation ( $\lambda > 300$  nm) of thin films was performed in a SEPAP  
109 12/24 unit, which was designed for studying polymer photodegradation during artificial  
110 ageing with medium-accelerated conditions (Philippart, Sinturel & Gardette, 1997). The  
111 chamber consisted of a square reactor equipped with four medium-pressure mercury lamps  
112 situated vertically at each corner of the chamber. Wavelengths below 300 nm were filtered by  
113 the borosilicate envelope of the lamps, acting as a UV filter below 300 nm (see Fig S1). In the  
114 centre of the chamber, the samples were fixed on a 13 cm-diameter rotating carousel that can  
115 hold 24 samples. The temperature in the chamber of SEPAP 12/24 is controlled at the surface  
116 of a polymer film through a Pt 1000 probe and continually regulated by air circulation. The  
117 temperature at the surface of the samples was set to 60°C.

118

## 119 **2.3. Characterization Techniques**

### 120 *Spectroscopic analysis*

121 Changes in the UV-visible spectra were monitored with a Shimadzu 2600 plus  
122 spectrophotometer equipped with an integrating sphere. IR spectra were recorded in  
123 transmission mode with a Nicolet 6700 Fourier transform infrared (FTIR) spectrophotometer  
124 operated with OMNIC software. The spectra were obtained with 32 scan summations at 4 cm<sup>-1</sup>

125 <sup>1</sup> resolution. IR analysis of chitosan powder was performed by mixing 2 mg of this powder  
126 with 200 mg of KBr.

127

### 128 *Chemical derivatization reaction*

129 Most of the oxidation products were identified by performing chemical derivatization  
130 treatments that selectively convert oxidation products into chemical groups with different IR  
131 characteristics (Wilhelm & Gardette, 1994). Ammonia (NH<sub>3</sub>) reacts with carboxylic acids to  
132 generate carboxylate ions and with esters to generate amides. NH<sub>3</sub> treatment was performed at  
133 room temperature in simple flow reactors that could be sealed off to allow the reaction to  
134 proceed. 2,4-Dinitrophenylhydrazine (2,4 DNPH) was used to identify aldehydes and ketones.  
135 The formation of 2,4-dinitrophenylhydrazone can be detected by two IR absorption bands at  
136 1595 cm<sup>-1</sup> and 1620 cm<sup>-1</sup> and a very intense UV-Vis absorption band at 365 nm.

137

### 138 *Solid Phase Micro Extraction (SPME)*

139 Films were irradiated in sealed vials to collect the volatile photodegradation products.  
140 SPME analysis was performed using Carboxen–PDMS fibres (75 mm) from Supelco  
141 (Bellefonte, PA, USA) with an extraction time of 5 min at 60°C, and gas chromatography-  
142 mass spectrometry (GC-MS) was performed with a PEG Supelcowax® 10 column (30 m x  
143 0.25 mm x 0.25 mm) from Supelco.

144

### 145 *Gel fraction*

146 Crosslinking of polymers was evaluated by gravimetric measurements of the gel fraction (Gf).  
147 Irradiated films (approximately 5 mg) were immersed in acidified water (acetic acid 1% v/v)  
148 (5 mL) for 48 h. The solutions were then filtered using a Büchner funnel. The insoluble  
149 fractions were dried at 60°C and then weighed. The gel fraction is defined by equation 1:

$$Gf = \frac{M_{\text{insoluble}}}{M_{\text{initial}}} \quad (1)$$

150

### 151 *Atomic force microscopy*

152 AFM measurements were performed in tapping mode using an atomic force microscope  
153 (AFM) Bruker Multimode driven by a Nanoscope V control unit. The surface topographical  
154 images were analysed by the Nanoscope 7.20 software. Images were obtained at three  
155 different locations, and the average roughness was determined from these images at four

156 different locations for each image. The tip was an RTESPA-300 cantilever (Bruker) with a  
157 spring constant of approximately 40 N/m and a radius of curvature of approximately 8 nm.

158

159

#### 160 *AFM nano-indentation*

161 The force–distance curves were measured at a constant deflection (or constant load). Nano-  
162 indentations were performed using a diamond tip (64.05 kHz) with a spring constant of 234.5  
163 N/m and a curvature radius of 25 nm. Three tests of nano-indentation were performed on  
164 different areas of the polymer film. Each test corresponded to nine indentations; thus, 3×9  
165 force curves were used with a load that varied from 1 to 5 μN. The force-displacement curves  
166 obtained via AFM nano-indentation measurements were analysed using the model proposed  
167 by Oliver and Pharr (Oliver & Pharr, 1992) (procedure (O&P)).

168

#### 169 *AFM nanoscale thermal analysis*

170 Variations in the thermal properties of the films were monitored at the surface using an  
171 AFM nanoscale thermal analysis module (Vita). The probe was heated from 20°C to 300°C at  
172 a rate of 5 °C/s, and each measurement was performed 3 times to ensure good reproducibility.  
173 The Vita probes can also be used to obtain AFM images of the surface (Collin et al., 2012).

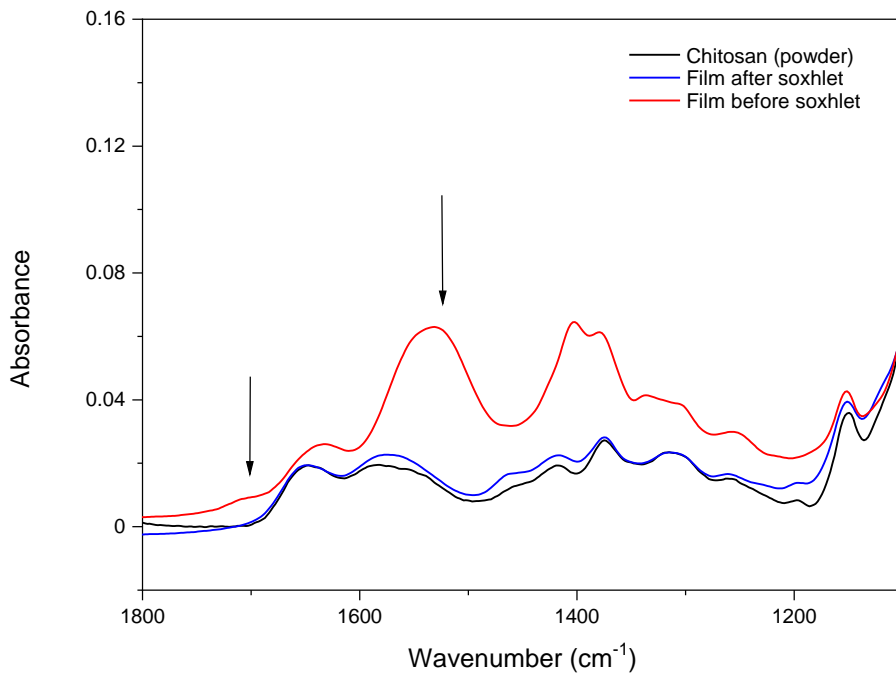
174

### 175 **3. Results and discussion**

#### 176 **3.1. Characterization of chitosan films before irradiation**

177 The main spectral features of chitosan and assignment of the IR bands according to the  
178 literature (Mucha & Pawlak (2002)(Valentin, Bonelli, Garrone, Di Renz &, Quignard, 2007)  
179 are the OH and NH stretching bands at 3435 cm<sup>-1</sup> and between 3300-3150 cm<sup>-1</sup>, respectively,  
180 the C=O stretching band at 1655 cm<sup>-1</sup>, the NH<sub>2</sub> scissoring and NH deformation bands at 1590  
181 cm<sup>-1</sup> and 1560 cm<sup>-1</sup> and the C-O-C stretching band attributed to the osidic bond or pyranose  
182 ring at 1150 cm<sup>-1</sup>. Figure 1 compares the carbonyl domain in the IR spectrum of the  
183 commercial chitosan powder pressed into a KBr pellet to the same domain in the IR spectra of  
184 the pristine films before and after Soxhlet extraction in MeOH for 24 h.

185



186  
 187 Figure 1: IR spectra of chitosan films ( $e = 25 \mu\text{m}$ ) before and after 24 h Soxhlet extraction in  
 188 MeOH and the chitosan powder in KBr pellets.

189  
 190 The IR spectra shown in Figure 1 indicate that Soxhlet extraction of the chitosan film  
 191 in MeOH provokes the disappearance of three IR bands at  $1710 \text{ cm}^{-1}$ ,  $1550 \text{ cm}^{-1}$  and  $1410 \text{ cm}^{-1}$ .  
 192 This finding can be attributed to the removal of acetic acid and acetate ions. These two  
 193 products are retained from the process that was used to obtain the films and involved  
 194 preliminary dissolution in acetic acid-containing solution, which could therefore be trapped  
 195 within the films at low concentrations even after drying. Acetate ammonium groups are likely  
 196 generated as a result from the reaction of acetic acid with the amine functions of chitosan  
 197 (Rinaudo, Pavlov & Desbrieres, 1999). The IR spectrum of chitosan film after Soxhlet  
 198 extraction is similar to the spectrum of chitosan powder that was pressed into KBr pellets. The  
 199 experiments reported in this paper were performed for chitosan films that were purified by  
 200 Soxhlet extraction in MeOH for 24 h.

201  
 202 **3.2. Photooxidation of chitosan**

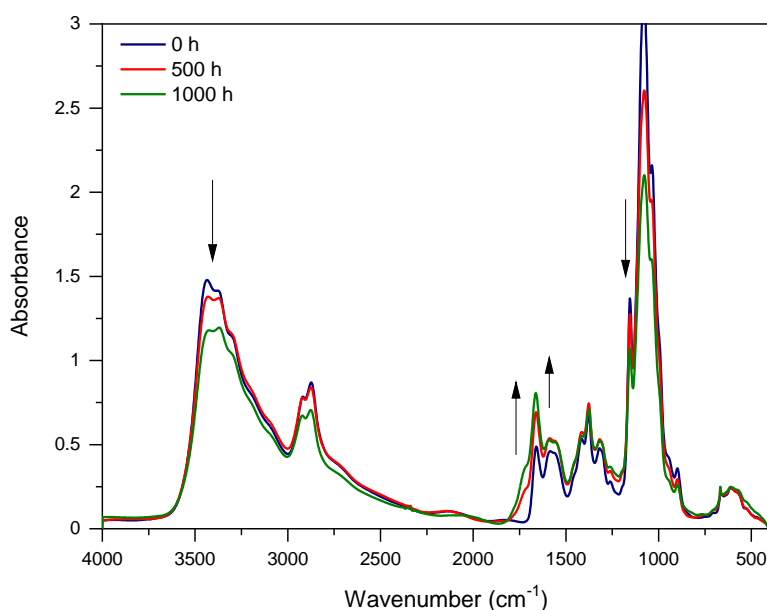
203 **3.2.1. Analysis of chemical variations by IR spectroscopy**

204 Irradiation in accelerated artificial conditions at  $\lambda > 300 \text{ nm}$  in the presence of  $\text{O}_2$   
 205 (photooxidation) resulted in noticeable modifications to the IR spectra (Figure 2a). Irradiation  
 206 provoked a marked decrease in the absorption in the domain  $3500 - 3300 \text{ cm}^{-1}$ , which

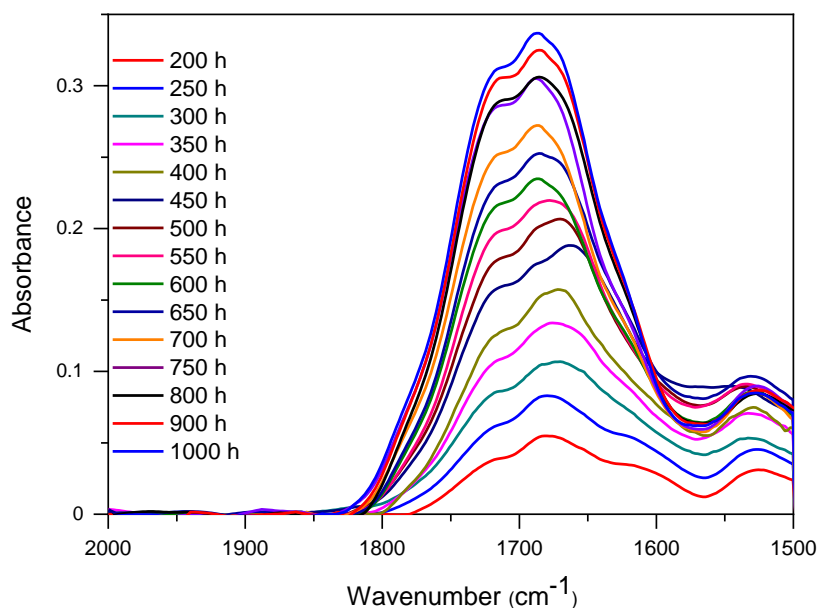


207 indicates a loss of constitutive water or water generated from the dissolution process and/or  
208 breaking of hydrogen bonds present in the chitosan film (Ogawa et al., 2004). A decrease in  
209 the IR absorption band of C-O-C bonds at  $1150\text{ cm}^{-1}$  was also observed, which could reflect  
210 chain scission reactions and/or ring opening of glucosamine and N-acetyl glucosamine. To  
211 visualize the changes in the IR spectra in the carbonyl domain, the spectra were subtracted  
212 from those of the unexposed film in the domain  $2000\text{-}1500\text{ cm}^{-1}$  and are presented in Figure  
213 2b. A relatively broad band was observed, with a maximum at  $1675\text{ cm}^{-1}$  and shoulders at  
214  $1730\text{ cm}^{-1}$  and  $1780\text{ cm}^{-1}$ . This result is similar to the  $254\text{ nm}$  irradiation-induced spectral  
215 variations reported in the literature, which proposed chain scission reactions and cleavage of  
216 glycosidic bonds (Andrady et al. 1996) (Mucha & Pawlak, 2002) (Wasikiewicz, Yoshii,  
217 Nagasawa, Wach & Mitomo, 2005) (Wang, Huang & Wang, 2005).

218 a)  
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Figure 2: a) IR spectra of a chitosan film ( $e = 25 \mu\text{m}$ ) during photooxidation  
b) Subtracted IR spectra in the carbonyl domain

233 The decrease in absorbance at  $1150 \text{ cm}^{-1}$  (Figure 2) reflects the disappearance of the  
234 pyranose ring/acetal bond and can be correlated with the accumulation of oxidation products  
235 at  $1730 \text{ cm}^{-1}$ . As proposed by several authors (Mucha & Pawlak, 2002) (Sionkowska, 2011),  
236 the accumulation of oxidation products at  $1730 \text{ cm}^{-1}$  could be related to the formation of an  
237 ester function and simultaneous disappearance of the  $\beta$ -1,4 osidic bond. Taking into account  
238 the correlation between the increase in absorbance at  $1730 \text{ cm}^{-1}$  and the decrease at  $1150 \text{ cm}^{-1}$ ,  
239 which reflects the disappearance of the pyranose ring/acetal bond (hydrogen abstraction on  
240 carbon atom (b) in Scheme 2), this ester bond formation would be consistent with the  
241 generation of gluconolactone.

242 The IR band at  $1675 \text{ cm}^{-1}$  can be attributed to the vibration  $\nu\text{C}=\text{O}$  of a carbonyl function  
243 of amide I. Considering the chemical structure of chitosan, the formation of a primary amide  
244 can also be explained by a mechanism involving oxidation in the  $\alpha$ - position of the amine  
245 function (hydrogen abstraction on carbon atom (c)). This mechanism is represented in  
246 Scheme 2 and discussed at a later point in the manuscript.

247

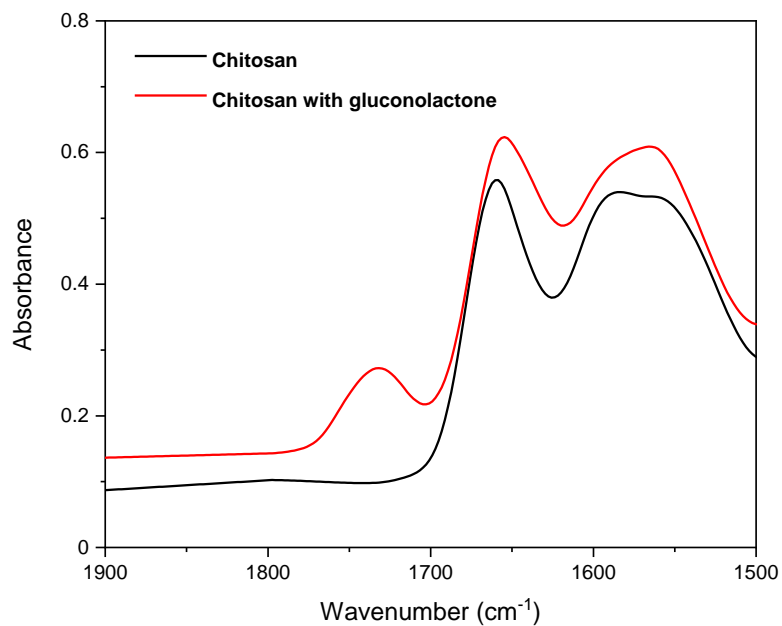
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### 249 **3.2.2. Derivatization treatment**

250 Chemical derivatization treatments with  $\text{NH}_3$  were performed to identify the  
251 degradation products observed by IR analysis at  $1730\text{ cm}^{-1}$ .  $\text{NH}_3$  reacts with carboxylic acids  
252 to generate carboxylate ions and reacts with esters, lactones and anhydrides to generate  
253 amides. First, it was verified that no reaction with  $\text{NH}_3$  occurred with pristine chitosan.  $\text{NH}_3$   
254 treatment of photooxidized films provoked a decrease in the broad absorption at  $1730\text{ cm}^{-1}$   
255 and the formation of two weak bands at  $1640\text{ cm}^{-1}$  and  $1595\text{ cm}^{-1}$  (see Fig. S2). These bands  
256 indicate the formation of amides ( $1640\text{ cm}^{-1}$ ) and ammonium carboxylates ( $1595\text{ cm}^{-1}$ ).  
257 Amides were formed from the reaction of  $\text{NH}_3$  with esters or anhydride, and ammonium  
258 carboxylates resulted from the reaction of  $\text{NH}_3$  with carboxylic acid or anhydrides.  $\text{NH}_3$   
259 treatment allows the identification of esters and carboxylic acids as oxidation photoproducts.

260 Ester formation was suggested by several authors (Sionkowska et al., 2013), (Andrady  
261 et al., 1996) (Mucha & Pawlak, 2002) (Wasikiewicz et al., 2005), but to the best of our  
262 knowledge, direct evidence of gluconolactone formation has not yet been reported. For  
263 comparison and to confirm the presence of gluconolactone in the photooxidized chitosan film,  
264 molecular gluconolactone was blended with pristine chitosan, and the blend was analysed by  
265 IR spectroscopy. The IR spectrum is shown in Figure 3a, which clearly confirms the existence  
266 of the absorption band at  $1730\text{ cm}^{-1}$  attributed to the  $\nu(\text{C}=\text{O})$  vibration band of  
267 gluconolactone.

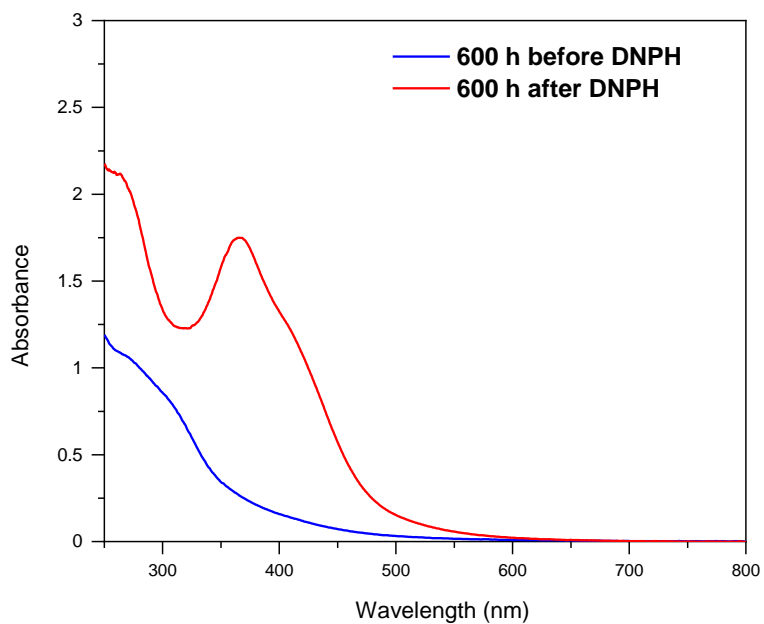
268 a)



269

270 Figure 3a: IR spectrum of a film ( $e=25\ \mu\text{m}$ ) of chitosan blended with gluconolactone.

271 b)



272

273 Figure 3b: UV-visible spectra of a 600 h photooxidized chitosan film before and after  
274 treatment with 2,4-DNPH.

275

276 To complete the identification of the oxidation photoproducts, chemical treatment  
277 using 2,4-DNPH was performed on photooxidized chitosan films. The UV-visible spectra of  
278 photooxidized chitosan films after 2,4-DNPH treatment (Figure 3b) show a broad band with a  
279 maximum at 365 nm corresponding to the phenylhydrazone derivative. This result indicates  
280 that aldehydes and/or ketones were formed by the photooxidation of chitosan.

281

282

### 283 ***3.2.3. Analysis of volatile products***

284 Photooxidation of polymers is known to be responsible for the formation of low molecular  
285 weight products that are likely to migrate from the solid polymer samples and are not detected  
286 by the analysis of the films by the spectroscopic methods reported above (Gaume, Wong-  
287 Wah-Chung, Rivaton, Therias, Gardette, 2011). To identify the volatile degradation products,  
288 SPME-GC/MS was performed. Formamide and acetamide were detected as the main  
289 degradation products (see Figure S3). It is also important to note the presence of acetic acid,  
290 which was used to solubilize chitosan. As formamide and acetamide molecules contain  
291 nitrogen atoms in their structure and therefore are not formed by photooxidation of the  
292 cellulose backbone, these products can be attributed to the degradation of the pending groups  
293 of chitosan. As a consequence, the deacetylation degree of the chitosan film is changed.

294 The formation of volatile products (such as acetamide) and the accumulation of  
295 ketone/aldehyde groups can be connected to the cleavage of pending groups on carbon atoms  
296 (a), following the mechanism given in Scheme 2.

297

### 298 ***3.2.4. Characterization of chain scission/crosslinking***

#### 299 *Gel fraction*

300 To provide evidence of the variations in polymer architecture, the insoluble/gel  
301 fractions of photooxidized chitosan films were measured for irradiation times as long as 400  
302 hours. The gel fraction of photooxidized chitosan films increased progressively from 0 (no  
303 insoluble fraction before irradiation) to 96 % after 400 hours of irradiation, which indicates  
304 that the polymer becomes almost insoluble. This high gel fraction value can be attributed to  
305 the formation of a three-dimensional network from crosslinking during the irradiation of  
306 chitosan.

307

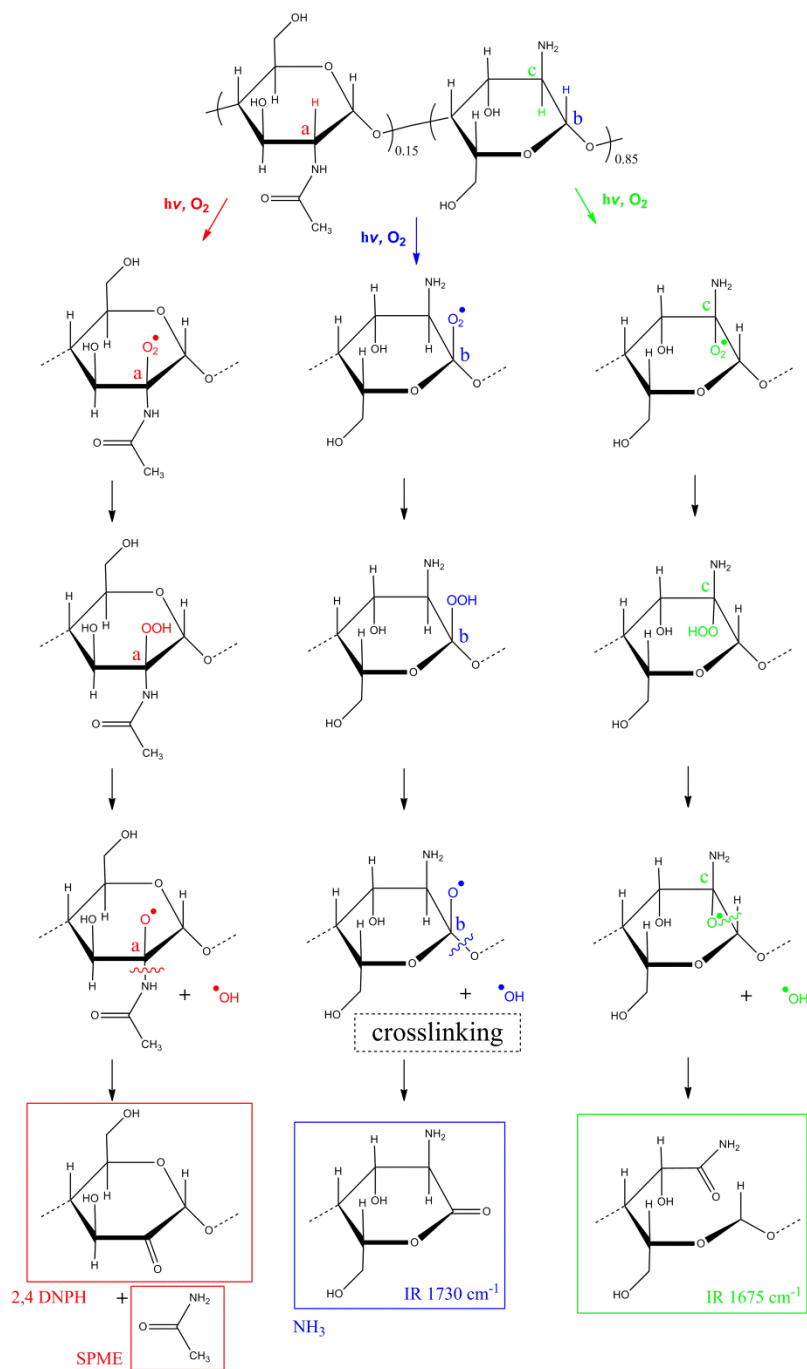
### 308 **3.3 Degradation mechanism**

309 A photooxidation mechanism that explains the formation of the identified  
310 photoproducts was proposed (Scheme 2). This mechanism is based on a radical chain reaction  
311 with initial hydrogen abstraction on the carbon atoms noted (a), (b) and (c) by radicals from  
312 chromophoric impurities, or defects that in commercial chitosan could include proteins and  
313 metals. The photodissociation of acetamide groups attached to glucosamine unit, (broad band  
314 from 250 till 350 nm) could also be a potential source of radicals able to initiate the chain  
315 reaction. However, despite the many articles published in the literature, no real proof was  
316 brought that this reaction occurs on exposure to wavelengths above 300 nm. (Rideal &  
317 Mitchell, 1937). However, no evidence was obtained that this reaction would occur (Sharkey  
318 & Mochel, 1959). (Moore, 1963). Radical Oxygenated Species such as  $\text{HOO}\cdot$ ,  $\text{HO}\cdot$ ,  $\text{R-OO}\cdot$   
319 can be formed and initiate the oxidation by hydrogen abstraction on the polymer chains. The  
320 initiation processes in polymer degradation have been reviewed many times in the literature  
321 (see for example Scott, 1995). This is followed by the addition of dioxygen, the formation of  
322 hydroperoxides and the decomposition of hydroperoxides to generate macroalkoxy radicals  
323 (Scheme 2). Hydrogen abstraction from the carbon atom (a) generates the macroalkoxy  
324 radical A, which may decompose to generate gluconolactone (its IR band at  $1730\text{ cm}^{-1}$  was  
325 identified by  $\text{NH}_3$  treatment). This reaction generates lactones such as gluconolactone and is  
326 accompanied by chain scissions. Hydrogen abstraction from carbon (c) leads to the formation  
327 of radical C, which may decompose to form amide groups via ring-opening (IR band at  $1675$   
328  $\text{cm}^{-1}$ ).

329 An important issue is the role played by the temperature in the photooxidation performed at  
330  $60^\circ\text{C}$ . Thermal ageing at  $60^\circ\text{C}$  in a ventilated oven in absence of light (dark control) during  
331 the same time (up to 420 h) was then performed. Comparing the IR spectra after exposure for  
332 420 h to thermooxidation at  $60^\circ\text{C}$  or 400 h to photooxidation at  $\lambda > 300\text{ nm}$ - $60^\circ\text{C}$  (see Fig.  
333 S4) clearly indicates that the IR spectra was only slightly modified after thermooxidation for  
334 420 h, whereas exposure to light at  $\lambda > 300\text{ nm}$ - $60^\circ\text{C}$  resulted in dramatic modification of the  
335 infrared spectra with the formation of the photoproducts which formation has been described  
336 above (part 3.2.1). This result clearly indicates that in the current ageing conditions, the  
337 oxidation mainly results from irradiation.

338

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342

Scheme 2: Simplified photooxidation mechanism of chitosan

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344

### 345 3.4 Consequences of photooxidation on the properties of chitosan

#### 346 3.4.1 UV-visible analysis

347 Analysis of a chitosan film by UV-visible spectroscopy was carried out during  
 348 photooxidation (Figure 4).

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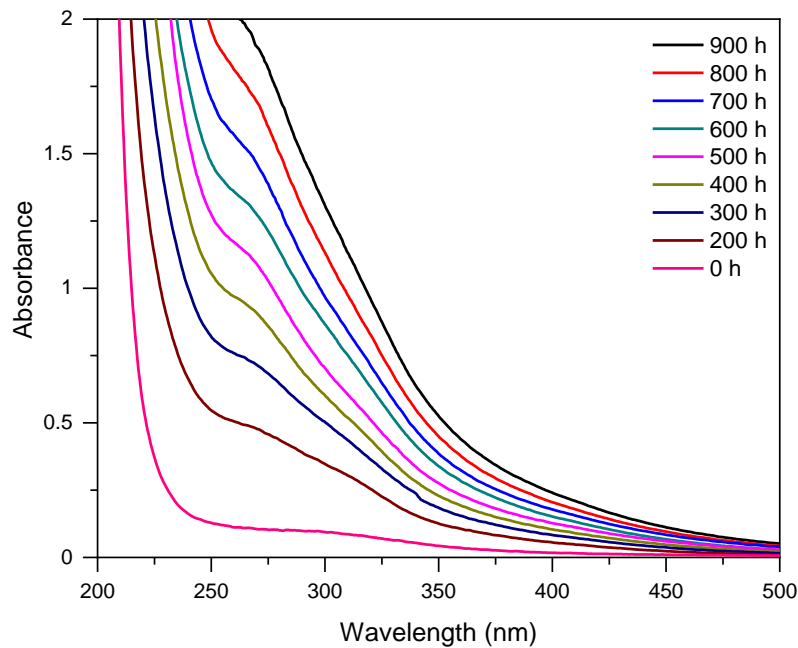


Figure 4: UV-Visible spectra of chitosan films during photooxidation ( $e = 25 \mu\text{m}$ )

The absorption front of the UV-vis spectra progressively shifted towards longer wavelengths during photooxidation, leading to yellowing of the chitosan film, and a shoulder with an absorption maximum could be observed at 270 nm. This feature was attributed to the formation of carbonyl groups and the cleavage of glycosidic bonds by several authors (Synytsya et al., 2012) (Wang et al., 2005).

### 3.4.2 Mechanical properties

Variations in the mechanical properties at the surface of chitosan films during photooxidation were assessed by elasticity measurements at the nanoscale (Table 1). To confirm the behaviour at the nanoscale, the polymer surface was analysed by AFM nano-indentation measurements. The loading/unloading indentation curves were measured for two irradiation times (see Figure S5).

The distance or displacement to reach the maximum force decreased after 200 h of irradiation. This is characteristic of an increase in the surface nano-hardness and Young's modulus. The increase in the nano-hardness of chitosan observed under irradiation can be correlated to crosslinking reactions.



371 One can also notice a small negative deflection after 200 h of irradiation that is  
372 consistent with the increase in the adhesion work. The increase in the adhesion work with the  
373 exposure time has already been connected (Bussiere, Rivaton, Therias & Gardette, 2012) to  
374 the accumulation of polar species at the surface of the sample and thus to the formation of  
375 oxidation products. In addition, the nano-hardness of the polymer sample can be calculated  
376 from these loading-unloading curves.

377

### 378 *AFM nanoscale thermal analysis*

379

380 In addition, variation in the glass transition temperature resulting from UV light  
381 exposure were monitored by AFM thermal analysis (Vita). The deflection-temperature curves  
382 (Figure S6) after 100 h and 300 h of irradiation were compared with the deflection-  
383 temperature curve before exposure. Nano- $T_g$  values are reported in Table 1. After irradiation,  
384 the deflection shifts to higher temperatures. As  $T_g$  is related to the deflection, this result  
385 indicates that  $T_g$  increased with irradiation time. The Flory theory (Fox & Flory, 1950) relates  
386  $T_g$  to the molecular weight, reflecting crosslinking reactions.

387

388 Table 1: Evolution of nano-hardness (nano-Hv) and nano- $T_g$  as a function of  
389 irradiation time.

Irradiation time	Nano-Hv (at 160 nm)	Nano- $T_g$ (K)
0 h	$90 \pm 8$ MPa	$430 \pm 5$
100 h	$150 \pm 15$ MPa	$465 \pm 5$
200 h	$230 \pm 25$ MPa	$495 \pm 10$
300 h	$280 \pm 25$ MPa	$510 \pm 5$
400 h	$330 \pm 45$ MPa	$530 \pm 15$

390

391 The increase in nano-hardness and nano- $T_g$  are consistent with crosslinking reactions  
392 leading to higher molecular weights, with a marked increase in the gel fraction (chitosan that  
393 became almost insoluble after 400 h of irradiation at  $\lambda > 300$  nm in the presence of oxygen).

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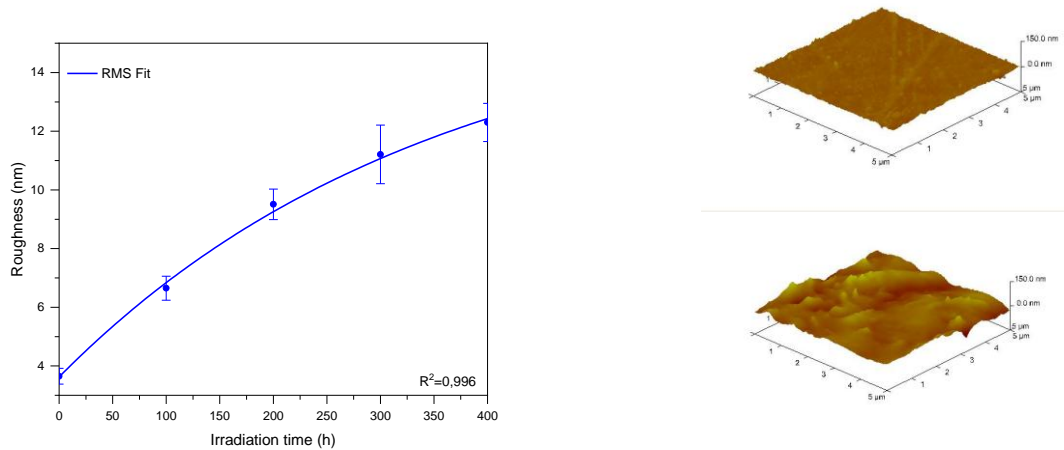
395

### 396 *3.4.3 Surface properties*

397 Roughness values (RMS) of a chitosan film surface were calculated before and after  
398 irradiation. Modifications of the roughness (RMS) displayed in Figure 5 show that RMS  
399 increased during irradiation.

400

401



402 Figure 5: a) Change in roughness (■) versus irradiation time

403 (line is obtained by an exponential fit).

404 b) Image of the chitosan film surface before and after 300 hours of irradiation.

405

406 As previously reported, these variations arise from the formation of oxidation products  
407 of the polymer sample and of volatile products (Berthumeyrie, Collin, Bussiere & Therias,  
408 2014).

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### 411 3.5 Multiscale correlation

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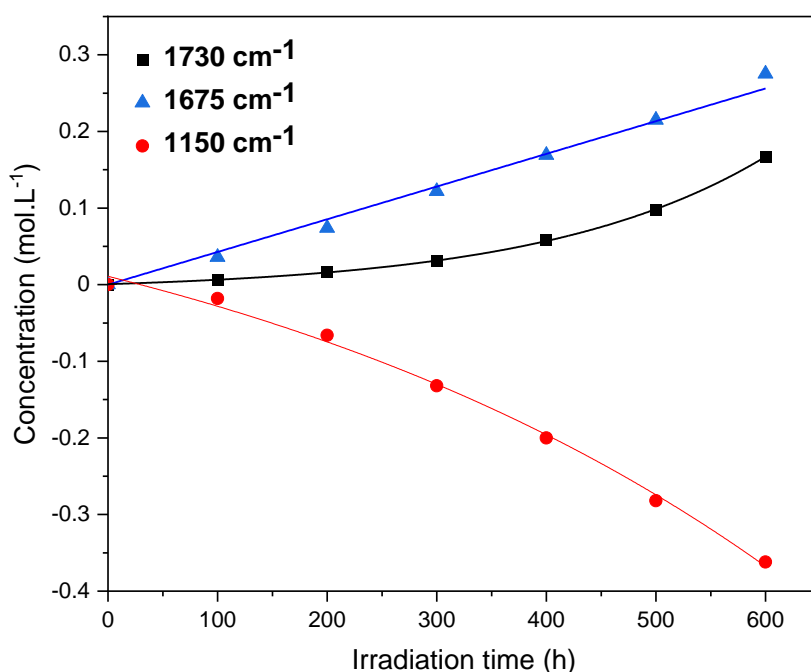
413 As shown above, the degradation of chitosan provoked by exposure to UV light in the  
414 presence of air led to variations in the following:

- 415 • chemical structure (oxidation, chain scission/crosslinking)
- 416 • macromolecular architecture (increase in network density, i.e.,  $T_g$ )
- 417 • mechanical properties (increase in hardness)
- 418 • surface properties (increase in roughness and yellowing).

419

420 Therefore, the degradation caused by exposure to UV light can be characterized by the  
421 following parameters: amide and gluconolactone formation, C-O-C conversion rate,  $\tan\delta$ ,  
422 nano-indentation and increased hardness. To check the inter-connection between the  
423 evolutions of these parameters during irradiation, a quantitative description was attempted.

424  
425 Figure 6 shows the variations in the concentrations of amide groups (IR band at  $1675\text{ cm}^{-1}$ ),  
426 gluconolactone (IR band at  $1730\text{ cm}^{-1}$ ), and ethers (IR band at  $1150\text{ cm}^{-1}$ ) during irradiation  
427 (the film thickness was 25 microns, and the absorption coefficients were as follows: CONH:  
428  $340\text{-}380\text{ L.mol}^{-1}.\text{cm}^{-1}$ , gluconolactone:  $650\text{-}700\text{ L.mol}^{-1}.\text{cm}^{-1}$ , COC:  $40\text{-}50\text{ L.mol}^{-1}.\text{cm}^{-1}$ )  
429 (from the literature) [Okamba-Diogo et al., 2014].



430  
431 Figure 6: Concentration of the oxidation products (detected at  $1675\text{ cm}^{-1}$  and at  $1730$   
432  $\text{cm}^{-1}$  in IR spectra) and disappearance of the C-O-C bond (detected at  $1150\text{ cm}^{-1}$  in IR spectra)  
433 versus irradiation time.

434 Figure 6 shows that the concentration of amide groups ( $1675\text{ cm}^{-1}$ ) produced during  
435 photooxidation was higher than the concentration of gluconolactone ( $1730\text{ cm}^{-1}$ ). Indeed, the  
436 concentration of gluconolactone generated after 600 hours of ageing corresponded to the  
437 concentration of amide group after 100 hours of ageing.

438

439 To connect all the degradation criteria from the molecular scale to the macroscopic  
 440 scale, we aimed to find kinetic information from the different parameters responsible for the  
 441 degradation versus exposure time. We used a phenomenological model (Rose, Le Bras  
 442 Bourbigot & Delobel, 1994) (Musto, Ragosta, Abbate & Scarinzi, 2008) to describe the  
 443 kinetic processes of degradation as follows:

$$\frac{X(t)}{X(t=0)} = 1 - kt$$

$$X(t) = X(t=0). \exp(-kt)$$

444 where X(t) is the value of a selected parameter obtained for various times t, X (t=0) is  
 445 the parameter value at t = 0 and k is the Arrhenius-type kinetic constant. The equation was  
 446 applied to the experimental parameters X, such the absorbance A at 1730 cm<sup>-1</sup>, nano-  
 447 hardness, and T<sub>g</sub>.

448 Table 2: Degradation rate (k) for the main degradation criteria of photooxidation

Parameters	Kinetic model	k (h <sup>-1</sup> .10 <sup>-3</sup> )	standard error (h <sup>-1</sup> .10 <sup>-3</sup> )	C.coeff χ <sup>2</sup>
α C=O (1675 cm <sup>-1</sup> )	$\frac{A(t)}{A(t=0)} = 1 - kt$	0.475	0.05	0.999
Yellowing (A 400 nm)	$\frac{A(t)}{A(t=0)} = 1 - kt$	0.475	0.07	0.989
α C=O (1730 cm <sup>-1</sup> )	$A(t) = A(t=0). \exp(-kt)$	2.8	0.1	0.996
α COC	$A(t) = A(t=0). \exp(-kt)$	1.6	0.1	0.995
nano-Hardness/ Hardness	$A(t) = A(t=0). \exp(-kt)$	1.7	0.2	0.992
nano-Tg	$A(t)$	2.5	0.1	0.98
Roughness	$A(t)$	2.8	0.15	0.996

449

450

451 The results in Table 2 indicate that only the appearance of amide groups and the  
452 increase in yellowing follow zero-order kinetics. The accumulation of gluconolactone and the  
453 various parameters that were selected in an attempt to establish a correlation between the  
454 variation in chemical structure and mechanical properties follow a first-order kinetic, and the  
455 k values for the main parameters are similar (the average k value was  $2 \cdot 10^{-3} \cdot \text{h}^{-1}$ ). The half-life  
456 time was calculated from the kinetics, and the average  $t_{1/2}$  value was approximately 200 hours.  
457 The good agreement between the degradation-rate k-values of the different criteria confirms  
458 the cross-correlation established. These results underline the difference in reactivity of all the  
459 detected degradation species and confirm diverse routes of degradation.

460

#### 461 **4. Conclusion**

462 Photooxidation of chitosan resulted in yellowing, crack formations, and an increase in  
463 roughness. The increases in network density and nano-hardness are relevant in terms of a  
464 reduction in the material's functional properties. A mechanism accounting for the main  
465 degradation routes for chitosan photooxidation at the molecular scale was proposed. We  
466 demonstrated the consequences of the chemical modifications on the material properties, and  
467 we showed correlations between these chemical modifications and the evolution of the  
468 mechanical properties. Our results allowed a « quantitative » correlation between the relevant  
469 degradation criteria.

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471

#### 472 **Declaration of Interest**

473 None

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### Figure captions

Figure 1: IR spectra of chitosan films ( $e = 25 \mu\text{m}$ ) before and after 24 h Soxhlet extraction in MeOH (domain  $1800\text{-}1000 \text{ cm}^{-1}$ ) and the chitosan powder in KBr pellets.

Figure 2: a) IR spectra of chitosan film ( $e = 25 \mu\text{m}$ ) during photooxidation, b) Subtracted IR spectra in carbonyl domain.

Figure 3: a) IR spectrum of a film ( $e = 25 \mu\text{m}$ ) of chitosan blended with gluconolactone. b) UV-visible spectra of a 600h photooxidized chitosan film before and after treatment with 2,4-DNPH.

Figure 4: UV-Visible spectra of chitosan film during photooxidation ( $e = 25 \mu\text{m}$ ).

Figure 5: a) Change in roughness ( $\blacksquare$ ) versus irradiation time (line is obtained by an exponential fit). b) Image of the chitosan film surface before and after 300 hours of irradiation.

Figure 6: Concentration of the oxidation products (detected at  $1675 \text{ cm}^{-1}$  and at  $1730 \text{ cm}^{-1}$  in IR spectra) and loss of the C-O-C bond (detected at  $1150 \text{ cm}^{-1}$  in IR spectra) versus irradiation time.

Table 1: Evolution of nano-hardness (nano-Hv) and nano- $T_g$  as a function of irradiation time

Table 2: Degradation rate (k) for the main degradation criteria of photooxidation

Scheme 1: Chemical structure of chitosan

Scheme 2: Simplified photooxidation mechanism of chitosan

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