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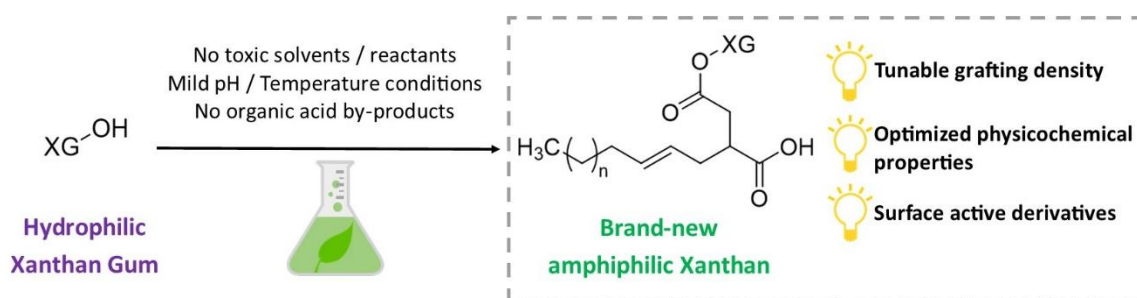
Original tools for xanthan hydrophobization in green media: Synthesis and characterization of surface activity

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Original tools in xanthan hydrophibization



Abstract

Xanthan gum (XG) was modified through esterification using various alkenyl succinic anhydrides in green media. FTIR and NMR spectroscopy confirmed that the alkenyl succinic hydrophobic groups were successfully grafted on octenyl-grafted (XG-C8), dodecenyl-grafted (XG-C12), and hexadecenyl-grafted (XG-C16) xanthan backbone. Grafted chains were evaluated by degree of succinylation (DS). Modification enhance the surface activity of xanthan by decreasing surface tension from 72 until 37 and 58 mN/m due to respectively long alkenyl chain for XG-C16 (DS = 2.2 ± 0.2 %) and high grafting rate for XG-C12 (DS = 16.9 ± 1.9 %). These original tools are of great interest to optimize both the DS and the properties of xanthan gum by varying reaction parameters like pH range, reaction time and the mole excess of the grafting reagents to xanthan monomeric units.

Keywords: Xanthan, green chemical modification, alkenyl succinic anhydrides, 1H NMR spectroscopy, surface active derivatives

1. Introduction

Polysaccharides are widely used in many industrial areas due to their availability, low cost, biodegradability, non-toxicity and functional properties (Barclay et al., 2019). Among them, xanthan is well known for its outstanding thickening capacities and ability to stabilize dispersions. However, due to poor interfacial properties xanthan has to be used combined with conventional surfactants, these latter low molecular weight compounds owning negative toxicological and environmental impacts (Johnson et al., 2017; Olkowska et al., 2011). To overcome these problems, intensive researches have been devoted to polysaccharides chemical modification as reported for chitosan (Babak et al., 2005), inulin (Stevens et al., 2001), alginate (Yang et al., 2012) or xanthan (Mendes et al., 2011) to increase

30 their potentialities as amphiphilic biopolymers combining both texture and interfacial properties, thus
31 giving access to innovative formulations.

32 Xanthan is an anionic bacterial heteropolysaccharide produced by *Xanthomonas campestris*. Its
33 structure consists of a linear cellulosic backbone of β -D-glucose substituted on every two units with a
34 pendant trisaccharidic side chain containing a β -D-glucuronic acid between an inner α -D-mannose and
35 a terminal β -D-mannose (Jansson et al., 1975) with acetate and pyruvate functionalities varying with
36 fermentation conditions (Casas et al., 2000).

37 Due to its structure, xanthan can undergo various modification treatments to acquire new
38 functionalities. Literature reports different methods through physical, chemo-enzymatic (Arimura et
39 al., 2011), and chemical (Mendes et al., 2011; Sara et al., 2020) pathways or plasma irradiation (Hua et
40 al., 1997).

41 Many studies regarding chemical grafting on xanthan polysaccharide have been carried out, mainly
42 with grafting onto hydroxyl functions by alkylation, acetylation, etherification, acetylation, acylation,
43 esterification, oxidation or copolymerization reactions (Patel et al., 2020; Salazar et al., 2019; Sara et
44 al., 2020), and functionalization onto carboxyl groups allowing selective grafting such as thiolation and
45 amidation reactions (Fantou et al., 2017; Roy et al., 2014; Mendes et al., 2013).

46 Nevertheless, modifications are mainly performed using either (1) toxic and expensive solvents, (2)
47 derivatization with coupling agents, (3) high temperature, (4) prolonged reaction time or all together.

48 Among these reactions, Roy and Fantou succeeded in modifying xanthan under both its ordered (Roy,
49 2015) and disordered (Fantou, 2018) conformations via carbodiimide-mediated amidation.
50 Corresponding hydrophobically modified xanthans (HMX) were demonstrate as promising in emulsion
51 stabilization (Fantou et al., 2019a, 2019b).

52 Nevertheless, such a grafting reaction is unfortunately considered as polluting and presents several
53 drawbacks according to green chemistry principles: use of carbodiimide, multi-step synthesis carried
54 out at high temperature, use of organic solvent etc. thus impeding industrial development. Therefore,
55 there is a need to develop more environmentally respectful methods. The present work aims grafting
56 hydrophobic alkyl residues onto xanthan backbone using green and sustainable protocol.

57 According to literature, using cyclic carboxylic acid anhydrides as biopolymers grafting agents is
58 advantageous due to milder reaction conditions and absence of organic acid by-products (Shah et al.,
59 2018). Different polysaccharides such as alginate (Falkeborg et al., 2015), starch (Ren et al., 2014),
60 karaya (Padil et al., 2015), inulin (Morros et al., 2011) were successfully chemically modified by octenyl
61 succinic anhydride (OSA) or dodecenyl succinic anhydride (DDSA) in aqueous alkaline medium.
62 Esterification reaction takes place between polysaccharide hydroxyls and anhydrides carboxylic end.
63 Corresponding derivates show better dispersibility and homogeneity in hydrophobic medium (Shah et
64 al., 2018) but also non-toxicity (Venkataraman et al., 2013) and good biodegradability due to easy ester
65 bonds cleavage (Morros et al., 2011); a remarkable example are octenyl starch derivatives approved
66 by US FDA for food applications with grafting around 3 % (w/w) (Shah et al., 2018).

67 Regarding such ecological and efficient reactions (Kokubun et al., 2013; Renault et al., 2012), the aim
68 of the present project was to develop an innovative HMX through esterification using different alkenyl
69 succinic anhydrides (ASA) as grafting agents. A protocol for xanthan chemical modification in aqueous

70 medium at low temperature was first developed and optimized. Then chemical and physicochemical
71 properties of HMX were characterized and finally, unprecedented interfacial properties were
72 evidenced.

73 **2. Materials and methods**

74 **2.1. Materials**

75 Xanthan Gum (XG) was kindly offered by Cargill (France). Substitution degrees of acetate and pyruvate
76 as determined by ^1H NMR were 0.56 and 0.59 per monomeric unit, respectively. Moisture content
77 (11.3 %) was measured by thermogravimetry, and protein content as determined by the Bradford
78 procedure was 0.95 %. 2,2,2,3,3-d(4)-3-(trimethylsilyl) propionic acid sodium salt (TSP) was purchased
79 from Alfa Aesar (USA), hydrochloric acid and sodium hydroxide from Fisher Scientific (USA), (2-
80 Dodecen-1-yl) succinic anhydride (DDSA) and 2-Octen-1-ylsuccinic anhydride (OSA) from Sigma Aldrich
81 (USA), Hexadecenyl succinic anhydride (HDSA) from TCI chemicals (Japan). Ultra-pure water (16.8
82 $\text{M}\Omega\cdot\text{cm}$ resistivity) was used. All chemical reagents were analytical grades.

83 **2.2. Synthesis**

84 Full details are given in Results and Discussion section (3.1).

85 **2.3. Confirmation of structure modification by FT-IR spectroscopy**

86 FT-IR measurements were recorded in absorbance mode over the $4000\text{-}650\text{ cm}^{-1}$ region at a resolution
87 of 8 cm^{-1} , using a Perkin Elmer Frontier FT-IR spectrometer (PerkinElmer, USA) with ATR mode
88 (Diamond/Zinc Selenide crystal). Spectrum analysis was performed on the Spectrum program Version:
89 10.4.00.0190.

90 **2.4. Determination of the degree of succinylation (DS) by ^1H NMR**

91 DS of modified xanthan was quantified by proton nuclear magnetic resonance (^1H NMR) analysis. First
92 xanthan solutions (either native or modified) were prepared by dissolving gum powder at $5\text{ g}\cdot\text{L}^{-1}$ in
93 H_2O ; then NMR samples were prepared according to the following protocol adapted from (Esquenet,
94 2003).

95 First, 1 mL of xanthan solution was freeze-dried to obtain 5 mg of polymer powder. Then, the polymer
96 powder was dissolved in 1 mL of D_2O at room temperature (RT) and the corresponding solution was
97 then freeze-dried again. This later step was repeated twice before dissolving the resulting powder in 1
98 mL of D_2O solution containing $5 \times 10^{-3}\text{ M}$ of TSP as internal quantification reference. This procedure
99 allowed the exchange of labile protons of hydroxyl groups by deuterium atoms in order to reduce the
100 residual water signal intensity, thus improving both the spectrum quality and interpretation.

101 All ^1H NMR spectra were recorded using a Bruker Avance 300 MHz spectrometer (Bruker, Germany) at
102 $80\text{ }^\circ\text{C}$ to reduce the solutions viscosity thus allowing adequate protons relaxation. WATERGATE W5
103 sequence (WATER suppression by GrAdient Tailored Excitation) was used to eliminate the solvent peak
104 (Piotto et al., 1992) and to improve the spectra quality. 256 scans were run for all samples, which
105 led to obtain a high enough signal-to-noise ratio for quantitative exploitation. All ^1H NMR spectra
106 were analyzed using MestReNova software (14.1). Integration was done according to a deconvolution

107 based on a mathematical model (Laplacian) using the Global Spectral Deconvolution (GSD) algorithm
108 of the MestReNova software.

109 Degree of succinylation was calculated as follows:

$$110 \quad \%_{ASA} = \frac{n_{ASA}}{n'_{RU}} \quad (1)$$

111 With:

- 112 - n_{ASA} the number of moles of ASA calculated by comparing the integration of the methylene
113 protons group of grafted alkenyl (1.28 ppm) and $C_6H_9D_4NaO_2Si$ (TSP as internal reference at 0
114 ppm).
- 115 - n'_{RU} the number of moles of modified repeating units of xanthan. Its value was calculated by
116 dividing the introduced mass of polymer by its repeating unit molecular weight.

117 M'_{RU} was determined by incrementing calculations using equations (1) and (2).

$$118 \quad M'_{RU} = M_{RU} + \%_{ASA} \times M_{ASA} \quad (2)$$

119 With M_{RU} the molecular mass of non-modified xanthan and M_{ASA} the molecular mass of ASA.

120 All measurements were conducted in duplicates/triplicates, and the results are reported as means \pm
121 standard deviations (SD).

122 **2.5. Surface tension measurements**

123 Surface tension (ST) was measured using the pendant drop method on a Digidrop GBX goniometer
124 (GBX, France). To this purpose, a drop of solution was suspended from a vertical syringe and then kept
125 in balance between gravity and surface forces (Berry et al., 2015). Surface tension was monitored over
126 time using a high-speed camera (25 images/s). ST calculation was performed with using Windrop++
127 software; ST was followed over a period of time until reaching stabilization.

128 **3. Results and discussion**

129 **3.1. Xanthan succinylation**

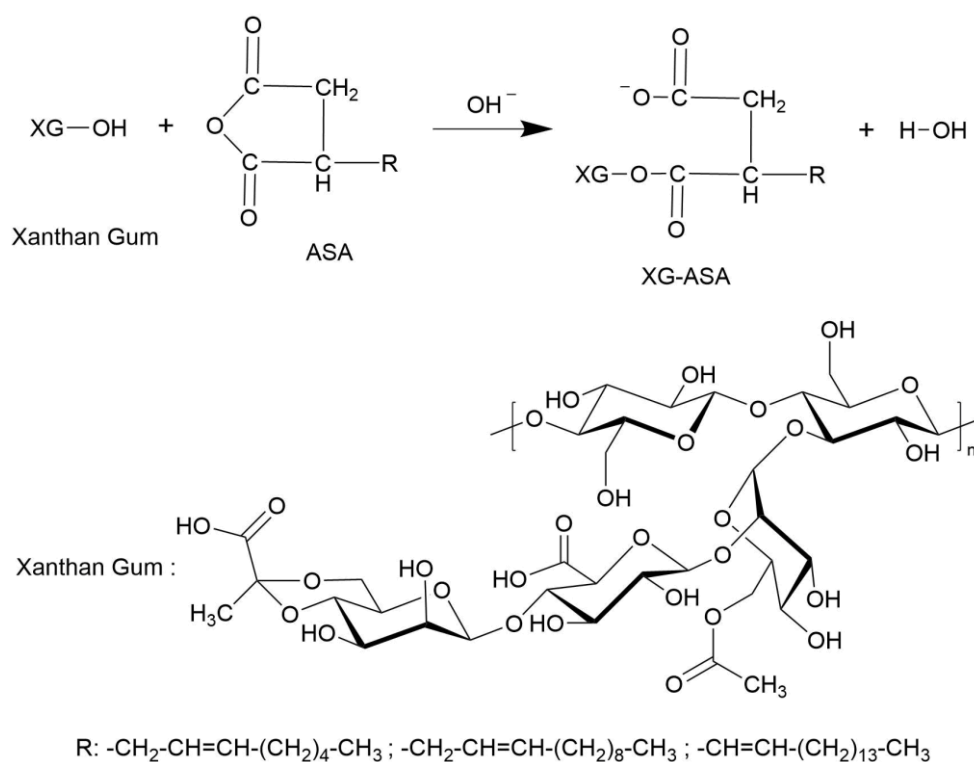
130 A series of xanthan derivatives was prepared as illustrated in Fig. 1, using OSA, DDSA or HDSA inspired
131 from previously reported methods (Falkeborg et al., 2015; Jeon et al., 1999; Sweedman et al., 2013).
132 The reaction proceeded according to the following general methodology: xanthan aqueous solution
133 (0.25 % w/v) was prepared by xanthan powder dissolution in ultrapure water under mechanical stirring
134 at RT until complete dissolution; then polysaccharide solution was stored overnight at 4 °C. OSA was
135 used as is; DDSA and HDSA being solids were dispersed in isopropyl alcohol at 1:3 ratio (w/w).

136 Succinylation was performed on 300 mL polymer solution in a 500 mL multi-necked flask at 30 °C during
137 24 h under mechanical stirring (Heidolph-TORQUE Core, Germany) with PTFE anchor shaft. Grafting
138 agent was progressively added to the xanthan solution and then pH was maintained between 8.5 and
139 9.0 with adding 2 M NaOH solution using an automated control system for the whole reaction duration.
140 Indeed, M300 process transmitter constantly checks the pH of the mixture through connected digital
141 ISM pH/ORP sensor (InPro 3253i/SG/225; Mettler Toledo, Switzerland). Any pH change induces the

142 start of the peristaltic pump (Preciflow; Lambda Laboratory Instruments, Czech Republic) thus injecting
143 NaOH to get constant pH value.

144
145 At the end of reaction, pH was brought to 6.0 with HCl solution to convert all carboxylate groups to
146 acid form. Succinylated product was then precipitated in isopropanol excess (1.8 L) and washed once
147 with acetone and twice with isopropanol in order to remove any unreacted acid. HMX residue was
148 dried at 40 °C for 24 h and then stored at 4 °C. Resulting HMX were noted as **XG-C8**, **XG-C12** and **XG-**
149 **C16** for octenyl-grafted, dodecenyl-grafted and hexadecenyl-grafted xanthan, respectively.
150 All HMX remained fully water soluble thus allowing pouring back in water for further physicochemical
151 characterizations. Samples degree of succinylation (DS) was determined using ¹H NMR analysis.

152 Reaction optimization was performed by varying either the reaction pH range, the reaction time and
153 the grafting reagents molar excess. Precursor refers to samples subjected to the same treatment apart
154 from adding OSA, DDSA or HDSA.



155
156 Fig. 1. Reaction scheme of succinylation of xanthan with ASA

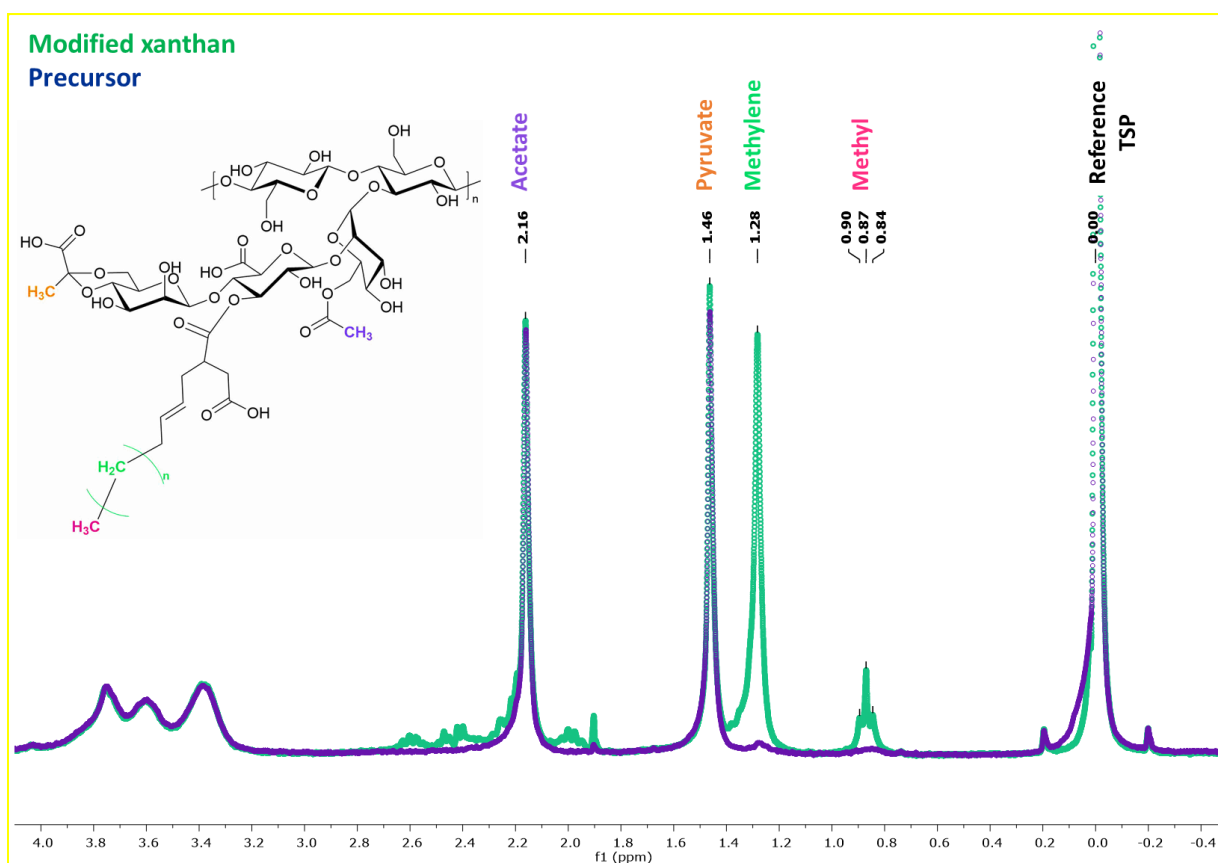
157
158 3.2. Samples chemical analysis

159 HMX were first analyzed using either FT-IR and ¹H NMR.

160 To this end, FT-IR spectra of native xanthan, HMX and grafting agents (OSA; DDSA and HDSA) were
161 recoded and compared (data not shown). A crucial outcome is the absence of anhydride moieties on
162 any of the FTIR analysis, thus demonstrating efficient washing and purification steps whatever the
163 grafting agent used. In addition, grafting was confirmed with two weak peaks at 1561 cm⁻¹ and 1733
164 cm⁻¹ corresponding to asymmetric COO⁻ stretching and ester carbonyl stretching on HMX spectra,

165 respectively; this is in accordance with the literature (Kokubun et al., 2013; Padil et al., 2015). In
166 addition, stretching bands of alkane moieties (CH₂ and CH₃) at 2924-2858 cm⁻¹ slightly increased after
167 alkenyl succinic group grafting a mentioned by (Bai et al., 2009). Thus, FTIR spectra allowed confirming
168 the effective grafting of alkyl moieties onto the polysaccharide backbone; nevertheless, due to low
169 spectra resolution impeding any quantitative analysis, ¹H NMR analysis were envisaged to get both
170 qualitative and quantitative information on HMX samples.

171 HMX degree of succinylation was quantified by ¹H NMR at 80 °C in the presence of TSP as internal
172 reference. Figure 2 reports the NMR spectra of a xanthan derivative grafted with DDSA, superimposed
173 with the one for unmodified xanthan (precursor). Firstly, one can notice that the xanthan precursor
174 spectrum shows no additional peaks to those attributed to native xanthan, namely, acetate at 2.16
175 ppm and pyruvate at 1.46 ppm (Rinaudo et al., 1983). This confirms that no polysaccharide degradation
176 occurred due to either reaction conditions and precipitation and purification steps, and this was also
177 checked through a comparison of intrinsic viscosity determination for both the precursor and the
178 native xanthan (data not shown).



179
180 Fig. 2. ¹H NMR spectra of the precursor (bleu) and modified xanthan (green) in D₂O at 80 °C

181 NMR spectrum of XG-C12 displayed two specific peaks in the upfield region: peaks at 0.87 ppm and
182 1.28 ppm corresponding to the dodecyl methyl and methylene protons, respectively. These
183 observations validate the effectiveness of the grafting method; it also allows grafted chains
184 quantification, either C8, C12 or C16, by quantifying either the methyl peaks which integrates for 3
185 protons or the methylene peaks which integrates for 8, 14, 24 protons respectively for XG-C8, XG-C12
186 or XG-C16. Resulting DS (%) represents the number of alkenyl chains grafted per 100 repeat units.

187 Proceeding to methyl triplet integration is theoretically the most suitable for grafted chain percentage
188 determination since it is the unique alkenyl moiety terminal unit. However, succinylated xanthan NMR
189 spectra often present weak methyl group signals since it only integrates for 3 protons; thus, accurate
190 distinguishing of the 3 peaks triplet can be very tricky especially for low grafting densities. Hence, it
191 was decided to integrate the intense peaks corresponding to methylene since they integrate for 8, 14,
192 24 protons for octenyl, dodecenyl or hexadecenyl substituents, respectively.

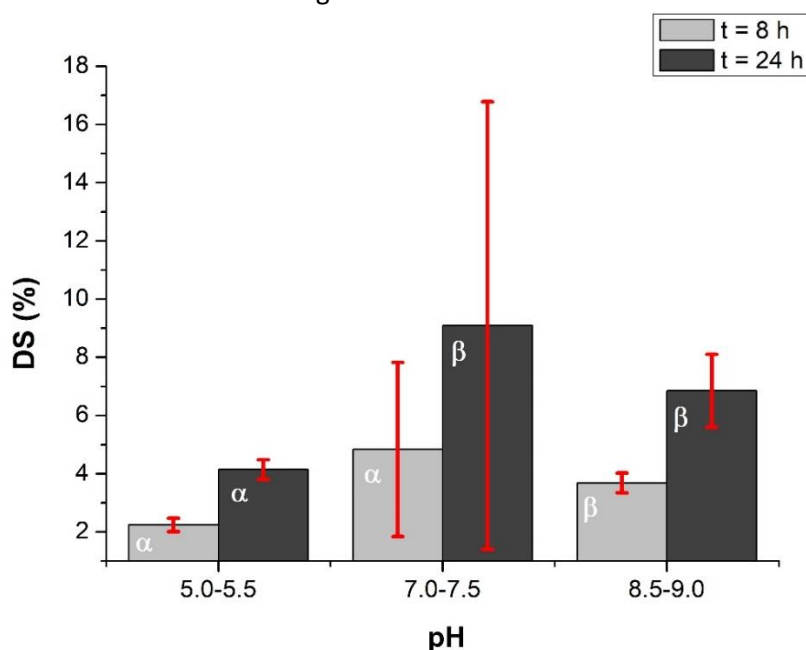
193 Calculations based on methylene peaks integration were performed using GSD method. This method
194 is more suitable than areas below the peaks integration since the methylene and pyruvate signals
195 appear quite close thus making it difficult to precisely delineating them. DS was calculated either from
196 a range of analysis carried out on different HMX samples issued from the same reaction product (so
197 called intra-reaction (α)) or from repetitions carried out on samples issued from different preparation
198 under the same grafting conditions, so called inter-reaction (β).

199 DS calculation made it possible the optimization of the reaction conditions; then, results allowed
200 targeting the desired grafting rates by modulating reaction conditions such as pH, xanthan/ASA ratio,
201 alkenyl chains length and the duration of the reaction. The following parts of the paper highlight the
202 effects of reaction parameters on HMX grafting rates.

203 3.3. Effect of pH range

204 Given that succinylation reaction depends on the pH of the medium, we first investigated the effect of
205 pH of the reaction on the reaction rate and efficacy focusing on DDSA. For this series of experiments,
206 the molar ratio (xanthan monomer: DDSA) was fixed at (1:15) with two different reaction times,
207 namely 8 h and 24 h. Three distinct pH ranges were tested: 5.0-5.5; 7.0-7.5 and 8.5-9.0. DS as a function
208 of both reaction time and pH are reported on figure 3.

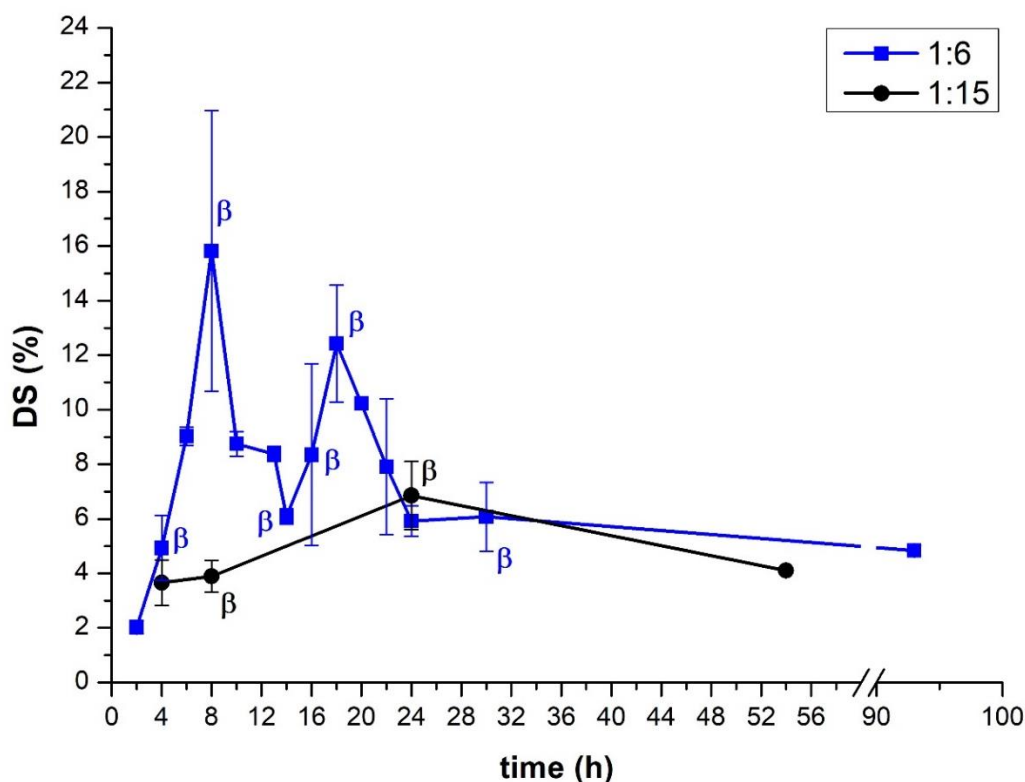
209 The highest DS values were obtained at pH range of 7.0-7.5. However, corresponding SD values were
210 high in spite of repetitions, for either intra or inter reaction. On the opposite, other tested conditions
211 gave DS with much less variation thus indicating a better control for the reaction conditions.



212

241 efficient becomes the reaction. Indeed, for a given xanthan concentration, a higher DDSA amount
 242 induces a more hydrophobic fatty phase, which decreases the DDSA availability and, consequently, its
 243 diffusion into the aqueous phase so its reactivity decreases. This is consistent with previous
 244 observations (Jeon et al., 1999).

245 Secondly, it is striking that DS values for the ratio (1:15) keeps fairly low over the whole period of
 246 reaction. So, the reaction duration effect on the grafting rate remains almost insubstantial for this
 247 ratio. On the opposite, for 1:6 ratio, degree of succinylation significantly increases when the reaction
 248 is monitored from 2 to 8 h. Thus, it comes that prolonging the period of time until 8 h enhances the
 249 dodeceny succinylation reaction by promoting diffusion between the grafting agents and polymer
 250 molecule as it was already described elsewhere (Bhosale & Singhal, 2006).



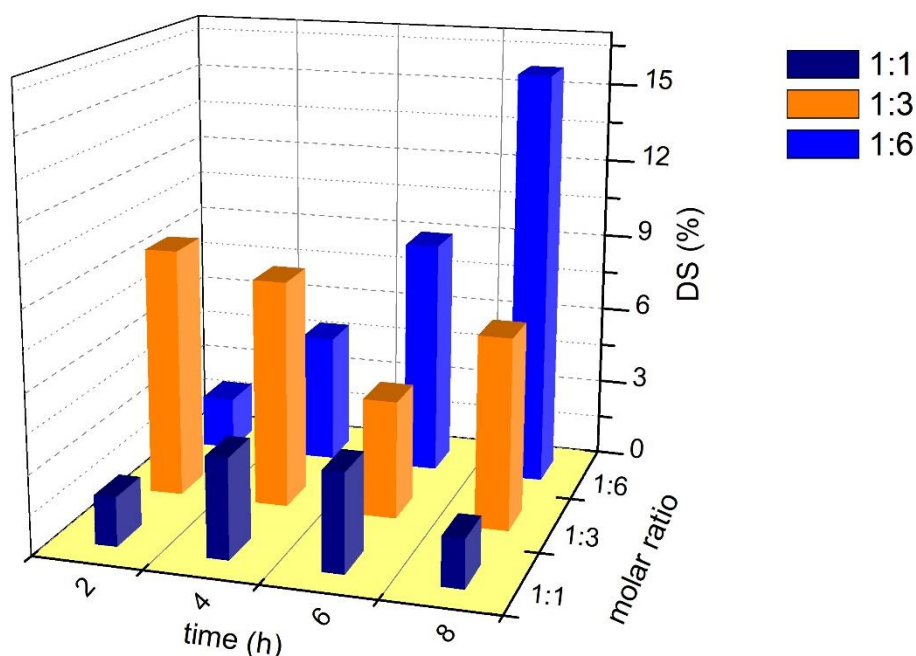
251
 252 Fig. 5. Effect of increasing the reaction duration on DS for two molar ratio (xanthan monomer: DDSA)
 253 1:15 (black) and 1:6 (blue). Inter-reaction (β) and intra-reaction (no symbol) repetitions. (30 °C, pH
 254 8.5-9.0)

255 Then, still for the (1:6) ratio above 8 h up to 24 h, DS fluctuates between 5 and 12 %. This could be
 256 related to disturbances generated by the successive sampling procedure as leading to volume and
 257 medium composition changings. Finally, over 24 h, the degree of succinylation slightly decreases or
 258 remains fairly constant. An explanation may be that as the reaction progresses, DDSA is depleted due
 259 to succinylation and hydrolysis. Thus, reverse reaction corresponding to ester side-chain hydrolysis
 260 (Fig. 4.a) becomes predominant and induces a DS decrease; this is in agreement with observations by
 261 (Jeon et al., 1999) and (Song et al., 2006) when DDSA and OSA grafting on starch.

262 **3.5. Effect of polymer:ASA ratio**

263 On the basis of the results exposed above, it was decided to investigate the effect of xanthan:DDSA
 264 molar ratio within the first 8 h of reaction time. As 1:15 ratio appeared less efficient to reach high DS,
 265 further ratios corresponding to lower DDSA quantities were tested, namely 1:3 and 1:1.

266 Fig. 6. gives the effect of xanthan to DDSA molar ratio on the DS values over the first 8 h of reaction.
 267 Corresponding DS values with SD are also reported in Table 1.



268
 269 Fig. 6. Effect of decreasing the polymer:DDSA molar ratio on DS for a reaction time up to 8 h.
 270 (30 °C; pH 8.5-9.0).

271 Table 1
 272 Means and standard deviations of DS associated to figure 6.

Time \ Molar ratio	2 h	4 h	6 h	8 h
1:6	2.0 ± 0.2	4.9 ± 1.2*	9.0 ± 0.3	15.8 ± 5.1*
1:3	9.5 ± 0.9	8.7 ± 1.2	4.5 ± 0.2	7.3 ± 0.3
1:1	1.87**	3.8 ± 0.5	3.7 ± 0.1	1.8 ± 0.5

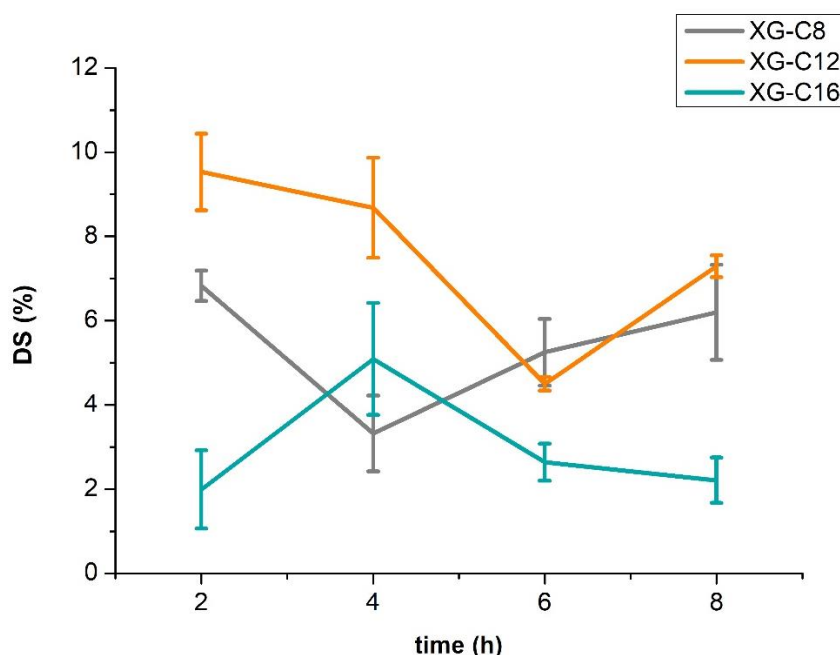
273 *inter-reaction repetitions

274 **no repetition

275 It is striking that 1:1 ratio leads to low degree of succinylation whatever the reaction time within the
 276 first 8 h. This can be interpreted as the result of low availability of non-hydrolyzed DDSA reactant thus
 277 limiting the possibility of reaction with xanthan hydroxyl groups. On the contrary, for 1:3 ratio, higher
 278 DS values are reached thus confirming that DDSA molecular species diffusion in the aqueous media
 279 towards xanthan molecules is easier in such a way that the succinylation reaction becomes more
 280 efficient from the first hours of reaction. Nevertheless, DS tends to decrease when reaction time is
 281 prolonged for the same reasons as mentioned above, namely the domination of reverse reactions
 282 following the DDSA depletion.

283 **3.6. Alkenyl chain length effect**

284 To go further, the effect of the ASA grafting agents alkenyl chain length was investigated. Thus, in
285 addition to DDSA, this part describes the grafting of both OSA and HDSA. Based on the results discussed
286 in the previous paragraph, reactions were carried out at 1:3 molar ratio with kinetic monitoring
287 between 2 h and 8 h, using successive sampling of 15 mL from the reaction medium to perform
288 chemical analysis over the period of time explored. Figure 7 refers to the study of the effect of alkenyl
289 chain length over 8 h of reaction.



290
291 Fig. 7. Effect of varying hydrophobic chain length on DS for a reaction time between 2 h and 8
292 h of reaction. Molar ratio (xanthan monomer: DDSA) 1:3; inter-reaction repetitions. (30 °C, pH 8.5-
293 9.0)

294 DS values are higher for XG-C12 compared to XG-C16 for all the reaction duration. An explanation is
295 that the ASA fatty phase is more hydrophobic when alkenyl chains is longer. Hence, HDSA owns a
296 slower diffusion capacity into the aqueous phase than DDSA, leading to less efficient grafting reaction.
297 In addition, grafting longer chain alkenyl onto xanthan polymer could make inaccessible other hydroxyl
298 to react with further anhydride moieties. Similar result was reported by (Jeon et al., 1999) for starch
299 succinylation.

300 This phenomenon should lead to an increase in the grafting rate for the less hydrophobic compound
301 such as OSA, but the opposite is actually observed. Nevertheless, there is another factor that should
302 be considered, related to ASA solubility and stability differences in the aqueous medium. Indeed,
303 anhydrides are unstable in aqueous medium so they hydrolyze and lose reactivity due to the ring
304 opening (Falkeborg et al., 2015). Therefore, they are inactive in relation to the nucleophilic attack of
305 hydroxyl groups. OSA, whose alkenyl chain is shorter, being more soluble than DDSA in aqueous media
306 (Shah et al., 2018), makes it less resistant to hydrolysis and therefore less available for succinylation
307 reaction. Hence the degree of succinylation of XG-C8 are higher than those of XG-C12.

308

309 3.7. HMX surface activity

310 Xanthan gum is described as hydrophilic non-adsorbing polysaccharide owning poor or no surface
311 activity in aqueous solution (Tempio & Zatz, 1981). Nevertheless, it is obvious that chemical
312 modification of such a polysaccharide with hydrophobic groups brings amphiphilic properties, thus
313 theoretically leading to surfaces properties changings. When dissolved in water, amphiphilic molecules
314 are well-known to diffuse at the air/water interface whereby hydrophilic polysaccharide backbone
315 extends into water while hydrophobic alkenyl chains are oriented toward the air phase (Sweedman et
316 al., 2013). Therefore, dynamic ST measurements are useful to evidence surfaces properties changings
317 between precursor and HMX; in particular, monitoring ST over time has to be carefully considered as
318 it may evidence species diffusion motion depending on different parameters such as concentration,
319 temperature and degree of substitution.

320 In the present work ST measurements over time were performed on different HMX samples issued
321 from the ASA grafting. ST was measured using pendant drop method, thus allowing monitoring the ST
322 evolution over a period of time up to 20 minutes to prevent any artefact such as evaporation. A series
323 of HMX derivatives owning various DS were selected (table 2); experiments were performed at 0.2 %
324 (w/w) polymer concentration in deionized water. Xanthan precursor molecule (e.g. without chemical
325 grafting) was taken as reference. Results are reported on figure 8.

326 Table 2: DS value of the selected HMX.

HMX	XG-C8	XG-C12	XG-C16
DS (mean ± SD%)	/	2.2 ± 0.2	2.2 ± 0.5
	4.1 ± 0.9	4.1 ± 0.3	/
	/	8.4 ± 0.8	/
	/	16.9 ± 1.9	/

327
328 First, ST for both native and precursor xanthan remains constant (72 ± 1 mN/m) over the whole period
329 of measurement thus unambiguously confirming the absence of xanthan surface activity as currently
330 reported in the literature (Curves are not shown to ensure good readability).

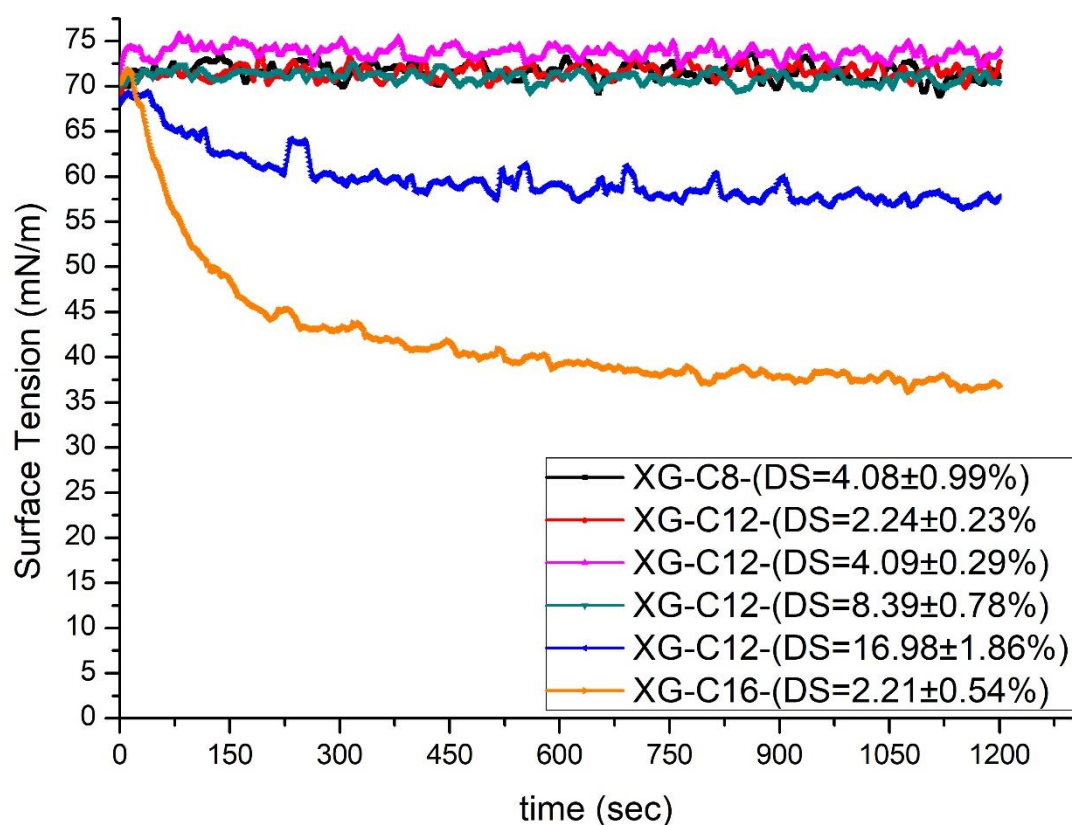
331 As visible in Figure 8, no surface activity is observed for low grafted XG-C12 (DS < 9 %) and for XG-C8
332 xanthan derivatives as ST remains constant, equal to water value. On the contrary, a slow but marked
333 ST decrease is observed for both XG-C16 and high grafted XG-C12 (DS = 16.9 ± 1.9 %) derivatives, thus
334 illustrating the occurrence of marked surface activity for the corresponding HMX specimen. Indeed, ST
335 first sharply decreases during the first 10 minutes before stabilizing, with a constant value much lower
336 than the one for pure water. This clearly demonstrates that both highly grafted XG-C12 and XG-C16
337 molecules do gradually diffuse into the air/water interface since they are large and bulky molecules
338 (Tømmeraas et al., 2011). Then, one can establish that HMX surface activity behaves as follow:

- 339 - For low DS and short alkenyl moieties, no surface activity is observed for either XG-C8 and XG-
340 C12. This can be explained by low hydrophobicity provided to the polymer, thus remaining
341 insufficient to modify polysaccharide interfacial properties. It can be assumed that in such
342 conditions macromolecules mainly interact through intramolecular interactions.

343 - On the opposite either for long alkenyl moieties or high DS, ST significantly decreases to $37 \pm$
344 1 mN/m in the presence of XG-C16 while it reaches 58 ± 1 mN/m for XG-C12 owning high DS.
345 Thus, the longer the alkenyl chain or the higher the grafting rate, the lower the final ST as a
346 result of hydrophobicity increasing (Lin & Chen, 2006; Rouzes et al., 2002).

347 These results are in good accordance with previous observations establishing that the hydrophobicity
348 increasing is suitable to reduce ST (Aziz et al., 2020). Another striking point is that XG-C16 ST lowering
349 occurs rapidly within the first 100 seconds, thus illustrating high HMX molecules initial diffusion rate
350 in water. This allows understanding that such an amphiphilic polymer is effective in stabilizing
351 interfaces without molecular surfactants as previously reported (Fantou et al., 2019a).

352 Finally, HMX show significant capability to lower air/water ST as far as grafted residues own long alkyl
353 residues and/or minimum grafting rate. This is in good accordance with results reported for other
354 polymers modified with ASA such as pullulan (Aziz et al., 2020), sugar beet pectin (Chen et al., 2015)
355 and hyaluronic acid (Tommeraas & Eenschooten, 2012).



356
357 Fig. 8. Surface tension against time for aqueous HMX solutions (0.2 % w/w, RT); curves
358 smoothed via 15 points Savitzky-Golay filter.

359 4. Conclusion

360 In this study, xanthan gum was chemically modified with three different alkenyl succinic anhydrides,
361 namely octenyl, dodecenyl and hexadecenyl, in green conditions. Grafting one-step reaction with only
362 two compounds took place in water at low temperature and slightly alkaline conditions. Moreover it
363 required no catalysts, no organic or toxic solvents, and did not generate any organic by-products.

364 Optimal conditions for each hydrophobically modified xanthan (HMX) derivatives (C8, C12 and C16)
365 were investigated through NMR analysis, thus allowing accurate control of degree of succinylation (DS)
366 ranging from 1 to 16 % depending on the pH, reaction duration, molar ratio and alkyl chain length. The
367 highest DS was obtained with grafting DDSA (C12) to xanthan under the following reaction conditions:
368 30 °C, pH 8.5-9.0, molar ratio (xanthan monomer: DDSA) 1:6, 8 h of reaction. The hydrophobic succinic
369 alkenyl grafted moieties induce surface activity for HMX owing either longer alkenyl chains or higher
370 grafting rate. Interestingly, for XG-C16 (DS = 2.21 ± 0,54 %) the water surface tension was better
371 lowered compared to all other derivatives thus offering significant potentialities to stabilize interfaces
372 with such a low modified polymer. Furthermore, it could be possible to investigate the grafting of other
373 alkenyl succinic anhydrides such as nonanyl, tetradecenyl etc. These original tools to obtain
374 hydrophobically new-branded modified xanthan gums is of great interest to acquire new functional
375 material with promising potential applications in interfaces and emulsions stabilization offering both,
376 academic and industrial applications. Indeed, the process that has been developed, involving mild
377 reaction conditions, a short reaction time and cheap chemicals, could be easily optimized and
378 transposed on a large scale. The effective capacity to stabilize emulsions with these new classes of
379 xanthan gum derivatives will be studied soon.

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