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1 **H₂O₂-Sensitive Delivery Microparticles based On the Boronic acid**
2 **Chemistry: (Phenylboronic –Alginate Derivative / Dextran) System**

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14

15

16 **Abstract.**

17 Oxidative stress is mainly due to **the** accumulation of hydrogen peroxide (H₂O₂), enabling to
18 distinguish between inflamed tissues from a healthy one. Hydrogen peroxide is then a very
19 promising stimulus for targeted drug delivery to diseased tissue. Herein, we design a new bio-
20 compatible and -degradable polymeric multilayer film stable in aqueous solution at
21 physiological pH, but which is destabilized upon exposure to a critical hydrogen peroxide
22 concentration. More precisely, we exploit the possibility to form “boronate ester” linkage
23 between a boronic derivative (the phenylboronic acid-modified alginate) and a diol
24 biopolymer (the dextran) as a driving force for the multilayer build-up. We show that the
25 resulting LbL films present H₂O₂-responsiveness at physiological pH. The film disruption is
26 induced in response to H₂O₂ addition which causes the breakage of the boronate ester bond
27 under this oxidative condition. This strategy is then extended for the fabrication of H₂O₂-
28 responsive microparticles (coating of **the** calcium carbonate microsphere). The porosity of
29 these microparticles **has have** been judiciously employed to load a probe (rhodamine b). This
30 approach represents a complementary approach to biodegradable H₂O₂-triggered release
31 systems.

32

33 **Keywords:** Layer-by-Layer films; H₂O₂-responsive; phenylboronic acid chemistry;
34 polysaccharide; dextran

35

36 1. Introduction

37 Reactive oxygen species (ROS) are implied in numeral physiological processes. One may cite
38 apoptosis, cell signaling or even proliferation. Excess ROS production is often associated with
39 diseases such as tumors and inflammatory pathologies.[1, 2] ROS-specific detection systems
40 [3-7] have gained an increase of interest due to the high impact of oxidative stress and
41 reactive oxygen species on several diseases.[8-13] Oxidative stress is correlated to the balance
42 of oxidative and reducing species within the cellular medium. Cellular components can be
43 damaged out of the balance due to ROS such as hydrogen peroxide, superoxide or even
44 hydroxide radicals.[14] Consequently, some ROS can be considered as a key to cell signaling
45 [15] and defense mechanisms. Furthermore, these species may also contribute to various
46 diseases.[16-18]

47 Smart polymer-based materials (films, particles...) are useful as they can easily be tailored to
48 degrade or release drug under the action of certain stimuli. [19, 20] ROS-responsive materials
49 have then been fabricated using poly(vanillin oxalate) [21], polyoxalate nanoparticles [22],
50 poly(propylene sulfide) polymersomes [23] or polysulfide nanoparticles [10]. These latter **has**
51 **been are** evidenced to enable response to ROSs such as hydrogen peroxide leading to the
52 release of encapsulated drugs. Developing new polymer-based materials sensitive to ROSs
53 enabling drug delivery undergoing oxidative stress are then highly attractive for instance for
54 treatment of many diseases marked by high concentrations of ROS.

55 Herein, we particularly pay attention to H_2O_2 for triggering the release of cargo or for
56 impacting the multilayer stability. Indeed, excessive production of H_2O_2 may show oxidative
57 stress leading to the development of many diseases such as Parkinson's disease, Alzheimer's
58 disease, Huntington's disease and cardiovascular disease [24, 25]. Stimuli-response to H_2O_2
59 may then be a key focus of research into the chemical mechanisms evidencing the
60 development and progression of disease. Therefore, the development of material/system
61 presenting sensitivity against H_2O_2 is of major interest.

62 Here, we report on polysaccharide multilayer film based on phenylboronic acid (PBA) moiety
63 and H_2O_2 -triggered release of a probe based on an oxidative decomposition reaction of
64 boronate ester moiety with H_2O_2 . Boronate esters can be transformed into phenol derivatives
65 due to an oxidative reaction [26]. H_2O_2 -sensitive materials have been developed using PBA
66 derivatives. [1, 2, 8, 27] However, these materials are (i) not entirely based on biopolymer, (ii)
67 sensitive to only a high amount of H_2O_2 (millimolar level) and (iii) the stimuli-response is
68 generally slow (several hours to a few days).

69 Towards this aim, we may think at using the LbL multilayer approach which has been proven
70 to be useful for drug delivery systems (DDS). [28] Functional layer-by-layer (LbL) multilayer
71 films on a solid substrate are typically prepared by exploiting the electrostatic interactions as
72 driving force.[29] However, various other interactions can also be involved in the elaboration
73 of LbL films. One may cite ~~interactions and~~ hydrogen bonding [30], avidin–biotin [31] or
74 even sugar–lectin. [32] Recently, phenylboronic acid and its derivatives ~~have been are~~
75 proposed for the elaboration of LbL films. [33-38] However, most of the polymers involved
76 are based on synthetic one.

77 In this paper, we present a complementary polymeric approach specifically sensitive to
78 biologically relevant concentrations of H₂O₂ and ~~presenting with~~ fast response to this
79 ~~chemical stimulus~~. Our research consists in the building-up and the characterization of
80 multilayer films based on phenylboronic-based alginate and dextran (a diol polymer). Indeed,
81 PBA and its derivatives spontaneously form a boronate ester bond with diol derivative. [39]
82 We show the multilayer disassembly upon H₂O₂ exposure. This strategy is then extended to
83 the elaboration of H₂O₂-responsive microparticles and ~~to for the~~ entrapment of a probe ~~inside~~
84 ~~the by involving a~~ porous matrix of the calcium carbonate microparticles.

85

86 2. Experimental

87 2.1. Materials

88 Alginate was provided from Cargill (Baupre, France) (alg⁻, mannuronic / guluronic ratio =
89 1.4, $\overline{Mn} = 2.7 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$, $\overline{D} = 2.3$), dextran ($1.1 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$, Biochemica), poly(ethylenimine)
90 (PEI, Sigma- ~~Aldrich aldrieh~~). All the chemicals were purchased from Sigma-Aldrich.

91 The phenylboronic-modified alginate labelled “alg-bor” (grafting ratio of phenylboronic acid
92 groups about 20%) was prepared by using the procedure developed by Belbekhouche *et al.*
93 [19].

94

95 2.2. Elaboration of H₂O₂-multilayer responsive on flat substrate

96 QCM measurement (Q-Sense E1, Q-Sense, Sweden) was performed to follow in situ all the
97 implemented strategy (i.e. multilayer buildup and H₂O₂-response). To clean the gold coated
98 QCM surface, this later was first flushed with two solvents (water and ethanol), ~~was~~ subjected
99 to a UV/ozone chamber for 20 min ~~and which was~~ followed by a final rinsing with ethanol.
100 Before introducing in the QCM flow chamber, the surface was overnight pretreated with a
101 solution of 4-mercaptophenylboronic acid (10 mg in 2 mL of ethanol). The surface was

102 conditioned in the solvent (PBS, pH 7.4) for 10 min. The self-assembly of alg-bor /dextran
103 was achieved onto the gold pretreated substrate through a flushing of stock polymers solutions
104 (1 mg.mL⁻¹, 15 min, flow rate of 250 µL.min⁻¹). A rinsing step was done with the buffer
105 between each polymer deposition step during 10 min.

106 Wetting contact angle measurements were performed with a Kruss goniometer (room
107 temperature, sessile drop configuration) on ~~the~~ gold modified surface.

108

109 **2.3. Elaboration of H₂O₂-responsive microparticles.**

110 Negatively charged CaCO₃ microparticles were fabricated following the process developed by
111 Volodkin et al. [37] Briefly, equal volumes of calcium chloride (CaCl₂, 0.33 M) and sodium
112 carbonate (Na₂CO₃, 0.33 M) solutions were mixed for 3 min under vigorous stirring. This
113 ~~enables~~ ~~enabled~~ rapid precipitation and then fabrication of the CaCO₃ microparticles
114 presenting negatively charged surface. The particles were washed two times with water and
115 then acetone to remove water.

116 10 mg of CaCO₃ particles ~~were was~~-dispersed in 500 µL of water, followed by ~~the~~ addition of
117 500 µL of a PEI (polycation, 1 mg.mL⁻¹) solution. The particles were shaken during 15 min.
118 The unadsorbed PEI was removed by a centrifugation process (5 min, 10 000 rpm). The
119 resulting particles were washed with water (5 min, two times). It deserves noting that the final
120 volume was kept during all the experiment at 1 mL. The PEI-coated CaCO₃ particles were
121 suspended in water and 500 µL of alginate derivative (1 mg.mL⁻¹) was added. The
122 polysaccharide excess was removed by centrifugation process (5 min, 10 000 rpm). The
123 particles were suspended in aqueous solution and 500 µL of dextran (1 mg.mL⁻¹) was added
124 and stirred for 15 min. This process presents the assembly of a single bilayer: (alg-bor
125 /dextran)₁. The adsorption of the polycation (PEI) and then the polyanion (alg-bor) on the
126 negatively charged CaCO₃ particles was followed by measurement of zeta potential (a
127 Zetasizer Nano-ZS, Malvern Instrumente). Scanning Electron Microscopy (SEM) was
128 performed to show the formation of microparticles and its shape (Leo Elektronenmikroskopie,
129 Oberkoche in Germany).

130

131 **2.3. Probes-loaded microparticles.**

132 A rhodamine b solution (0.5 mg in 5 mL of water) was mixed to 10 mg of bare CaCO₃
133 microparticles and stirred at room temperature for 24 h. The resulting microparticles were

134 centrifuged (5 min, 10 000 rpm) and washed with water to remove the untrapped rhodamine
135 (three times). The LbL process was then performed as discussed in section 2.3. The loaded
136 microparticles were subjected to absorbance measurement (554 nm, Varian, Cary 100).

137 Equations 1 and 2 enabled to estimate the probe loading as well as the entrapment efficiency.

138 Probe loading = (amount of probe in microparticles) / (amount of microparticles) x 100% (1)

139 Entrapment efficiency = (amount of entrapped probe) / (initial amount of probe) x 100% (2)

140

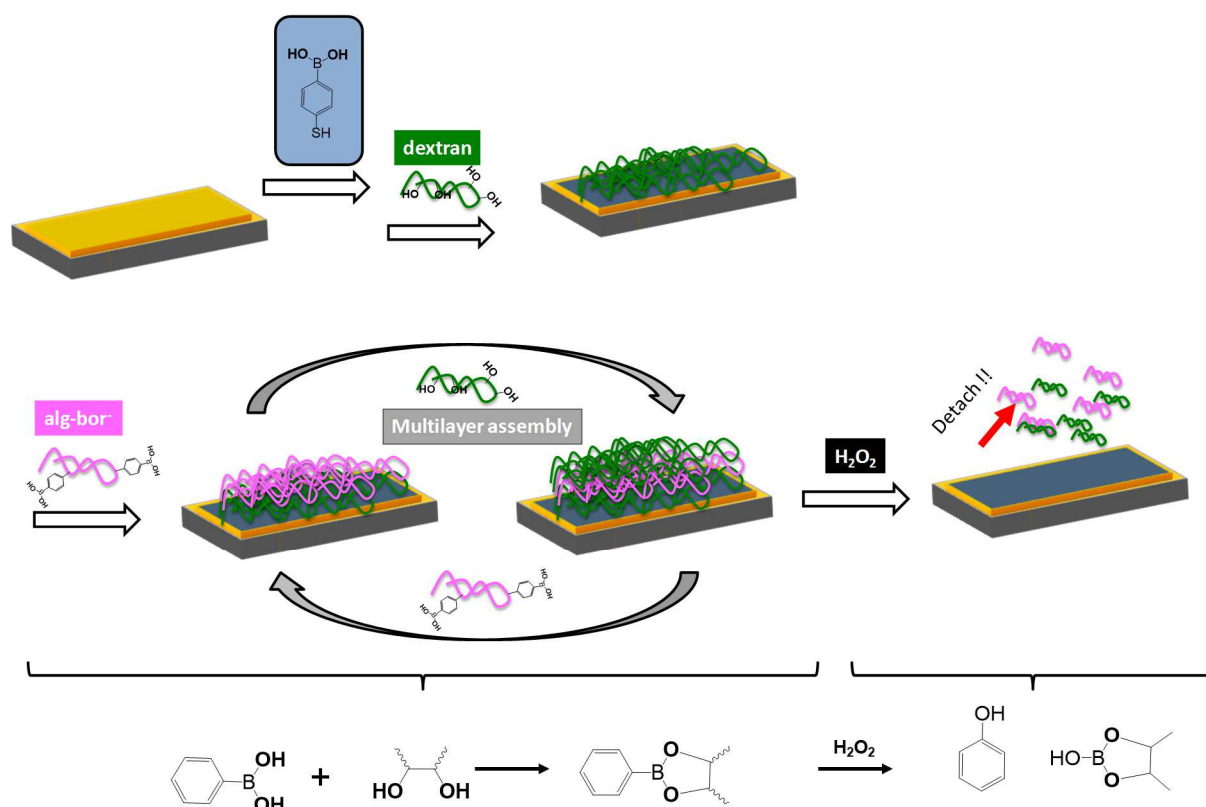
141 The release due to H₂O₂ of the as-obtained microparticle was then investigated. In this aim,
142 loaded microparticles were introduced in a dialysis bag and immersed in H₂O₂ solution at pH
143 7.4 (final dialysate volume = 400 mL). The used H₂O₂ concentration, named "critical H₂O₂
144 concentration" ~~will-be~~ was estimated by the quartz crystal microbalance study. At different
145 intervals, 1 mL of solution outside the dialysis membrane was removed and replaced with 1
146 mL of fresh buffer solution.

147

148 3. Results and discussion

149 As presented in Figure 1, the developed approach exploits the possibility to form "boronate
150 ester" linkage between a boronic derivative (phenylboronic acid-modified alginate) and a diol
151 polymer (dextran) as the driving force for the multilayer build-up. Their chemical structures
152 are shown in figure 2. We ~~will~~ then demonstrate that the resulting LbL films present H₂O₂-
153 responsiveness. The strategy ~~will-is further be~~ extended ~~for~~ to the elaboration of H₂O₂-
154 responsive microparticles. Toward this aim, calcium carbonate (CaCO₃) microparticles ~~will~~
155 ~~be~~ was prepared and modified. Calcium carbonate cores ~~have-been~~ were selected because of
156 its biocompatibility, low-cost and intrinsic porosity. The stability of the resulting multilayer
157 against H₂O₂ and a cargo entrapment and subsequent release ~~was are~~ finally investigated.

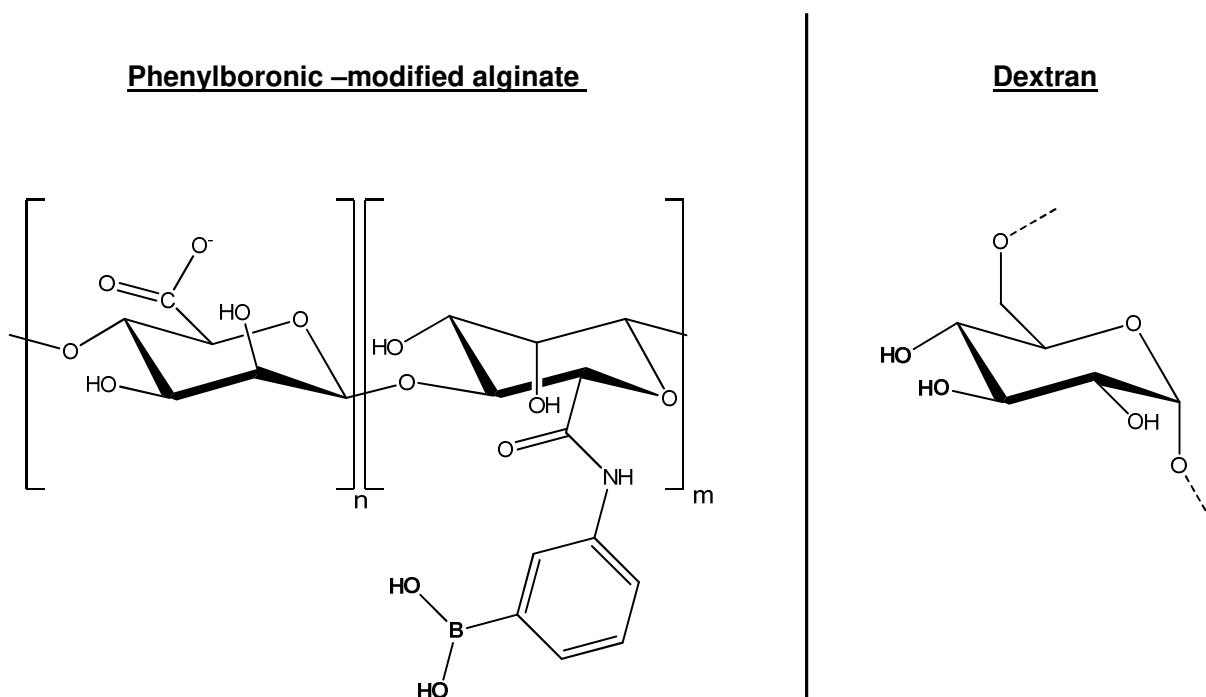
158



159

160 **Figure 1.** (Top) Representative scheme illustrating the preparation of H₂O₂-responsive
 161 multilayer films based on a phenylboronic acid polysaccharide derivative (alg-bor) and
 162 dextran. (Bottom) Illustration of the formation of boronate ester upon boronic acid and diol
 163 derivatives coupling followed by an oxidation of boronate ester to phenol by H₂O₂.

164



165

166 **Figure 2.** Chemical structures of phenylboronic acid modified alginate and dextran.

167 **3.1. Incorporation of phenylboronic acid moiety inside the alginate.**

168 Phenylboronic acid moiety ~~was inserting~~-was inserted inside the polysaccharide by reacting
169 the aminophenylboronic acid with the carboxyl functional group of the alginic acid (figure 2).
170 [40, 41] The carboxyl group of the alginate ~~reacts~~ reacted with 1-ethyl-3-(3-
171 dimethylaminopropyl)carbodiimide hydrochloride leading to an intermediate which is
172 unstable. An amine-reactive intermediate ~~is~~ was then obtained upon reacting with the NHS.
173 This later then immediately ~~reacts~~ reacted with the amine functionality of the
174 aminophenylboronic acid. The ¹H NMR spectra shows the protons chemical shifts of the
175 alginate (3 to 4 ppm) and phenyl backbone (6.7 to 7.2 ppm) (see SI, Figure S1). Herein, the
176 NMR measurement ~~allows~~-allowed qualitatively demonstrating that the as-obtained alginate
177 indeed contains aromatic groups. A Degree of Substitution "DS" ~~is~~-was qualitatively
178 estimated by using equation 3. [42]

$$179 \quad \text{DS} = (\text{I}_H \text{ in boronic unit in alg-bor}) / (\text{I}_H \text{ in alginate unit in alg-bor}) \quad (3)$$

180 The phenylboronic acid content ~~is~~-was then estimated to be about 0.20 ± 0.04 .

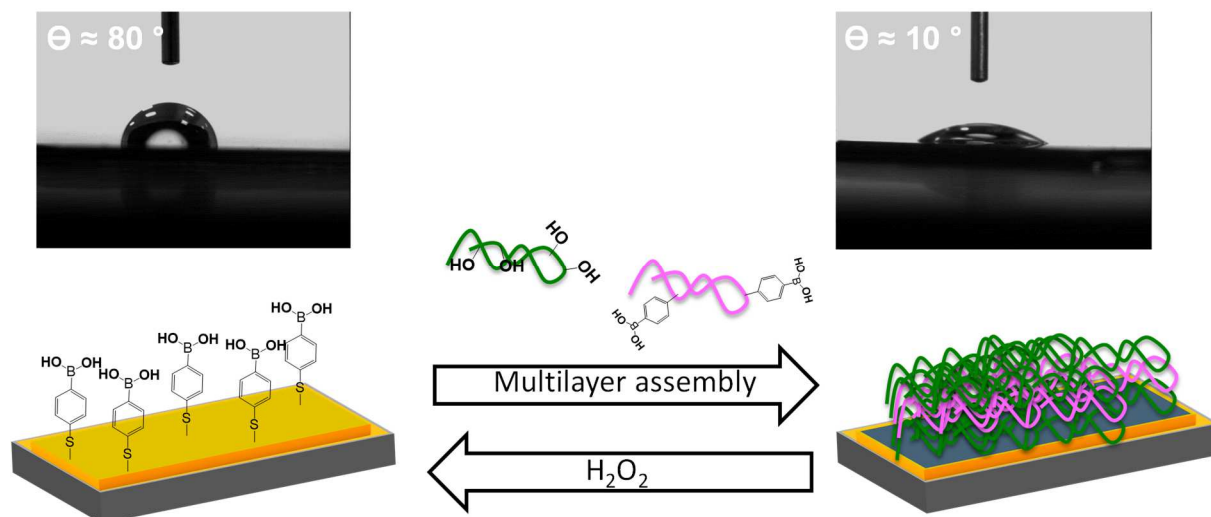
181 The FT-IR spectrum (see SI, Figure S2) of the alg-bor is in accordance with the success of the
182 chemical modification of the alginate (3300 cm^{-1} = -OH groups / 1740 cm^{-1} = -C=O due to the
183 amide / 1595 cm^{-1} and 1412 cm^{-1} = N-H deformation and C-N stretching vibrations / 1080 cm^{-1}
184 = C-N stretching vibration present after the chemical modification with the boronic
185 derivative / 1143 cm^{-1} = C-O stretching vibration of alcoholic hydroxyl group / 1023 cm^{-1} =
186 C-O-C stretching vibration / 867 cm^{-1} and 945 cm^{-1} = guluronate / mannuronate of (un-
187)modified alginate).

188

189 **3.2. Design of the H₂O₂-responsive multilayer film onto pretreated flat gold substrate.**

190 This implemented approach was first evidenced on a model substrate, i.e. on a pretreated flat
191 gold surface by wettability measurement and adsorption test. Static contact angle tests ~~are~~ was
192 done on the flat substrate before and after dipping in solutions containing polysaccharide
193 derivatives, i.e. (alg-bor/dextran)₁₀, to qualitatively show the building up of the multilayer
194 film onto the surface. The contact angle value diminished from 80° to 10° upon coating,
195 which could be correlated to the hydrophilic nature of these polysaccharides, adsorbed on the
196 surface (Figure 3). The variation of the number of bilayers ~~is~~ was found to have no impact on
197 the surface wettability behavior. Interestingly, after flushing with a solution of H₂O₂, the
198 contact angle value ~~is~~ was 80° which seems to be in accordance with multilayer removal
199 under this oxidizing condition. Note that a simple flushing with water or PBS solution (pH

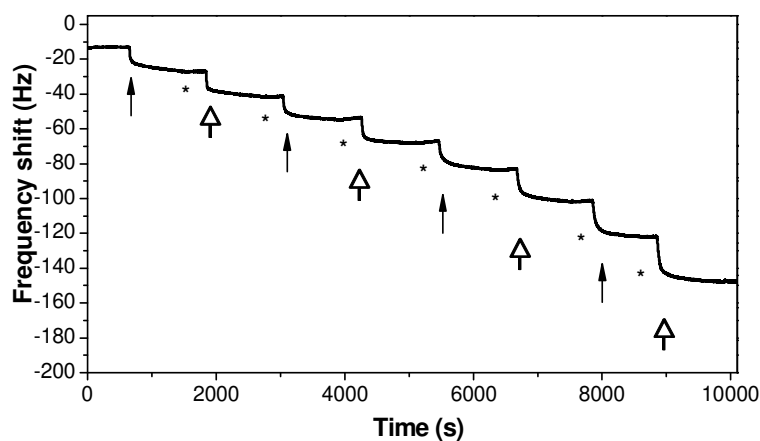
200 7.4) ~~has had~~ no impact on the contact angle value and then on the multilayer stability. The
 201 multilayer build-up could then be repeated on this pre-treated substrate. The same contact
 202 angle value ~~is was~~ then obtained - i.e. around 10° - and flushing again this modified substrate
 203 with a solution of H_2O_2 ~~leads led~~ to the multilayer removal. These results clearly show ~~n~~ the
 204 rapid and reversible H_2O_2 -responsive behavior of the developed system.
 205



206
 207 **Figure 3.** Reversible switching of the wettability property under oxidative condition: (A)
 208 precoated with a with 4-mercaptophenylboronic acid and (B) after the multilayer film build-
 209 up with (alg-bor/PVPON-dextran)₁₀.

210
 211 The opportunity to imply both boronic acid of the alginate and the diol from the dextran for
 212 forming boronate ester was then qualitatively demonstrated by wetting angle contact
 213 measurement and ~~will was~~ further quantitatively ~~be~~ evidenced by QCM measurements to (i)
 214 follow the multilayer build-up of the polysaccharides pair, i.e. alg-bor and dextran, and (ii)
 215 evidence the possible effect of H_2O_2 on the resulting LbL film. A gold-coated quartz substrate
 216 ~~is was~~ first pretreated with a 4-mercaptophenylboronic acid to impart boronic acid moiety on
 217 the surface. This ~~will~~ enabled the adsorption of the first layer of the diol containing polymer
 218 (dextran) followed by alg-bor adsorption (boronate ester interaction). Note that the presence
 219 of boronic acid groups was ensured by performing the build-up at pH 7.4 (pKa of phenylboric
 220 acid ~ 8.9). [43] As evidenced in Figure 4, the multilayer film build-up was monitored on the
 221 pre-treated surface of a quartz resonator. This figure shows the change in the resonance
 222 frequency during the QCM measurement upon successive deposition of dextran followed by
 223 phenylboronic acid-modified alginate. The variation of the frequency values decreased upon
 224 each polysaccharide deposition. This result strongly suggests that an increase of the mass onto

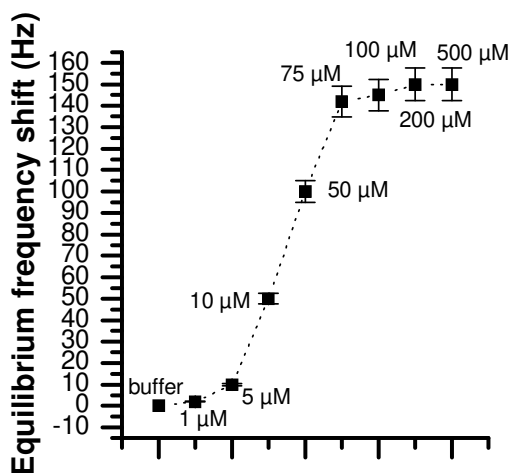
225 the surface upon the polymer addition and then demonstrates the multilayer buildup (i.e four
 226 bilayers of alg-bor/dextran). Figure 1 (bottom) proposes a plausible binding mode between
 227 phenylboronic acid-modified and the diol of the dextran in the film [39]. The result strongly
 228 suggests that there is formation of boronate ester bonds during the LbL build-up which is
 229 involved as the driving force for the preparation of LbL films. As a reference, test of
 230 multilayer build-up ~~has been~~ was performed by using native alginate, i.e. (dextran/alginate)
 231 pair. Only dextran ~~is~~ was adsorbed as first layer. This ~~is~~ was due to its interaction with
 232 boronic acid moieties present on the pretreated surface. Addition of native alginate ~~leads~~ led
 233 to non deposition of this polymer. This comforts our hypothesis, i.e., the boronate ester bonds
 234 act as the driving force during the multilayer build-up of (alg-bor/dextran) film.



235
 236 **Figure 4.** Evolution of the frequency shift values as a function of time as measured during the
 237 build up at pH 7.4 with (alg-bor/dextran) films (filled arrow: addition of dextran and unfilled
 238 arrow: alg-bor , "*" rinsing step).

239
 240 The impact of H₂O₂ concentration on the multilayer stability was investigated with QCM
 241 device. Towards this aim, the (alg-bor/dextran)₄ multilayer film was exposed to H₂O₂
 242 solutions ranging from 0.1 to ~~20 mM~~ 500 μM. From Figure 4, it ~~can~~ could be noted that at the
 243 end of the dextran/alg-bor build-up the final equilibrium frequency shift values were -150 ± 5
 244 Hz. These frequency values ~~can~~ could be used as reference to study the H₂O₂ sensitivity of the
 245 films. Thus, the film degradation or removal should be correlated to a final value of about
 246 +150 ± 5 Hz. As seen from figure 4, ΔF increases upon exposing the film to H₂O₂ to finally
 247 attain stable value. The critical H₂O₂ concentration ~~is~~ was found to be around 75 -100 μM,
 248 meaning near biologically relevant H₂O₂ concentrations. This evidences the film
 249 decomposition upon the increase of the concentration of H₂O₂. In contrast, in a solution

250 without H₂O₂, the frequency shifts ~~does did~~ not vary, the multilayer film remained then stable
251 (see supporting information, figure S3). These results clearly evidenced that the (alg-
252 bor/dextran)_n film is sensitive under oxidizing conditions and more precisely decomposes in
253 H₂O₂ solutions at pH 7.4. The boronate ester bond of alg-bor and dextran ~~can~~ could be
254 reversibly cleaved under oxidative condition [44] as illustrated in figure 1 (bottom).

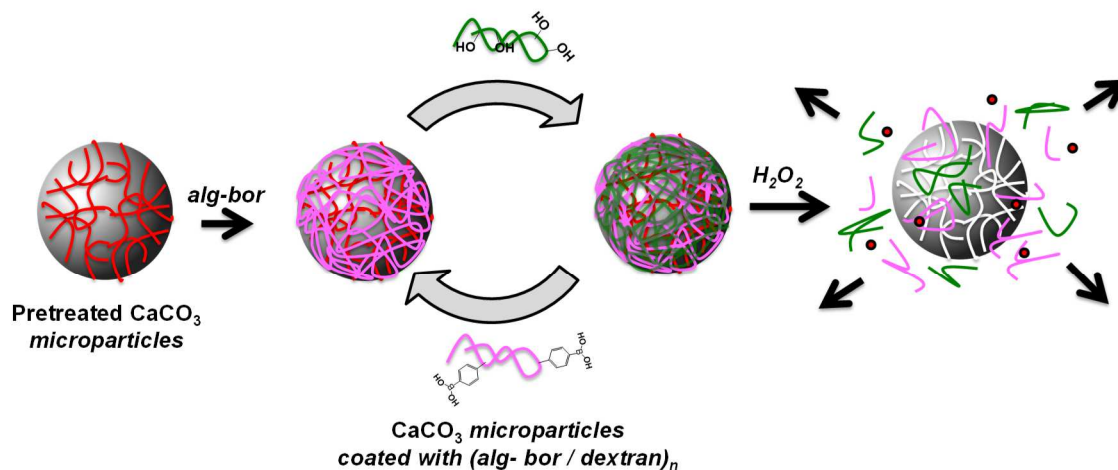


255
256 **Figure 5.** Effect of increasing concentrations of H₂O₂ at pH 7.4 on (alg-bor/dextran)₄ film.

257
258 It has then been proven that the (phenylboronic acid modified alginate /dextran) could form
259 multilayer formation in appropriate condition and to exhibit H₂O₂-responsive behaviour at pH
260 7.4. We then proposed to extend the process for the fabrication of microparticles pre-loaded
261 with a cargo and exhibiting H₂O₂-response.

262 263 **3.3. Elaboration of the H₂O₂ responsive particles based on (phenylboronic acid modified** 264 **alginate /dextran)**

265 We ~~have~~ used CaCO₃ microparticles as support for the deposition of these biopolymers films,
266 mainly because of its easy elaboration, biocompatibility and intrinsic porosity. [19]
267 (phenylboronic acid-modified alginate/dextran) pair ~~will~~ has then be deposited on this
268 microparticles, used as template. The stability of coated microparticles against H₂O₂ and the
269 cargo entrapment and subsequent release ~~will be~~ have then been investigated.

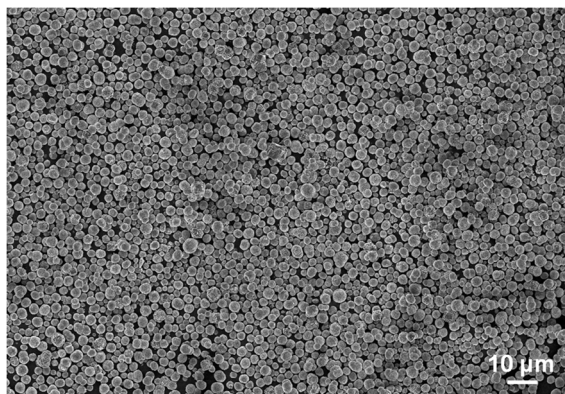


270
 271 **Figure 6.** Preparation of the H₂O₂-responsive microparticles based on phenylboronic acid
 272 containing polysaccharide (alg-bor) and a diol biopolymer (dextran) loaded with probes (The
 273 red lines in the left particle represent the loading of the probe inside the porosity of the
 274 microparticle. The white lines in the right particle indicate the release of the probe from the
 275 particle porosity).

276
 277
 278 The CaCO₃ microparticles present a negatively charged surface (-7 ± 1 mV) and is then pre-
 279 treated with the poly(ethylene imine) used as anchoring layer for enabling the subsequent
 280 multilayer build-up. The zeta-potential value switched to a positive one after the adsorption
 281 of the PEI (+ 50 mV). This allows the adsorption of the alg-bor (-23 ± 2 mV). After this step,
 282 the boronate ester-bonded layer-by-layer (LbL) approach was applied to prepare the
 283 multilayer (particle shell) with the phenylboronic acid modified alginate and dextran and
 284 remained stable up to 2 months.

285 Preloading enables to greatly increase the efficiency of cargo loading as lower chemicals is
 286 are necessary. One may cite, the work of Caruso et al. who presented that this approach
 287 allowed reported to significantly concentrate DNA from a dilute solution onto particles used
 288 as a matrix to fabricate capsules. [45] In their study, this polyanion was electrostatically bound
 289 to the surface of particles and then multilayers film were elaborated.

290 We have then used this strategy to load a probe (rhodamine derivative rhodamine b) inside the
 291 particle. The incorporation of rhodamine does did not significantly impact the zeta-potential
 292 value of the loaded CaCO₃ particle. SEM images show the spherical form and micrometer
 293 size of the fabricated microparticles (Figure 7).



294

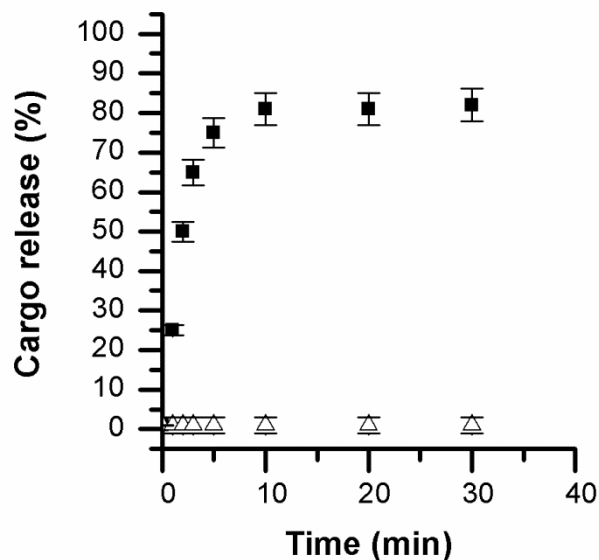
295 **Figure 7.** SEM images of modified microspheres composed by (alg-bor/dextran)₄
296 biopolymers pair.

297

298 The efficiencies entrapment ~~are~~ **was** found to be greater than 60% and the rhodamine loading
299 ~~is~~ **was** above 7%, evidencing that this chemical could be easily entrapped inside the CaCO₃
300 particles' matrix due to the intrinsic porosity.

301 When the **rhodamine-loaded** microparticles **composed by (alg-bor/dextran)₄** were suspended
302 **in into** an aqueous solution at pH 7.4 **and without hydrogen peroxide**, they ~~stay~~ **staid** stable
303 and intact, **i.e. the multilayer was not impacted by this condition (Figure S3)**. Then, when
304 these **(alg-bor/dextran)₄** microparticles were suspended in a solution containing H₂O₂ at pH
305 7.4 and at the critical H₂O₂ concentration determined during the QCM assay, the rhodamine ~~is~~
306 **was** gradually released in the medium (see Figure 8) which ~~is~~ **was** due to the destabilization of
307 the (alg-bor/dextran) multilayer. **Indeed, high release rate of rhodamine b was initially**
308 **observed (within the first 5 minutes) and then, release rate gradually decreased and levels off.**
309 **Upon oxidative condition, the release of rhodamine b from the inner microparticle cavity**
310 **could be correlated to the multilayer film decomposition as evidenced by QCM measurement**
311 **(Figure 5, H₂O₂-triggered release of the rhodamine b due to the oxidative decomposition**
312 **reaction of boronate ester moiety with H₂O₂).**

313



314

315 **Figure 8.** Release of rhodamine derivatives from (alg-bor/dextran)₄ microparticles with ■ and
 316 without H₂O₂ △ solution (100 μM) at pH 7.4.

317

318

319 4. Conclusion

320 H₂O₂-sensitive assemblies based on dextran (diol biopolymer) and phenylboronic acid
 321 containing polysaccharide ~~have been~~ **was successfully** prepared. This bioresponsiveness of the
 322 resulting multilayer film ~~is~~ **was** due to the formation of boronic ester during the build-up
 323 which is triggering groups impacted upon exposure to H₂O₂. Advantages of our system **are**
 324 **consisted in** biocompatibility, synthetic accessibility, reversibility and fast H₂O₂ triggered
 325 cleavage kinetics. Cargo-loaded microparticles were then formulated based on this multilayer
 326 film. The morphology of the microparticles was evidenced by SEM. A probe was loaded by
 327 taking advantage of the **porous** calcium carbonate **microparticles as** template **porosity**. The
 328 fast release contents upon exposure to H₂O₂ were shown at a biologically relevant H₂O₂
 329 concentrations (100 μM). **Herein, the H₂O₂ scavenging by the film was not possible, thus the**
 330 **developed system may mainly be used as H₂O₂ sensor.** Ongoing researches are dedicated to
 331 evidence the potential of these multilayer films to deliver therapeutic specifically to tissues
 332 undergoing oxidative stress.

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334 5. References

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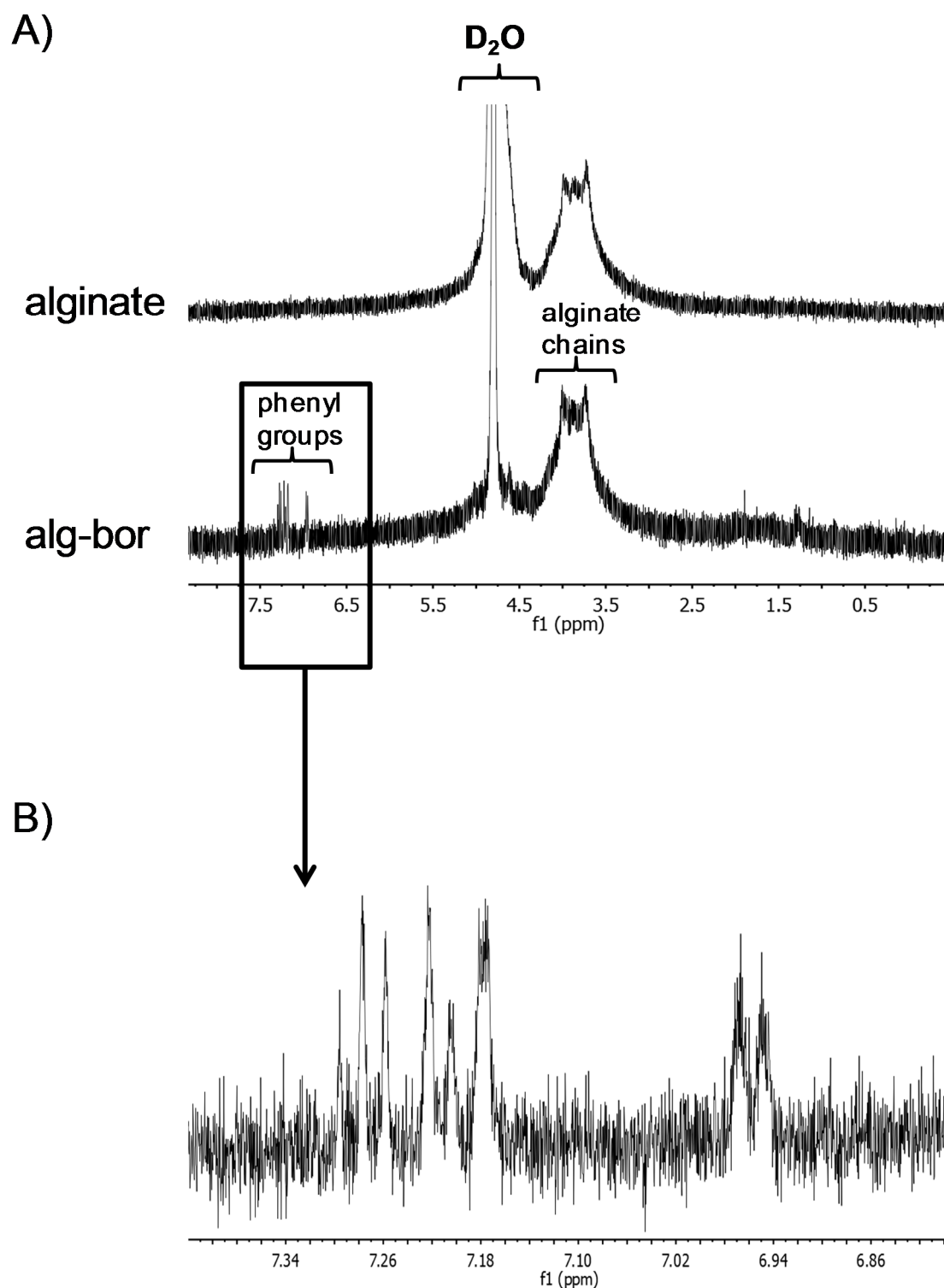
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Supporting information

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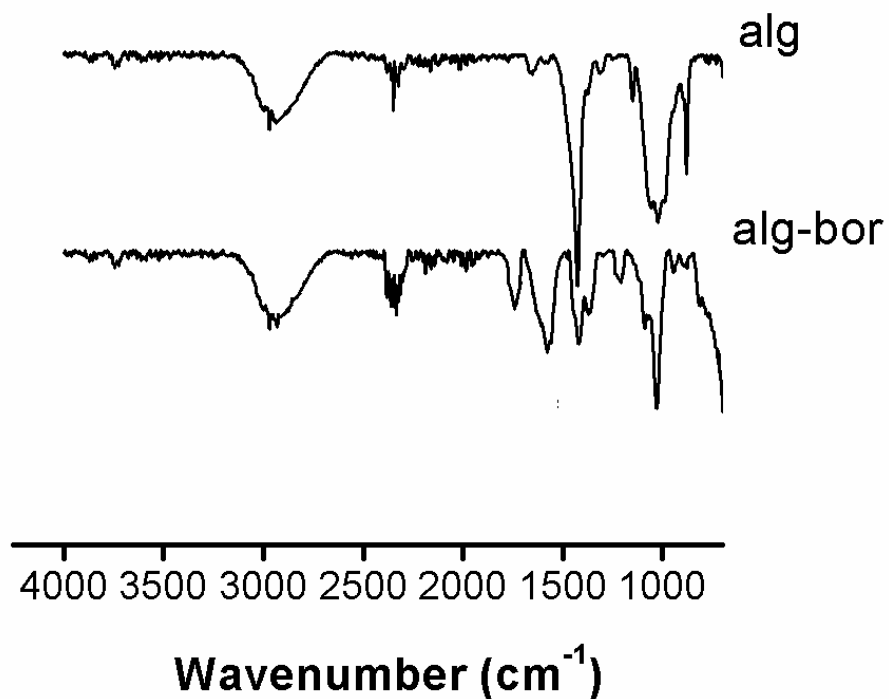


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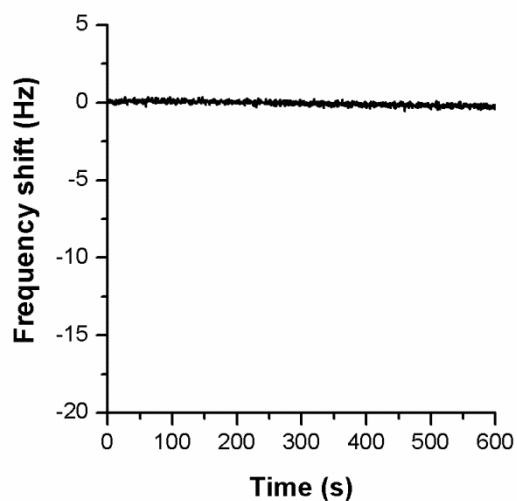
482 **Figure S1.** A) ¹H NMR spectrum (D₂O) of alginate and alg-bor, B) Zoom-in of graph A.

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Figure S2: FTIR spectra of alginate (alg) and modified alginate (alg-bor)



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Figure S3: QCM-D measurements. Flushing of a gold sensor coated with (alg-bor/ dextran)₄ film at pH 7.4. The non variation of the frequency evidences the stability of the multilayer films under such condition.