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1 **Glucose- and H₂O₂- Dual Responsives Drug Delivery Particle Based on**
2 **The Boronic Acid Chemistry**
3

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36 **Abstract.**

37 Herein, we report on responsive systems presenting dual response to two different stimuli,
38 namely glucose and hydrogen peroxide which are interesting for biological applications. New
39 chemical stimulus-responsive multilayer assemblies have been designed through the layer-by-
40 layer deposition of oppositely charged biopolymer on either flat or spherical surfaces.
41 (diethylaminoethyl-dextran hydrochloride/alginate derivative) polyelectrolytes pair was
42 selected for hydrogen peroxide-response and (polylysine/alginate derivative) polyelectrolyte
43 pair for glucose-response. Concentration-dependent glucose and hydrogen peroxide
44 responsiveness were reached via the incorporation of phenylboronic acid moieties inside the
45 alginate chain. Wettability and quartz crystal microbalance measurements showed that the
46 alginate derivate self-assembles though electrostatically-driven interactions with the
47 polycation. These techniques also evidenced that the multilayer assemblies present tuneable
48 behaviour according to the addition of glucose- and/or- hydrogen. This approach was further
49 extended to the preparation of smart microparticles using calcium carbonate as initial
50 substrate. The porosity of these microparticles has been advantageously used to entrap a
51 probe. Its release could then be triggered via glucose or hydrogen peroxide. The present
52 system may be used to targeted drug delivery to diseased tissue or as sugar-sensing device for
53 diabetic treatment.

54

55 **Keywords:** particle; glucose-responsive; polysaccharides; layer-by-layer; phenylboronic acid
56 chemistry; hydrogen peroxide-responsive

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68 **1. Introduction**

69 Stimuli-responsive particles have still attracted scientific interest as for diagnosis, sensing, or
70 drug delivery. [1, 2] This undeniable success can be rationalized by the possibility to tune the
71 resulting property by changing the environmental condition as pH or ionic strength. This
72 allows a control and a specific release of the drug.

73 Several methods have been successfully developed to produce particles. One may cite pH
74 induced micellization of a grafted polymer, [3] emulsion polymerization, crosslinking of
75 micelles, self-assembly and layer-by-layer (LbL) deposition of polyelectrolytes onto a
76 sacrificial template. [4, 5] Herein, we focus on the LbL process [6] as it is proved to be
77 efficient for the chemical modification of a plethora of substrates. [7, 8]

78 This approach allows developing particles with well-tuned properties. The overall strategy
79 combines particles templating and self-assembly methods. More precisely, it lies on the
80 alternative deposition of charged polyelectrolyte to form a multilayer film on particles used as
81 templates. The polyelectrolyte excess could easily be removed by a simple washing step
82 before the deposition of the next layer. This strategy enables to easily and efficiently control
83 the size and the film thickness of the as-obtained material. Herein, we focus on calcium
84 carbonate microparticle cores because it presents several advantages as biocompatibility, low
85 cost, mild decomposition and its intrinsic porosity which can be beneficial for providing high
86 drug loading. [9-13] Moreover, the CaCO_3 micro-sized particles are easily obtained, for
87 instance by mixing calcium chloride and sodium carbonate.[14]

88 For promising applications in the drug-delivery field, the most fundamental requirement is the
89 use of biocompatible and nontoxic polymer. In this aim, polysaccharides reveal to be very
90 competitive candidates. Their hydroxyl- or amine- rich chemical structure enables to bring
91 new interesting functionality as stimuli-responsiveness. They present also the interest of being
92 pH-dependent charged which is interesting for LbL assembly. We can cite the alternative
93 deposition of polysaccharides to form multilayer walls on microparticles, as dextran
94 sulfate/protamine.[15]

95 Bringing stimuli-responsiveness to drug system may open new routes to trigger the drug
96 release. Numerous studies reported on responsive particles to a broad range of external stimuli
97 such as pH, ionic strength or even temperature. [16-18] Although glucose has a crucial
98 biological function in living systems, only few groups have been working on this chemical.
99 [19, 20] For instance, cancer cells contain a very high glucose concentration in comparison to
100 normal cells [21] or we may simply think about diabetes treatment. The design of glucose-
101 responsive systems is then still a challenging and interesting task. [22] The phenylboronic

102 acids chemistry could be successfully implied for developing glucose-responsive system. [23]
103 Indeed, this functional group is recognized to form covalent bonds with polydiols as
104 polysaccharides. [19] The complexation occurs mainly in a pH range above the pKa of the
105 boronic moieties (pKa~9). [19, 22, 24] Levy et al. have prepared glucose-sensitive capsules,
106 stabilized by the formation of ester bonds between mannan and phenylboronic acid moieties
107 grafted onto a polyacid. [25] Glucose-sensitive capsules have been produced by De Geest et
108 al. by the layer-by-layer assembly of a copolymer of dimethylaminoethylacrylate and
109 acrylamidophenylboronic acid and poly(styrene sulfonate). They demonstrated glucose-
110 induced change in the electrostatic interactions between the polyanion and the phenylboronic
111 acid-containing polycation. [19] Belbekhouche et al. reported assemblies based on alginate
112 derivative containing phenylboronic acid side group and chitosan [20] or
113 poly(vinylpyrrolidone).[26]

114 Furthermore, it is important to note that phenylboronic acid chemistry may also be used for
115 developing hydrogen peroxide responsive system. This could be interesting to develop
116 reactive oxygen species detection systems. [27-31] Such systems are implied in several
117 physiological processes such as apoptosis or proliferation Excess ROS production is often
118 correlated to diseases such as tumors and inflammatory pathologies.[32, 33] Cellular
119 components can be damaged out of the balance due to ROS, e.g. hydrogen peroxide,
120 superoxide or even hydroxide radicals.[34] Thus, some ROS can be considered as a key to
121 cell signalling [35] for instance.

122 In this paper, the boronic acid chemistry is used to develop a new smart responsive material
123 presenting a dual response to glucose and hydrogen peroxide. Our work consists in the
124 building-up and the characterization of multilayer films on flat substrate based on either
125 ((diethylaminoethyl-dextran hydrochloride/alginate derivative) polyelectrolytes pair for
126 hydrogen peroxide-response or (polylysine/alginate derivative) polyelectrolytes pair for
127 glucose-response. This strategy is then extended to the fabrication of stimuli-responsive
128 microparticles which can be loaded with a probe and further be triggered released according
129 to glucose or hydrogen peroxide.

130

131 **2. Experimental parts.**

132 **2.1. Chemicals:**

133 These chemicals were purchased diethylaminoethyl-dextran hydrochloride (Sigma-aldrich),
134 polylysine (Sigma-aldrich), alginate with mannuronic / guluronic ratio 1.4 (Cargill (Baupte,
135 France, Table 1), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC,

136 Sigma-aldrich, > 98.5%), N-hydroxysuccinimide (NHS, Sigma-aldrich, > 98%),
 137 mercaptosulfonate (Sigma-aldrich, > 89.5%), calcium chloride (CaCl₂, rectapur, 99%),
 138 sodium nitrate (rectapur, 99%), 4-aminophenylboronic acid hydrochloride (Alfa Aesar, 97 %),
 139 acetic acid (Alfa Aesar,+99%), sodium acetate (Alfa Aesar, 99%) and
 140 ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich, 98%).

141

142 **Table 1:** Characterization of the alginate derivative.

Samples	\overline{Mn} (g·mol ⁻¹)	\overline{Mw} (g·mol ⁻¹)	DS*
alginate	2.7.10 ⁵ (± 1.3 %)	3.8.10 ⁵ (± 0.4 %)	-
alginate-g-boronic	2.5.10 ⁵ (± 1.3 %)	6.2.10 ⁵ (± 0.4 %)	0.20 ± 0.04

143 * grafting ratio of phenylboronic acid groups (estimated from ¹H NMR from Pettignano et al.
 144 [36]).

145

146 2.2. Introduction of phenylboronic group on polyanionic polysaccharide

147 Phenylboronic-modified alginate derivative was obtained by adding an amine-containing
 148 phenylboronic groups onto the carboxylic acid groups of the alginic acid via the formation of
 149 amide linkage through the EDC/NHS coupling reagent. The used EDC/NHS ratio is the
 150 optimized condition for this alginate modification. More precisely, 500 mg of alginate was
 151 dissolved in 100 mL of aqueous solution (potassium phosphate buffer (0.1 M, pH 7.2))
 152 overnight. Further, a mixture of 10 mg EDC and 10 mg NHS was added on the solution of
 153 alginate and stirred for 45 min. A solution of 4-aminophenylboronic acid (100 mg in 1 mL of
 154 methanol) was then dropwise added to the solution of alginate (mixing 1 day). A dialysis step
 155 was implied to purify the product (6 kDa cut off). The product was obtained via freeze-drying
 156 (yield 90%). [20]

157

158 2.3. Monitoring of the multilayer buildup and stimuli-response with QCM measurement

159 After cleaning (rinsing with water and ethanol, treatment in a UV/ozone chamber for 20 min
 160 and additional flushing with ethanol), the gold coated QCM Sensor Crystal was pretreated
 161 with sulfonated thiol (5 g.L⁻¹ in water). 1 mg.mL⁻¹ of polyelectrolytes was injected into the
 162 cell with a flow rate of 500 μL.min⁻¹. The following polyelectrolytes pair were used
 163 ((diethylaminoethyl-dextran hydrochloride/alginate derivative) and (polylysine/alginate
 164 derivative).

165 After the multilayer built-up, the glucose- or hydrogen hydroxide- response was investigated
 166 by flushing the resulting film by solution containing this chemical at different concentration.

167 **2.4. Fabrication of glucose- and H₂O₂-responsive[particles.**

168 CaCO₃ microparticles were fabricated according to Belbekhouche et al. [26] Multilayer
169 buildup was done via the LbL technique from 1 mg.mL⁻¹ of each polyelectrolytes (namely
170 diethylaminoethyl-dextran hydrochlorid, polylysine and alginate derivative). After each
171 adsorption process, the excess of polyelectrolyte was removed via two washing steps. A
172 centrifugation process (5 min, 10 000 rpm) was implied after each adsorption process. The
173 particles were washed with water two times during 5 min.

174

175 **2.5. Probe-loaded hybrid microcapsules.**

176 10 mg.mL⁻¹ of a suspension of microparticles were added into the solution containing a probe
177 (0.5 mg rhodamine b in 5 mL of deionized water). After 12 h, the loaded-CaCO₃
178 microparticles were centrifuged and washed to remove free rhodamine derivative. The LbL
179 process was done as explained in section 2.4. The probe loading and the entrapment
180 efficiency were calculated by using equations 1 and 2.

181

182 probe loading = $\frac{\text{amount of probe in microparticles}}{\text{amount of microparticle}} \times 100\%$ (1)

183

184 entrapment efficiency = $\frac{\text{amount of entrapped probe}}{\text{initial amount of probe}} \times 100\%$ (2)

185

186 The release behavior was investigated by introducing in a dialysis bag the probe-loaded
187 microcapsules and then immersing it in glucose or hydrogen peroxide solution. The probe
188 concentration was determined by absorbance measurements at 550 nm.

189

190 **2.6. Methods**

191 Fourier transform infrared spectra were recorded on a Bruker Tensor 27 instrument equipped
192 with a Digi TectDLatGS detector. ¹H NMR spectra were recorded on a Bruker Avance 300
193 NMR spectrometer. Zeta potential values of microparticles were determined on a Zetasizer
194 Nano-ZS (Malvern Instrument) equipped with a He–Ne laser ($\lambda = 633$ nm; scattering angle,
195 173°). Average molar masses and molar masses distributions were determined by size
196 exclusion chromatography (SEC) coupled online with multi-angle light scattering (MALS)
197 and differential refractive index (DRI) detectors. The MALS apparatus is the DAWN Heleos-
198 II from Wyatt Technology (Ca, USA) filled with a K5 cell and a Ga–As laser ($\lambda = 690$ nm).
199 The DRI detector is a Shimadzu RID-10A (Japan). Columns [Shodex SB OHpak 804 and 806

200 HQ for alginate] were eluted with 0.1mol.L^{-1} LiNO_3 at 0.5 mL min^{-1} . Eluent was filtered
201 through $0.1\text{ }\mu\text{m}$ filter unit (Millipore, USA), degassed (Shimadzu DGU3, Japan). The sample
202 was injected ($100\text{ }\mu\text{L}$) with an automatic injector (Shimadzu SIL-20A). A Q-Sense E1 (Q-
203 Sense, Sweden) system employing ATcut gold-coated quartz crystal sensors (Lot-Oiel,
204 France) with a nominal frequency of about 5 MHz was used. Absorbance spectra were
205 recorded with a UV-Visible spectrophotometer from Varian Cary 50 Bio. Scanning Electron
206 Microscopy (SEM) with line scanning of energy-dispersive X-ray spectroscopy was produced
207 Leo Elektronenmikroskopie, Oberkoche in Germany. The non-conducting surface of the
208 calcium carbonate particles and capsules was covered with palladium before subject to
209 analysis.

210

211

212 **3. Results and discussion**

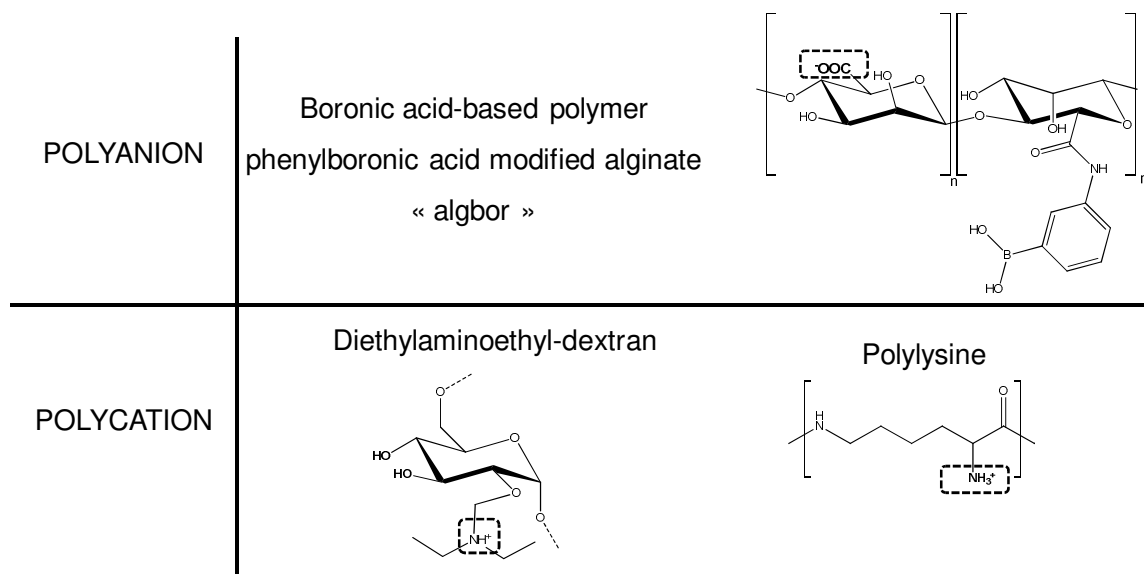
213 As shown in figures 1 and 2 A, our overall strategy consists in the layer-by-layer assembly of
214 charged biopolymer, namely diethylaminoethyl-dextran hydrochloride or polylysine
215 (polycation) and an alginate derivate (polyanion). For providing glucose- and hydrogen
216 peroxyde sensitivity, phenylboronic acid moiety is incorporated inside the alginate. Subjected
217 to these stimuli (figure 2B), the as-obtained multilayer is supposed to totally be removed from
218 the substrate. Then, this strategy is transposed to spherical substrate leading to stimuli-
219 responsive particle.

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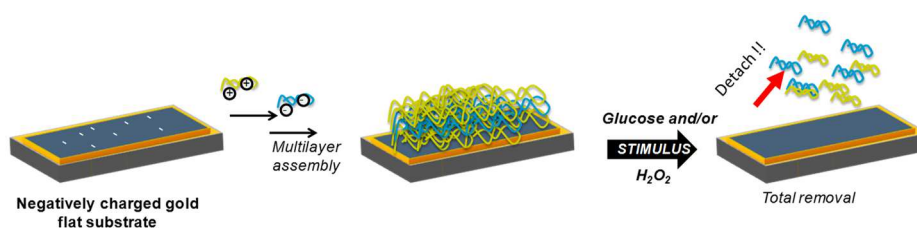


224
225

226 **Figure 1.** Biopolyelectrolytes used for the elaboration of glucose- and/or hydrogen peroxide
 227 responsive substrate namely phenylboronic acid modified alginate as polyanion and
 228 diethylaminoethyl-dextran hydrochloride or polylysine as polycation.

229
230

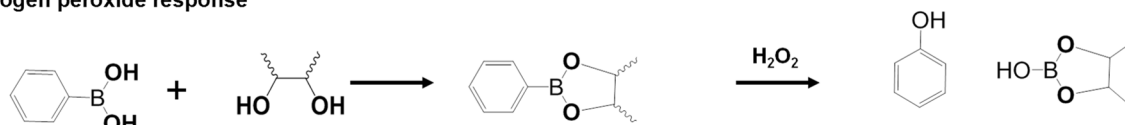
A)



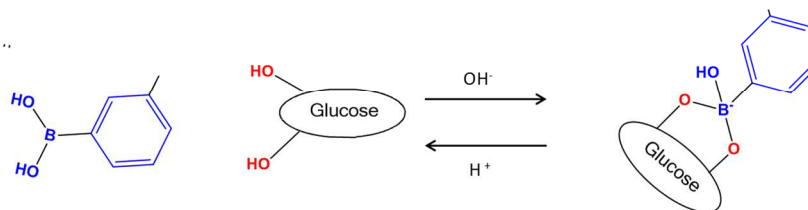
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B)

Hydrogen peroxide response



Glucose response

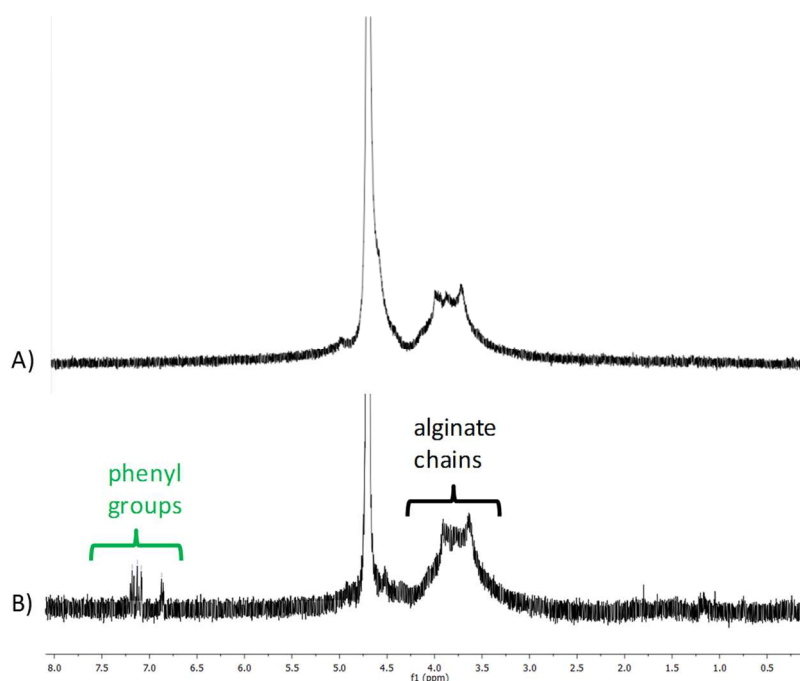


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Figure 2. A) Glucose- and/or hydrogen peroxide responsive multilayer, B) Boronic acid chemistry for obtaining glucose- or hydrogen peroxide response.

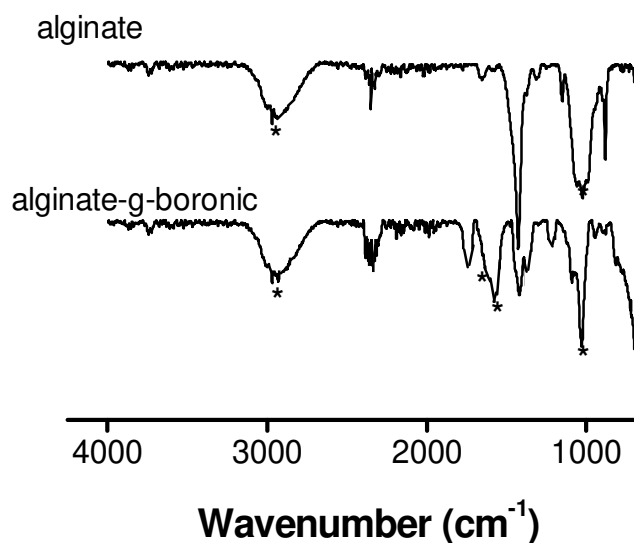
236 Phenylboronic acid pending group was incorporated in the alginate chain by coupling
237 aminophenylboronic acid with the carboxylic acid function of the alginic acid with a
238 condensation reagent namely the [1-ethyl-3-(dimethylamino)propyl]carbo-diimide
239 hydrochloride. [37, 38] After a purification process (dialysis and freeze drying), a white
240 chemical is obtained and labeled alg-bor (Figure 3A). The ^1H NMR spectra show the presence
241 of both chemical shift of the protons of the alginate chain (from 3 to 4 ppm) and phenyl
242 groups (from 6.7 to 7.2 ppm) (Figure 3A). From this technique, the presence of the aromatic
243 group is qualitatively checked and allows estimating a degree of substitution which is found
244 to be around 20%. [20] From FTIR spectra (Figure 3B), we show the presence of two peaks at
245 867 cm^{-1} and 945 cm^{-1} which are assigned respectively to the guluronate and mannuronate
246 residues of alginate and alg-bor samples and a band around 3300 cm^{-1} attributed to the -OH
247 groups. The C-O stretching vibration of alcoholic hydroxyl group and C-O-C stretching
248 vibration remain unchanged, i.e. at 1143 cm^{-1} and 1023 cm^{-1} respectively. Moreover, a new
249 peak attributed to the C-N stretching vibration (at 1080 cm^{-1}) appeared after the chemical
250 modification. At 1595 cm^{-1} and 1412 cm^{-1} peaks obtained are correlated to N-H deformation
251 and C-N stretching vibrations. The presence of the carbonyl group from the amide is observed
252 at around 1740 cm^{-1} .

253 A)



254
255
256
257

258 B)



259

260 **Figure 3.** A) ¹H NMR spectrum (D₂O) of (A) alginate and (B) phenylboronic acid modified
261 alginate; B) FTIR spectra of alginate and phenylboronic-modified alginate derivative.

262

263

264 **Multilayers self-assembly on flat gold surfaces**

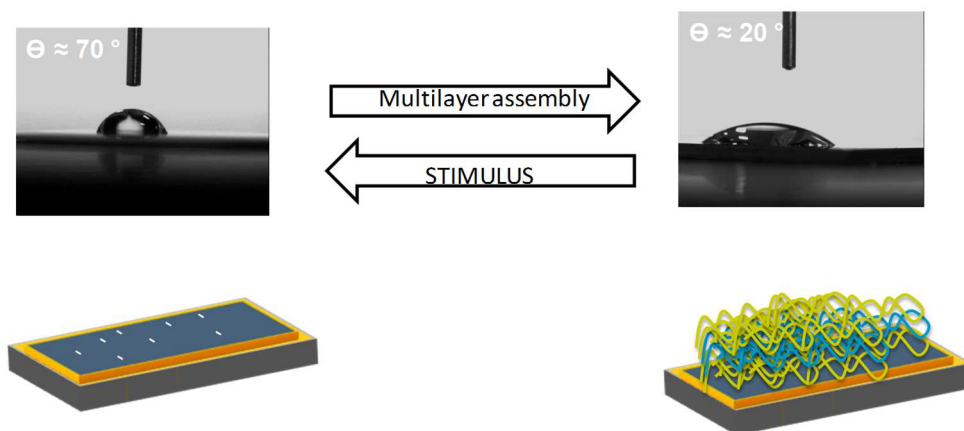
265 The Lbl process is performed in an aqueous solution between obtain alginate derivative
266 (presence of carboxylate groups) and diethylaminoethyl-dextran hydrochloride or polylysine
267 (presence of ammonium group) as oppositely charged polyelectrolytes. Electrostatic
268 attractions are then implied as the driving force during the multilayer assembly. First, the gold
269 substrate is pretreated with a sulfonated thiol derivative leading to high density of negative
270 surface charges. This enables to adsorb the polycation as the first layer, and then this is
271 followed by the adsorption of the alginate derivative (polyanion). This step is repeated to form
272 the multilayer build-up.

273 To demonstrate the efficiency of this method, the LbL assembly is investigated by wettability
274 and then in real time by QCM experiments on a flat surface.

275 Static contact angle measurement are performed on the flat gold pretreated substrate before
276 and after the multilayer build-up with (alg-bor/diethylaminoethyl-dextran hydrochloride)₅ or
277 (alg-bor/polylysine)₅ to show qualitatively the elaboration of the multilayer film onto the
278 substrate. The contact angle values decreased from 70°±2° to 20°±3° upon the coating
279 independently of the polyelectrolytes pair. This result could be correlated to the hydrophilic
280 nature of these polysaccharides (Figure 4). Interestingly, the flushing of this modified
281 substrate with a solution of glucose or hydrogen peroxide results in a contact angle values
282 above 70°±2° (i.e. removal of the multilayer from the surface). Note that flushing this

283 modified substrate with the buffer does not lead to a change in the contact angle value (i.e.
284 remains near $20^{\circ} \pm 3^{\circ}$).

285



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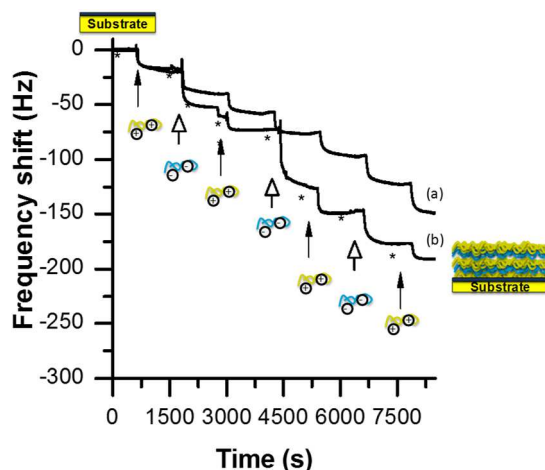
287 **Figure 4.** Wettability measurement on bare gold substrate ($70^{\circ} \pm 2^{\circ}$) and after the multilayer
288 film build-up ($20^{\circ} \pm 3^{\circ}$).

289

290

291 Then, the LbL assembly was monitored by QCM experiments to (i) demonstrate the stepwise
292 adsorption of polycation/ phenylboronic acid-containing alginate polyelectrolytes pair, (ii)
293 investigate the adsorption kinetics and (iii) evidence the impact of glucose or hydrogen
294 peroxide on the resulting multilayer film. In aqueous solution, the implied build-up procedure
295 ensures both the presence of oppositely charged carboxylate and ammonium groups and, that
296 boronic acid function remained uncharged during the layer-by-layer buildup (pK_a of
297 phenylboronic acid 8.9). [39] Upon addition of the polyelectrolytes, a decrease of the frequency
298 signal is seen, showing that mass was being added onto the surface (Figure 5). Seven layers of
299 the polyelectrolytes were then successfully deposited onto the quartz substrates. After
300 adsorption, the final frequency shift is around -150 Hz and -200 Hz for (phenylboronic acid
301 modified alginate/ polylysine)_n and (b) (phenylboronic acid modified alginate/
302 diethylaminoethyl-dextran hydrochloride)_n respectively.

303



304

305 **Figure 5.** Evolution of the frequency shift values vs time during the successive
 306 polyelectrolytes addition, (a) (phenylboronic acid modified alginate/ polylysine)_n and (b)
 307 (phenylboronic acid modified alginate/ diethylaminoethyl-dextran hydrochloride)_n (standard
 308 deviation was always lower than 5%).

309

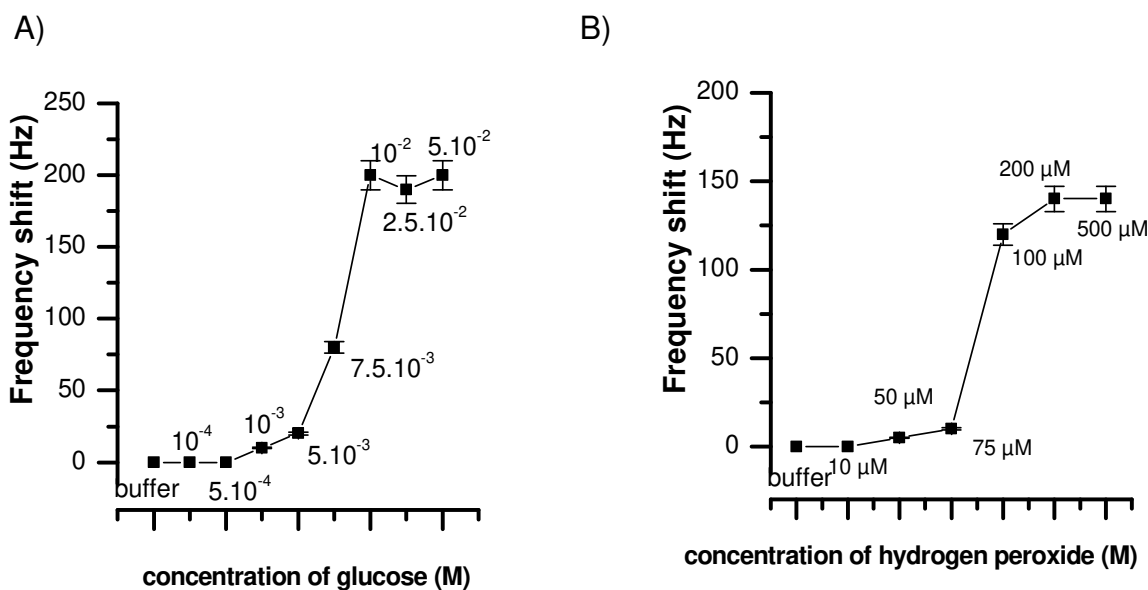
310 To demonstrate the glucose and/or hydrogen peroxide sensitivity, various concentrations of
 311 these chemicals were flushed on the resulting multilayer film (Figure 6). As discussed before,
 312 the final equilibrium frequency shift values after the LbL build-up of the dextran
 313 derivative/alg-bor films were -200 Hz. These values can then be used as references to show
 314 either the glucose or hydrogen peroxide response of the films. Indeed, the total destabilization
 315 of the films should involve a variation of the equilibrium frequency shift of about +200 Hz.
 316 The same hypothesis could be used if implying (phenylboronic acid modified alginate/
 317 polylysine)_n as polyelectrolytes pair.

318 As observed from figure 6A and 6B, the frequency shift parameter increases upon exposing
 319 the film to both glucose and H₂O₂ to finally reach a stable value. The critical concentration is
 320 found to be less than 100 μM for H₂O₂ and around 10⁻² M for glucose, meaning near
 321 biologically relevant chemical concentrations. Our results are in accordance with the results
 322 obtained by (i) Levy et al. who studied glucose-sensitive system based on mannan and
 323 phenylboronic acid moieties grafted onto a polyacid, [25] (ii) De Geest et al. who fabricated
 324 glucose-responsive layer-by-layer assembly of a copolymer of dimethylaminoethylacrylate
 325 and acrylamidophenylboronic acid and poly(styrene sulfonate) and (iii) Sato et al. who
 326 designed hydrogen peroxide responsive multilayer film based on phenylboronic acid-bearing
 327 poly (allylamine) and poly (vinyl alcohol). [40] The film decomposition upon the addition of
 328 these chemicals is then successfully demonstrated. In contrast, in a buffer solution meaning
 329 without H₂O₂ nor glucose, the frequency shifts does not change indicating that the multilayer
 330 film remained stable on the substrate. These results clearly pointed out that these films are

331 sensitive under oxidizing condition and glucose condition (explained through the suggested
332 mechanism in Figure 2B).

333 To our point of view, the as-observed mass behavior change is mainly due to the
334 incorporation of glucose within the multilayer films through complexation with the phenyl
335 boronic moieties. This assumption is confirmed by the fact that no significant frequency shift
336 was seen upon flushing glucose solutions with high concentration (up to 0.1 M) over
337 multilayer films composed by diethylaminoethyl-dextran hydrochloride and native alginate.

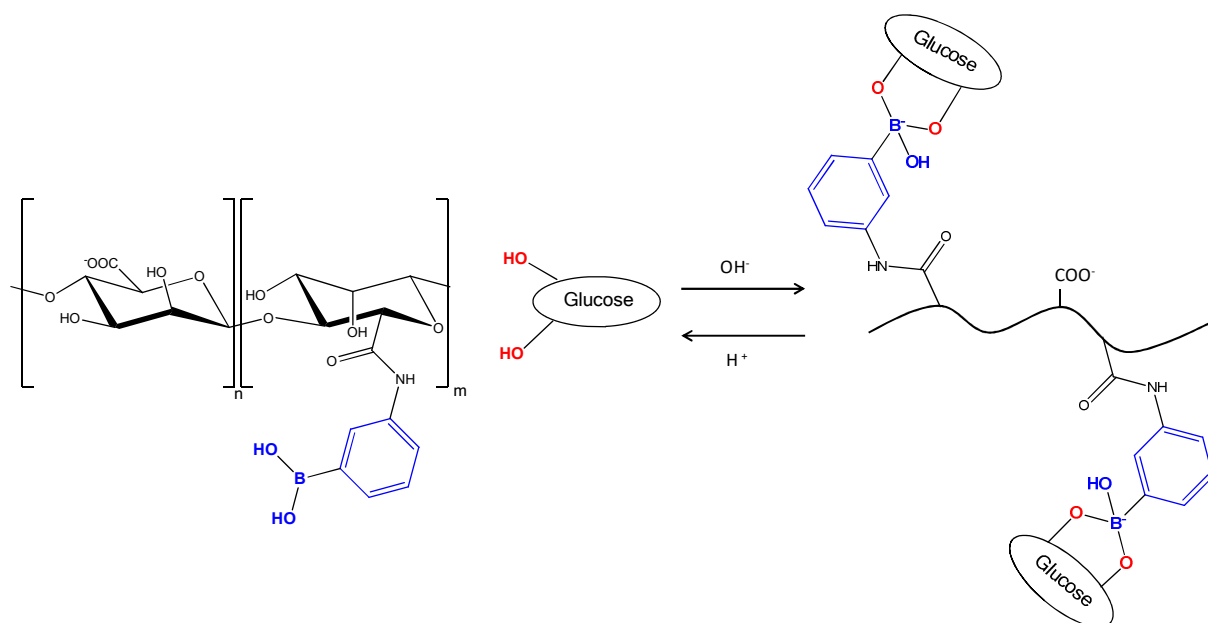
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341
342 **Figure 6.** Effect of increasing concentrations of (A) glucose and (B) hydrogen peroxide (NB:
343 no variation of the frequency without glucose or hydrogen peroxide)

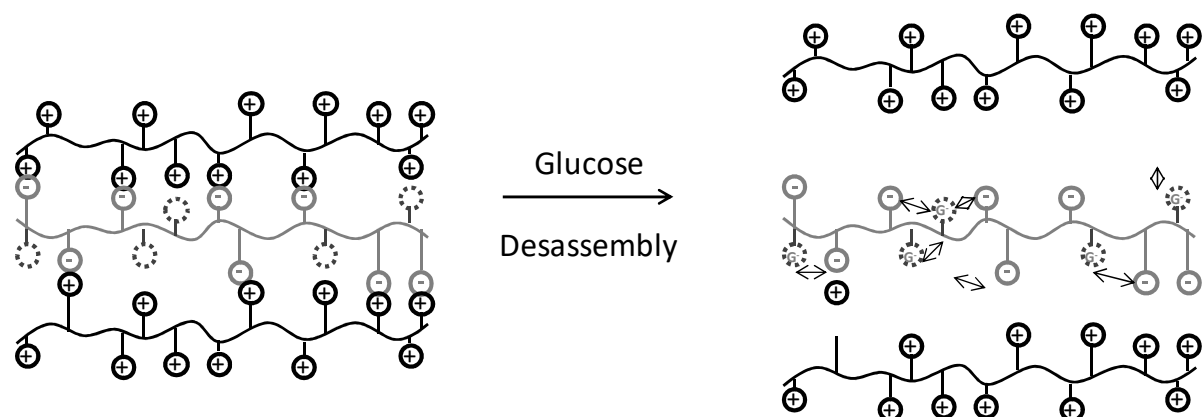
344
345
346 Figure 7 shows how phenylboronic acid- containing alginate interacts with the diol of the
347 glucose.[41] The result strongly suggests that there is formation of boronate ester bonds
348 during the contact with the flushing with glucose which leads to (i) the complexation caused
349 by the electrostatic interactions in the anionic glucose/borate complex and (ii) repulsion
350 between the carboxylate groups of the alginate and the glucose/borate complex. The
351 combination of these two phenomena seems to result in repulsion phenomena between the
352 multilayers and then to the multilayer removal (Figure 8).

353



354
 355 **Figure 7.** Equilibrium between phenylphenylboronic acids- containing alginate and diols-
 356 based glucose in aqueous solution.

357
 358

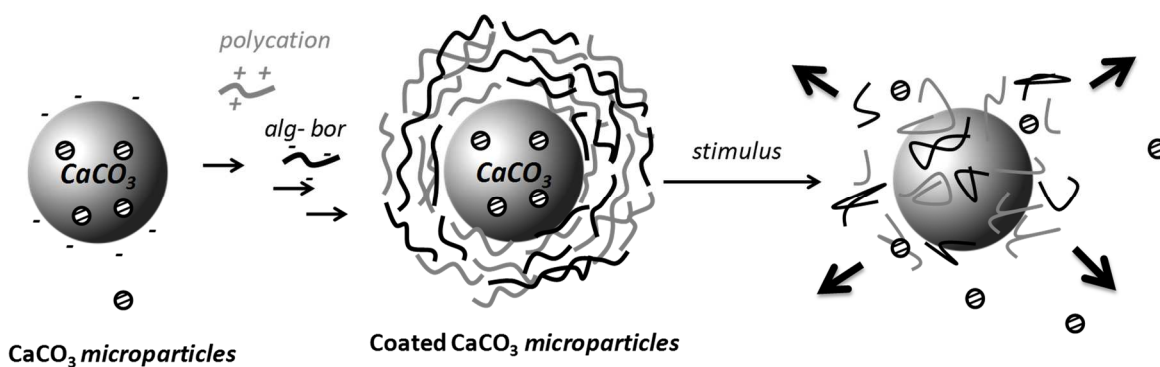


359
 360 **Figure 8.** Proposed mechanism of the glucose-induced disassembly of (phenylboronic acid
 361 modified alginate/ polylysine)_n

362
 363 The hydrogen peroxide response could be explained by the scheme presented in Figure 2B.

364
 365 **Multilayers self-assembly onto spherical surfaces**
 366 (diethylaminoethyl-dextran hydrochloride / phenylboronic acid-based alginate) and
 367 (polylysine / phenylboronic acid-based alginate) polyelectrolytes pairs have been evidenced to
 368 form multilayer film and to present glucose- or hydrogen peroxide responsive behavior.
 369 Further, these conditions will be implied for the fabrication of smart particles (Figure 9).

370



371

372 **Figure 9.** Schematic illustration of the elaboration of the stimuli-responsive microcapsules
373 based on phenylboronic acid containing polyanion.

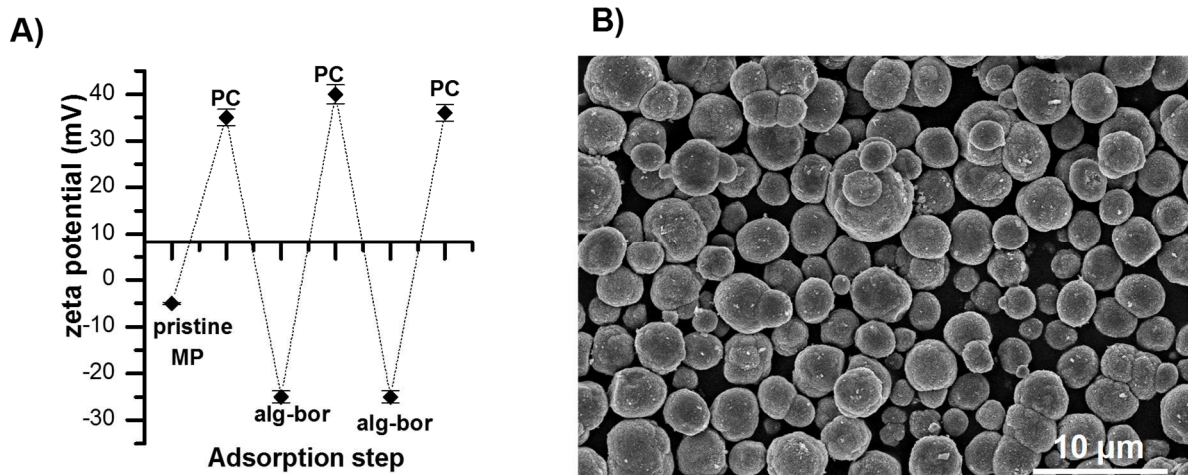
374

375 CaCO₃ particles have been used for the fabrication of responsive microparticles due to their
376 intrinsic porosity which may enable the entrapment of small molecules. They can easily
377 synthesize in a reproducible and size-controlled way. Furthermore, they are non-toxic. As
378 shown in Figure 9, the CaCO₃ core particle was coated with the studied polyelectrolyte
379 bilayers. The conditions of the multilayer deposition were identical to those implied during
380 the coating of the quartz surfaces but no pre-coating step with the thiol derivative was
381 required. Indeed, pristine CaCO₃ particles present negative surface charges allowing the direct
382 deposition of positively charged polyelectrolytes as pointed by the measured zeta-potential
383 value of -5 mV for the uncoated calcium carbonate material (Figure 10A). This parameter was
384 then measured after each adsorption step to evidence the successive deposition of each
385 polyelectrolyte on the particles surface. [42] A variation of the zeta-potential value is
386 observed i.e. between +40 and -20 mV upon successive adsorption of polycation and alg-bor,
387 respectively. As seen from SEM picture, the as-obtained microparticles present well-defined
388 spherical shape and micrometer size (Figure 10B).

389

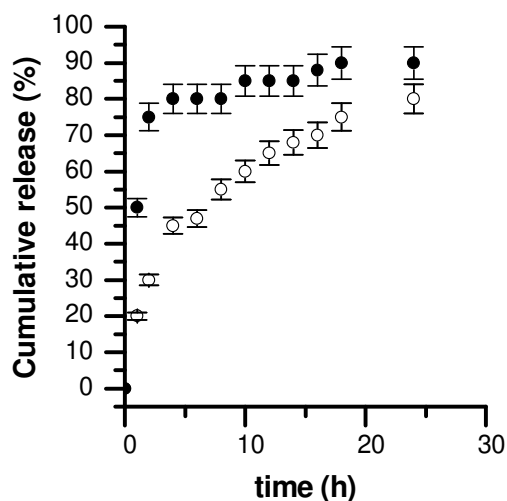
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392
 393 **Figure 10.** A) Zeta potential variation according to the adsorption step during the film build
 394 up on the CaCO_3 microparticles (PC = polycation and MP = microparticles). B) SEM images
 395 of modified microspheres constituted by (polycation/alg-bor)_n polyelectrolytes pair.
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 398
 399 The release rate of the entrapped probe within the interior cavity of the LbL coated
 400 microparticles may be highly impacted by the permeability of the material. For
 401 polyelectrolytes LbL assemblies, the inter-polyelectrolyte interactions can be tuned by various
 402 stimuli as pH or ionic strength. One may cite as example, particles based on
 403 alginate/polylysine or DNA/spermidine which were evidenced to provide a cargo release due
 404 to change in salt concentration. [43] In the present work, the release behavior of the coated
 405 microparticles loaded with the rhodamine b derivative inside its cavity is supposed to be
 406 triggered by both glucose and hydrogen concentration. Initially, the rhodamine b was loaded
 407 within the porous structure of the CaCO_3 material prior to coat it with the multilayer film. The
 408 entrapment efficiencies efficiency of the material are is up to 60% and the probe loading is
 409 above 4 % (determined via equations 1 and 2). Without chemical stimulus the coated
 410 microparticles stay stable meaning that the remained loaded with the probe and those even
 411 after more than two weeks. However, when the coated microparticles are suspended in
 412 glucose- or hydrogen peroxide containing solution a release of the rhodamine derivative is
 413 evidence. It deserves noting that the threshold concentrations of each of this chemical has
 414 been estimated during the QCM experiment. These concentrations were used for monitoring
 415 the rhodamine b release. A fast probe release of 85% is observed after 24 h up to this critical
 416 concentration. The probe is progressively released below this critical concentration. This
 417 result is illustrated in Figure 11.



418
 419 *Figure 11: Release of rhodamine b from ((phenylboronic acid modified alginate/ polylysine)_n*
 420 *particles in presence of glucose solution at ● $7.5 \cdot 10^{-3} M$ and ○ $10^{-2} M$.*

421
 422

423 **4. Conclusion**

424

425 Novel multilayer assemblies based on alginate derivative (chemically modified to introduce
 426 phenylboronic acid group) have been implied for the design of both glucose- and hydrogen
 427 peroxide responsive system. Herein, the implemented strategy is based on the alternative
 428 adsorption of the polycation and the phenylboronic acid-containing polyanion on the surface
 429 of CaCO₃ microparticles. After each deposition, the reversal of the surface charge was shown
 430 by zeta potential measurements, which is in accordance with the successful multilayer
 431 assemblies. Spherical shape was evidenced by SEM measurements. The glucose- and
 432 hydrogen peroxide sensitive character was related to these chemicals concentration in the
 433 surrounding buffer and implied due to the phenylboronic acid incorporation inside the
 434 polymer chain. The present work mainly focuses on the chemical modification and the
 435 physico-chemical aspect of the systems, a biological study will further be done. Indeed, the
 436 novel smart microparticle may further be employed for specific biological applications. One
 437 may cite diabetes disease (glucose-sensing device) or to detect oxidative stress (hydrogen
 438 peroxide-sensing system).

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446 **References**

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