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1 **Glucose-Sensitive Capsules Based on Hydrogen-bonded** 2 **(Polyvinylpyrrolidone / Phenylboronic –modified Alginate) System**

3 *Sabrina Belbekhouche,* Saddam Charaabi, Benjamin Carbonnier*

4
5 Université Paris Est, ICMPE (UMR 7182), CNRS, UPEC, F-94320 Thiais, France

6
7 Corresponding authors:

8 * Sabrina Belbekhouche

9 Université Paris Est, ICMPE (UMR7182), CNRS, UPEC, F-94320 Thiais, France

10 Tel : +33 (0)1 49 78 11 49.

11 Fax: +33 (0)1 49 78 12 08.

12 E-mail address: belbekhouche@icmpe.cnrs.fr

13
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15 **Abstract.** (~~167~~ 191 words)

16 Glucose-responsive multilayer capsules have been produced through the layer-by-layer
17 deposition of biodegradable polymers (~~alginate derivative and polyvinylpyrrolidone, PVPON~~)
18 based on hydrogen bonding. The used polymers are alginate derivative and
19 polyvinylpyrrolidone (PVPON). Concentration-dependent glucose responsiveness was reached
20 through chemical modification of alginate, selected as polyanion, with phenylboronic acid
21 moieties (known to enable complexes with diols derivative). ~~This strategy was implemented~~
22 ~~for the fabrication of smart capsules employing calcium carbonate microparticles as a~~
23 ~~removable core template. Glucose responsiveness was induced in the polymer assembly~~
24 ~~through chemical modification of alginate with phenylboronic acid moiety.~~ Herein, we **Quartz**
25 ~~crystal microbalance measurements~~ demonstrated that the alginate derivative self-assembles
26 with the PVPON in accordance with (i) the formation of hydrogen bonding layer-by-layer
27 assembly, and (ii) also evidence an enhancement of the stability **versus the pH**. We
28 demonstrate that incorporating boronic acid moieties inside a polymer chain allows (i)
29 glucose-responsiveness and (ii) using boronic moieties as hydrogen bond acceptor. ~~This point~~
30 ~~is very appealing since H-bonds are classically stable in acidic media.~~ This strategy was
31 implemented for the fabrication of smart capsules employing calcium carbonate
32 microparticles as a removable core template. This later ~~strategy~~ enables to consider the
33 loading of two probes (~~rhodamine b and methylene blue hydrate~~). The porosity of the calcium
34 carbonate microparticles ~~this~~ template has been used to load the first probe (rhodamine b) and
35 the other one has been loaded through electrostatic interaction with the carboxylate moiety in
36 the alginate (**methylene blue hydrate**). Insulin has also been loaded as chemical of interest to
37 demonstrate the interest of the present strategy.

38
39 **Keywords:** capsule; glucose-responsive; poly(vinylpyrrolidone); phenylboronic acid
40 chemistry; alginate; hydrogen bonding

41 1. Introduction

42 The layer-by-layer (LbL) technique for the production of polymeric multilayer microcapsules
43 has attracted great attention in the development of drug delivery systems (DDS). Indeed, LbL
44 self-assembly is a versatile method enabling the production of multilayered thin films with
45 controlled structure, thickness and composition. [1] A key challenge for the application of
46 capsules as a responsive DDS is to stabilize them at physiological conditions, as in an
47 intracellular environment, while also being able to trigger film disassembly with an external
48 stimulus, releasing the encapsulated substances. The driving force of LbL deposition is not
49 restricted to ~~attraction-by~~ electrostatic forces,[2] but can include others ~~binding-interactions~~
50 such as covalent bonding, biological affinity and hydrogen bonding. [3].

51 Hydrogen-~~bonding bonded~~ LbL assembly was first highlighted by Rubner and Zhang
52 simultaneously in 1997. [4, 5] This assembly method is based on alternate deposition of
53 polymers with either a hydrogen bond donor or a hydrogen bond acceptor. [6]

54 An important characteristic of hydrogen-~~bonding bonded~~ LbL multilayer films is that they are
55 very sensitive to ionic strength and pH, which can be an advantage for the loading and release
56 of chemicals of interest from these films. [7] Since hydrogen-~~bonding bonded~~ LbL films are
57 often composed of a polyacid (carboxylic acid moiety) as hydrogen bond acceptor, increasing
58 the pH above the pKa of the polyacid leads to the disruption of hydrogen bonding by
59 deprotonation of the carboxylic acid group. [6] Consequently, most of the hydrogen-~~bonding~~
60 ~~bonded~~ LbL multilayers are not stable under physiological conditions, which might be an
61 issue. Some strategies have been developed to overcome this problem. One example is
62 crosslinking the multilayer film [8] [3] [9, 10] However, developing a strategy to allow
63 stability of hydrogen-bonded multilayer films under physiological conditions is still of major
64 interest.

65 The glucose-sensitive drug delivery systems use a crucial biological function in living
66 systems and are still a challenging task [11]. ~~Only a few researchers have paid attention to~~
67 ~~glucose as a chemical stimulus for monitoring the physicochemical properties of polymer-~~
68 ~~based assemblies.~~ [12] Phenylboronic acids are recognized to be able to form covalent bonds
69 with polydiols such as polysaccharides under specific physicochemical conditions [12].
70 Indeed, the complexation is only possible in a pH range ~~above~~ of the pKa of the boronic
71 moieties (pKa~9) [11-13]. ~~Under this condition, there is an equilibrium between an uncharged~~
72 ~~and a charged form of this chemical (phenylboronic vs phenylboronate). Complexation with~~
73 ~~glucose is able to shift the equilibrium in the direction of increasing charged phenylborates~~

74 due to the fact that only charged borates can form a stable complex with glucose in aqueous
75 solution. Most of the developed strategies implied the total destabilisation of the film upon
76 contact with a sugar solution above a critical concentration.[12, 14] A complementary
77 approach to the sugar-induced total dissolution of multilayered has recently been proposed by
78 Belbekhouche et al. [15] We may then cite some example of work. Levy et al. reported on the
79 preparation of glucose-sensitive capsules, stabilized through ester bonds formed between
80 phenylboronic acid moieties grafted onto poly(acrylic acid) and mannan [14]. The
81 competitive binding of saccharides (~~fructose, glucose, mannose and galactose~~) and mannan
82 with the boronic acid moiety within the multilayer assembly induce destabilization of the
83 film. ~~Glucose sensitive capsules were also~~ De Geest et al. produced by ~~the layer by layer~~
84 ~~technique, i.e.~~ assembly of a poly(styrene sulfonate) ~~as the polyanion~~ and a copolymer of
85 acrylamidophenylboronic acid and dimethylaminoethylacrylate ~~as the polycation~~. ~~De Geest~~
86 ~~found evidence of~~ The glucose responsiveness due to a change in the electrostatic interactions
87 between the phenylboronic acid-containing polycation and the polyanion [12]. Belbekhouche
88 et al. reported ~~the glucose sensitive microcapsules~~ assemblies based on ~~biopolymers, i.e. an~~
89 ~~alginate derivative containing phenylboronic acid side group and chitosan.~~ [15] ~~The glucose-~~
90 ~~sensitive behaviour correlated to the glucose concentration in the surrounding buffer,~~
91 ~~involving~~ The glucose response was correlated to a variation of the swelling behaviour
92 ~~according to the crosslinking time, as well as the average chitosan molar mass and the~~
93 ~~acetylation degree~~. Herein, we suggest a complementary approach to the sugar-induced total
94 dissolution of multilayered films.

95 ~~In this context and with~~ With the final aim of creating a glucose-sensitive DDS system, our
96 research consisted of building-up and characterizing, degradable LbL thin films based on
97 phenylboronic-modified alginate derivative and PVPON, a pair of polymers that forms
98 hydrogen bonds. The chemical modification of this alginate is reported [15] but no study
99 explores the potential use of this modified alginate containing both carboxylic acid and
100 boronic acid moieties, according to the pH, for producing a hydrogen-bonded multilayer film
101 with new properties (i.e. both glucose responsiveness and enhanced stability against high pH).
102 ~~This research aims to increase the LbL multilayer film stability against high pH.~~ This is
103 crucial as the oral administration of glucose-responsive DDS containing a drug such as insulin
104 requires protection against acid condition as in the gastric environment, ~~i.e. for crossing the~~
105 ~~stomach, and release in intestinal media for promoting insulin uptake~~. [16]. In the present
106 work, in comparison with our previous study implying chitosan and phenylboronic acid-

107 containing alginate, [15], we produce a glucose-responsive system based on PVPON instead
108 of chitosan. ~~Employing this biopolymer requires a multilayer film crosslinking step to allow~~
109 ~~stability against high pH or ionic strength and swelling ability in the presence of glucose.~~
110 Herein, we propose to use PVPON as a hydrogen bond acceptor and to use the phenylboronic
111 -modified alginate for simultaneously furnishing two types of hydrogen bond donors
112 according to the pH, i.e. acid moiety (boronic acid and carboxylic acid). We expect an
113 increase of stability according to the pH will then solve one of the main issues of using
114 hydrogen-~~bonding bonded~~ LbL multilayers which has poor stability under physiological
115 conditions. ~~Moreover, attaining this stability against high pH does not require an additional~~
116 ~~processing step. As, the~~ driving force of LbL deposition is hydrogen bonding ~~if using PVPON~~
117 ~~instead of chitosan. Then,~~ , this results in particle without any electrostatic charge on the
118 surface (with the PVPON as the last layer). ~~Therefore, this~~ This may be useful to limit
119 interaction with the charged surface of cell walls for instance. ~~PVPON interacts with alginate~~
120 ~~derivative through polar interactions. Furthermore, this~~ multilayer may not be affected by the
121 increase of ionic strength ~~which is not the case if implying chitosan~~ as PVPON was proven to
122 ~~prevent particle aggregation in 0.5 M NaCl which might be correlated to a steric barrier.~~ [17]
123 ~~Additionally, new characteristics can be developed, such as control of the multilayer~~
124 ~~thickness, glucose-responsiveness or even the possibility to load another probe.~~

125 For the first time, we provide evidence that the incorporation of boronic moieties inside a
126 polymer backbone allows both (i) glucose-responsiveness and (ii) increasing stability of
127 hydrogen-~~bonding bonded~~ multilayer films. This is based on the following hypotheses: (i)
128 glucose-responsiveness is expected due to the incorporation of boronic acid moieties, which
129 are known to be able to form covalent bonds with diol derivatives such as saccharides and
130 (ii) the pKa of the boronic moieties is around 9, which is expected to enhance the film's
131 stability against high pH (i.e. use of boronic acid-as hydrogen bond acceptor instead of the
132 well-known carboxylic acid alone).

133 Moreover, the implemented strategy allows the loading of two probes: (i) by using the
134 calcium carbonate template porosity and (ii) inside the multilayer film by taking advantage of
135 the presence of the carboxylate group according to the pH. This second possibility of loading
136 is very appealing. Indeed, compared to reported glucose-systems, the possibility to
137 simultaneously load two probes, one inside the glucose-responsive capsule and the other one

138 in the shell, allows us to investigate the possibility of using the proposed system for
139 theranostic applications.

140

141 2. Experimental

142 2.1. Materials

143 The following chemicals were used as received: alginate (alg^- , according to the data provided
144 by the manufacturer, the mannuronic / guluronic ratio was 1.4 (Cargill (Baupre, France), \overline{Mn}
145 = 270 000 $\text{g}\cdot\text{mol}^{-1}$, $\text{Đ} = 2.3$), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
146 (EDC, Sigma-aldrich, > 98.5%), N-hydroxysuccinimide (NHS, Sigma-aldrich, > 98%),
147 poly(vinylpyrrolidone) (PVPON, $3.6 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$, Sigma-aldrich), poly(ethylenimine) (PEI,
148 Sigma-aldrich), calcium chloride (CaCl_2 , rectapur, 99%), [9-(2-carboxyphenyl)-6-
149 diethylamino-3-xanthenylidene]-diethylammonium chloride (rhodamine b, Alfa Aesar, >
150 98.5%), 4-aminophenylboronic acid hydrochloride (Alfa Aesar, 97 %), acetic acid (Alfa
151 Aesar, >99%), sodium acetate (Alfa Aesar, 99%), methylene blue hydrate (Sigma-aldrich),
152 insulin (bovine pancreas, Sigma-aldrich) and ethylenediaminetetraacetic acid (EDTA, Sigma-
153 Aldrich, 98%).

154 The phenylboronic-modified alginate (alg-bor) (grafting ratio of phenylboronic acid groups
155 about 20%, $\overline{Mn} = 2.5 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$, $\text{Đ} = 2.5$) was synthesized according to the procedure
156 developed by Belbekhouche *et al.* [15]. Average molar masses and molar masses distributions
157 were determined by size exclusion chromatography (SEC) coupled online with multi-angle
158 light scattering (MALS) and differential refractive index (DRI) detectors. The MALS
159 apparatus is the DAWN Heleos-II from Wyatt Technology (Ca, USA) filled with a K5 cell
160 and a Ga-As laser ($\lambda = 690 \text{ nm}$). The DRI detector is a Shimadzu RID-10A (Japan). Columns
161 [Shodex SB OHPak 804 and 806 HQ for alginate, ~~Eprogen-CATSEC-100 and 1000 for~~
162 ~~chitosan~~] were eluted with 0.1 $\text{mol}\cdot\text{L}^{-1}$ LiNO_3 ~~or acetate buffer 0.1M, respectively~~ at 0.5 mL
163 min^{-1} . Eluent was filtered through 0.1 μm filter unit (Millipore, USA), degassed (Shimadzu
164 DGU3, Japan). The sample (1 $\text{g}\cdot\text{L}^{-1}$ for alginate ($\text{dn}/\text{dc} = 0.140 \text{ mL}\cdot\text{g}^{-1}$ [18] filtered on 0.45 μm
165 unit filter (Millipore) was injected (100 μL) with an automatic injector (Shimadzu SIL-20A).
166 Water was purified with a Milli-Q reagent system (Millipore). ~~The modified alginate was~~
167 ~~characterized by FT-IR, ^1H NMR and Total Organic Carbon analyses (TOC) and Total~~
168 ~~Nitrogen (TN) analysis. Fourier transform infrared spectra were recorded on a Bruker Tensor~~
169 ~~27 instrument equipped with a Digi TectDLatGS detector. ^1H NMR spectra were recorded~~

170 on a Bruker Avance 300 NMR spectrometer. ¹H NMR (Bruker Avance 300MHz, Ge) was
171 used to estimate the Degree of Substitution (DS) of the modified alginate with solutions at 10
172 g/L in D₂O. Total organic carbon analyses (TOC) and total nitrogen (TN) analysis were
173 performed on a Shimadzu TOC-L CSN instrument (ten measurements were performed).

174

175 **2.2. Fabrication of hollow "glucose-responsive" capsules.**

176 Negatively charged CaCO₃ microparticles were prepared according to Volodkin et al. [19] ~~De~~
177 ~~Geest et al. [12]~~ Equal volume of 0.33 M calcium chloride (CaCl₂) and sodium carbonate
178 (Na₂CO₃) solutions were mixed under a vigorous stirring for 3 min. This method led to a rapid
179 precipitation of the CaCO₃ microparticles. A centrifugation process of the suspension during
180 10 min allows removal of the residual ions. Lastly, the resulting particles were rinsed several
181 times with water. Acetone was used to remove the water. Particles were dried under a stream
182 of filtered air.

183 The hydrogen-bonded LbL films were based on the alternate deposition of polymers with a
184 hydrogen bond acceptor (PVPON) and a hydrogen bond donor (phenylboronic –modified
185 alginate). As hydrogen bond acceptor, PVPON presented the carbonyl group. Interestingly,
186 according to the pH, phenylboronic –modified alginate furnished two types of hydrogen bond
187 donors, i.e. acid moieties (boronic acid and carboxylic acid). The pKa of alginate and boronic
188 acid are above 3-4 and 9, respectively. Two pH were then investigated, i.e. 2 or 8, to evidence
189 the possibility to use boronic acid moiety as hydrogen bond donors. Indeed, at pH 2, the
190 carboxylic and the boronic acid forms were obtained. Then, they both could be implied as
191 hydrogen bond ~~acceptor~~ donor. At pH 8, the carboxylate and boronic acid forms could be
192 considered. Therefore, only boronic acid moiety could be used as hydrogen bond ~~acceptor~~
193 donor.

194 ~~These~~ microparticles were first pretreated with PEI. More precisely, 10 mg of CaCO₃ particles
195 were incubated in 500 μL of water. The first layer of PEI (1 mg.mL⁻¹) was deposited onto the
196 surface of the calcium carbonate particles by adding 500 μL of this polycation stock solution
197 to the particles during 15 min. At pH 2 (below pKa of alginate), the alginate derivative (alg-
198 bor) ~~presenting then carboxylic acid~~ moiety will be adsorbed on the PEI layer through
199 hydrogen bond whereas at pH 8 (up to pKa of alginate) the polysaccharide derivative will be
200 absorbed through electrostatic interaction.

201 The excess of PEI was then removed by centrifugation process (5 min, 10 000 rpm) and
202 particles were washed two times with water (5 min). Note that the final volume was always

203 kept at 1 mL and the aqueous solution used for the rinsing is water adjusted at pH 2 for the
204 build-up at pH 2 with few droplets of HCl 100 mM and 8 for the build-up at pH 8. After the
205 second rinsing, the particles were suspended in aqueous solution and 500 μL of alg-bor (1
206 $\text{mg}\cdot\text{mL}^{-1}$) was added to the particles suspension and the mixture was stirred for 15 min for
207 alg-bor deposition on the previous PEI layer. The excess of polymers was removed by
208 centrifugation process (5 min, 10 000 rpm), after each adsorption process, by two washing
209 steps with aqueous solution each during 5 min. The particles were suspended in aqueous
210 solution and 500 μL of PVPON (1 $\text{mg}\cdot\text{mL}^{-1}$) was added and the mixture was stirred for 15
211 min. This process describes the assembly of a single bilayer: (alg-bor /PVPON)₁ and was
212 repeated to obtain different number of assembled bilayers.

213 ~~Multilayer buildup was then performed by a coating of hydrogen bonded film from aqueous~~
214 ~~solutions of 1 $\text{mg}\cdot\text{mL}^{-1}$ alginate derivative and 1 $\text{mg}\cdot\text{mL}^{-1}$ poly(vinylpyrrolidone). The excess~~
215 ~~of polymers was removed, after each adsorption process, by two washing steps.~~ After the LbL
216 construction with the desired number of (alg-bor/PVPON) bilayers, the CaCO_3 core was
217 dissolved overnight using 0.2 M EDTA followed by a dialysis washing step (3.5 kDa cut off)..
218 The adsorption of the PEI and then alg-bor (at pH 8) was monitored by zeta potential
219 measurement (a Zetasizer Nano-ZS, Malvern Instrumente, equipped with a He-Ne laser, i.e.
220 $\lambda = 633 \text{ nm}$; scattering angle, 173°).

221 For the proof-of-concept, wetting contact angle and QCM were used. This last technique was
222 employed to monitor the hydrogen-bonding-bonded multilayer buildup and to investigate the
223 glucose-response. A Q-Sense E1 (Q-Sense, Sweden) system employing AT_{cut} gold-coated
224 quartz crystal sensors (Lot-Oriel, France) with a nominal frequency of about 5 MHz was used.
225 The gold coated QCM Sensor Crystal was cleaned (i) with water and ethanol and (ii) in a
226 UV/ozone chamber for 20 min. After an additional rinsing with ethanol, surfaces were dried
227 under N_2 flow and pretreated overnight with a solution containing the anchoring layer
228 (solution of PEI, 1 $\text{mg}\cdot\text{mL}^{-1}$ prepared in water). After mounting in the QCM flow chamber,
229 the sensor was conditioned in the solvent (pH 2 or pH 8) for 10 min. The self-assembly of alg-
230 bor /PVPON was performed onto flat gold substrate through a flushing of stock solutions of
231 polymers (1 $\text{mg}\cdot\text{mL}^{-1}$) for 15 min injected into the cell with a flow rate of 500 $\mu\text{L}\cdot\text{min}^{-1}$. A
232 rinsing step with aqueous solution was performed between each polymer deposition step
233 during 10 min. ~~Stock solutions of polymers (1 $\text{mg}\cdot\text{mL}^{-1}$) were injected into the cell with a~~
234 ~~flow rate of 500 $\mu\text{L}\cdot\text{min}^{-1}$.~~ After the multilayer build-up, the resulting film was exposed to a
235 gradual increase of concentration of glucose. The measurements of wetting contact angle were

236 carried out at room temperature with a Kruss goniometer in the sessile drop configuration. For
237 the wettability study, the multilayer film was performed onto flat gold substrate pretreated
238 with PEI through the dipping of stock solutions of polymers (1 mg.mL^{-1}) for 15 min. A
239 rinsing step with aqueous solution was performed between each polymer deposition step. A
240 droplet ($5 \text{ }\mu\text{L}$) of Milli-Q water was deposited onto the resulting modified surface and the
241 recorded image was analyzed by the Drop Shape Analysis software to determine the contact
242 angle value. An average of three measurements was performed on each modified surface.

243

244 **2.3. Probes/Drug-loaded microcapsules.**

245 *Loading inside the capsule.* A 0.5 mg of rhodamine b was dissolved in 5 mL of deionized
246 water. 10 mg of microparticles were added to the solution and stirred for 12 h. The CaCO_3
247 microparticles were then centrifuged (5 min, 10 000 rpm) and washed with deionized water
248 three times to remove free probe. The LbL process was conducted as described in section 2.4
249 2.2. The same process was used if loading insulin instead of rhodamine derivative. Both was
250 loaded at pH = 4.

251

252 *Loading in the capsule wall.* Methylene blue hydrate was entrapped in the capsule ~~partiele~~
253 shell through a diffusion process/electrostatic interaction of this molecule from solution
254 through the $(\text{alg-bor/PVPON})_n$ multilayer at pH 8 ($\text{pH} > \text{pKa}_{\text{alginate}}$) (interaction between the
255 carboxylate group of the alginate and the cationic function of the methylene blue). A solution
256 containing $(\text{alg-bor/PVPON})_n$ ~~partieles~~ microcapsules were introduced into a dialysis tubing.
257 A concentrated methylene blue hydrate solution (0.5 g.L^{-1}) was used as dialysate for 48 h at
258 room temperature. ~~The unloaded probe was removed by a dialysis step.~~ The loaded
259 microcapsules were subjected to absorbance measurement at 663 nm (Varian, Cary 100).

260 The probe loading, as well as the entrapment efficiency, are estimated as follows (equations 1
261 and 2).

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

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

264

265 To investigate the release behavior, the loaded microcapsules were introduced in a dialysis
266 bag and immersed in glucose solution at pH 8 or 2, keeping the final dialysate volume equal
267 to 400 mL. The involved glucose concentration, named "critical glucose concentration" will

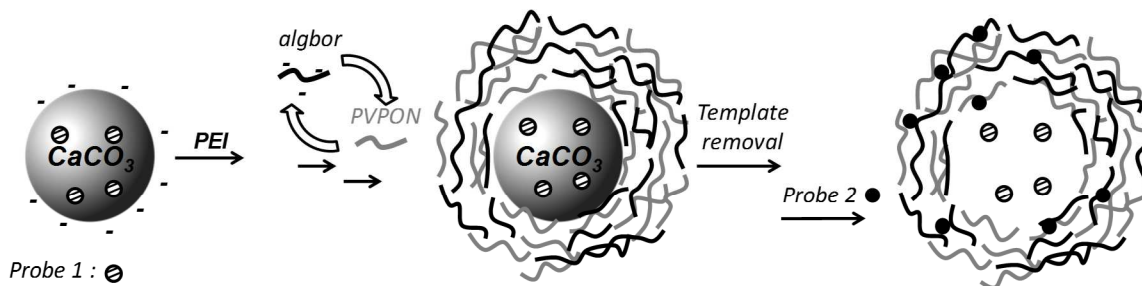
268 further be determined by QCM measurement. At predetermined intervals, 1 mL of solution
269 outside the dialysis bag was taken out and replaced with 1 mL of fresh buffer solution.

270 Absorbance spectra were recorded with a UV-Visible spectrophotometer from Varian Cary
271 100 (at 554 nm for the rhodamine derivative and 663 nm for the methylene blue hydrate
272 solution). Insulin concentration was estimated using the Bradford assay. [16, 20] Insulin
273 samples and Bradford reagent were admixed at 1:1 (v/v) ratio and incubated at room
274 temperature for 15 min. The absorbance was measured at 595 nm. Images of the capsules
275 were taken with an optical fluorescence ZEISS microscope equipped with a CCD camera and
276 a fluorescence illuminator and various mirror units. Images were recorded at $\times 630$
277 magnification and processed using the ZEN software. A 5 μL drop of capsules suspension
278 was placed on a coverslip to visualize the microcapsules. ~~To visualize the microcapsules, a 5~~
279 ~~μL drop of capsule suspension was placed on a cover slip. To assess the glucose-responsive~~
280 ~~behavior of the microcapsules, a~~ To assess the glucose-responsive behavior of the
281 microcapsules, a second 5 μL drop containing a solution of glucose was added gently
282 (preventing capsule movement as much as possible) to the drop of capsule suspension.
283 ~~preventing capsule movement as much as possible.~~ [12]

284

285 3. Results and discussion

286 As shown in Figure 1, the implemented strategy ~~combines both templating method-uses-of~~
287 ~~particles (CaCO_3) as a sacrificial template~~ and glucose-responsive polysaccharide. We focus
288 on calcium carbonate (CaCO_3) cores as sacrificial template due to its easy preparation, low
289 cost, biocompatibility, decomposition in mild conditions and intrinsic porosity. After
290 deposition of the desired number of polymer layers (PVPON/phenylboronic acid-modified
291 alginate), the sacrificial template is eliminated in the presence of ethylenediaminetetraacetic
292 acid. Its selective removal allows the formation of capsules (hollow particles) presenting
293 highly controlled dimensional features. ~~This latter~~ Glucose-response was obtained by the
294 chemical modification of the alginate (incorporation of phenylboronic acid moiety intended to
295 provide glucose sensitivity). The stability of microcapsules against glucose and the probe
296 loading/release properties are finally tested.



CaCO_3 microparticles

297
 298 **Figure 1.** Implemented strategy for the elaboration of the glucose-responsive microcapsules
 299 based on phenylboronic acid containing alginate (alg-bor) and poly(vinylpyrrolidone)
 300 (PVPON) loaded with two cationic probes.

301
 302 3.1. Glucose responsive alginate derivative, i.e. incorporation of phenylboronic acid as a
 303 pending group inside the alginate chain.

304 The coupling of the aminophenylboronic acid with the carboxylic acid groups of alginic acid
 305 was done using [1-ethyl-3-(dimethylamino)propyl]carbo-diimide hydrochloride as a
 306 condensation reagent.[21, 22] This allows obtaining an alginate derivative containing
 307 phenylboronic acid as a pending group. In this method, the carboxyl group of the alginate
 308 reacts with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) to form an
 309 unstable intermediate. Upon reacting with N-hydroxysuccinimide (NHS), a more stable,
 310 amine-reactive intermediate is formed. Finally, this intermediate is coupled to the amine
 311 groups of the aminophenylboronic acid, completing the polysaccharide modification. After a
 312 purification process (i.e. dialysis 6-8 kDa cut off and freeze-drying), a white compound was
 313 obtained and labelled as alg-bor. The ^1H NMR spectra show the chemical shifts of the protons
 314 of the alginate chain (from 3 to 4 ppm) and phenyl moiety (from 6.7 to 7.2 ppm) (Figure 2).
 315 Indeed, as seen from the spectra of the pristine alginate, all the chemical shifts of the protons
 316 in the alginate chain are above 3 to 4 ppm. Therefore, using this technique, the presence of
 317 aromatic groups is qualitatively checked for the modified alginate. Unfortunately, we could
 318 not distinguish, using this technique, whether the substitution occurred mainly on the
 319 guluronate or on the mannuronate residues of alginate. Indeed, all the protons of the alginate
 320 chain, i.e. protons from guluronate and mannuronate, all experience about the same chemical
 321 shift. From ^1H NMR, the presence of aromatic groups is qualitatively evidenced and will
 322 enable a qualitative determination of the Degree of Substitution "DS" (equation 4 3) by
 323 employing the method of Pettignano et al. [23], i.e. from the ratio between the integration

324 values of the phenylboronic acid unit those from the alginate and by considering the number
325 of hydrogens responsible for the integrated area.

$$326 \quad DS = (I_H \text{ in boronic unit in alg-bor}) / (I_H \text{ in alginate unit in alg-bor}) \quad (3)$$

327

$$328 \quad DS = \frac{(I_H \text{ in boronic unit in alg-bor})}{(I_H \text{ in alginate unit in alg-bor})}$$

329 The DS value was also estimated and confirmed by Total Organic Carbon analyses (TOC)
330 and Total Nitrogen (TN) analysis (equation 4).

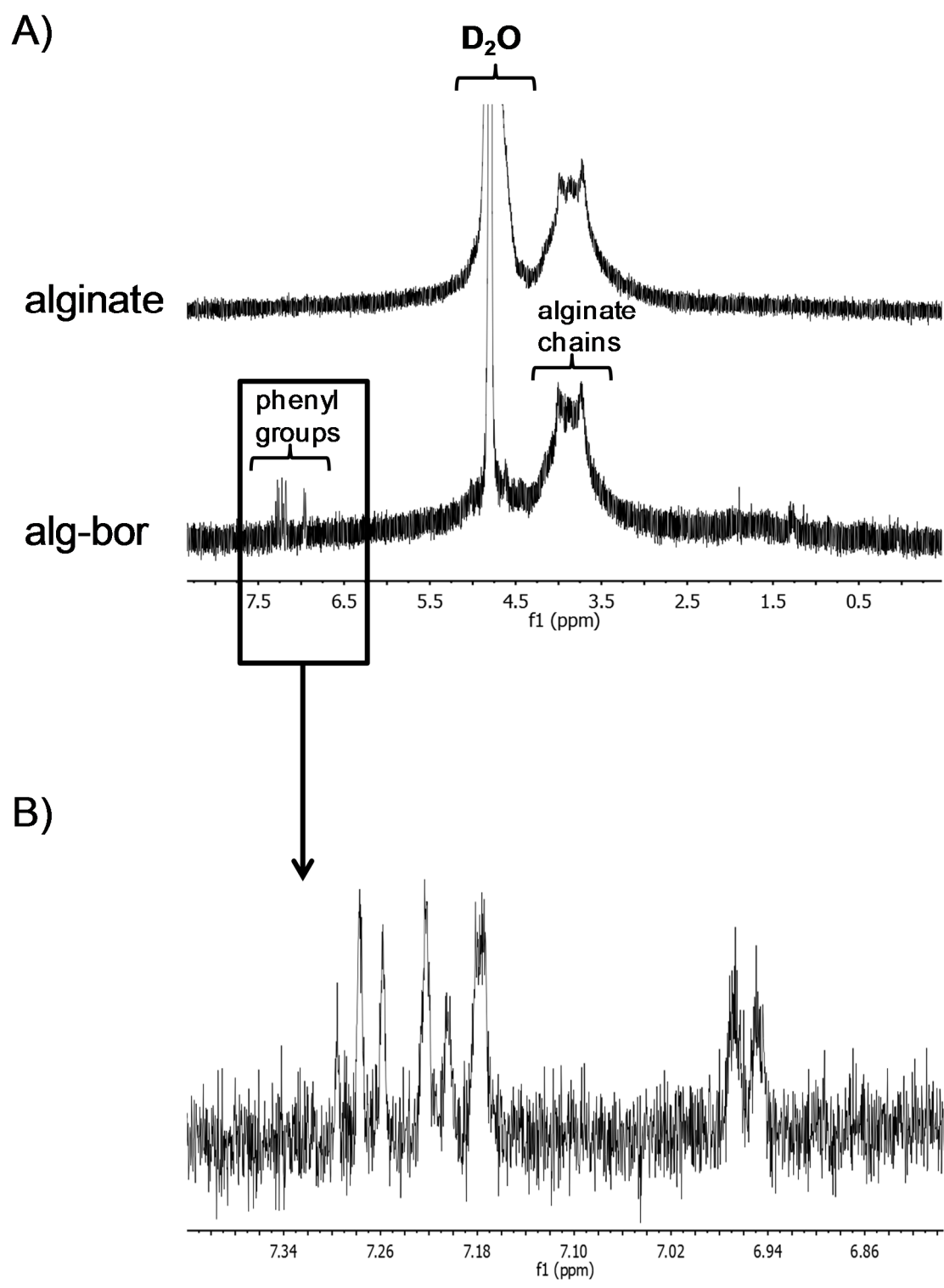
$$331 \quad \frac{C}{N} = \frac{(M_C(\text{alginate}) + DS \times M_C(\text{boronic acid}))}{M_N} \quad \text{i.e. } DS = 0.2 \frac{C}{N} - 1 \quad (4)$$

332 $M_C(\text{alginate})$: molar mass of carbon in the alginate unit; $M_C(\text{boronic acid})$: molar mass of carbon in
333 the boronic acid; M_N : molar mass of nitrogen due to the grafting of the boronic moiety, C/N =
334 carbon/nitrogen ratio. The value of the C/N ratio for the modified alginate is above 6.0 ± 0.2 .

335

336 The FT-IR spectrum (Figure S1) of the polymer shows peaks that confirm the presence of
337 functional groups in the alginate structure (867 cm^{-1} and 945 cm^{-1} = guluronate and
338 mannuronate residues of alginate and alg-bor samples / 3300 cm^{-1} = hydroxyl groups / 1143
339 cm^{-1} = C-O stretching vibration of alcoholic hydroxyl group / 1023 cm^{-1} = C-O-C stretching
340 vibration / 1080 cm^{-1} = C-N stretching vibration appeared after the chemical modification
341 with the aminophenylboronic acid / 1595 cm^{-1} and 1412 cm^{-1} = N-H deformation and C-N
342 stretching vibrations / 1740 cm^{-1} = carbonyl group from the amide). The experimentally
343 determined phenylboronic acid content (the DS) is estimated to be about 0.20 ± 0.04 . The
344 average molecular weights and dispersity are $\overline{Mn} = 2.5 \cdot 10^5 \text{ g.mol}^{-1}$ and $\overline{Mw} = 2.5$, respectively.

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Figure 2. A) ¹H NMR spectrum (D₂O) of alginate and alg-bor, B) Zoom-in of graph A.

3.2. Proof of concept: Elaboration of the glucose responsive multilayer film onto flat gold substrate based on alginate derivative and PVPON

354 The negatively charged surface of the CaCO_3 is first pre-treated with a polycation
355 (poly(ethylene imine)) leading to a positively charged surface and enabling the adsorption of
356 the polyanion (phenylboronic–modified alginate) at pH 8 or enabling the adsorption of a
357 polysaccharide derivative through hydrogen bonding at pH 2.

358 After this step, a hydrogen-bonded layer-by-layer (LbL) technique was used to build
359 multilayers (particle shell), i.e. between poly(vinylpyrrolidone) and the phenylboronic acid
360 modified alginate. Herein, the hydrogen-~~bonding~~ bonded LbL films are based on the alternate
361 deposition of polymers with a hydrogen bond acceptor and a hydrogen bond donor. As
362 hydrogen bond acceptor, poly(N-vinylpyrrolidone) (PVPON) presents the carbonyl group.
363 Interestingly, according to the pH, phenylboronic –modified alginate furnishes two types of
364 hydrogen bond donors, i.e. acid moieties (boronic acid and carboxylic acid) (Figure 3a). After
365 adding the desired number of polymer layers, the sacrificial core is removed. The stability of
366 microcapsules against glucose is then investigated. Furthermore, in the present study, it is
367 possible to load one or two probes according to the pH, i.e. (i) inside the capsule (no pH
368 restriction) and (ii) in the capsule shell (only possible at pH up to pKa of the alginate) (Figure
369 3b).

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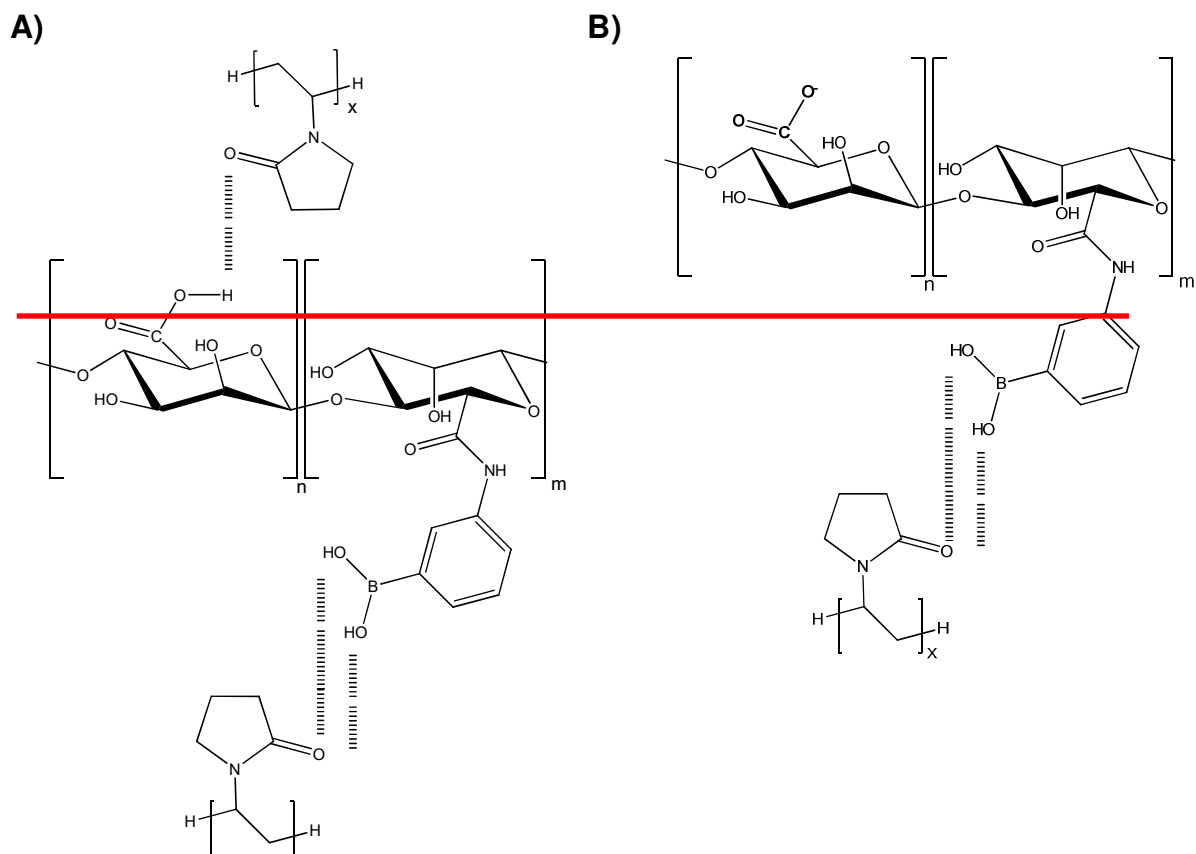
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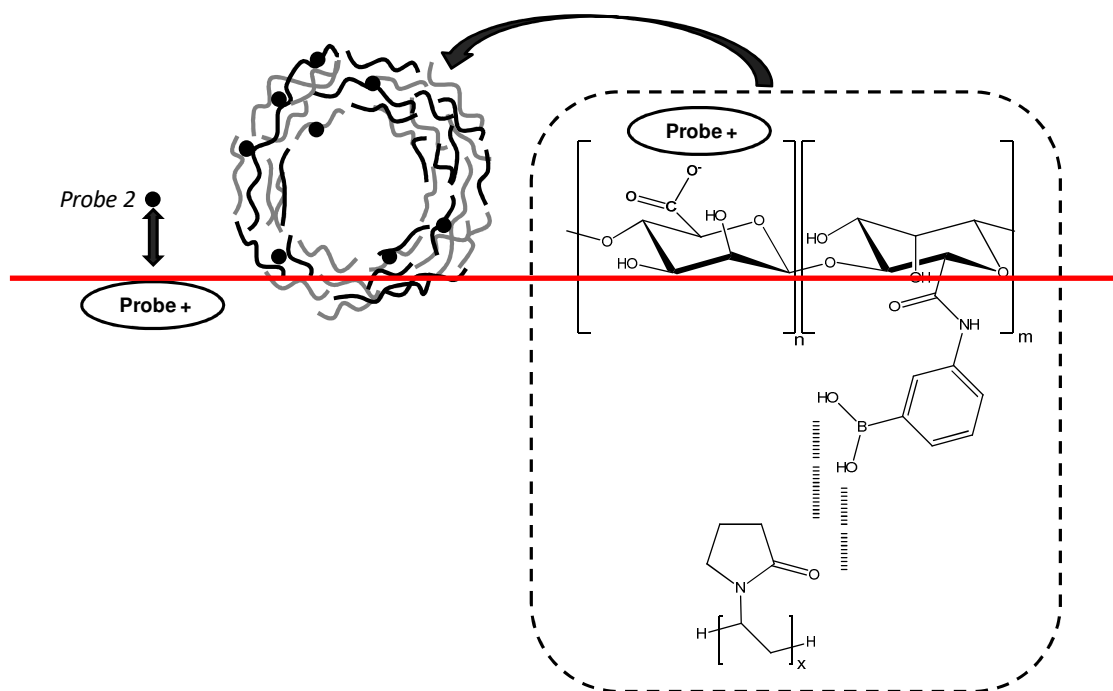
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383 a)



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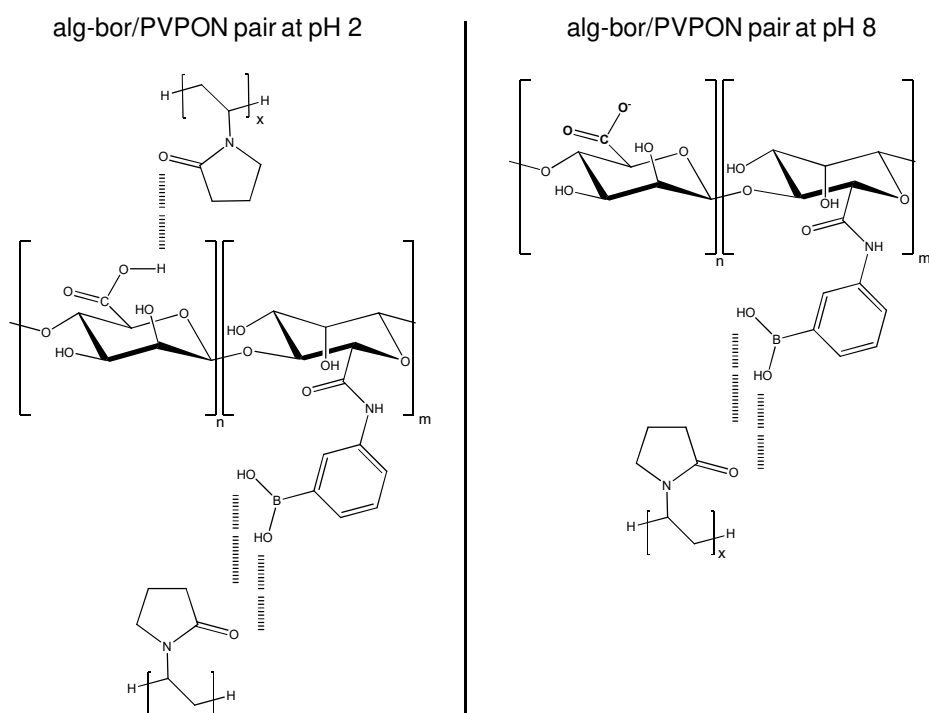
385 b)



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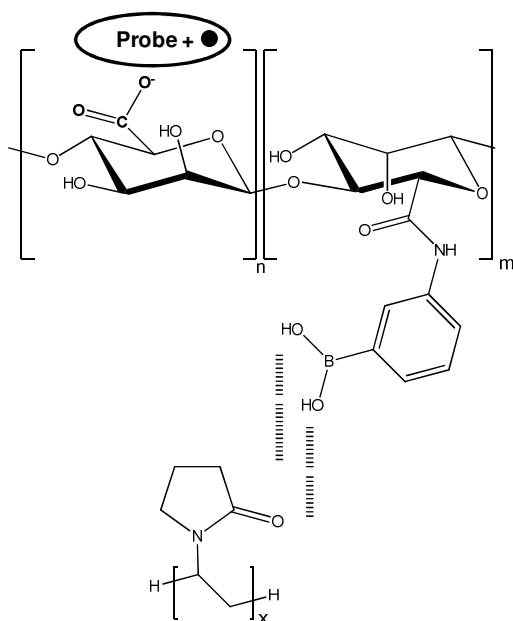
387 **Figure 3.** a) Suggested illustration of the implied hydrogen bonding interaction between alginate and PVPON pair at (A) pH 2, $\text{pH} < \text{pK}_a\text{-alginate}$ and $\text{pH} < \text{pK}_a\text{-boronic acid}$ (B) pH 8 and $\text{pH} >$
 388 $\text{pK}_a\text{-alginate}$ and $\text{pH} < \text{pK}_a\text{-boronic acid}$, b) Loading of a cationic probe inside the (PVPON/alg-bor)
 389 multilayer film. For clarity purpose, the scheme is not to scale.
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391 A)



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393 B)



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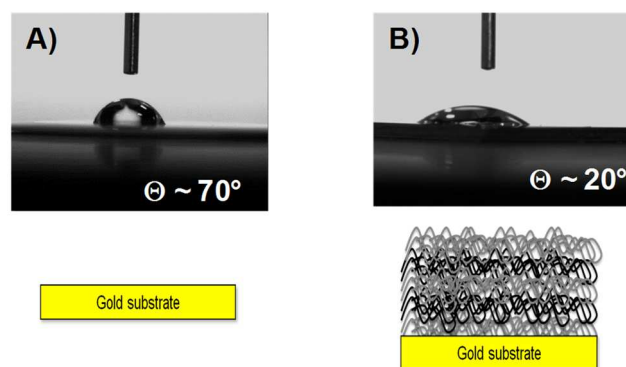
395 **Figure 3.** A) Suggested illustration of the implied hydrogen-bonded interaction between alg-
396 bor and PVPON pair at pH 2, $\text{pH} < \text{pK}_a$ alginate and $\text{pH} < \text{pK}_a$ boronic acid pH 8 and $\text{pH} > \text{pK}_a$
397 alginate and $\text{pH} < \text{pK}_a$ boronic acid, B) Loading of a cationic probe inside the (PVPON/alg-bor)
398 multilayer film. For clarity purpose, the scheme is not to scale.

399

400 In comparison with non-chemically modified polyanion (alginate), the electrolytic properties
401 associated with its functionalization, i.e non-zero zeta-potential and water solubility, are

402 conserved as evidenced by zeta-potential measurements at pH = 8, (-20 ± 3 mV for alg-bor).
403 This property allows us to (i) use this modified polyanion in the construction of multilayer
404 films via alternating adsorption of PEI and alginate derivative and (ii) load a positively
405 charged probe inside the multilayer film (Figure 3b).

406 For the proof-of-concept, the implemented strategy was first demonstrated on a flat substrate
407 through static contact angle and QCM measurements. Static contact angle measurements are
408 performed on flat gold surfaces before and after treatment by the polymers, i.e. PEI-(alg-
409 bor/PVPON)_n to qualitatively confirm the formation of the multilayer polymers onto the
410 substrate. The contact angle decreased from 70° to 20° upon coating, in agreement with the
411 hydrophilic nature of the introduced polysaccharide and PVPON (Figure 4). This variation in
412 surface wettability after LbL treatment is found to be independent of the number of bilayers
413 and the LbL procedure (pH 2 or 8).



414
415 **Figure 4.** Wetting angle contact value for (A) a flat gold bare surface and (B) gold surface
416 modified with (alg-bor/PVPON)₄.

417
418 The possibility to involve both carboxylic acid and boronic acid as hydrogen bond donors was
419 then demonstrated by the construction of multilayers under varying pH. To support this aim,
420 QCM measurements were performed (Figure 5a). This technique enables the monitoring of
421 the assembly in real time on a flat surface to (i) evidence the stepwise adsorption of (alg-
422 bor/PVPON) polymers, (ii) investigate the adsorption kinetics and (iii) illustrate the impact of
423 the pH (2 and 8) on the LbL build-up. First, a quartz substrate is precoated with a PEI layer,
424 enabling the adsorption of the polyanion (alginate derivative, i.e. electrostatic interaction at
425 pH 8 or H-bonding at pH 2) followed by PVPON adsorption (i.e. hydrogen bonding
426 interaction). The build-up procedure at pH 2 ensures, both the presence of carboxylic groups
427 and that boronic acid moieties remained uncharged during the layer-by-layer build-up since

428 the pKa of phenylboric acid is 8.9, [24] facilitating hydrogen bonds between the carbonyl
429 group of PVPON and the carboxylic acid and boronic acid groups of alginate. However,
430 preparing the assembly at pH 8 leads to the formation of carboxylate groups (pH > pKa of
431 alginate) and therefore there is only formation of hydrogen bonds between boronic moieties
432 and the carbonyl groups of PVPON. As evidenced in Figure 6 5a, a decrease of the frequency
433 signal by increasing the number of adsorption cycles is noticed regardless of the pH,
434 indicating that mass was being added onto the surface and then evidencing the successful
435 multilayer buildup (i.e four bilayers of alg-bor/PVPON) at pH 2 or 8. This result is very
436 interesting because hydrogen-bonded multilayers are typically only stable at low pH. Since
437 hydrogen-~~bonding bonded~~ LbL films are often composed of a polyacid (carboxylic acid
438 moiety) as the hydrogen bond acceptor, increasing the pH above the pKa of the polyacid leads
439 to the disruption of hydrogen bonding by deprotonation of the carboxylic acid group.[6]
440 When comparing films based on electrostatic interactions to the ones based on hydrogen-
441 ~~bonding bonded~~ interactions, the pH range at which hydrogen-~~bonding bonded~~ polymers can
442 interact and form stable films is restricted because it depends on the pKa of the polymers and
443 the strength of the interaction between the pairs of polymers which have hydrogen-~~bonding~~
444 ~~bonded~~ functional groups. Several researchers have reported the stabilization of hydrogen-
445 ~~bonding bonded~~ multilayers versus pH through crosslinking using either photochemical, [25]
446 chemical [8, 26] or even thermal [8] techniques. Herein, we then propose a new method based
447 on the incorporation of boronic acid moieties in a polymer backbone to tune the
448 responsiveness of pH-sensitive film.

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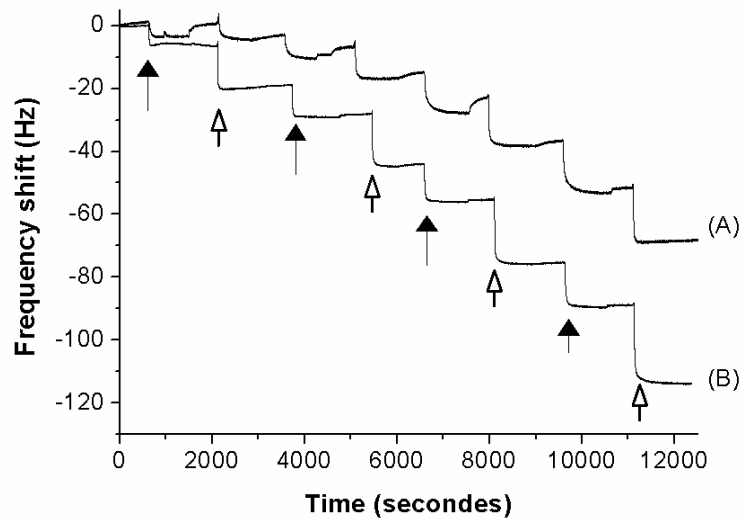
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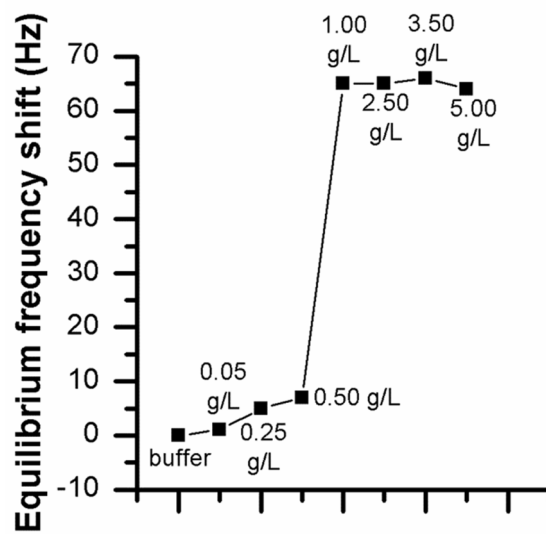
458 a)

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461 b)



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463 **Figure 5.a)** Evolution of the frequency shift values as a function of time for films build up at
464 (A) pH 8 and (B) 2 with $(\text{alg-bor/PVPON})_n$ as a function of bilayer number. (filled arrow
465 (alg-bor) addition) and unfilled arrow (PVPON addition), b) Effect of increasing
466 concentrations of glucose at pH 8 on $(\text{alg-bor/PVPON})_4$ film.

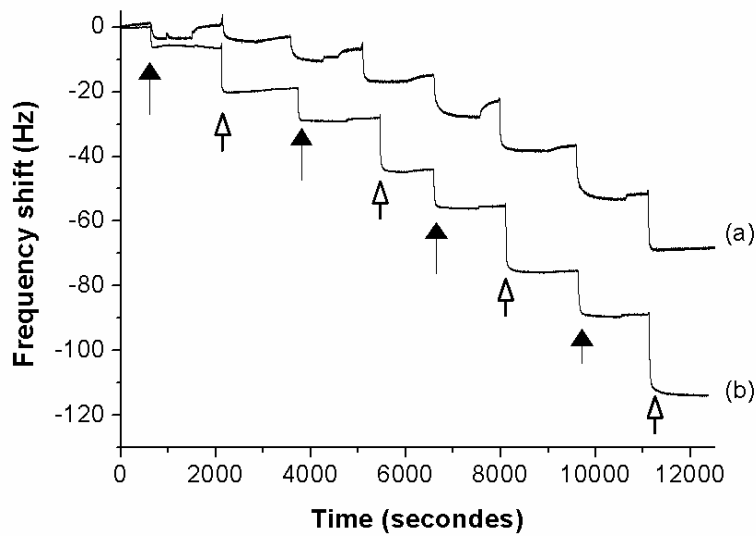
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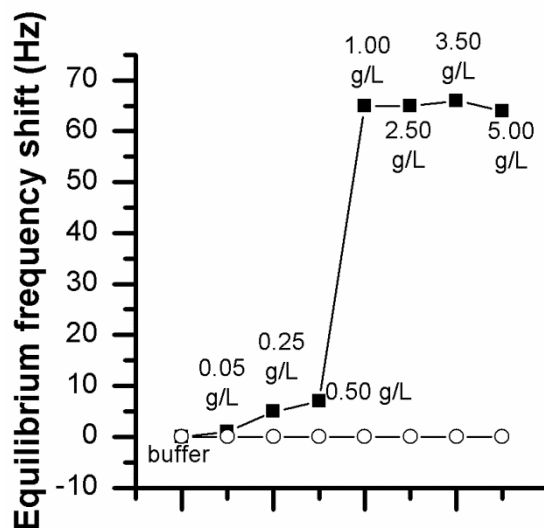
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471 A)



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473 B)



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475 **Figure 5.** A) Evolution of the frequency shift values as a function of time for films build up at
476 (a) pH 8 and (b) 2 with (alg-bor/ PVPON)_n as a function of bilayer number. (filled arrow (alg-
477 bor) addition) and unfilled arrow (PVPON addition), b) Effect of increasing concentrations of
478 glucose at pH 8 (■) and pH 2 (○) on (alg-bor/PVPON)₄ film.

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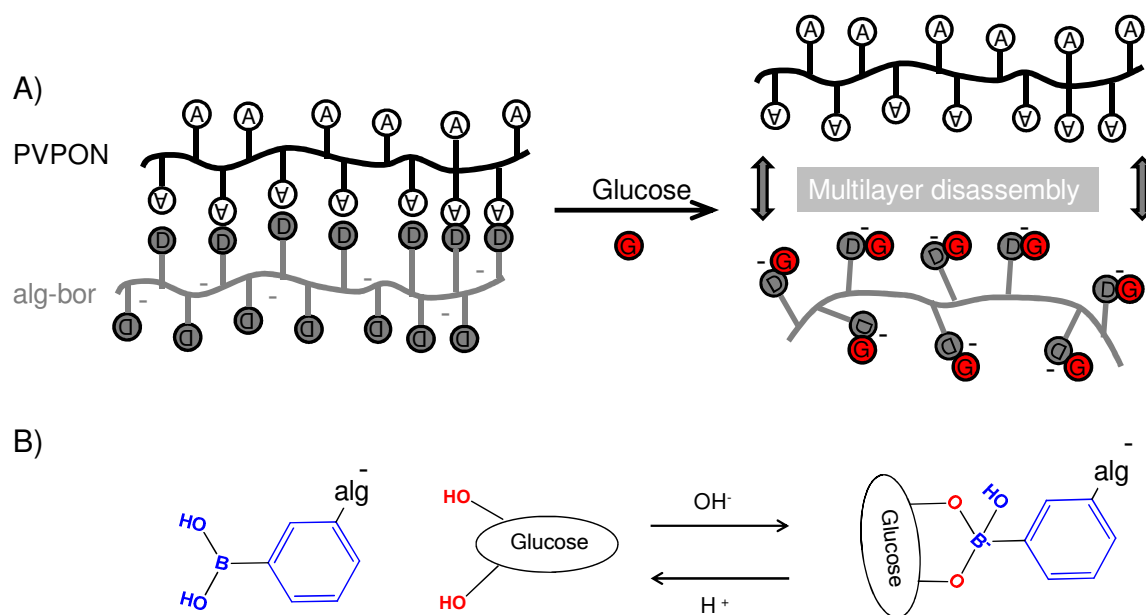
480 To demonstrate glucose sensitivity, different concentrations of glucose solution (from 0.05
481 g.L⁻¹ to 5 g.L⁻¹) at pH 8 or pH 2 are flushed through the (alg-bor/PVPON)₄ multilayer film. As
482 seen in Figure 5a, the final equilibrium frequency shift values after build-up of the
483 PVPON/alg-bor films were -60 ± 5 Hz and -115 ± 7 Hz for the build-up at pH 8 and at pH 2,
484 respectively. These values can be used as references to investigate the glucose response of the

485 films, in accordance with the swelling behaviour due to glucose complexation with the
486 boronic moieties, and the film's stability. For this last case, the film removal should lead to a
487 variation of the equilibrium frequency shift of about $+60 \pm 5$ Hz and $+115 \pm 7$ Hz for the
488 build-up at pH 8 and at pH 2. As seen in Figure 5b, increasing the glucose concentration leads
489 to an increase of the frequency signal which reaches to a plateau regime. The critical glucose
490 concentration is close to 1 g.L^{-1} . The same experiments were repeated (i) at pH 2 in presence
491 of glucose and (ii) at pH 8 without glucose, but no variation of the frequency shifts was
492 observed. Note that no variation of the frequency shift was observed upon the increased of the
493 ionic strength. Therefore, the multilayer stability was not affected by this parameter which is
494 in accordance by the work reported by Smith et al. [17]

495 Phenylboronic acids are known to form covalent bonds with polydiols such as saccharides
496 [12] and the complexation occurs efficiently in a pH range above the pKa of the boronic
497 moieties (pKa~9) [11, 13] This lead to the formation of anionic complex formed by
498 "glucose/borate" as presented in figure 6B. ~~The sensitivity of phenylboronic acid derivative to~~
499 ~~saccharide (as glucose) [27] is well known to be dependent on the pH of the surrounding~~
500 ~~medium, more precisely around the pKa of the phenylboronic acid.~~ From Springsteen and
501 Wang's work, the phenylboronic acid/polydiol complexes formation clearly induces a
502 decrease of 2-4 pKa units of the apparent pKa. [27] They explained the change in pKa to the
503 stronger acid behaviour of the polydiol complex compared to the phenylboronic acid
504 derivative. [27, 28] De Geest et al. [12] and Belbekhouche et al. [15] who prepared glucose-
505 responsive capsules at pH 8 and 9 explained their results based on Springsteen and Wang's
506 work, [27] **the as-observed glucose behavior arises mainly from incorporation of glucose**
507 **within the films through complexation with the phenyl boronic moieties.**

508 Herein, at pH 8, the multilayer build-up between PVPON and alg-bor is due to hydrogen
509 bonds between PVPON/carboxylic groups of alg-bor and between PVPON/ phenylboronic
510 acid moieties of alg-bor (near the pKa of phenylboronic acid), the decrease of the apparent
511 pKa upon addition of glucose involves a strong enhancement of borate anions. More
512 precisely, the borate groups are charged and complexation could occur between borate
513 moieties and glucose. Increasing the glucose concentration promotes an increase in the
514 number of borate moieties which begin to strongly interact with the polymers constituting the
515 multilayers. There is simultaneously (i) complexation caused by the electrostatic interactions
516 in the anionic **complex composed by glucose/borate** ~~complex~~ and (ii) disruption of the
517 hydrogen-~~bonding~~ **bonded** between the boronic acid and the PVPON. The synergy of these

518 phenomena seems to result in the disruption of the overall multilayer film. Therefore, This
 519 explains why at pH 8 the gradual increase of the glucose concentration leads to the frequency
 520 signal increases. This could be directly correlated to in accordance with a mass removal or
 521 multilayer disassembly, due to the addition of glucose, where the glucose concentration is
 522 gradually increased. Schemes presented in figure 6 illustrate this hypothesis.
 523 However, at pH 2 upon the increase of the addition of glucose concentration, the increase in
 524 borate anions does not occur. Therefore, no impact of the increase of the glucose
 525 concentration variation of the glucose is expected at this pH no variation of equilibrium
 526 frequency shift as a function of glucose concentration is expected at this pH. This is
 527 corroborated by the QCM results, after the multilayer build-up, no variation of the frequency
 528 signal or impact on the multilayer is observed (Figure 5b). As the layer-by-layer assembly
 529 was done under acidic conditions (At pH 2, the phenylboronic acid moieties are uncharged
 530 and participate in the hydrogen bond during the assembly along with the carboxylic groups.
 531 Furthermore, Complexation between glucose and borate is not favourable at this pH.
 532 Consequently, no significant impact on the hydrogen-bonding bonded assembly multilayers
 533 behaviour is observed due to the addition of glucose.



534
 535 **Figure 6.** A) Suggested mechanism of the glucose-induced decomplexation of (PVPON/alg-
 536 bor) bilayer at pH = 8. The circles containing the letter A represent the PVPON as H-acceptor,
 537 the one containing the letter D represent the boronic groups, i.e. as H-donor. The circles
 538 labeled D⁻ corresponds to the uncharged phenylboronic acid groups becoming anionic after
 539 addition of glucose (red circles with the letter G). B) Equilibrium between
 540 phenylphenylboronic acids- containing alginate and diols-based glucose in aqueous solution.

541 The alg-bor/PVPON pair has been evidenced to be suitable for multilayer formation and
542 exhibits glucose-responsive behaviour at pH 8. These conditions will further be employed for
543 the preparation of hollow polyelectrolyte microcapsules exhibiting glucose-response.

544

545 3.3. Elaboration of the glucose responsive capsules based on alginate derivative and PVPON

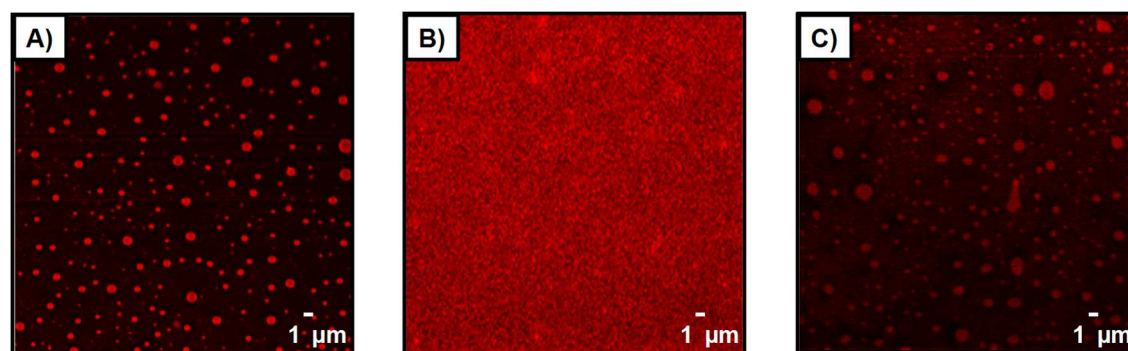
546 The zeta-potential value of the particles is measured after PEI and alg-bor addition for
547 monitoring their adsorption onto the microparticles. The zeta-potential value of the bare
548 (uncoated) calcium carbonate particles is -7 mV and switches to a positive value after the
549 initial layer of PEI adsorption (+ 50 mV) and a negative value after the subsequent layer of
550 alginate derivative deposition at pH 8 (-20 mV). This step is followed by hydrogen-~~bonding~~
551 ~~bonded~~ assembly on particle templates by sequential alternative deposition of PVPON and
552 alg-bor. The probe was preloaded on the particle templates prior to LbL film assembly.

553 Preloading allows to great enhancement of the efficiency of encapsulation as significantly less
554 amount of probe material is required in the adsorption/loading step. As a typical example,
555 Caruso et al. reported that a distinguishing feature of this method was the possibility to
556 concentrate DNA from a dilute solution onto the template particles to produce capsules with
557 increased concentration compared to the initial solution. [10] In their study, the DNA was
558 electrostatically bound to the surface of the particles and then multilayers were assembled.
559 Removal of the sacrificial silica particles results in the formation of capsules containing DNA.
560 We have then used this method to load a probe (i.e. rhodamine) inside the capsule. The
561 rhodamine loaded microparticles are prepared and then the CaCO₃ core is removed employing
562 a 0.2 M EDTA solution. The incorporation of rhodamine does not significantly impact the
563 zeta-potential of the loaded CaCO₃ template. As evidenced by fluorescent microscopy, even
564 after the core removal step, microparticles have a well-defined spherical shape and mainly
565 exist as single microparticles (Figure 9 7A). SEM and TEM images also confirm the spherical
566 shape and micrometer size of the microparticles and microcapsules (Figures S2 and S3). The
567 entrapment efficiencies of microcapsules are greater than 60% and the probe loading is
568 around 5%, indicating this probe could be readily loaded into the particles' matrix due to the
569 porous structure of CaCO₃.

570 When the rhodamine-loaded capsules were placed into a solution at pH 8, they remained
571 intact and stable (Figure 7 A). However, when the capsules were suspended in a solution
572 containing glucose at pH 8, the capsules were destabilized and rhodamine is released as
573 shown in Figure 9 7 B. For the release, the involved glucose concentration is the critical

574 glucose concentration determined during the QCM experiment. We repeated the same test at
575 pH 2 and, as expected, we observed that the capsules remained stable in the presence of
576 glucose (~~data not shown~~-Figure 7C).

577



578

579 **Figure 7.** Confocal microscopy image of microcapsules consisting of (alg-bor/PVPON)₅
580 loaded with rhodamine derivative A) before ~~and~~ - B) after addition of a solution containing
581 glucose at pH = 8 and C) after addition of a solution containing glucose at pH = 2 (630x
582 magnification).

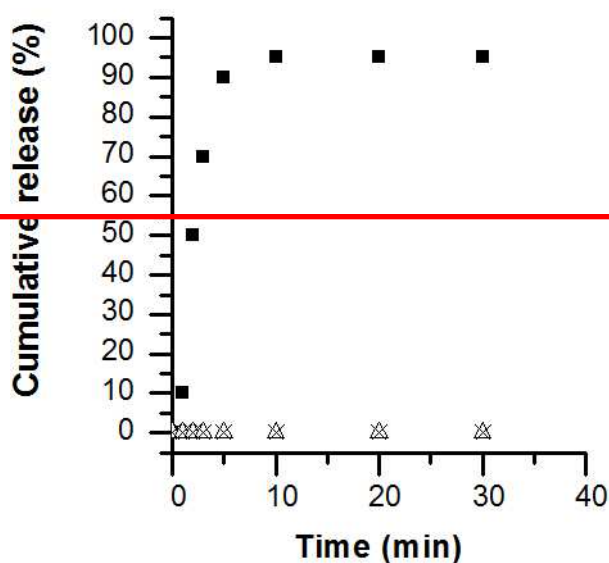
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584 The ability to load small molecules containing ionic groups in polyelectrolyte layers
585 (~~chitosan/poly(acrylic acid)~~) of a ~~particle~~ through simple diffusion/electrostatic entrapment is
586 highlighted in the work of Hu *et al.* [29] or Belbekhouche *et al.* ~~also evidenced the possibility~~
587 ~~to load an antibiotic (i.e. amoxicillin) through a polyelectrolyte multilayer film.~~ [30] Based on
588 these works, a second probe (methylene blue) has been entrapped in the multilayer shell. ~~This~~
589 ~~implies~~ i.e. through the electrostatic interaction of the methylene blue loading in a protonated
590 form and the carboxylate groups of alginate (Figure 4 3B). The methylene blue entrapment
591 efficiency is 70±3% and the probe loading is 3.4±0.2 % with (alg-bor/PVPON)₄. This was
592 determined by subjecting the loaded microcapsules to absorbance measurement at 663 nm.
593 ~~We then demonstrate the possibility to load two probes one inside the capsule (rhodamine~~
594 ~~derivative) and one in the multilayer shell (methylene blue).~~

595 After this demonstration of proof-of-concept, we then switch to loading insulin instead of
596 rhodamine derivative. The entrapment efficiencies of microcapsules and the ~~probe~~ insulin
597 loading are greater than 65% and 5.5 % respectively. At pH 2, in presence of glucose, no
598 release of insulin was observed in agreement with the proof-of-concept result (Figure 8). This
599 point is very appealing because resistance to acid pH is required to prevent the release of
600 insulin from the carriers when passing through the stomach to release this drug in intestinal
601 media and promote insulin uptake. [16]. However, when the PVPON/alg-bor microcapsules

602 were suspended in glucose solution at pH 8, the insulin is released in fewer 10 minutes. As
603 expected at pH 8 without glucose, no release is observed. Indeed as discussed during the
604 QCM study, for impacting the multilayer assembly, pH 8 and glucose are both required
605 simultaneously to allow (i) complexation caused by the electrostatic interactions in the
606 anionic glucose/borate complex and (ii) disruption of the hydrogen-bonding bonded between
607 the boronic acid and the PVPON. In these conditions, the insulin release from the
608 microcapsule might be correlated to the multilayer disassembly as discussed in section 3.2.

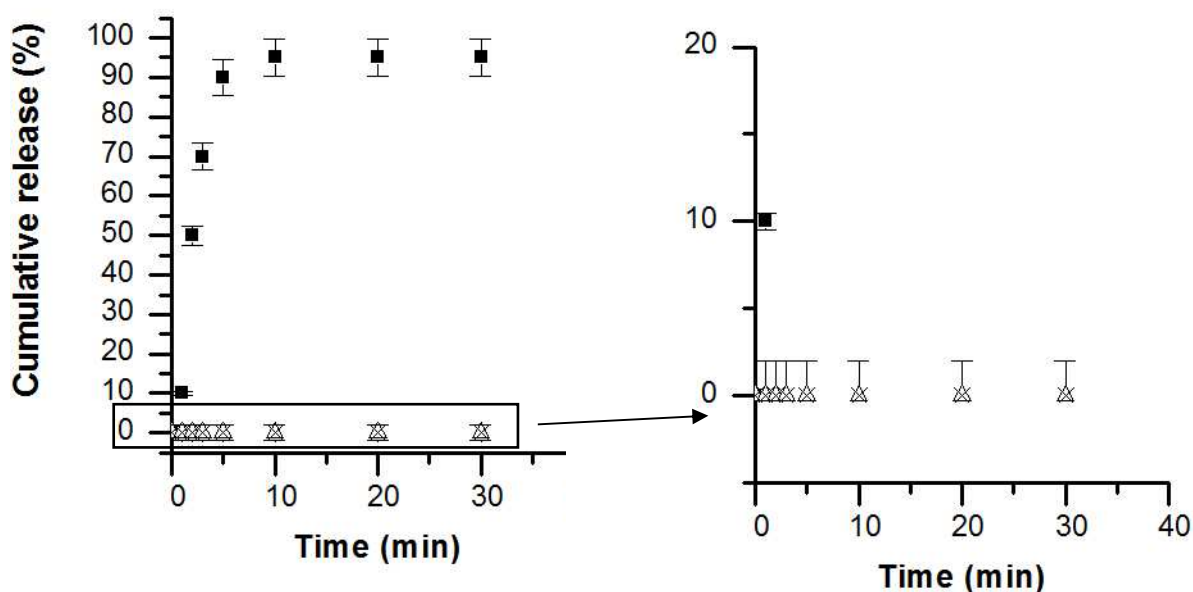
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A)

B)



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617 **Figure 8.** A) Release of insulin from (alg-bor/PVPON)₅ capsules pH 8 in presence of
618 glucose solution (1 g.L⁻¹) at Δ pH 2, ■ pH 8 and x pH 8 without glucose, B) Zoom-in of
619 graph A.

620
621
622 ~~The ability to load small molecules containing ionic groups in polyelectrolyte layers~~
623 ~~(chitosan/poly(acrylic acid)) of a particle through simple diffusion/electrostatic entrapment is~~
624 ~~highlighted in the work of Hu *et al.* [29] Belbekhouche et al. also evidenced the possibility to~~
625 ~~load an antibiotic (i.e. amoxicillin) through a polyelectrolyte multilayer film. [30] Based on~~
626 ~~these works, a second probe (methylene blue) has been entrapped in the multilayer shell. This~~
627 ~~implies the electrostatic interaction of the methylene blue loading in a protonated form and~~
628 ~~the carboxylate groups of alginate (Figure 4 3B). The methylene blue entrapment efficiency is~~
629 ~~70±3% and the probe loading is 3.4±0.2 % with (alg-bor/PVPON)₄. This was determined by~~
630 ~~subjecting the loaded microcapsules to absorbance measurement at 663 nm. We then~~
631 ~~demonstrate the possibility to load two probes one inside the capsule (rhodamine derivative)~~
632 ~~and one in the multilayer shell (methylene blue).~~

633

634 4. Conclusion

635 Novel assemblies based on PVPON and an alginate derivative containing phenylboronic acids
636 moieties have been employed to prepare glucose sensitive hollow microspheres. This
637 approach combines both templating method and hydrogen-bonded LbL multilayer. One of the
638 novelties of the present work is to provide evidence that the incorporation of boronic acid
639 moieties in the alginate backbone enables (i) production of multilayer films based on
640 hydrogen bonding that are stable under physiological conditions (Figure S4 S3) and even in
641 basic media (pH < 9) and (ii) glucose-responsiveness. This involves PVPON as a hydrogen
642 bond acceptor and phenylboronic modified alginate simultaneously furnishing two types of
643 hydrogen bond donors according to the pH, i.e. acid moieties (boronic acid and carboxylic
644 acid). Our strategy is based on the sequential surface deposition of the respective
645 phenylboronic acid containing polyanion and PVPON on pretreated, with PEI, CaCO₃
646 microparticles used as sacrificial templates. The hollow morphology of the microcapsules was
647 evidenced by fluorescence microscopy imaging and SEM. The glucose-sensitive character
648 was related to the glucose concentration in the surrounding buffer, involving a
649 disassembly/destructuring of the multilayer film. This phenomenon is related to the
650 competitive binding of glucose and boronic acid groups of the alginate within the multilayer
651 film, resulting in destabilization of the film. Furthermore, one of the novelties of the present

652 ~~work is to provide evidence that the incorporation of boronic acid moieties in the alginate~~
653 ~~backbone enables (i) production of multilayer films based on hydrogen bonding that are stable~~
654 ~~under physiological conditions and even in basic media (pH < 9) and (ii) glucose-~~
655 ~~responsiveness. The initial hypotheses are successfully validated, i.e. incorporating boronic~~
656 ~~acid moieties inside a polymer chain (herein a polysaccharide, the alginate) allows glucose-~~
657 ~~responsiveness and (ii) using boronic moieties as hydrogen bond acceptor. We then~~
658 ~~demonstrate an increase of stability according to the pH under neutral conditions and even in~~
659 ~~basic media and then propose a new approach to overcome the main issues due to hydrogen-~~
660 ~~bonding-bonded LbL multilayer, poor stability under physiological conditions. This is crucial~~
661 ~~as DDS must resist the acidic media in the gastric environment.~~ Moreover, this elegant
662 strategy enables the loading of two probes: (i) by using the calcium carbonate template
663 porosity and (ii) inside the multilayer film by taking advantage of the presence of the
664 carboxylate group according to the pH. ~~Indeed, the glucose-dependent stability of these~~
665 ~~capsules has been investigated by monitoring the loading and release of probes (rhodamine~~
666 ~~derivative inside the capsules and methylene blue inside the shells) and then insulin.~~ The
667 present capsules with dual functionality may be useful for theranostic applications for both
668 glucose detection and treatment.

669

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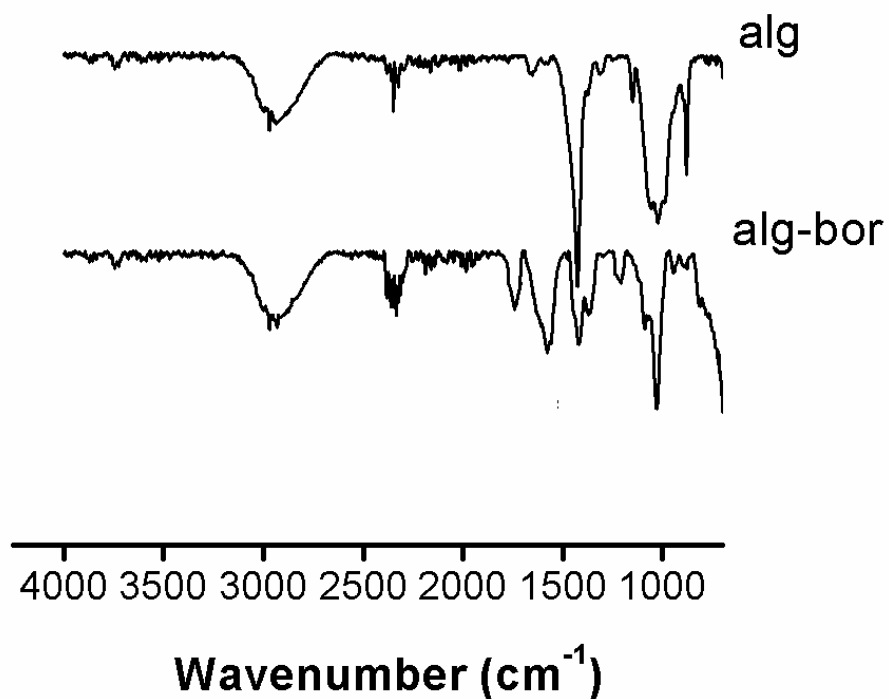
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Supporting information



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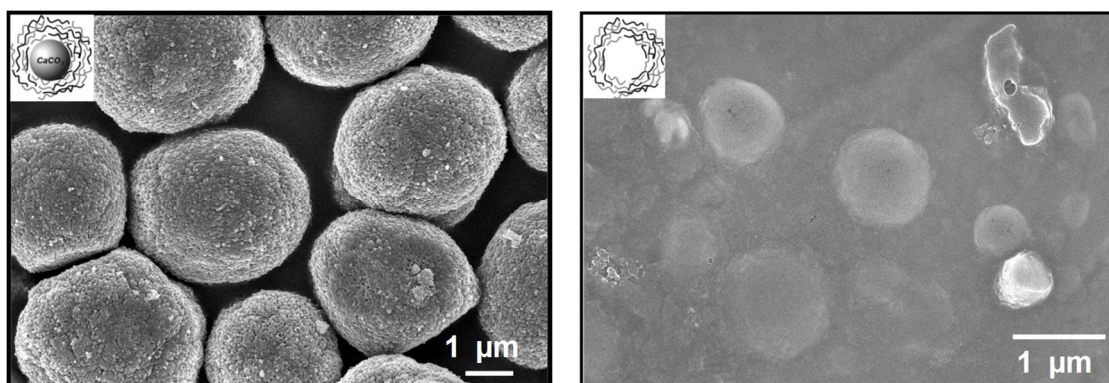
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Figure S1: FTIR spectra of alginate (alg) and modified alginate (alg-bor)

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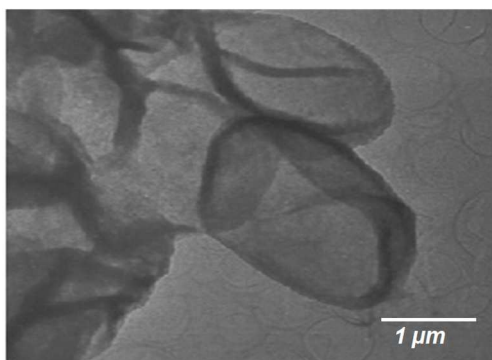
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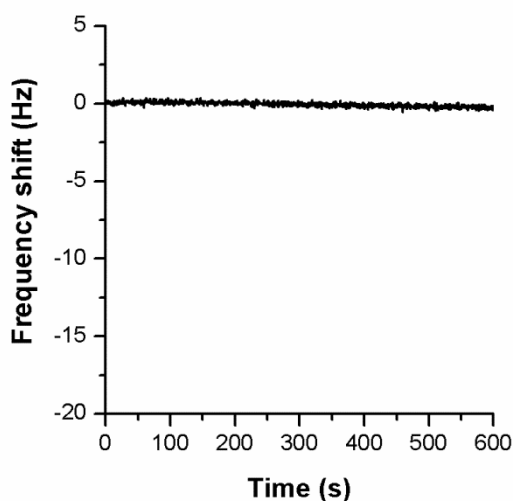
Figure S2. SEM images of modified microspheres (left panel) and microcapsules (C) (right panel) composed by (PVPO_n/alg-bor)₆ polymers pair. Scanning Electron Microscopy (SEM) with line scanning of energy-dispersive X-ray spectroscopy was produced Leo Elektronenmikroskopie, Oberkoche in Germany. The non-conducting surface of the modified calcium carbonate particles and capsules was covered with palladium before subject to analysis.

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Figure S3. TEM images of modified microcapsules composed by (PVPON/alg-bor)₆ polymers pair. Observations were conducted on a Tecnai F20 ST microscope (field-emission gun operated at 3.8 kV extraction voltage) operating at an acceleration voltage of 200 kV. The samples were prepared as follows: a droplet of capsule suspension and one of a solution of uranyle acetate (0.7 wt%) were deposited on a plasma pre-treated copper grid and left to dry at room temperature prior to analysing them on the copper grid.



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Figure S3 S4: QCM-D measurements. Flushing of a gold sensor coated with (alg-bor/PVPON)₄ film under physiological salt and pH condition (150 mM NaCl, 2 mM CaCl₂, TrisHCl, pH 7.0). The non variation of the frequency evidences the stability of the multilayer films under such condition.

