



HAL
open science

Glucose-Responsive Capsules Based on (Phenylboronic-modified Poly(Lysine)/Alginate) System

Ones Mansour, Taleen Peker, Sena Hamadi, Sabrina Belbekhouche

► **To cite this version:**

Ones Mansour, Taleen Peker, Sena Hamadi, Sabrina Belbekhouche. Glucose-Responsive Capsules Based on (Phenylboronic-modified Poly(Lysine)/Alginate) System. *European Polymer Journal*, 2019, 120, pp.109248 -. 10.1016/j.eurpolymj.2019.109248 . hal-03487770

HAL Id: hal-03487770

<https://hal.science/hal-03487770>

Submitted on 21 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Glucose-Responsive Particles Based on (Phenylboronic –modified** 2 **Poly(Lysine) / Alginate) System**

3 *Ones Mansour, Taleen Peker, Sena Hamadi, Sabrina Belbekhouche**

4
5 Institut de Chimie et des Matériaux Paris-Est, UMR 7182 CNRS-Université Paris-Est
6 Créteil Val-de-Marne, 2 rue Henri Dunant, 94320 Thiais, France

7
8
9 Corresponding authors:

10 * Sabrina Belbekhouche

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

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

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

14 15 **Abstract.**

16 Developing “smart” systems that respond to chemical stimuli is of major interest. The
17 physico-chemical properties of such a system would enable it to detect and respond to
18 changes in the concentrations of targeted molecules. Saccharides are one member of a
19 challenging class of chemical stimuli that are particularly important with respect to both
20 medical applications and industrial processes. In this context, a new glucose-responsive,
21 multilayer system based on electrostatic interaction has been created via the layer-by-layer
22 deposition of biopolymers. The studied polymers are poly(lysine) derivatives and alginate and
23 are used as polycation and polyanion respectively. The system detected glucose at levels
24 approximating 0.5 g.L^{-1} at which point the chemical modification of poly(lysine) with
25 phenylboronic acid groups (known to form complexes with diol derivatives) occurred. Herein,
26 we have shown, using wetting contact and quartz crystal microbalance (QCM) measurements,
27 that the poly(lysine) derivatives self-assemble with alginate through the formation of
28 electrostatic bonding throughout the layer-by-layer assembly. QCM measurement revealed
29 that introducing boronic acid moieties inside a macromolecular chain leads to glucose-
30 responsiveness. This strategy was then extended to the fabrication of responsive particles
31 using gold nanoparticles as a core template, and even to the fabrication of the capsule. As
32 evidenced by measuring zeta potential, electrostatic interactions between the protonated
33 amine groups of poly (lysine) derivative macromolecules (+ 40 mV) and the carboxylate
34 groups of alginate (-20 mV) are the driving attraction force driving the fabrication of well-
35 ordered multilayer films onto the spherical substrate. Transmission electron microscopy
36 (TEM) was used to confirm that nanocapsules had been formed. This novel responsive

37 capsule may further be used for specific biological purposes such as the detection of diabetes
38 and/or enhancing treatment of the disease through sensing sugar levels.

39

40 **Keywords:** capsule; glucose-responsive; alginate; phenylboronic acid chemistry; poly(lysine);
41 biopolymer, LbL

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66 **1. Introduction**

67 Fabrication of polymeric multilayer capsules using layer-by-layer (LbL) technique is an
68 appealing approach for the construction of customised drug delivery systems.[1-3] The
69 method is very versatile and facilitates the preparation of multilayered films of precise
70 structure. [4] A key requirement for the use of capsules as a smart drug delivery system is that
71 they must be stable at physiological conditions such as within intracellular media, while also
72 being able to trigger multilayer film disassembly as a result of an external stimulus. [5]
73 “Smart” capsules sensitive to a variety of external stimuli, including ionic strength,
74 temperature and pH, and have already been successfully reported. [6, 7] However, there have
75 been relatively fewer reports of smart capsules successfully responding to chemical stimuli in
76 the literature. These types of smart capsules work by changing a physico-chemical property of
77 the capsule in response to a sensed change in the concentration of a surrounding molecule(s)
78 of interest.

79 Saccharides are one of the most interesting and challenging classes of chemical stimuli.
80 Further, the ability to sense and respond accordingly to changes in saccharide concentrations
81 has the potential to very impactful with respect to biomedicine. Fabricated glucose-responsive
82 systems are potentially so important because of the critical role that the regulation of sugar
83 plays within living organisms [8-10].

84 Phenylboronic acids are well-known for their capacity to form covalent bonds with diol
85 derivatives such as polysaccharides [8]. However, the formation of complexes will only occur
86 in a range of pH that is near the pKa of the boronic moieties (pKa ~ 9) [8, 11, 12]. Under this
87 specific condition, an equilibrium between uncharged and charged (phenylboronic and
88 phenylboronate, respectively) forms of the chemical is possible. The formation of a complex
89 with a diol, such as glucose, shifts equilibrium toward the increased formation of charged
90 phenylborates. This can be explained by the presence charged borates which can form a stable
91 complex with glucose in aqueous medium. Most of previously-developed approaches have
92 relied on the total removal of the film as result of exposure to a critical concentration a sugar
93 solution.[8, 13] For instance, in work reported by Levy et al., researchers were able to
94 demonstrate the fabrication of glucose-sensitive particles, stabilised via ester bonds, that were
95 formed between mannan and phenylboronic acid moieties grafted onto poly(acrylic acid) [13].
96 The competitive bindings of saccharides and mannan with the boronic acid group inside the
97 multilayer film lead to the complete destabilization of the film. Responsiveness to glucose can
98 be explained by a changes in the electrostatic interactions between the phenylboronic acid-

99 containing polycation and the polyanion [8]. Recently, a complementary approach has been
100 shown by Belbekhouche et al. [9] This work reported the multilayer assembly of
101 polysaccharide derivatives containing phenylboronic acid side groups and chitosan. [9] The
102 glucose response was correlated to a change in swelling behaviour. An elegant approach has
103 also been recently proposed in which hollow microspheres are formed based on
104 polyvinylpyrrolidone (PVPON) and phenylboronic acid-containing alginate. [10] Their
105 strategy combined both the templating method and hydrogen-bonded LbL multilayer method
106 to create multilayer films based on hydrogen bonding. Researchers were able to demonstrate
107 that incorporation of boronic acid within the polysaccharide chain produced (i) films that are
108 stable under both physiological and basic conditions due to phenylboronic components, which
109 can simultaneously provide two types of hydrogen bond donors depending on pH, and (ii) the
110 ability to respond to glucose.

111 With the aim of designing a new glucose-sensitive drug delivery system, the present research
112 has been focused on the creation and characterization of multilayer films comprised of a
113 phenylboronic-modified poly(lysine) derivative and alginate. This pair of polymers interacts
114 through electrostatic interactions. Our work demonstrates that incorporation of boronic
115 moieties inside the poly (lysine) backbone produces glucose-sensitivity. We hypothesised that
116 the response to stimuli was due to the boronic acid moieties present in the film, which are
117 known to form of covalent bonds with diols such as saccharides. Further, this work aims to
118 show how controlling electrostatic interactions facilitates the development of new, elegant
119 glucose-responsive systems.

120

121 **2. Experimental**

122 **2.1. Materials**

123 The following chemicals were purchased: alginate (alg^-), 1-ethyl-3-(3-dimethylaminopropyl)
124 carbodiimide hydrochloride (EDC, Sigma-Aldrich, > 98.5%), N-hydroxysuccinimide (NHS,
125 Sigma-Aldrich, > 98%), poly (lysine) (PLL), gold nanoparticles (Sigma-Aldrich) and 3-
126 carboxyphenylboronic acid hydrochloride (Sigma- Aldrich), and 11-mercaptoundecanoic acid
127 (Sigma- Aldrich).

128

129

130

131 **2.2. Chemical modification of poly (lysine)**

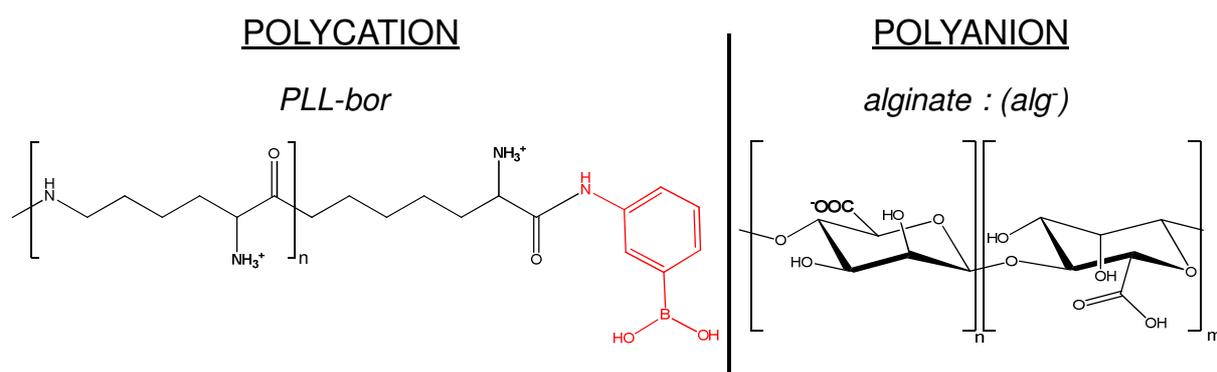
132 The carboxyl group of the phenylboronic acid derivative reacted with 1-ethyl-3-(3-
133 dimethylaminopropyl)carbodiimide hydrochloride (EDC) to form an unstable intermediate.
134 Upon reacting with N-hydroxysuccinimide (NHS), a more stable, amine-reactive intermediate
135 was formed. Finally, this intermediate was coupled to the amine groups of the poly(lysine),
136 completing the process of biopolymer modification. More precisely, 200 mg of PLL was
137 dissolved in aqueous medium and stirred for 24 h. 5 mg of 3-carboxyphenylboronic acid was
138 dissolved in aqueous medium followed by addition of EDC (90 mg) and NHS (50 mg). This
139 reaction mixture was stirred at room temperature for 45 min to fully activate the carboxylic
140 groups on phenylboronic acid derivative molecules. The solution was then added to the PLL
141 solution and stirred for 24 h at room temperature. The resulting product was purified using a
142 dialysis process (4 d) to remove the unreacted reactants (3.5 kDa cut-off). The polymer was
143 isolated via freeze-drying. The synthesised phenylboronic acid derivative that was modified
144 poly(lysine) was denoted as PLL-bor.

145

146 **2.2. Fabrication of glucose-responsive materials-**

147 Phenylboronic acid derivative-modified poly (lysine), (PLL-bor) and alginate (alg^-)
148 compounds were used as polyelectrolyte pairs (figure 1) for the coating of multilayer films
149 onto gold substrates.

150



151

152 **Figure 1.** Chemical structure of the cationic poly (lysine) derivative (PLL-bor) and the
153 polyanionic alginate (alg^-).

154

155 **2.2.1. Deposition of PLL-bor/alginate multilayers films onto a flat, gold substrate**

156 Wetting contact angle and quartz-crystal microbalance measurements (QCM) were used to
157 show that derivatives self-assemble. A Q-Sense E1 (Q-Sense, Sweden) system employing

158 ATcut, gold-coated quartz crystal sensors (Lot-Oriel, France) with a nominal frequency of
159 about 5 MHz was used to monitor the *in situ* formation of the (PLL-bor and alg-) self-
160 assembled film. Further, the same system was used to assess the impact of the glucose on the
161 resulting multilayer film. The gold-coated QCM Sensor Crystal was cleaned (i) with water
162 and ethanol and (ii) in a UV/ozone chamber for 20 min. After an additional ethanol rinse,
163 surfaces were dried under N₂ flow and pre-treated overnight with a solution of 11-
164 mercaptoundecanoic acid, 5 mg.mL⁻¹ prepared in ethanol, which contained the anchoring
165 layer. Self-assembly was initiated on a flat, gold surface via successive flushing of stock
166 solutions of the polymer (1 mg.mL⁻¹ in a PBS buffer at pH 8) for 15 min injected into the cell
167 with a flow rate of 250 μL.min⁻¹. A rinsing step (PBS buffer at pH 8) was performed between
168 each polymer deposition step for 5 min. The final multilayer build-up film was exposed to a
169 glucose solution of gradually increasing concentration. For the wettability study, the
170 multilayer film was formed on a pre-treated, flat, gold substrate through the successive
171 dipping of stock solutions of polymers (1 mg.mL⁻¹) for 15 min. A rinsing step with aqueous
172 PBS buffer at pH 8 solution was performed between each polymer deposition step. The
173 wetting contact angle measurements were performed with a Kruss goniometer at room
174 temperature in a sessile drop configuration. A droplet (5 μL) of Milli-Q water was deposited
175 onto the gold modified surface and the recorded image was analysed by the Drop Shape
176 Analysis software to determine the value of the contact angle. An average of three
177 measurements was calculated for each modified surface

178

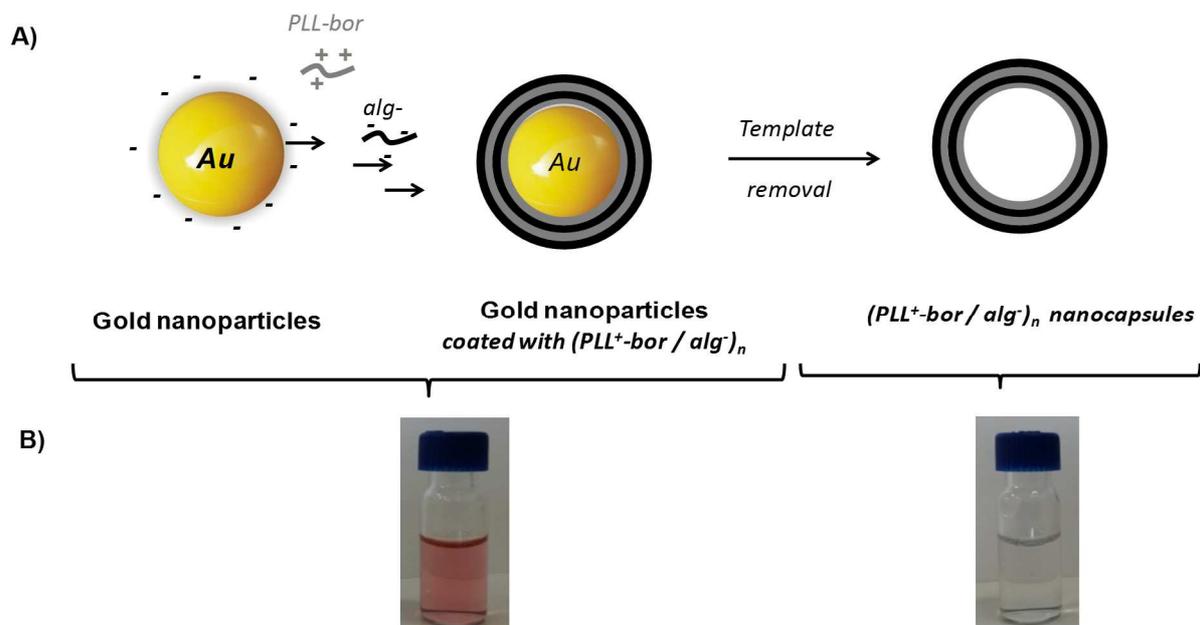
179 *2.2.2 Fabrication of phenylboronic acid derivative-modified poly (lysine)-based* 180 *nanomaterials*

181 To enable the successive adsorption of the cationic-poly (lysine) and the polyanionic alginate
182 on the spherical nanomaterial, the colloidal gold nanoparticles were preliminary treated with a
183 thiol derivative that facilitated the formation of carboxylate groups on the surface. 700 μL of a
184 filtered suspension of gold nanoparticles, 200 μL of aqueous solution and 100 μL of 11-
185 mercaptoundecanoic acid solution (5 g.L⁻¹, 10 mg in 2 mL of ethanol) were mixed overnight.
186 Ungrafted thiol was removed via centrifugation (17968 g for 15 min). Then, 100 μL of
187 cationic-poly (lysine) derivatives (1 g.L⁻¹ solubilised in water) were added to the pre-treated,
188 gold nanoparticles suspension, after treatment with 11-mercaptoundecanoic acid. After 15
189 min, the unadsorbed cationic-poly(lysine) derivatives were removed from the supernatant
190 fraction via washing (PBS buffer at pH 8) and centrifugation (17968 g for 15 min). This

191 washing process was repeated one more times and the volume of the solution was kept at 1
192 ml. Further, 100 μL of alginate solution (1 g.L^{-1} prepared in water) was added to the
193 suspension of cationic-poly(lysine)-modified gold nanoparticles and a rinsing step was
194 performed in order to remove the unadsorbed alginate. This procedure produced a single
195 bilayer comprised of cationic-poly(lysine) derivatives and anionic-alginate (PLL-bor/alg^-)₁
196 and was repeated until the desired number of layers was reached. After the creation of the
197 multilayer film zeta potential and absorbance measurements were taken. The zeta potential
198 measurement was performed on a Zetasizer Nano-ZS (Malvern Instrument). Absorbance
199 spectra were recorded with a UV-Visible spectrophotometer from Varian Cary 50 Bio.

200 From this coated gold nanomaterial, nanocapsules could be obtained by dissolving the gold
201 core in a 100 μL a potassium cyanide solution (created from a stock solution prepared by
202 dissolving 70 mg of KCN in 15 mL of water). Dialysis enabled researchers to remove the gold
203 complex (3.5 kDa cut off). The absence of the gold complex was investigated both in the last
204 dialysate and in capsules using the inductively coupled plasma optical emission spectrometry
205 (ICP-OES spectrometer, Simultaneous Varian Vista Axial) analyses. The capsules were
206 viewed with transmission electron microscopy (TEM). A droplet of the nanomaterial
207 suspension in PBS buffer at pH 8 and one of a solution of uranyl acetate (0.7% by wt) were
208 deposited on a plasma pre-treated copper TEM grid and allowed to air dry at room
209 temperature prior to analysis. TEM analyses were conducted using a Tecnai F20 ST
210 microscope (field-emission gun operated at 3.8 kV extraction voltage) operating at an
211 acceleration voltage of 200 kV.

212



213

214 **Figure 2.** A) Schematic illustration of the strategy for the fabrication of glucose-responsive
 215 nanomaterials comprised of phenylboronic acid containing poly (lysine) (PLL-bor) and
 216 alginate (alg^-) and B) Suspensions of bare and coated gold nanoparticles (red vial on the left).
 217 Removal of the gold core is results in a loss of colour (colourless vial on the right).

218

219

220 3. Results and discussion

221 As shown in Figure 2, the present strategy requires a combination of both a glucose-
 222 responsive biopolymer and a templating method. The successive adsorption of poly (lysine)
 223 derivative /alginate, (chemical structures shown in figure 1) polyelectrolyte pairs onto gold
 224 materials has been investigated. A glucose-response was obtained though the chemical
 225 modification of poly (lysine) (incorporation of phenylboronic acid moiety intended to provide
 226 glucose sensitivity). As reference a multilayer film was prepared using unmodified poly
 227 (lysine). This aimed to demonstrate the specific effects of the presence of boronic acid
 228 moieties. The stability of resulting materials in the presence of glucose was subsequently
 229 tested and researchers were able to show that it is possible to obtain hollow nanoparticles
 230 (capsule).

231

232

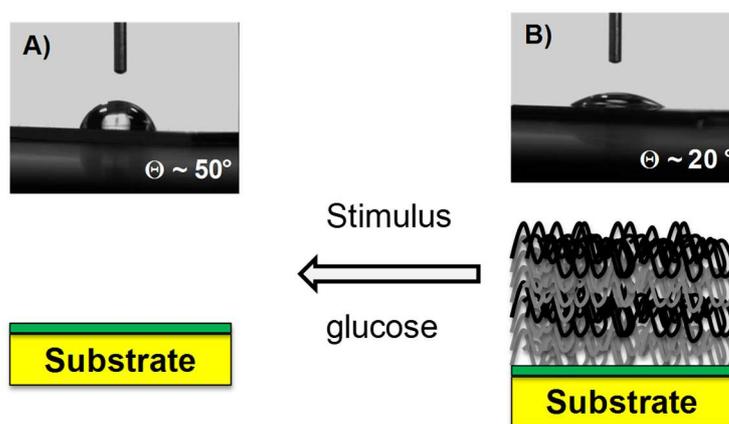
233

234

235 **3.1. Proof of concept: Fabrication of a glucose-sensitive film onto flat gold surface based**
236 **on phenylboronic acid derivative-modified poly (lysine)**

237 Compared to the non-chemically modified poly(lysine) (polycation), the electrolytic
238 properties of PLL-bor are retained, for instance it has a non-zero zeta-potential value ($+ 38 \pm$
239 4 mV for PLL-bor). This characteristic enables researchers to use this modified polymer for
240 the creation multilayer films. Indeed, under experimental conditions alginate and the poly
241 (lysine) derivative were oppositely charged and electrostatic attraction between the two types
242 of macromolecules was considered the driving force for their LbL assembly. First, the gold
243 substrate was pretreated with a carboxylate thiol derivative bringing a high density of negative
244 surface charges and thus allowing the robust anchoring of PLL-bor. Notably, fabrication was
245 performed near the optimal pH for the formation of a complex between the phenylboronic
246 acid moiety and diol derivatives, i.e. near to 9.

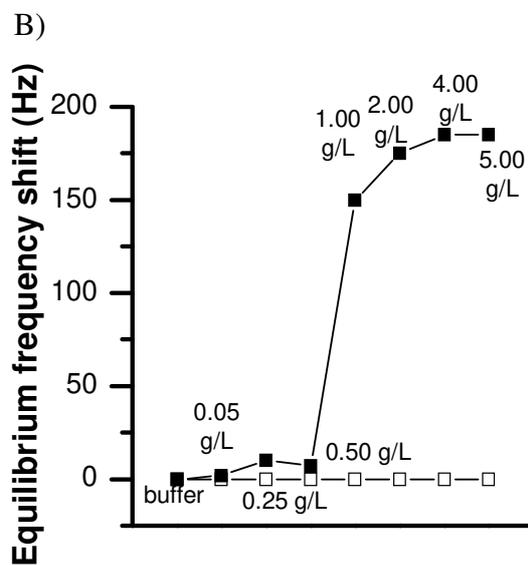
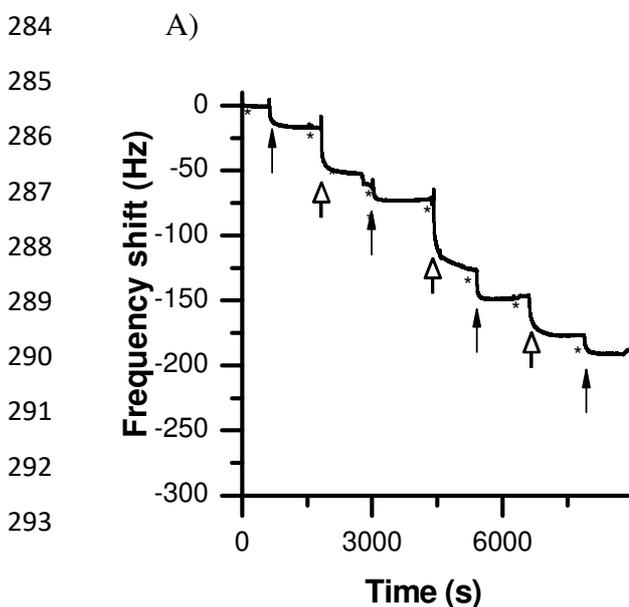
247 The feasibility of this strategy was assessed using static contact angle and QCM
248 measurements on the flat gold surface. Static contact angle measurements were performed on
249 flat gold pretreated substrates before and after treatment with polyelectrolyte pairs, (PLL-
250 bor/alg)_n to qualitatively demonstrate the presence of the multilayer film on the substrate. The
251 contact angle value diminished from initial values of 50° to final values of 20° upon coating,
252 in accordance with the hydrophilic nature of the incorporated biopolymer (Figure 3). This
253 variation in surface wettability is independent of the number of bilayers. Interestingly, the
254 addition of a solution of glucose leads to variability in wettability measurement, which varies
255 between 20° and 50° . Notice that the largest value is equal to the one obtained the gold
256 pretreated surface. This phenomenon may be correlated to the removal of the multilayer from
257 the surface. Note that using a non-modified poly(lysine) instead of PLL-bor leads to the same
258 wettability value (20°). No change of in this particular parameter was observed upon
259 subjecting the newly-formed surface to a solution of glucose.



260

261 **Figure 3.** Wetting angle contact value for (A) a flat, gold surface and (B) a flat, gold surface
262 modified with (PLL-bor/alg⁻)₄. Addition of a solution of glucose leads to the multilayer
263 removal.

264
265 The QCM technique was used to follow, in real time, (i) the stepwise adsorption of (PLL-
266 bor/alg⁻) polyelectrolytes pairs, (ii) the kinetics of adsorption and (iii) the impact of the
267 concentration-dependence glucose solution on the LbL build-up. As show in Figure 4A, a
268 stable baseline measurement was taken prior to the adsorption of the polyelectrolytes. After
269 10 min, a solution of PLL-bor (1 mg.mL⁻¹) was added to measurement chamber of the QCM-
270 D. A decrease in frequency shift was observed in accordance with the adsorption of the
271 cationic polymer to the anionic gold surface. PLL-bor was then incubated for 900 s before the
272 beginning of the rinsing step. It has been suggested that this adsorption is sufficient for the
273 sufficient surface coverage of the charged gold substrate with PLL-bor to affect a charge
274 reversal of the interfacial layer that is a prerequisite for the further adsorption of the polyanion
275 (i.e. the alginate). A slight increase in the frequency shift is seen upon rinsing, which could be
276 attributed to the removal of excess polyelectrolyte from the surface. The polyanionic alginate
277 (1 mg.mL⁻¹) was then flushed and a decrease in the resonance frequency was observed due to
278 its adsorption to the cationic, PLL-adsorbed layer. Once again, excess of free polyelectrolyte
279 chains were removed in a rinsing step. Typical decreases in frequency signals to -190 Hz were
280 observed by increasing the number of polyelectrolyte adsorption cycles. These results
281 demonstrate that mass was being added to the substrate and indicated that multilayer build-up
282 had occurred successfully (i.e (PLL-bor/alg⁻)₃- PLL-bor)). Note that the same frequency shift
283 variation is observed whether using PLL or PLL-bor.



294 **Figure 4.** A) Monitoring of $(\text{PLL-bor/alg}^-)_n$ build-up on flat substrate using QCM-
295 measurements (filled arrow = flushing with PLL-bor and unfilled arrow = flushing with alg^-),
296 B) Effect of gradually increasing the concentrations of glucose on $(\text{PLL-bor/alg}^-)_3$ - PLL-bor,
297 \blacksquare and $(\text{PLL/alg}^-)_3$ - PLL, \square multilayer films.

298

299 We then evaluated the impact of the addition of glucose solutions on the multilayer film
300 stability with and without boronic acid moieties (PLL-bor vs PLL) using QCM
301 measurements. To accomplish this aim, various concentrations of glucose solution (from 0.05
302 g.L^{-1} to 5 g.L^{-1}) are flushed through the $(\text{PLL-bor/alg}^-)_3$ - PLL-bor or $(\text{PLL/alg}^-)_3$ -PLL
303 multilayer film. As seen in Figure 4A after the build-up of the (PLL-bor/alg^-) films, the value
304 of the final equilibrium frequency shift was -190 ± 5 Hz. This value can be used as a reference
305 when examining how the film responds to glucose where the boronic moieties of the PLL, in
306 combination with the sugar, affect the stability of the film. Complete film removal should
307 induce a change in the equilibrium frequency resulting in a shift of frequency above $+190 \pm 4$
308 Hz. As depicted in Figure 4B, enhancing the glucose concentration results in an increase in
309 the value of the frequency signal. The critical glucose concentration is near to 0.5 g.L^{-1} . Note
310 that the same experiments were performed with (PLL/alg^-) and in this case, no frequency shifts
311 variations were observed.

312 Phenylboronic acid derivatives are known to be able to form covalent bonds with diols
313 derivatives, especially saccharides. [8] This anionic complex formed between glucose and
314 borate forms efficiently at the pH equal to the pKa of the boronic moiety (pKa ~ 9) and above
315 [11, 12] Springsteen and Wang's work have been able to demonstrate that the formation of
316 phenylboronic acid/diol derivative complexes clearly leads to a decrease equal to 2-4 pKa
317 units below the original pKa. [14] According to the authors, changes in pKa can be explained
318 by the stronger acid behaviour of the diol complex in comparison to the phenylboronic acid
319 derivative. [14, 15] Belbekhouche et al. [9, 10], who developed glucose-sensitive capsules,
320 used Springsteen and Wang's work [14] to describe their own results in which observed
321 glucose behavior mainly arose from the addition of glucose within the multilayer films
322 through the formation of a complex with phenyl boronic moieties.

323 A very simplified explanation for this type of phenomenon is diagrammed in Figure 5. The
324 multilayer film is formed as a result of the electrostatic interaction between the anionic
325 carboxylate groups of the alginate components and the ammonium groups of the PLL. Under
326 the conditions studied here, the phenylboronic acid groups were not charged and, therefore do
327 not take part in the formation of electrostatic complexes that occurring as the film is created.

328 The addition of glucose leads to the formation of negatively-charged phenylboranate groups
329 that may strongly interfere with self-assembled polyelectrolytes. As a result, there is first an
330 intermolecular repulsion between the carboxylate groups of the alginate and the complex
331 formed by interactions between glucose and phenylboronic acid. Therefore, there is a strong
332 repulsion that exists between successive multilayers constituting the film. Further,
333 electrostatic interactions between the ammonium groups of PLL and the anionic
334 glucose/phenylboronic acid both occur within the PLL-bor chain. All of these phenomena
335 contribute to the disassembly of the film, and show that varying the electrostatic interaction
336 between the PLL-bor and alginate in the multilayer assembly has a crucial impact on
337 multilayer stability. This phenomenon may explain why gradual increases in the concentration
338 of glucose solutions increase the frequency signal and are directly correlated to the removal of
339 mass and, subsequently, multilayer disassembly.

340

341

342

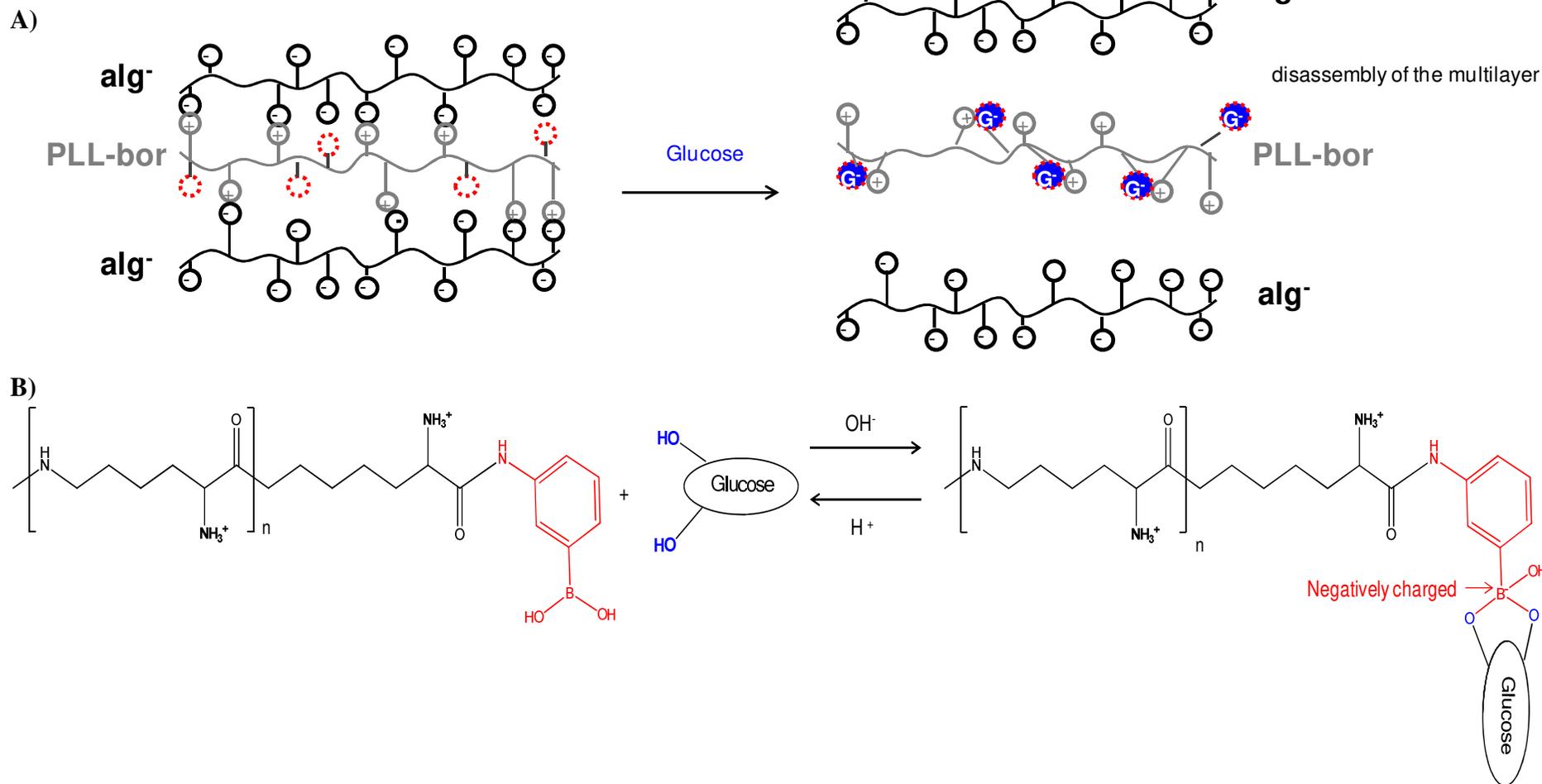
343

344

345

346

347
348
349
350
351
352
353
354
355
356
357
358
359



360
361
362
363
364
365
366

Figure 5. A) Suggested mechanism of glucose-induced decomplexation of the (PLL-bor/alg⁻) bilayer. (G = glucose). The black circles labelled with a (-) sign represent the carboxylate group of the alginate, and the grey circles with a (+) sign represent charged amino groups. The circles labeled G⁻ correspond to uncharged phenylboronic acid groups that becoming anionic after the addition of glucose (red circles with the letter G). B) Equilibrium between phenylboronic acid- containing poly(lysine) and diol-based glucose in aqueous solution.

367 The PLL-bor/alg⁻ polyelectrolyte pairs studied here enable the formation of multilayer films
368 that are responsive to glucose. Further, conditions used here to fabricate glucose-sensitive
369 nanomaterials can also be applied to form hollow polyelectrolyte nanocapsules capable of
370 responding to glucose.

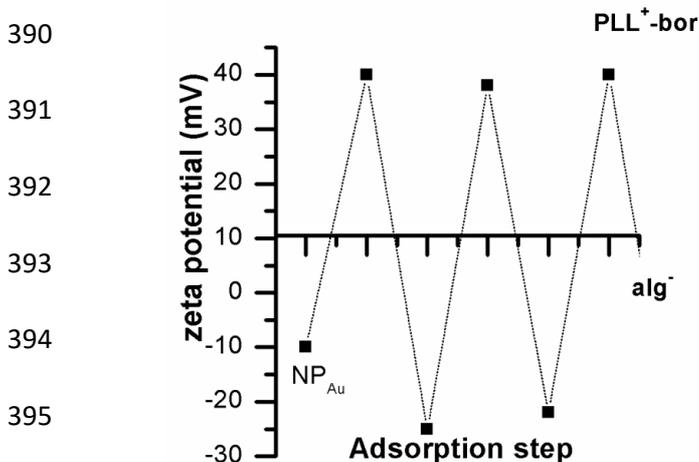
371

372

373 3.2. Glucose sensitive multilayer films comprised of phenylboronic acid containing poly 374 (lysine) deposited on a spherical substrate

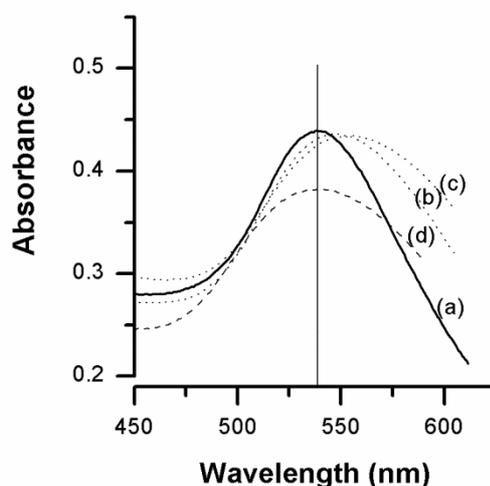
375 The zeta-potential value of gold coated nanomaterial was measured after pretreatment with
376 thiol derivatives and after the alternative coating with PLL-bor and alg⁻ particles. The zeta-
377 potential value of pre-treated gold particles is above -10 mV and becomes a positive value
378 after PLL-bor adsorption (+ 40 mV) and then becomes negative after subsequent adsorption
379 of alginate layer (-20 mV) (Figure 6A). This observed alternating variation of zeta potential
380 values clearly demonstrates the stepwise growth of (PLL-bor/alg) onto gold particles. [2, 6,
381 16-18] Furthermore, adsorption onto the gold nanoparticle surface has also been successfully
382 shown through absorbance measurements via exploiting the sensitivity of the surface Plasmon
383 band of the gold nanoparticle.[6, 19] As seen in figure 6B, gold coated nanoparticles with 4
384 two and four bilayers (PLL-bor/alg⁻) promote a shift toward red relative to the non-modified
385 nanoparticles (Figure 6B, curves a, b and c). This confirms that colloidal particles were
386 successfully coated. Interestingly, addition of glucose to the (PLL-bor/alg⁻)₄- coated gold
387 nanoparticles results in the same absorbance signature as bare particles. This result could be
388 correlated to multilayer removal upon saccharide treatment (Figure 6B, curves d and a).

389 A)



396

B)



397 **Figure 6.** A) Variation of the zeta potential values due to the alternative adsorption of PLL-
398 bor and alginate onto colloidal gold nanoparticles. B) UV-vis spectra of polyelectrolyte-
399 coated gold nanoparticle as a function of the number of adsorbed polymer layers. Curves a, b,
400 c and d depict zeta potentials for uncoated gold nanoparticles, (PLL-bor/alg⁻)₂, (PLL-bor/alg⁻)₄
401 and addition of glucose to the coated particle.

402

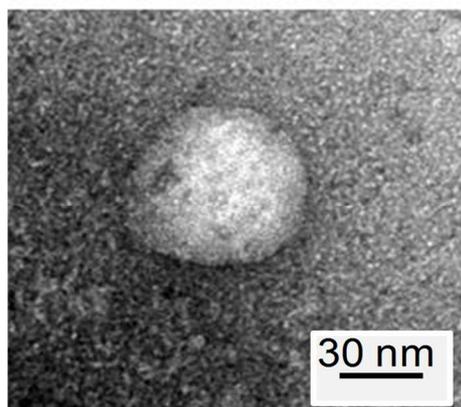
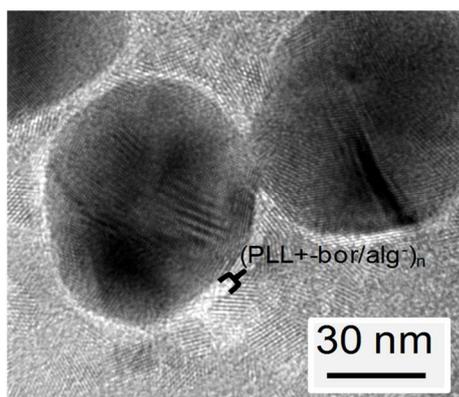
403

404 Finally, nanocapsules were obtained by removing gold cores via cyanide etching ($4 \text{ Au} + 8$
405 $\text{KCN} + 2 \text{ H}_2\text{O} + \text{O}_2 \rightarrow 4 \text{ KOH} + 4 \text{ KAu}(\text{CN})_2$). Direct visualization of the morphology of
406 coated nanoparticles and nanocapsules was accomplished using TEM micrographs of air-
407 dried polymer capsules (figure 7B). Note that no Au% was detected in the last dialysate via
408 ICP measurements.

409

410 A)

B)



411

412 **Figure 7.** TEM micrographs of A) (PLL-bor)/alg⁻)₄-coated gold nanoparticles and B)
413 nanocapsules.

414

415

416 4. Conclusion

417 Novel assemblies based on poly(lysine) derivative containing phenylboronic acid moieties
418 and an alginate can be used to create glucose-sensitive materials. This procedure route
419 combines both the templating method and electrostatic-bonded LbL multilayer method. The
420 glucose-sensitive behavior of the film is likely strongly be related to the competitive binding

421 of glucose and the boronic acid groups of the poly(lysine) within the multilayer film that
422 result in the destabilization of the film.

423 In fact, the film comprised of PLL-bor and alginate is formed due to electrostatic interactions
424 between the ammonium groups of the poly (lysine) derivative and the carboxylate moiety of
425 alginate. Under conditions studied here, addition of glucose increases the number of borate
426 moieties which begin to strongly interact with the poly (lysine) derivative. There are
427 simultaneously (i) new complexes formed from electrostatic interactions within the anionic
428 complex of glucose and borate, (ii) interaction between this complex and the ammonium
429 moiety of the poly (lysine) and (iii) disruption of the electrostatic interaction between the poly
430 (lysine) derivative and the alginate. The synergy of all of these phenomena seems to lead to
431 the total disassembly of the multilayer film. This elegant strategy could be used to obtain
432 hollow particles, namely capsules.

433

434

435 **5. Acknowledgment**

436 The authors would like to thank the UPEC and CNRS for financial help.

437

438

439 **6. References**

440 [1] C.S. Peyratout, L. Dähne, Tailor-made polyelectrolyte microcapsules: from multilayers to
441 smart containers, *Angewandte Chemie International Edition* 43(29) (2004) 3762-3783.

442 [2] F. Caruso, Hollow capsule processing through colloidal templating and self-assembly,
443 *Chemistry-A European Journal* 6(3) (2000) 413-419.

444 [3] S. Belbekhouche, N. Bousserhine, V. Alphonse, B. Carbonnier, From beta-cyclodextrin
445 polyelectrolyte to layer-by-layer self-assembly microcapsules: From inhibition of bacterial
446 growth to bactericidal effect, *Food Hydrocolloids* 95 (2019) 219-227.

447 [4] E. Donath, G.B. Sukhorukov, F. Caruso, S.A. Davis, H. Möhwald, Novel hollow polymer
448 shells by colloid-templated assembly of polyelectrolytes, *Angewandte Chemie International
449 Edition* 37(16) (1998) 2201-2205.

450 [5] A.P.R. Johnston, C. Cortez, A.S. Angelatos, F. Caruso, Layer-by-layer engineered
451 capsules and their applications, *Current Opinion in Colloid & Interface Science* 11(4) (2006)
452 203-209.

453 [6] S. Belbekhouche, O. Mansour, B. Carbonnier, Promising sub-100 nm tailor made hollow
454 chitosan/poly (acrylic acid) nanocapsules for antibiotic therapy, *Journal of colloid and
455 interface science* 522 (2018) 183-190.

456 [7] M. Sauer, W. Meier, Responsive nanocapsules, *Chemical Communications* (1) (2001) 55-
457 56.

458 [8] B.G. De Geest, A.M. Jonas, J. Demeester, S.C. De Smedt, Glucose-responsive
459 polyelectrolyte capsules, *Langmuir* 22(11) (2006) 5070-4.

- 460 [9] S. Belbekhouche, S. Charaabi, L. Picton, D. Le Cerf, B. Carbonnier, Glucose-sensitive
461 polyelectrolyte microcapsules based on (alginate/chitosan) pair, *Carbohydrate Polymers* 184
462 (2018) 144-153.
- 463 [10] S. Belbekhouche, S. Charaabi, B. Carbonnier, Glucose-sensitive capsules based on
464 hydrogen-bonded (polyvinylpyrrolidone / phenylboronic –modified alginate) system, *Colloids
465 and Surfaces B: Biointerfaces* 177 (2019) 416-424.
- 466 [11] Q. Wu, L. Wang, H. Yu, J. Wang, Z. Chen, Organization of Glucose-Responsive
467 Systems and Their Properties, *Chemical Reviews* 111(12) (2011) 7855-7875.
- 468 [12] W.L.A. Brooks, B.S. Sumerlin, Synthesis and Applications of Boronic Acid-Containing
469 Polymers: From Materials to Medicine, *Chemical Reviews* 116(3) (2016) 1375-1397.
- 470 [13] T. Levy, C. Déjugnat, G.B. Sukhorukov, Polymer Microcapsules with Carbohydrate-
471 Sensitive Properties, *Advanced Functional Materials* 18(10) (2008) 1586-1594.
- 472 [14] G. Springsteen, B. Wang, A detailed examination of boronic acid–diol complexation,
473 *Tetrahedron* 58(26) (2002) 5291-5300.
- 474 [15] J. Yoon, A.W. Czarnik, Fluorescent chemosensors of carbohydrates. A means of
475 chemically communicating the binding of polyols in water based on chelation-enhanced
476 quenching, *Journal of the American Chemical Society* 114(14) (1992) 5874-5875.
- 477 [16] F. Caruso, H. Lichtenfeld, E. Donath, H. Möhwald, Investigation of Electrostatic
478 Interactions in Polyelectrolyte Multilayer Films: Binding of Anionic Fluorescent Probes to
479 Layers Assembled onto Colloids, *Macromolecules* 32(7) (1999) 2317-2328.
- 480 [17] F. Caruso, R.A. Caruso, H. Möhwald, Nanoengineering of inorganic and hybrid hollow
481 spheres by colloidal templating, *Science* 282(5391) (1998) 1111-1114.
- 482 [18] G. Sukhorukov, A. Fery, H. Möhwald, Intelligent micro- and nanocapsules, *Progress in
483 Polymer Science* 30(8–9) (2005) 885-897.
- 484 [19] D.I. Gittins, F. Caruso, Multilayered polymer nanocapsules derived from gold
485 nanoparticle templates, *Advanced Materials* 12(24) (2000) 1947-1949.

486

487

488

489

490

491

492

493

494

Graphical Abstract

