

Multilayer films based on host–guest interactions between biocompatible polymers†

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Multilayer films are formed using host–guest interaction between two derivatized chitosans, one, with β -cyclodextrin cavities and the other with adamantyl moieties.

Hydrogels that undergo swelling changes in response to subtle variations in environmental temperature, pH, ionic strength or to specific molecules, among other stimuli, have attracted considerable attention over the past decade, due to their numerous potential applications in the biochemical and biomedical fields. More recently, there has been interest in developing stimuli-responsive gels with confined dimensions such as substrate-supported films. By restricting the volume-phase transitions of these systems to fewer dimensions, it is possible to create stimuli-responsive surfaces of which interactions with biological molecules or tissues could be modulated. Among the different methods used to fabricate structured and functional thin films on solid substrates, the layer-by-layer electrostatic self-assembly has emerged as an efficient, versatile, yet simple technique. This method, based on the alternate adsorption of polycations and polyanions, has been successfully extended to various other attractive forces such as hydrogen bonding,^{1,2} charge-transfer interaction,^{3,4} specific molecular recognition (sugar–lectin or biotin–avidin interaction)^{5–7} or host–guest interaction.⁸

Recently, we have exploited guest complex formation to develop new chitosan-based supramolecular assemblies in aqueous media, exhibiting a gel-like behavior.⁹ Chitosan is the main derivative of chitin, a natural polysaccharide found in crustacean shells. This biopolymer has important biological properties but also good film and gel forming characteristics in relation with its molecular structure (β 1 \rightarrow 4 D-glucosamine backbone), giving a semi-rigid character but also allowing a cooperative H-bond network formation.

The supramolecular-structured hydrogels were obtained from mixtures of two derivatized chitosans, one, denoted Chit-CD, with β -cyclodextrin (CD) cavities and the other, denoted Chit-AD with

adamantyl moieties (AD), randomly distributed along the chains. The bulk properties of these original systems in which cyclodextrin–adamantane complexes play a role of interchain junctions were shown to be highly sensitive to the addition of competitive molecules and also, to the ionic strength of the aqueous medium as a result of the polycationic nature of chitosan.⁹ In the present study, we describe the buildup of new self-assembled multilayer films by alternate multivalent complexation of adamantane- and cyclodextrin-grafted chitosan. The adsorption steps as well as the response of the thin films to the presence of competitive host molecules and to external salt concentration were investigated using a quartz crystal microbalance with dissipation monitoring (QCM-D).

While some examples of multilayer films based on inclusion complexes and elaborated from single molecules and/or grafted nanoparticles are related in literature,^{8,10,11} this work represents to our knowledge, the first example of the buildup of stimuli-responsive multilayer films based on host and guest biopolymers. Huskens and co-workers have adsorbed adamantyl-modified polymer on pure β -CD self-assembled monolayer (SAM) *via* host–guest interactions and demonstrated that adsorption of a second layer of β -CD-covered nanoparticles on the first adamantyl-modified polymer layer is not stable upon rinsing by water.¹² The problem of the vacancy of the complementary complexation sites was then suggested. Indeed, the driving force of the assembly, *i.e.* the inclusion complexation, requires a perfect recognition between the host cavities and the guest moieties and availability of each partner during the assembly growth. Another point to mention is that both polymers used in our study are positively charged, conferring electrostatic repulsions between polymers and hence, adding a supplementary difficulty to the construction of the assembly.

The multilayer formation was performed in the usual solvent (solvent A) used for chitosan (0.3 M AcOH–0.03 M

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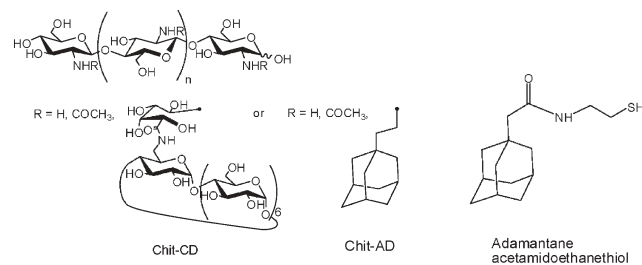


Fig. 1 Chemical structure of (A) adamantane- and cyclodextrin-grafted chitosan and (B) of the thiol-adamantane used to functionalize the gold-coated quartz crystal.

AcOONa)^{13–15} by alternated deposition of chit–CD and chit–AD (Fig. 1) onto a self-assembled monolayer (SAM) exhibiting a variable density of adamantyl groups. SAMs were prepared by diluting adamantane acetamidoethanethiol (AD-SH) with ethanethiol (EtSH) to obtain the desired AD/SH/EtSH molar ratios 5, 25 and 50%.[†] Fig. 2 shows the variation of the QCM-D signals, normalized frequency ($\Delta F_n/n$) and dissipation (D_n) shifts, recorded during the assembly construction on a 50% adamantyl-coated surface (see ESI[†]).

The adsorption of chit–CD on this SAM gives rise to a large $\Delta F_n/n$ decrease and D_n increase. The mass $\Delta\Gamma$ obtained for each adsorption by fitting the QCM-D curves according to the viscoelastic model¹⁷ are reported in Table 1 (see ESI[†] for the value of the shear elastic modulus (μ) and shear viscosity (η) of the adsorbed layer). Mass values obtained on the three SAMs containing AD after chit–CD adsorption and subsequent rinsing with solvent indicate a chit–CD layer formation. Moreover the shear viscosity values ($\sim 1.3 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$) are in agreement with the hydrogel layer values reported in literature.¹⁸ Chit–CD layers formed on the three SAMs are stable by rinsing with solvent.

To confirm the inclusion complex formation between the polymer and the surface, two tests were additionally carried out: first, non-specific adsorption was checked *via* adsorption of

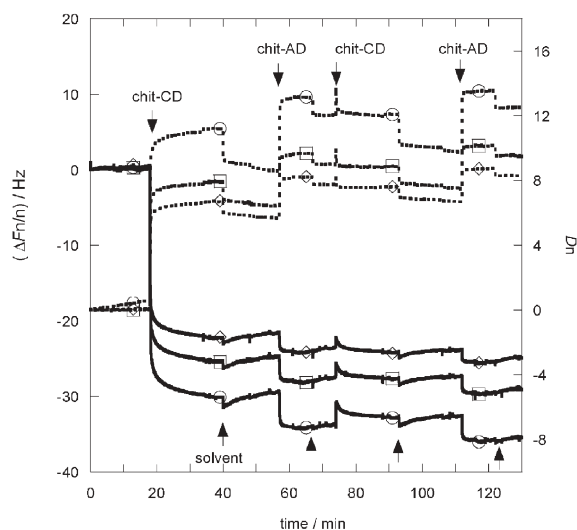


Fig. 2 Typical QCM-D response, normalized frequency $\Delta F_n/n$ (solid line) and dissipation D_n (dashed line) at three overtones $n = 3$ (\diamond), 5 (\square) and 7 (\circ) recorded during the multilayer construction on a 50% adamantyl-functionalized SAM (see ESI[†]).

Table 1 Mass $\Delta\Gamma$ of the adsorbed layers, obtained by fitting the QCM-D response according to the Voinova's model¹⁷

| Layer | Polymer | AdSH molar ratio of the adsorption solution | | |
|-------|---------|---|-----|-----|
| | | 50% | 25% | 5% |
| 1 | Chit-CD | 1368 | 907 | 951 |
| 2 | Chit-AD | 530 | 657 | 946 |
| 3 | Chit-CD | -288 | 437 | 85 |

chit–CD onto a 0% adamantyl SAM, lacking the guest moieties (*i.e.* formed from the 100% EtSH solution). The mass adsorbed was 460 ng cm^{-2} . Secondly, competition experiments with 5 mM solution of free β -CD were conducted on the different chit–CD layers grafted to the 5, 25 and 50% adamantyl SAMs. For all surfaces, only partial desorption of the chit–CD layer was involved by β -CD (ESI[†]). These last two results suggest that two phenomena take place during the chit–CD adsorption. The first one is the occurrence of non specific chit–CD adsorption. In addition to non-specific adsorption, another phenomena occurs: part of the adsorbed polymer is strongly anchored to the adamantyl-coated surface through multivalent specific host–guest interaction. Indeed, the multiple presentation of the guest sites offered by the polymer likely induces a higher affinity to the surface compared to the monovalent β -CD.^{19–21} This may be the reason why the mass remaining on the surface after rinsing with the β -CD increases with the SAM AD/SH/EtSH ratios and is higher, especially for SAM exhibiting high adamantyl ratios, than the mass adsorbed by non-specific interaction. These results thus confirmed that the assembly is mainly based on host–guest interactions between the cyclodextrin inclusion sites of the chit–CD polymer and the adamantyl moieties immobilized on the surface.

Comparing the three surfaces, similar masses of CD-grafted polymer are adsorbed on the SAMs. The ratio of adamantyl moieties exhibited by the surface has no sharp influence on the adsorption of this first layer. This observation could be explained by the fact that enough hydrophobic sites are offered by the surface to graft the first layer *via* host–guest interaction.¹²

As mentioned above, the difficulty in the elaboration of multilayers based on host–guest interaction is related to the availability of the host or guest moieties coupled to the charge repulsion between the polymers. In our work, we observed that on the first chit–CD layer, a second layer of chit–AD could be adsorbed, overcoming the electrostatic repulsion between the positively charged chitosan polymers (Table 1). This result confirmed that the supramolecular assembly is based on host–guest interaction between the chit–CD and chit–AD polymers. Indeed, by contrast to the results obtained for the first layer, the masses of the adsorbed chit–AD second layers are function of the initial adamantyl molar fraction in the SAMs: the more AD guests are on the surface, the lower is the mass of the second layer (Table 1). Since the chit–AD layer is supposed to be separated from the SAM by the chit–CD layer, this result leads to the conclusion that first layers anchored on the three SAMs do not present the same organization. The mass differences between the second layers adsorbed on the three SAMs could be explained by a difference in the availability of host sites offered by the chit–CD polymer first layer (Fig. 3). Chit–CD polymer seems to adapt its conformation to the SAM adamantyl density; a low proportion of

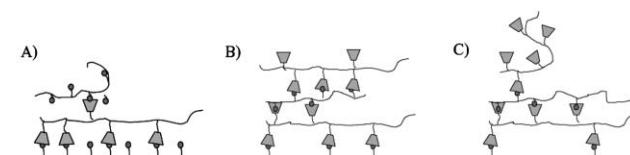


Fig. 3 Cartoon representing the influence of the SAMs adamantyl densities on the self-assembly growth: (A) high, (B) medium and (C) low adamantyl ratio exhibited by the SAM.

host sites of the chit-CD polymer is engaged for adsorption onto a adamantyl-diluted surface, meaning that a large proportion of sites is available for further inclusion complexation, *i.e.* for a second layer adsorption (Fig. 3(C)). By comparison, on a high-density surface, the contrary is observed; most of the inclusion sites of the first host polymer layer are involved in complexation with the surface leaving only a relatively few free sites for complexation to the second layer adsorption (Fig. 3(A)).

The chit-CD deposition to form a third layer corroborates our hypothesis on the major role played by the ratio of available host or guest sites for the multilayer elaboration. On the two-layer assembly built on a 50% functionalized surface, chit-CD polymer acts as a competitor. Instead of forming a third layer, chit-CD leads to partial desorption ($\sim 288 \text{ ng cm}^{-2}$) of the chit-AD second layer (Table 1). This observation could be attributed to the low amount of inclusion complexes involved in the adhesion between the first and the second layer of polymers. Moreover, the same alternated deposition carried out on a 5% functionalized surface, instead of desorbing the second layer as previously, leads to a low third layer adsorption, confirming the stability of the two previous layers. Indeed, on the 5% adamantyl-coated SAM, chit-CD comprising the first layer may not be grafted to the surface with a large amount of inclusion complexes. Most of the CD cavities should be involved in complexation with the chit-AD second layer that has almost no more guest sites available for further complexation. The two first layers are strongly grafted leaving few complexation sites for the third layer adsorption. All these results point out an optimum ratio of adamantyl moieties offered by the initial surface to multilayer elaboration based on host-guest interactions. In our experiments, this optimum value might be near 25%. Indeed, for this surface, the complexation sites between each layer are well distributed, allowing better third layer adsorption.

The effect of increasing the ionic strength on the three-layer assemblies built on the 5 and 25% adamantyl surfaces is shown in Fig. 4. The mass variations of the film alternatively exposed to solvent A and to a solvent B of higher ionic strength (0.3 M AcOH-0.1 M AcOONa) clearly indicate the swelling/deswelling capacity of the multilayers (see ESI†). This capacity appears to be conditioned by the adamantyl surface ratio and is probably linked to the number of host-guest complexation pairs between each layer of the assemblies (Fig. 3). The mass decrease observed in the saline solvent suggests the compression of the assembly occurring with release of the water trapped in the multilayer.²² Moreover, the viscosity of the multilayer is higher in the saline solvent ($\sim 1.8 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$) than in solvent A ($\sim 1.4 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$), see ESI.† This result is in agreement with the deswelling of an hydrated film.¹⁸ Solvents exhibiting higher ionic strength certainly contribute to lower the electrostatic repulsive effect between the layers, screening the positive charges of the polymers. Another point to underline is the good reversibility of the response offered by the assemblies formed on both surfaces. Although the electrostatic repulsions between the polycationic chitosan polymers are overcome by inclusion complexation between their respective residues, they play a crucial role in the self-assembly, and especially modulate its thickness.

In summary, the present work establishes the feasibility of polymer multilayer formation based on host-guest interaction using β -cyclodextrin- and adamantyl-grafted chitosans. While stability of the self-assembly is conferred by multivalent

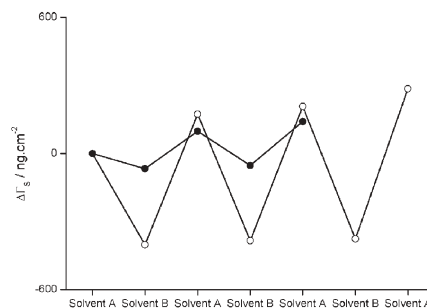


Fig. 4 Mass variation $\Delta\Gamma_s$ of the three-layer assemblies built on a 5% (○) and on a 25% (●) adamantyl SAM exposed to solvents of different ionic strength. $\Delta\Gamma$ is the mass difference between the assembly at each solvent exposure and the initial assembly in solvent A (see ESI†).

complexation occurring at each step of the construction, our results reveal that the assembly growth is mainly governed by the availability of the complexation sites offered by each layer. This preliminary study shows that the assembly obtained is stimuli-responsive and so paves the way for the use of host-guest interaction into bottom-up elaboration of nanodevices based on biopolymers.

Notes and references

- 1 Y. Zhang, S. Yang, Y. Guan, X. Miao, W. Cao and J. Xu, *Thin Solid Films*, 2003, **437**, 280–284.
- 2 D. M. DeLongchamp and P. T. Hammond, *Langmuir*, 2004, **20**, 5403–5411.
- 3 Y. Shimazaki, M. Mitsuishi, S. Ito and M. Yamamoto, *Langmuir*, 1997, **13**, 1385–1387.
- 4 X. Wang, K. Naka, H. Itoh, T. Uemura and Y. Chujo, *Macromolecules*, 2003, **36**, 533–535.
- 5 J.-i. Anzai and Y. Kobayashi, *Langmuir*, 2000, **16**, 2851–2856.
- 6 J.-i. Anzai, Y. Kobayashi, Y. Suzuki, H. Takeshita, Q. Chen, T. Osa, T. Hoshi and X.-y. Du, *Sens. Actuators, B*, 1998, **52**, 3–9.
- 7 T. Hoshi, S. Akase and J.-i. Anzai, *Langmuir*, 2002, **18**, 7024–7028.
- 8 I. Suzuki, Y. Egawa, Y. Mizukawa, T. Hoshi and J.-i. Anzai, *Chem. Commun.*, 2002, 164–165.
- 9 T. Lecourt, P. Sinaye, C. Chassenieux, M. Rinaudo and R. Auzély-Velty, *Macromolecules*, 2004, **37**, 4635–4642.
- 10 O. Crespo-Biel, B. Dordi, D. N. Reinhoudt and J. Huskens, *J. Am. Chem. Soc.*, 2005, **127**, 7594–7600.
- 11 C. A. Nijhuis, F. Yu, W. Knoll, J. Huskens and D. N. Reinhoudt, *Langmuir*, 2005, **21**, 7866–7876.
- 12 O. Crespo-Biel, M. Peter, C. M. Bruinink, B. J. Ravoo, D. N. Reinhoudt and J. Huskens, *Chem. Eur. J.*, 2005, **11**, 2426–2432.
- 13 R. Auzély-Velty and M. Rinaudo, *Macromolecules*, 2001, **34**, 3574–3580.
- 14 R. Auzély-Velty and M. Rinaudo, *Macromolecules*, 2002, **35**, 7955–7962.
- 15 A. Charlot, R. Auzély-Velty and M. Rinaudo, *J. Phys. Chem. B*, 2003, **107**, 8248–8254.
- 16 J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo and G. M. Whitesides, *Chem. Rev.*, 2005, **105**, 1103–1169.
- 17 M. V. Voinova, M. Rodahl, M. Jonson and B. Kasemo, *Phys. Scr.*, 1999, **59**, 391–396.
- 18 P. Asberg, P. Bjork, F. Hook and O. Inganas, *Langmuir*, 2005, **21**, 7292–7298.
- 19 J. Huskens, A. Mulder, T. Auletta, C. A. Nijhuis, M. J. W. Ludden and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2004, **126**, 6784–6797.
- 20 A. Mulder, T. Auletta, A. Sartori, S. Del Ciotto, A. Casnati, R. Ungaro, J. Huskens and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2004, **126**, 6627–6636.
- 21 J. D. Badjic, A. Nelson, S. J. Cantrill, W. B. Turnbull and J. F. Stoddart, *Acc. Chem. Res.*, 2005, **38**, 723–732.
- 22 F. Hook, B. Kasemo, T. Nylander, C. Fant, K. Sott and H. Elwing, *Anal. Chem.*, 2001, **73**, 5796–5804.