



Short communication

Nanostructured self-assembled films containing chitosan fabricated at neutral pH

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ABSTRACT

The layer-by-layer (LbL) approach enables the design of highly engineered nanostructured surfaces aimed for a variety of applications. Here, the formation of chitosan–alginate assemblies was followed *in situ* by quartz crystal microbalance with dissipation monitoring (QCM-D). For the case of biomedical and biological applications, it would be desirable to process the film near physiological pH. Water-soluble chitosan was used to allow the build-up of the multilayers at neutral pH. Distinct structural characteristics were found between films built at different pH values. Specifically, films assembled at a pH of 5.5 exhibit a more rigid behaviour and compact state, whereas at a pH of 7.0 the layers display more viscoelastic properties and higher thicknesses. These results confirmed the key role of electrostatic interactions during polyelectrolyte adsorption and the influence of chitosan charge density in the formation and structure of multilayers.

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1. Introduction

Surface modification of biomaterials is a well known approach for preserving the bulk properties of the underlying material while enabling an adequate biointerface between the implant and the surrounding tissue. One promising way to functionalize the biomaterials surface in a controllable and versatile manner can be achieved with the layer-by-layer (LbL) technique. LbL build-up proceeds by alternating adsorption of oppositely charged polyelectrolytes until a charge inversion occur on the surface assembly (Ai, Jones, & Lvov, 2003). The power of this technique relies mainly on the wide choice of molecules available, the flexibility and simplicity of construction, the structural precision and the ability to coat materials of any shape and dimension. Multilayered assemblies involving weak polyelectrolytes can be molecularly tailored by simply adjusting the solution pH and ionic strength (Boddohi, Killingsworth, & Kipper, 2008).

Over the past years polysaccharides, such as chitosan, have been proposed for biomedical applications due to their biocompatibility and non-toxic character. Recent studies have explored the development of chitosan-based self-assemblies to enhance the biocompatibility of biomaterials (Song et al., 2009), in the preparation of insulin-loaded nanoparticles (Fan, Wang, Fan, & Ma, 2006) or core-shell structures (Shenoy, Antipov, Sukhorukov, & Mohwald, 2003)

for local drug delivery and as bioactive nanocoatings for endovascular devices (Thierry, Winnik, Merhi, Silver, & Tabrizian, 2003). Concerning these chitosan-based multilayers, previous reports have explored the difficulty of working near physiological conditions mostly because the solubility of chitosan is pH-dependent (Lawrie et al., 2007). Picart et al. have showed that a preincubation with the crosslinking system 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/*N*-hydroxysuccinimide (EDAC/NHS) promotes covalent binding between activated carboxylic sites and the primary amine groups on deacetylated chitosan residues, enhancing the film stability (Picart et al., 2005). Also, chemical modifications have been incorporated into chitosan such as phosphorylcholine (Kujawa, Schmauch, Viitala, Badia, & Winnik, 2007) and quaternary ammonium (Channasanon, Graisuwan, Kiatkamjornwong, & Hoven, 2007) substituents to achieve coatings more stable over a broader pH range.

The main goal of this work was to demonstrate the possibility of producing biocompatible nanostructured surfaces containing chitosan at neutral conditions. The step-by-step build-up of alginate and chitosan multilayers was monitored *in situ* by means of quartz crystal microbalance with dissipation monitoring (QCM-D). The combination of normalized resonant frequency ($\Delta f/n$) and energy dissipation (ΔD) measurements available with this technique gives information about the adsorbed amount (via $\Delta f/n$) and the variation of the viscoelastic properties (via ΔD) of the formed film in real time. The influence of pH (5.5 and 7.0) on the LbL deposition process was investigated. Since the ultimate purpose of these multilayers is their use in biomedical applications,

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one has to take into account the film stability and grow-up in physiological environment. Ultrapure chitosan polymers have already been incorporated in multilayered assemblies in acidic conditions (Richert et al., 2004). By choosing a water-soluble chitosan salt as a weak polyelectrolyte, we were able to expand the pH range of functionality necessary for the build-up and simultaneously maintain the mild environmental conditions during nanofabrication. Moreover, the versatile thin films produced can be further functionalized by inclusion of active drugs, proteins and growth factors at physiological conditions, expanding their applicability.

2. Experimental

2.1. Materials

Ultrapure chitosan salt (CHI, viscosity <20 mPa s, molecular weight, M_v <150 kDa, degree of deacetylation, DDA 80–90%) was purchased from NovaMatrix and used without purification. This water-soluble chitosan chloride salt (PROTASAN UP CL 113) is obtained by the substitution of the amine moiety at the C-2 position of chitosan to form amine salts, such as NH_3^+Cl^- and the manufacturing process include the following steps: dissolution in acid, depyrogenization, filtration and spray drying. The DDA of chitosan, 84–85%, was determined by proton nuclear magnetic resonance (H NMR) spectroscopy (Lavertu et al., 2003). Sodium alginate derived from brown algae (ALG, low viscosity) was obtained from Sigma and used as received. Chitosan is a weak polybase with a pK_a around 6.5 (Rinaudo, 2006) and alginate is a weak polyacid with a pK_a between 3 and 4 (de Kerchove & Elimelech, 2006). Fresh solutions of chitosan and alginate were prepared in saline conditions (0.15 M NaCl) to yield a final polymer concentration of 0.5 mg/mL. Experiments were performed at two different pH values, 5.5 and 7.0. The pH of the solutions was adjusted with acetic acid and sodium hydroxide solutions.

2.2. Quartzcrystal microbalance with dissipation monitoring (QCM-D)

The multilayer build-up was followed *in situ* by quartz crystal microbalance with dissipation monitoring (Q-Sense, E4 system). QCM-D has the ability of simultaneously measuring the normalized resonant frequency ($\Delta f/n$) and energy dissipation (ΔD) shifts. This technique has been extensively described in detail elsewhere (Marx, 2003). Very briefly, AT-cut quartz crystals with gold plated polished electrodes (Q-Sense) can be excited at its fundamental frequency ($f_0 \approx 5$ MHz) as well as at the 3rd, 5th, 7th, 9th and 11th overtones, corresponding to 15, 25, 35, 45 and 55 MHz, respectively. The piezoelectric properties of the quartz crystal changes its oscillation frequency as the mass is deposited onto the crystal surface. If the adsorbed material is rigidly attached and is much thinner than the crystal, the resulting decrease in the oscillation frequency is proportional to the mass of the applied layer and thus can be quantitatively interpreted using the Sauerbrey equation (Michael, Fredrik, Anatol, Peter, & Bengt, 1995). However, when using polymers, a mass load introduces dissipative energy damping and the previous relation is no longer valid. These hydrated films exhibit a typical viscoelastic behaviour that can be used to characterize structural reorganisation of layers in real time by applying, for instance, a Voigt based model. Very briefly, in this model, the adsorbed film is represented by a single Voigt element that consists of a parallel combination of a spring and dashpot to represent the elastic (storage) and inelastic (damping) behaviour of a material, respectively (Voinova, Rodahl, Jonson, & Kasemo, 1999).

The gold coated crystals were cleaned with sequential sonication in water, ethanol and water and then dried with flowing nitrogen gas. QCM-D experiments were started with a 0.15 M NaCl

solution (buffer baseline). Then, the multilayer films were built by alternating chitosan and alginate depositions onto quartz crystal. The gold substrates were firstly coated with a polycation layer (chitosan). Fresh polyelectrolyte solutions were injected into the measurement chamber for 10 min at a flow rate of 100 $\mu\text{L}/\text{min}$ and a washing step of 10 min with a 0.15 M NaCl solution was included after the adsorption of each polyelectrolyte. Two different pH were used to assembly the multilayered chitosan–alginate system, 5.5 and 7.0. During the whole process, $\Delta f/n$ and ΔD variations were continuously recorded as a function of time. For all measurements, the temperature was set up at 25 °C. The experiments were performed at least in triplicate.

The QTools software (version 3.0.6.213) provided with the equipment (Q-Sense) was used to model our data according to the above mentioned Voigt model. $\Delta f/n$ and ΔD shifts were fitted for three overtones (7th, 9th and 11th).

3. Results and discussion

Figs. 1 and 2 compare the build-up of chitosan–alginate multilayered assemblies with 5 bilayers at different pH, 5.5 (squares) and 7.0 (circles). The decrease of $\Delta f/n$ after each polymer adsorption step evidences that mass is being deposited at the crystal surface for both pH. Also, the sequential deposition process was generally stable and reproducible. Although it can be seen some differences for the $\Delta f/n$ shifts, the major distinction between the films built at a pH of 5.5 and 7.0 concerns their mechanical and structural organization. Fig. 1B shows that assemblies built at a pH of 7.0 present a pronounced viscoelastic behaviour as each polymer deposition is accompanied by an increase of ΔD and their overtones are separated (a typical characteristic behaviour of soft and hydrated systems). For the system built at a pH of 5.5, dissipation displays a distinct behaviour in which the overall change in ΔD is almost inexistent upon the deposition of the two first polysaccharide bilayers. Plotting ΔD versus $\Delta f/n$ eliminates the time dependence and displays how much energy is dissipated by unit of mass adsorbed (or frequency shift). So, the analysis of Fig. 1C reveals that ΔD shifts are all positive meaning that both pH-systems (5.5 and 7.0) do not experience any kind of stiffening. Moreover, for the build-up at a pH of 7.0 the plot's slope is steeper as an indication of a softer and less compact layer. Experimental data concerning film build-up were fitted according to the previous mentioned Voigt model by assuming a fluid density of 1000 kg/m^3 , a fluid viscosity of 0.0020 kg/ms and a layer density of 1200 kg/m^3 . Fig. 2 represents the system thickness grow-up as a function of the number of layers. It was obtained a value of ≈ 56 nm for the final thickness of the films built at a pH of 7.0 and ≈ 34 nm for multilayers assembled at a pH of 5.5. This last result is in agreement with earlier investigations conducted with a similar system using conventional chitosan (Alves, Picart, & Mano, 2009).

Since polyelectrolyte deposition is primarily governed by electrostatic interactions, the degree of ionization of the charged molecule is of prime importance. The thickness and the organization of these sequentially adsorbed layers are extremely sensitive to the pH solution since the charge density and conformation of weak polyelectrolytes, like chitosan, is influenced by the changes of pH. The degree of dissociation of protonated amines in chitosan chains can also be ruled by the degree of deacetylation and the ionic strength of the medium. The degree of deacetylation determines the density of the amine groups that can carry a charge and the solution pH determines the degree of ionization of the amine groups. An earlier study has reported that the zeta potential of 0.1% (w/w) solution of chitosan reached zero at pH 7.5 (Mounsey, O'Kennedy, Fenelon, & Brodkorb, 2008). It has also been shown that at a pH of 6.5 (pK_a of chitosan) only 50% of the polymer's func-

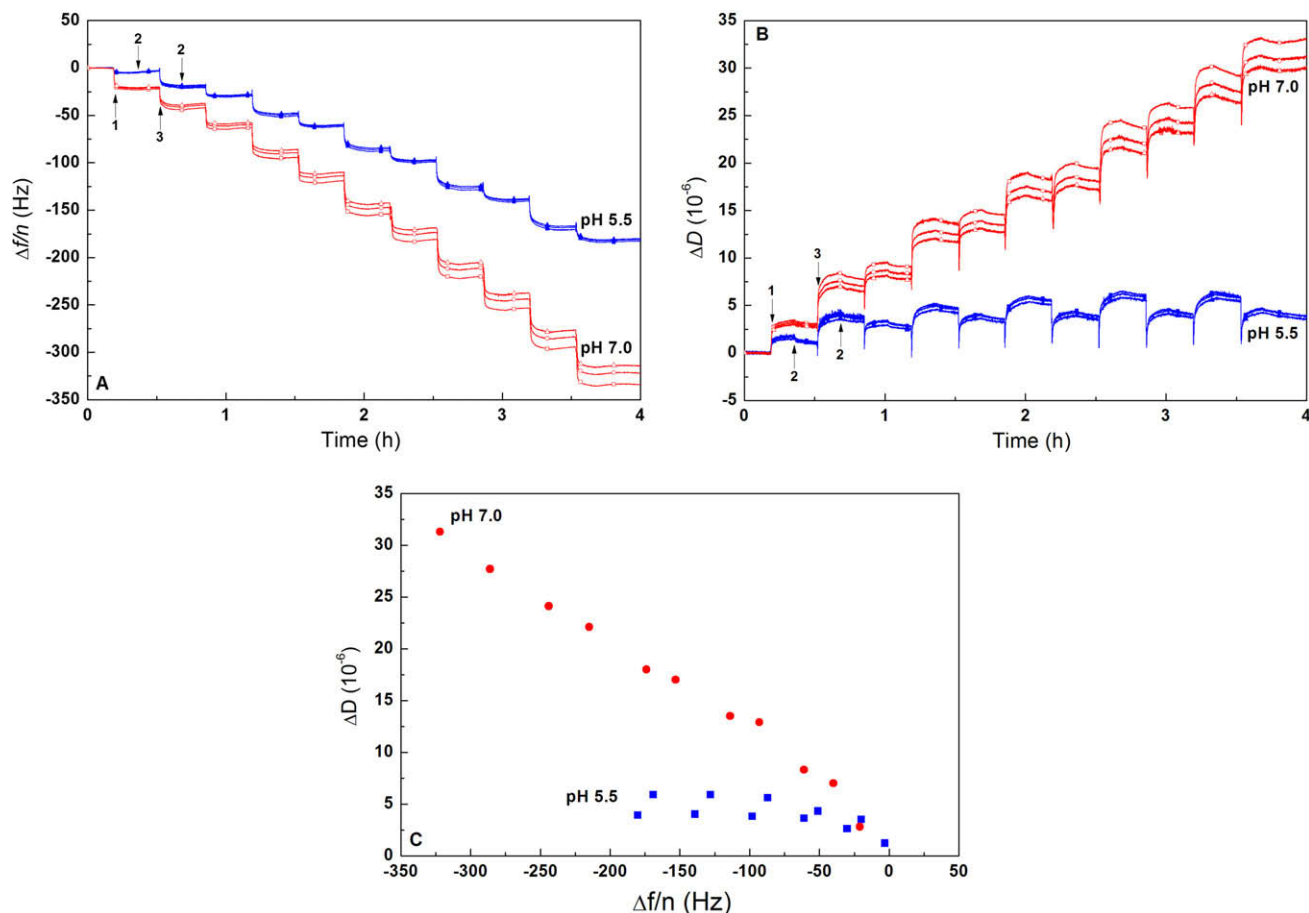


Fig. 1. QCM-D results showing the *in situ* build-up of (CHI/ALG)₅CHI films at a pH of 5.5 (blue line) and at a pH of 7.0 (red line). Steps 1 and 3 represent chitosan and alginate deposition, respectively and step 2 relates to the rinsing with NaCl solution. (A) Normalized frequency ($\Delta f/n$) and (B) dissipation (ΔD) changes are recorded as a function of time. Plots represent 7th (■), 9th (●) and 11th (▲) at pH 5.5 and 7th (□), 9th (○) and 11th (△) at pH 7.0. (C) Variation in the ΔD as a function of the $\Delta f/n$, for the 9th harmonic, at a pH of 5.5 (squares) and at a pH of 7.0 (circles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

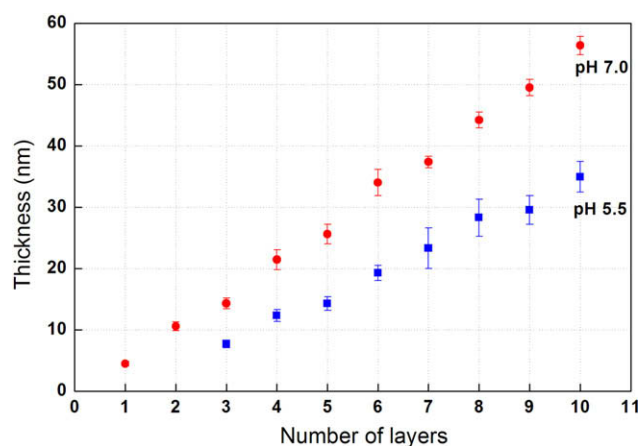


Fig. 2. Thickness grow-up of the chitosan–alginate multilayered system at a pH of 5.5 (squares) and at a pH of 7.0 (circles) as a function of the number of layers, calculated with the Voigt model. The odd numbers correspond to thickness values of the film after the addition of chitosan and the even numbers concern alginate introduction.

tional groups are ionized resulting in a low positive charge density (Song et al., 2009). So, it is expected an increase of the charge density of this weak polycation at a pH of 5.5 (below chitosan pK_a) due to the protonation of the amine groups (Boddohi et al., 2008), enhancing electrostatic interactions with the carboxylate groups

of alginate. Moreover, the assembled system exhibits a more compact and stiff behaviour as a result of a preferential flat conformation (Fig. 3A). On the other hand, at a pH of 7.0 chitosan will mostly prevail in a non-protonated form and thus, the build-up of the chitosan–alginate films can involve additional mechanisms besides electrostatic interactions. Previous studies have reported that hydrogen bonding can also play an important role in the LbL assembly (Gergely et al., 2004; Kharlampieva & Sukhishvili, 2006). The existence of intermolecular hydrogen bonding in chitosan–alginate based-systems has already been acknowledged by means of fourier transform infrared spectroscopy (FTIR) (Xiaowen, Yumin, Liping, Baozhong, & Abo, 2006). As expected, higher pH values contribute to the formation of hydrogen bond (Sorlier, Denuziere, Viton, & Domard, 2001). Another explanation can arise from the fact that at a pH of 7.0 chitosan bears less charged groups to interact with alginate and, hence, the film structure is more loosely and dissipates more energy (Fig. 3B). Charge overcompensation is the key rule for the formation of polyelectrolyte multilayers. When the charge density of the adsorbing species decreases (i.e., chitosan at a pH of 7.0), more polyelectrolyte chains are needed to overcompensate and invert the surface charge, explaining the higher thickness associated with the deposition of polymers at pH 7.0. Schoeler et al. have reported the build-up of PAH-based films with higher thickness values at pH 10 due to the lower degree of ionization of the weak polycation (Schoeler et al., 2006). This is in agreement with the different thickness values found at different pH, 56 nm at a pH of 7.0 and 34 nm at a pH of 5.5. Other multilayered assemblies have also reported a similar behaviour claiming also that with a

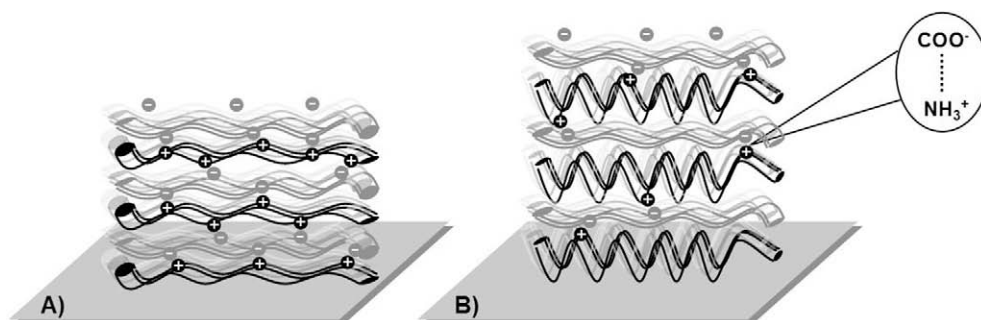


Fig. 3. Schematic representation concerning the build-up of chitosan (polycation) and alginate (polyanion) multilayers at different pH, 5.5 (A) and 7.0 (B).

decreasing degree of charge (lower degree of ionization) polyelectrolytes loose their flat conformation adopting a more coiled one due to the decreasing electrostatic repulsion between the charges along the chain (Steitz, Jaeger, & Klitzing, 2001). In addition, data showed that the adsorption of alginate is responsible for higher changes of dissipation possibly as a result of alginate having a more hydrophilic character than chitosan (Lawrie et al., 2007). Due to this water-rich nature (hydrogel like), alginate deposition can be accompanied by solvent entrapment and, hence, resulting in a more viscoelastic multilayered system. Thus, the following adsorption of chitosan is accompanied by a decrease in the dissipation and resulting in a “stiffening” of the multilayer assembly (Notley, Eriksson, & Wågberg, 2005).

4. Conclusions

In conclusion, we have reported the design of a polyelectrolyte multilayer of natural-origin polymers, chitosan and alginate, at neutral pH. It was further showed that the density charge of chitosan has a profound influence on the film formation and on its structural properties. Also, the pH-dependent character of polyelectrolytes enables the production of multilayered systems with different viscoelastic behaviour. These results may open the possibility of processing multilayered films containing chitosan at physiological conditions that could enable the introduction of pH sensitive molecules, including proteins and growth factors, or the interaction of the films with cells during the build-up.

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