Polyelectrolyte Multilayers Composed of Chitosan and Poly(acrylic acid)

I. Deposition conditions and post-treatment strategies

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Polyelectrolyte multilayers composed of chitosan and poly(acrylic acid) were constructed onto silicon wafers from solutions of different concentrations of polymer and NaCl. To modulate the film properties, the multilayers were subject to three post-treatment strategies: (i) immersion in an aqueous solution with pH = 2.4 for 1 min, followed by a 15 s immersion step in water and drying at 80 °C for 60 min; (ii) immersion for 30 min in water and (iii) immersion in an aqueous solution with pH = 2.4 for 5 min, followed by immersion for 1 min in water and thermal treatment at 120 °C for 60 min. The changes of the film morphology, thickness, and wetting state as a function of deposition conditions and post-treatment strategies used were followed by atomic force microscopy, spectroscopic ellipsometry and contact angle measurements.

Keywords: polyelectrolyte multilayer; chitosan; poly(acrylic acid); post-treatments

Layer-by-layer (LbL) polyelectrolyte thin films have been often used in the last decades to generate nanostructured coatings with tailored properties. This type of nanocoatings are often applied in areas like controlled release of drugs, dyes, genes or proteins [1, 2], sensors [3-5], modulation of membranes selectivity [6, 7], control of cell interactions [8, 9], enzymatic and non-enzymatic catalysis [10, 11] etc.

In the LbL strategy the deposition conditions (polymer concentration, *p*H, ionic strength, charge density, temperature), as well as the nature of building blocks (synthetic/natural polyelectrolytes, inorganic nanoparticles, biomacromolecules, dyes) have an important role on the driving forces which lead to the formation of polyelectrolyte multilayers (electrostatics, hydrogen bonds, hydrophobic interactions, specific biointeractions) [12-15]. The subsequent changes in the environment can induce modifications in the chemical structure, as well as in the morphology of multilayers. Concerning the applications of polyelectrolyte multilayer thin films, it is very important to achieve a sufficient mechanical stability under external stimuli and a significant loading capacity for different active species [16]. Rubner and coworkers presented a novel strategy for the construction of porous multilayers, based on the *p*H and ionic strength responsiveness of weak polyelectrolytes [17, 18]. They constructed polyelectrolyte multilayers using poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), which afterwards were briefly immersed into acidic aqueous solutions. This post-treatment strategy led to substantial and irreversible transformations of the multilayers by generating pores into their matrix with diameter up to a few hundreds of nanometers. Later on, they showed that these systems can be successfully used to entrap and release drugs [19]. Caruso *et al*. used poly(4-vinylpyridine) as template inside PAH/PAA multilayers to generate nanoporous thin film [20]. Also, Zhang et al. obtained porous multilayers by a dewetting process of the films constructed by alternate deposition of poly(sodium 4-styrenesulphonate) with PAH-PAA nonstoichiometric complexes [21].

Our group has recently reported another strategy to design porous multilayer films, which consisted of the alternately deposition of poly(vinyl amine) (PVAm) and PAA onto silica microparticles and silicon wafers, followed by selective chemical cross-linking of polycation layers and the removal of PAA chains, a single component cross-linked multilayer of $(PVAm)_n$ remaining at the end [22]. One of the main building blocks for the construction of polyelectrolyte multilayer biointerfaces is chitosan (CHI), a biocompatible polysaccharide with amine and hydroxyl functionalities [3, 4, 9, 19].

The aim of the present work was to investigate the influence of different deposition conditions and post-treatment strategies onto the properties of CHI/PAA multilayer films. In this regard, CHI/PAA multilayers have been constructed onto silica substrates using deposition solutions having different concentrations of polymer and NaCl. As post-treatment strategies, the films were immersed: (i) in an aqueous solution with *p*H = 2.4 for 1 min, followed by a 15 s immersion step in water and drying at 80 °C for 60 min; (ii) in water for 30 min and (iii) in an aqueous solution with *p*H = 2.4 for 5 min, followed by immersion for 1 min in water and thermal treatment at 120 °C for 60 min. The influence of the post-treatment strategies onto the topography, thickness and wetting properties of the multilayers deposited onto silicon wafers were monitored by atomic force microscopy (AFM), spectroscopic ellipsometry and contact angle measurements.

Experimental part

*Materials and method***s**

CHI was purchased from Sigma-Aldrich and used without further purification. The degree of deacetylation, established by infrared spectroscopy, was approximately 85%. The average molar mass of CHI was determined by

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viscometry. The intrinsic viscosity of CHI solved in a mixture of 0.3 mol/L acetic acid and 0.2 mol/L sodium acetate (1:1, v/v) was measured with an Ubbelohde viscometer at 25 ± 0.1 °C. The viscometric average molar mass (M_v) of CHI was estimated using Equation 1 [23]:

$$[\eta] = 1.38 \cdot 10^{-4} M_v^{0.85} \qquad (1)$$

The M_v of CHI used in this study thus determined was 467000 g/mol. PAA, with molar mass of approximately 58000 g/mol, was synthesized in our laboratory [24]. NaCl analytical grade, purchased from Chimopar (Romania), was used without further purification. The chemical structures of the polyelectrolytes are presented in figure 1.

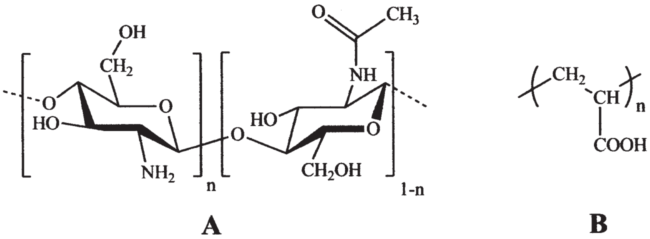

Fig. 1. Chemical structures of chitosan (A), poly(acrylic acid) (B).

As deposition substrates, silicon wafers were used. Prior to multilayers deposition, the silicon wafers were carefully cleaned according to the following procedure: (i) immersion for 20 min in Piranha solution (70:30 v/v H_2SO_4/H_2O_2 mixture), followed by intensive rinsing with water, and (ii) immersion in a mixture of $NH_4OH/H_2O_2/H_2O$ (1:1:1) and ultrasonication at 70°C for 60 min, followed by intensive rinsing with water. For all experiments Millipore grade water with a conductivity of 0.055 μS/m was used.

Construction of CHI/PAA polyelectrolyte multilayers

The multilayer films were constructed onto silicon wafers by alternated deposition of CHI and PAA from aqueous solutions with concentration of 10^{-3} mol/L or 10^{-2} mol/L, taking into account the molar mass of the polymer repeat unit. The deposition procedure was performed using PAA solution with *p*H = 3.5 (obtained by dissolving the PAA in distilled water) and CHI solution with *p*H = 5.5. The CHI solution was prepared using the following procedure: (i) a corresponding amount of CHI powder was dissolved in an aqueous solution with *p*H = 2 (adjusted using 0.1 mol/L HCl solution); (ii) after CHI dissolution, the *p*H was adjusted to 5.5 using a 2 mol/L NaOH solution. The ionic strength of the deposition solutions was adjusted to 10^{-2} mol/L or 10^{-1} mol/L by adding appropriate amounts of NaCl.

The deposition procedure of CHI/PAA multilayers onto silicon wafers was performed at room temperature in open beakers. The substrates were first immersed in the CHI solution for 20 min, followed by three rinsing steps, each 1 min. Then, the substrates were immersed in the PAA solution for 20 min, followed by a similar rinsing cycle. This procedure led to the assembly of one double layer. The water used in the rinsing cycles contained the same amount of NaCl as the deposition solutions. The entire process was repeated until the desired number of double layers was deposited.

Post-treatment strategies

After the construction of CHI/PAA multilayers, three post-treatment strategies were applied in order to generate pores onto their surface. The first one, denoted as T_1, consisted of immersing the multilayers in an aqueous solution with *p*H = 2.4 for 1 min, followed by a 15 s immersion in water and drying at 80°C for 60 min. The second post-treatment, denoted as T_2, consisted of immersing the multilayers for 30 min in water without NaCl. The third post-treatment, denoted as T_3, consisted of immersing the CHI/PAA multilayers in am aqueous solution with *p*H = 2.4 for 5 min, followed by immersion for 1 min in water and thermal cross-linking at 120 °C for 60 min.

Characterization methods

Atomic force microscopy (AFM) was employed to examine the topography of the CHI/PAA multilayers, before and after post-treatments. AFM images were recorded with a SPM Solver Pro-M device. All images were acquired in air, at room temperature, using a high-resolution no contact “Golden” silicon NSG10/Au/50 cantilever, with Au conductive coating. Surface parameters were obtained using WSxM 5.0 Develop 4.1 software [25].

Spectroscopic ellipsometry was performed using a null-ellipsometer in a polarizer-compensator-sample-analyzer mode . As light source, a red He-Ne laser with a wavelength of 632.8 nm was used. The accuracy for Δ and ψ angles was ± 0.005°. Relative thicknesses were determined assuming an average index of refraction of 1.46.

Contact angle measurements were realized by the sessile drop method using a conventional drop shape analysis technique (Kruss DSA10, Hamburg, Germany) using the following conditions: initial drop volume = 3 μL; added volume = 10 μL (0.1 μL/s); receding volume = 13 μL (0.1 μL/s); tip diameter = 0.205 mm. The advancing and receding contact angles were determined for a constant volume of the drop of 3 μL.

The contact angle measurements were also used to determine the work of adhesion (W_{adh}) of CHI/PAA multilayers towards water using the following equation [26]:

$$W_{adh} = \gamma_l(1 + \cos\theta) \qquad (2)$$

where γ_l represents the superficial tension of water (which has a values of 72 dynes/cm), and θ is the advancing contact angle for a drop of 3 μL.

Results and discussions

Some previous studies carried out by Rubner and coworkers showed that, when PAH/PAA multilayers, constructed using deposition solutions in which the polyelectrolytes had a low degree of ionization (e.g., *p*H = 3.5 for PAA and *p*H = 7.5 for PAH), were exposed to acidic solutions with *p*H = 1.7 – 2.6, their morphology changed from a smooth one to a microporous one [17, 18]. The respective transformations occurred due to the reduction of ionic cross-links between the polymer chains inside the multilayers via a spinodal decomposition process. Our work aims to follow the influence of similar post-treatment strategies onto the properties of polyelectrolyte multilayers constructed with CHI and PAA as a function of the polymer concentration and ionic strength.

The morphological transformations of the CHI/PAA multilayers, before and after the post-treatments, were investigated by AFM. Figure 2 shows the AFM images of $(CHI/PAA)_n$ multilayers, deposited from polymer solutions with concentration $C_P = 10^{-3}$ mol/L and $C_{NaCl} = 10^{-2}$ mol/L, before and after the post-treatment T_1.

From figure 2 it can be seen that the surface of silicon wafers was well covered by the CHI/PAA multilayers, the films being characterized by low values of root-mean-square roughness (RMS). The RMS values decreased with increasing the number of polyelectrolyte double layers, which shows that the films with a higher number of double layers were smoother. Also, it can be noticed that the

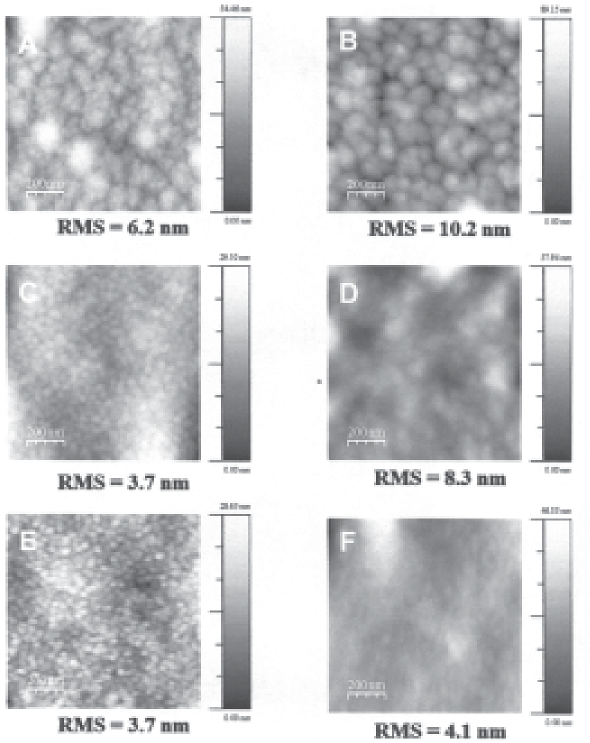

Fig. 2. AFM images (1 x 1 μm^2) of $(CHI/PAA)_n$ [n = 5.5 (A and B), 10.5 (C and D) and 15.5 (E and F)] multilayers deposited onto silicon wafers from solutions with C_P = 10^{-3} mol/L and C_{NaCl} = 10^{-2} mol/L, before (A, C and E) and after (B, D and F) post-treatment T_1.

surfaces of the films were covered by numerous granular structures, which could be assigned to the semi-rigidity of CS chains [27]. The morphology of the LbL films changed after the post-treatment T_1, this being accompanied by the increase of RMS. The difference between the RMS values of post-treated and untreated multilayers increased up the 10.5 double layers, and then significantly decreased, suggesting that the efficiency of the post-treatment is influenced by the number of double layers deposited.

Because the morphology of the CHI/PAA multilayers obtained using low concentrations of polymer and NaCl did not significantly change after applying the post-treatment T_1, similar films were constructed, using polymer and NaCl solutions with concentrations of 10^{-2} mol/L and 10^{-1} mol/L, respectively, which were further subject to post-treatments T_2 and T_3. The difference between post-treatment T_3 and post-treatment T_1 consisted of the immersion duration in *p*H = 2.4 (5 min in the case of T_3 compared with 1 min in the case of T_1) and drying conditions (60 min at 120 °C in the case of T_3, compared with 60 min at 80 °C in the case of T_1). The AFM images of $(CHI/PAA)_{5.5}$ multilayers, deposited onto silicon wafers using solutions of C_P = 10^{-2} mol/L and C_{NaCl} = 10^{-1} mol/L, before and after the post-treatments T_2 and T_3, are presented in figure 3.

Before post-treatments, the surface of CHI/PAA multilayers was covered with larger granular structures than in the case of the films constructed using previous deposition conditions. This could be explained by the presence of a high concentration of NaCl in the deposition solutions, which formed ion-pairs with the oppositely charged ionic groups on the polyelectrolyte chains and reduced the intrachain electrostatic repulsions, thus forming more compact conformations with more loops and coils [28]. After both post-treatments the morfology of the CHI/PAA multilayers surface changed significantly (figs. 3B and 3C), being accompanied by the decrease of RMS values. Considering that the gradient of NaCl concentration between the interior of the multilayers and the external post-treatment solutions was very high, it can be assumed that a part of the small counter-ions, which formed ion-pairs with the charged groups of the polyelectrolytes, diffused towards the external post-treatment solutions. This process favoured the rearrangement of polyelectrolyte chains inside the multilayers and the intrinsic compensation between oppositely charged groups of CHI and PAA chains.

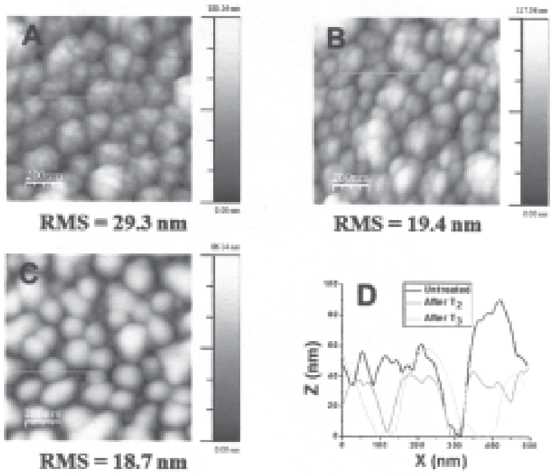

Fig. 3. AFM images (1 x 1 μm^2) of $(CHI/PAA)_{5.5}$ deposited onto silicon wafers from solutions with C_P = 10^{-2} mol/L and C_{NaCl} = 10^{-1} mol/L, before (A) and after post-treatment T_2 (B) and T_3 (C). Image D shows the cross section profiles which were drawn in images A, B and C.

Figure 3D shows a comparison of the profile lines drawn onto the AFM images (fig. 3A, B and C). When the multilayers were immersed in water with *p*H = 5.5 (post-treatment T_2), the diameter of the granular shapes onto the multilayers surface slightly decreased. However, when the multilayers were immersed in an aqueous solution with *p*H = 2.4, some channels appeared on the surface of the multilayers, with a width of approximately 100 nm. This indicates that the *p*H of the post-treatment solutions has a more pronounced effect in the morphological transformations of CHI/PAA multilayers than the lack of NaCl.

As Rubner and coworkers showed, immersion of the PAH/PAA multilayers in an aqueous solution with *p*H = 2.4 led also to a significant increase of the film thickness [17]. Therefore, the thickness evolution of $(CHI/PAA)_n$ multilayers deposited from solutions with C_P = 10^{-3} mol/L and C_{NaCl} = 10^{-2} mol/L, before and after the post-treatment T_1, was investigated by spectroscopic ellipsometry as a function of the number of double layers deposited (fig. 4).

Figure 4 shows that the thickness of the $(CHI/PAA)_n$ multilayers increased exponentially with increasing the number of double layers deposited, both before as well as after post-treatment T_1. Also, it was noticed that the thickness of the multilayers treated by post-treatment T_1 was lower than that of the untreated films. This behaviour was different comparing with the results reported for PAH/PAA multilayers, when after a similar post-treatment the thickness increased [31]. In a similar way, we found that the thickness of $(CHI/PAA)_{5.5}$ multilayers deposited from solutions with C_P = 10^{-2} mol/L and C_{NaCl} = 10^{-1} mol/L decreased from 58.8 nm before treatment, to 52.9 nm and 35.8 nm after the post-treatments T_2 and T_3, respectively. Moreover, comparing the thickness of $(CHI/PAA)_{5.5}$ multilayers constructed using both deposition conditions, it was noticed that the multilayers assembled from

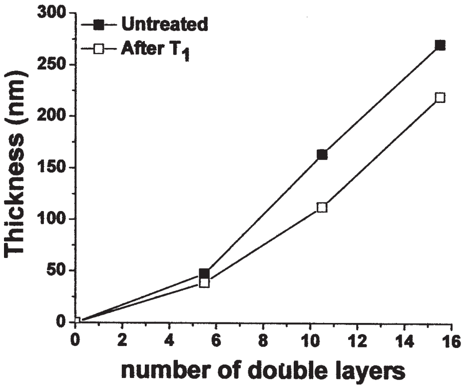

Fig. 4. Thickness of $(CHI/PAA)_n$ multilayers deposited from solutions with $C_P = 10^{-3}$ mol/L and $C_{NaCl} = 10^{-2}$ mol/L, before and after post-treatment T_1

Table 1
CONTACT ANGLES OF $(CHI/PAA)_N$ MULTILAYERS, BEFORE AND AFTER POST-TREATMENTS.

Sample	Θ_a (°)	Θ_r (°)	H (°)	W_{adh} (mJ/m^2)
silicon wafers	46.23	23.4	22.8	121.8
5.5^a	37.41 ± 0.95	13.36 ± 3,19	24.05	129.19
10.5^a	46.49 ± 2,75	12.95 ± 1.53	33.54	121.57
15.5^a	43.40 ± 3.62	12.95 ±1.48	30.55	124.31
5.5 T_1^{a}	46.83 ± 4.32	15.12 ± 1.63	31.71	121.26
10.5 T_1^{a}	35.65 ± 0.41	10.42 ± 0.53	25.23	130.50
15.5 T_1^{a}	42.63 ± 3.17	14.52 ± 0.56	28.11	124.97
5.5^b	34.12 ± 0.09	4.86 ± 2.23	29.26	131.60
5.5 T_2^{b}	48.22 ± 2.65	13.55 ± 0.41	34.67	119.97
5.5 T_3^{b}	40.69 ± 1.27	13.23 ± 0.85	27.72	126.59

[a] CHI/PAA multilayers deposited using solutions with $C_P = 10^{-3}$ mol/L and $C_{NaCl} = 10^{-2}$ mol/L;

[b] CHI/PAA multilayers deposited using solutions with $C_P = 10^{-2}$ mol/L and $C_{NaCl} = 10^{-1}$ mol/L.

solutions with higher C_P and C_{NaCl} were thicker (≈ 58.8 nm) than those assembled using solutions with lower C_P and C_{NaCl} (≈ 47.4 nm). This could be attributed to the fact that in the presence of a high amount of NaCl, the polyelectrolytes adopt a coiled conformation which, upon adsorption onto solid surfaces, leads to thicker layers.

The lower thickness of CHI/PAA multilayers after post-treatments could be attributed to the conformational modifications of both polyelectrolytes upon immersion in the post-treatment solutions. The deposition conditions of the polyelectrolytes, i.e. *p*H 3.5 for PAA and *p*H 5.5 for CHI, when both polymers were partially ionized, as well as the presence of a certain amount of NaCl, renders them to adopt a coiled conformation upon deposition onto the solid substrate [29, 30]. After the immersion of $(CHI/PAA)_{5.5}$ multilayers (constructed from solutions with $C_P = 10^{-2}$ mol/L and $C_{NaCl} = 10^{-1}$ mol/L) in water (post-treatment T_2), the thickness decreased from 58.8 nm to 52.9 nm. According to Moya and coworkers this behaviour could be explained by the reorganization of the polyelectrolyte layers due to partial diffusion of the salt ions from the multilayers and the simultaneous regeneration of the electrostatic interactions between the ionic groups of the polyelectrolytes. At the same time, a certain amount of water was expelled from the multilayers [31]. After the immersion of the same type of films in aqueous solution with *p*H = 2.4, the thickness decreased to 35.8 nm, indicating that the *p*H of the post-treatment solution affected in a larger extent the internal structure of CHI/PAA multilayers. Rubner and coworkers showed that the thickness of the multilayers constructed using weak polyelectrolytes depended strongly on the ionization degree of the polyelectrolytes [29]. The thickness had the highest value when both polyelectrolytes were weakly charged, while it decreased when at least one of them was strongly charged. In our case, at *p*H = 2.4 only a small number of carboxylate groups existed on the PAA chains, while CHI was strongly charged. Thus, the lower thickness of the CHI/PAA multilayers after post-treatment can be attributed to the reduction of the thickness of CHI layers. Consequently, due to the difference between the molar masses of CHI (M_v ≈ 467000 g/mol) and PAA (M_v ≈ 58000 g/mol), some PAA chains might diffuse outside the multilayer matrix.

The contact angle method was used to investigate the wetting characteristics of the CHI/PAA multilayers surface, before and after post-treatments (table 1).

As table 1 shows, the surface of all studied CHI/PAA multilayers was hydrophilic, both before and after post-treatments. The contact angle values were irregular, which could be related to the local roughness of the multilayers on the drop landing spot. From the contact angle values presented in table 1 it can be concluded that post-treatment T_1 did not affect in a significant way the contact angles of CHI/PAA films, while after post-treatments T_2 and T_3, the contact angles of CHI/PAA films slightly increased. This could be attributed to the influence of thermal treatment, which might induce a slight cross-linking between the amino groups of CHI and carboxilic groups of PAA with the formation of amide bonds, thus reducing the number of hydrophilic groups onto the surface of the multilayers.

W_{adh} is a measure related to the surface wettability and is widely used in practice for predicting the potential bonding properties of materials. It is inversely proportional with the contact angle, meaning that a hydrophilic surface will have a high W_{adh}, while a hydrophobic surface will have a low W_{adh}. In the case of CHI/PAA multilayers, the W_{adh} values were high, which indicates that the film surface has a high tendency to interact with different molecular species dissolved in aqueous media [32].

Conclusions

The influence of different deposition conditions and post-treatment strategies on the properties of CHI/PAA polyelectrolyte multilayers was studied by AFM, spectroscopic ellipsometry and contact angle. It was shown that the morphology and the thickness of CHI/PAA multilayers were strongly influenced by the polymer and NaCl concentrations of the deposition solutions. Immersion of CHI/PAA multilayers in aqueous solutions with *p*H = 2.4 produced significant transformations of the films morphology with the appearance of pores onto their surface. These transformations were accompanied by the decrease of multilayers thickness, which were ascribed to the conformational rearrangements of CHI and PAA chains inside the multilayer matrix. Contact angle measurements showed that the surface of CHI/PAA multilayers was hydrophilic, both before as well as after post-treatments, and that they had a high tendency to interact with other species from aqueous media.

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