

## Novel biocompatible chitosan based multilayer films

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### Abstract

*The formation of novel biocompatible multilayer films by the interactions between complementary polyelectrolytes, on planar surfaces, by layer-by-layer self-assembly, was studied. For this purpose, chitosan and two polyanions, poly(acrylic acid) and poly(2-acrylamido-2-methyl propanesulfonic acid - co - acrylic acid), were used. To get a direct image on the polyelectrolyte multilayers formation and properties, infrared spectroscopy, gravimetry and atomic force microscopy have been used. A special attention was paid to the responsiveness of the new biocomposite materials to the pH of the environment. Thanks to biocompatibility properties of CS and polyanions used in this study, these multilayer films may be potentially useful for biomedical and environmental protection applications.*

**Keywords:** surface modification, biocompatibility, chitosan, pH responsiveness, infrared spectroscopy, atomic force microscopy

### Introduction

In recent years, many procedures based on surface modification aimed to improve the biocompatibility of materials which interact with cells and tissues through their interfaces. Different processing strategies have been employed to transpose the use of traditional materials into the biomedical field [1-3]. The alternate deposition of polyanions and polycations onto a solid substrate has been proposed as a reliable, flexible and simple method to build-up multilayered polyelectrolyte films on planar surfaces or three-dimensional substrates, with fine control over thickness, composition, morphology, and chemical functionality [4]. As a function of the last adsorbed layer, with positive or negative charges, by simple contact, the films can adsorb a great variety of compounds such as dyes, particles, or clay microplates [5-7].

Among the vast choice of polyelectrolytes the use of natural-based polymers in the biomedical field has been considered as an attractive option as such systems exhibit often similarities with the extracellular matrix, they are chemically flexible and show typically good biological performance [8,9]. Most of natural polyelectrolytes that have been used are polysaccharides, such as dextran derivatives, chondroitin sulfate, hyaluronan and chitosan (CS). In this context, CS, the copolymer of  $\beta$ -(1-4) linked 2-acetamido-2-deoxy-D-glycopyranose and 2-amino-2-deoxy-D-glycopyranose) produced by deacetylation of chitin from crustacean exoskeletons and fungal cell walls, has attracted numerous scientists due to its outstanding biological properties [10]. Sustainable interest in the biomedical application of CS to discourage bacterial adhesion and implant infection is stimulated strongly by its no toxicity, biodegradability, and strong antibacterial properties [11]. Also, CS has valuable biomedical applications such as membranes for dialysis, packaging, coatings and wounds dressing, polyelectrolyte complex beads for controlled delivery of proteins, drugs, vaccines [10,12,13].

The cationic nature of CS is primarily responsible for electrostatic interactions with anionic glycosaminoglycans, proteoglycans and other negatively charged molecules. This property is of great interest because a large number of cytokines (growth factors) are linked to glycosaminoglycans, and a scaffold or other biomedical device incorporating a chitosan-glycosaminoglycans complex may retain and concentrate growth factors secreted by any colonizing cells. Moreover, the N-acetylglucosamine moiety in CS is active in the specific interactions with growth factors, receptors, and adhesion of proteins. Poly(acrylic acid) (PAA) is a weak polyanion, well-known for its biocompatibility, and is widely used as polyelectrolyte in different biomedical applications [14]. The presence of carboxylic groups in PAA allows it to be functionalized with bioactive molecules such as proteins, growth factors, peptides, etc. In the last period, for biological applications, a special attention was paid to copolymers of AA, especially copolymers with 2-acrylamido-2-methylpropanesulfonic acid (AMPS) because of their anticoagulant properties [15].

Multilayer systems have been proposed for different biomedical applications, including for biomimetic composite-like coatings, to control the release of drugs, DNA or peptides/proteins, or to manipulate the adhesion, differentiation, proliferation and even function of attached cells [8,16-18]. CS containing multilayer films prepared at different ionic strengths showed different bacterial resistance properties [19], and the LbL process solution pH had a remarkable effect on the antibacterial properties of polyelectrolyte multilayer nanofilms [20]. Moreover, CS-containing multilayer thin films showed high resistance to bacterial adhesion and led to a substantial decrease in *Escherichia coli* [19,20] adhesion compared to control bare substrates. Multilayers built up from CS and *k*-carrageenan exhibit an exponential type of growth with pH-dependent thickness increment per double layer [21]. These multilayers represent promising systems for development of antiadhesive coatings (against strains of *E. faecalis*) on biomaterials surface, which are in addition bioinert or show mild antibacterial properties depending on the nature of terminating polymer.

The aim of this study was the formation of polyelectrolyte multilayers on planar substrate (silicon wafers), by assembling CS and two weak polyanions, PAA and poly(2-acrylamido-2-methylpropanesulfonic acid - co - acrylic acid) (PAMPSAA). The influence of polyanions structure and the number of double layers, on the properties of new nanostructured materials were monitored by infrared spectroscopy, gravimetry, and atomic force microscopy (AFM). A special attention was paid to the responsiveness to pH of environment on the swelling degree of the new biocomposite materials.

## Materials and Methods

CS sample was kindly provided by Yaizu Suisankagaku Ind., Japan, as powder, and was used without further purification. The CS viscometric molar mass is 80000 g/mol (estimated using the method described in ref [22]), and the deacetylation degree 82.5 % (determined by  $^1\text{H-NMR}$  [23]).

PAA with a molar mass  $M_w = 70000$  g/mol was synthesized and purified according to refs. [24]. The copolymer PAMPSAA, which contains 55 mol.-% 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and 45 mol.-% acrylic acid (AA), was synthesized and purified according to refs. [25]. The intrinsic viscosity of the copolymer, determined in 1M NaCl at 25 °C, was  $[\eta] = 0.72$  dL/g.

The concentration of polyelectrolytes used for multilayer construction was 2 mg/ml in 0.02 M NaCl. CS solution was prepared in 1 vol.-% acetic acid solution and 0.02 M NaCl, and intensive stirring for at least 48 h. The polyanion solutions were prepared by dissolving the appropriate polymer amounts in 0.02 M NaCl aqueous solution.

Slices of 2x2 cm<sup>2</sup> silicon wafers were used as substrates for the multilayer deposition. Prior to use, the silicon wafers substrates were carefully cleaned in two steps as described elsewhere [26] and were kept under distilled water, in refrigerator, before the start of multilayer deposition. Polyion adsorption was carried out at pH 5.5, at room temperature in open Petri boxes for 20 min every layer. After every polyion adsorption step, the silicon wafers were rinsed three times (each 1 min) with 0.02 M NaCl aqueous solution. The procedure was repeated until the desired number of layers was achieved. The composite materials were not dried between the adsorption steps. The dry steps were necessary only to monitor the adsorbed polymer amount in dependence on the layer pair number and to examine the surface morphology.

Polyelectrolyte and potentiometric titrations were performed with the particle charge detector Mutek PCD 03 (BTG Instruments GmbH, Herrsching, Germany). The concentration of the charged groups of each solution was evaluated by titration with a standard solution of a strong oppositely charged polyelectrolyte, poly(sodium ethylenesulfonate) or poly(diallyldimethyl-ammonium chloride), with a concentration of 10<sup>-3</sup> M. The concentration of the charged groups in the examined solution was calculated from the amount of standard solution needed to reach the zero value of the streaming potential. Potentiometric titration of the polyions was performed between pH 2 and 10, using 0.1 M NaOH and HCl, respectively.

FT-IR spectra of multilayers were recorded with DIGILAB spectrometer Scimitar Series, USA, resolution of 4 cm<sup>-1</sup>, in the range of 4000-400 cm<sup>-1</sup>.

The amount of polyions deposited after a certain number of adsorbed layer pairs, P, was determined as follows:

$$(1) \quad P = (m_n - m_i) / m_i, \text{ g polymer/g support}$$

where  $m_n$  is the weight of the composite materials (silicon wafers with  $n$  adsorbed layer pairs) and  $m_i$  is the weights of the silicon wafers. The value of  $m_n$  corresponds to the constant weight after the drying of the composite materials in air (about four days). Four samples were measured for each specimen.

To examine the topography of the multilayers adsorbed on silicon wafers, a SPM Solver PRO-MAFM (NT-MTD Co. Zelenograd, Moscow, Russia) was used. AFM images were taken in the tapping mode at room temperature. All images were acquired using a high resolution 'Golden' silicon NSG10/Au/50 cantilever with an Au conductive coating.

SD of multilayers was calculated using equation (2):

$$(2) \quad SD = (m - m_0) / m_0, \text{ g water/g polymer}$$

where  $m_0$  and  $m$  are the weights of the dry and the swollen composite materials, respectively. Four samples were measured for each specimen.

## Results and discussion

### *Formation of polyelectrolyte multilayer films*

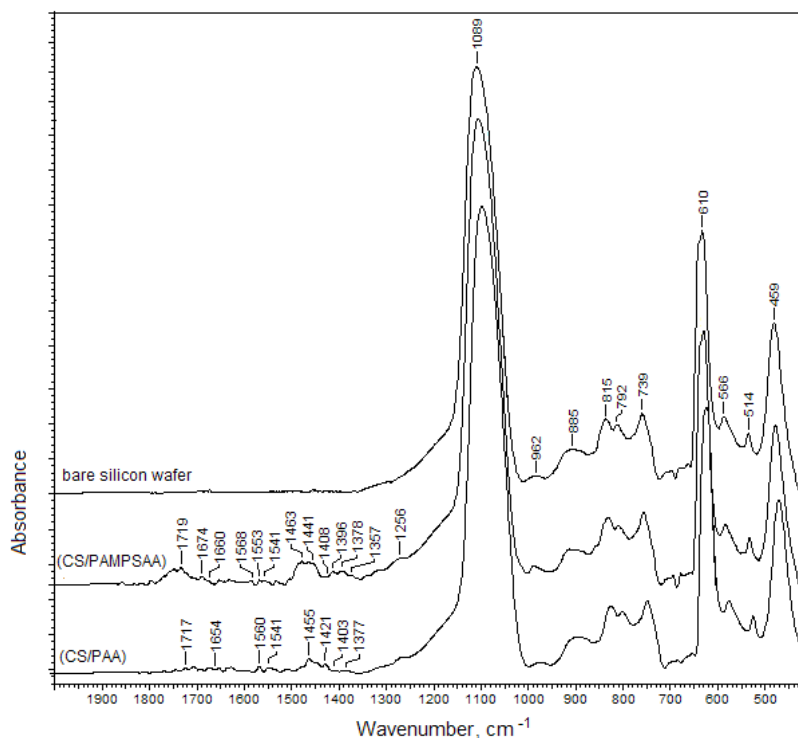
The formation of polyelectrolyte multilayer films was first monitored by FT-IR spectra performed on multilayers having eight double layers adsorbed (Figure 1).

The FT-IR spectrum of bare silicon wafers contains the following characteristic bands: at 1089 cm<sup>-1</sup> (asymmetric) and 459 cm<sup>-1</sup> (symmetric) assigned to the interstitial oxygen in the silicon (Si-O-Si), and at 610 cm<sup>-1</sup> assigned to the substitutional carbon (Si-C) absorption [27]. Furthermore, no characteristic bands appeared in the region 2000-1200 cm<sup>-1</sup>.

After multilayer adsorption, the spectra contain the silicon wafers characteristic bands and also a number of bands located between 2000 and 1200 cm<sup>-1</sup>, characteristics for the adsorbed polymers [28]. Thus, the presence of CS in multilayers is clearly demonstrated by the following characteristic bands: 1463 - 1455 cm<sup>-1</sup> assigned to the protonated primary

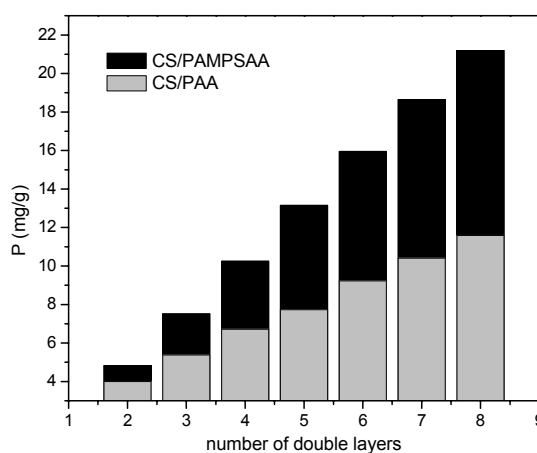
amino groups ( $-\text{NH}_3^+$ ),  $1674 - 1654 \text{ cm}^{-1}$  assigned to amide I,  $1541 \text{ cm}^{-1}$  for amide II, and  $1378 \text{ cm}^{-1}$  amide III bands.

The copolymer PAMPSAA presence is shown in FT-IR spectra by the following characteristic bands:  $1719 \text{ cm}^{-1}$  ( $\text{C}=\text{O}$  of AA units stretching vibration),  $1660 \text{ cm}^{-1}$  (amide I),  $1553 \text{ cm}^{-1}$  (amide II) and  $1357 \text{ cm}^{-1}$  ( $\text{CH}_2$  bending). The band due to the presence of  $-\text{SO}_3\text{H}$  groups of AMPS units is at  $1256 \text{ cm}^{-1}$ . The  $\text{COO}^-$  stretching bands are located at  $1568 \text{ cm}^{-1}$  (asymmetric) and  $1408 \text{ cm}^{-1}$  (symmetric). The characteristic stretching frequencies of PAA include the carbonyl stretch ( $\text{C}=\text{O}$ ) at  $1717 \text{ cm}^{-1}$ , and  $\text{CH}_2$  stretching between  $1377$  and  $1455 \text{ cm}^{-1}$ . The characteristic stretching frequencies include the symmetric and anti-symmetric stretching frequencies of the carboxylate ion ( $\text{COO}^-$ ) at  $1403$  and  $1560 \text{ cm}^{-1}$ , respectively.



**Figure 1.** FTIR spectra of bare silicon wafer and after deposition of eight double layers.

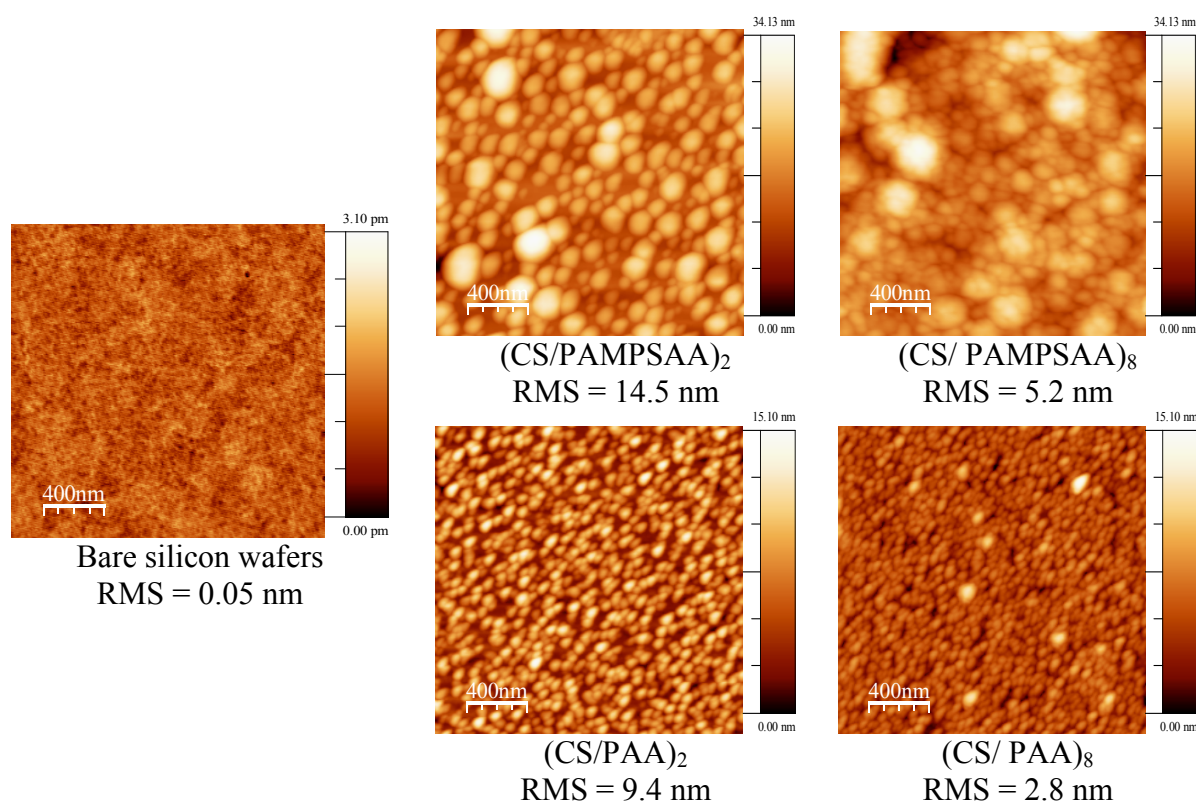
The formation of polyelectrolyte multilayer films was also followed by the variation of the amount of adsorbed polymer,  $P$ , on the silicon wafers surface as a function of the number of polyion double layers and polyanions structure (Figure 2).



**Figure 2.** Polymer amount adsorbed,  $P$ , at  $\text{pH} = 5.5$ , as a function of the number of polyions double layers.

As Figure 2 shows, an almost linear increase of the adsorbed polymer with the increase of number of double layers took place, suggesting a regular increase of the multilayers. The influence of polyion pair structure on the multilayer formation is also evidenced. The highest amount of polymer was adsorbed in the case of CS/PAMPSAA pairs and the lowest when the polyanion PAA was used, for the same number of double layers. PAA has the ionic groups located at regular intervals on the macromolecular backbone, with high ability to compensate the positive centers of CS. The copolymer PAMPSAA contains 55 % AMPS units, with the ionic group attached by a longer spacer to the macromolecular backbone, with high flexibility. Thus, probably part of the AA ionic sites are hindered, and remain extrinsic compensated by small ions, leading to thicker layers. Moreover, the charge density of the polyelectrolyte solutions involved in the multilayer preparation, determined by polyelectrolyte titration, was  $6.22 \text{ meq L}^{-1}$ ,  $4.64 \text{ meq L}^{-1}$ ,  $6.98 \text{ meq L}^{-1}$  for PAA, PAMPSAA and CS, respectively. Thus, to reach the interpolyelectrolyte charge stoichiometry a higher amount of PAMPSAA is necessary, comparative with CS/PAA films where the complementary polyions have almost the same charge density. Similar results have been obtained for interpolyelectrolyte complexed as colloidal dispersions, formed by the same polyions [29].

The surface topography of the composite materials, with different number of double layers, was investigated using AFM (Figure 3).



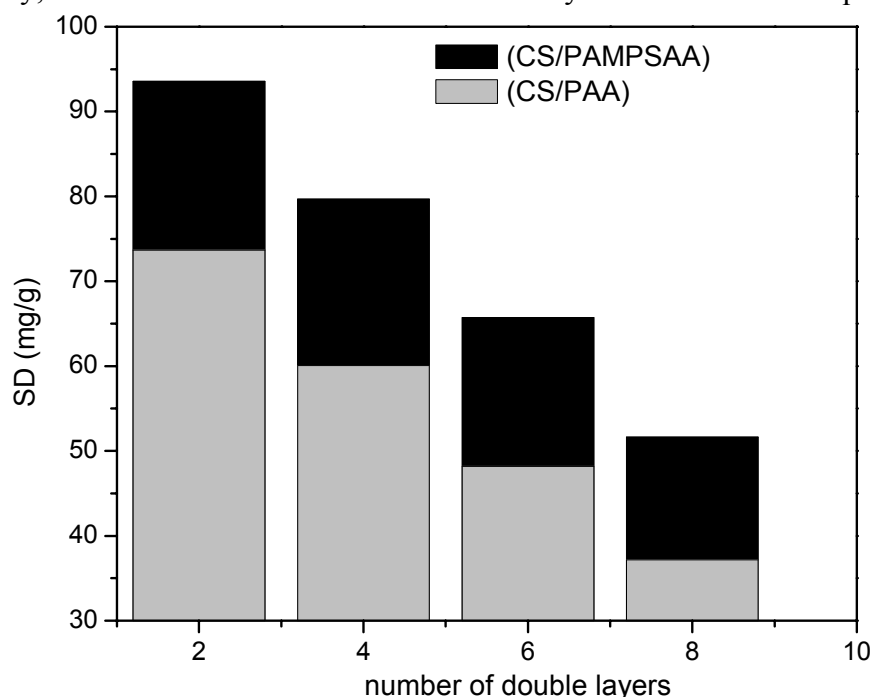
**Figure 3.** Height tapping mode AFM images and root mean square roughness (RMS) of bare silicon wafers and after adsorption of [CS/polyanion] films with different number of layers.

As Figure 3 shows, the bare silicon wafers have a smooth surface (RMS = 0.05 nm), without irregularities, the maximum z-range being 3.1 nm. The influence of the polyanions structure on the thin films surface morphology is clearly reflected in AFM images showed in Figure 3. Thus, the most uniform layers have been observed when PAA was used, with a maximum z-range of 15.1 nm, irrespective of number of adsorbed double layers, suggesting a uniform compensation of complementary ionic sites. When the copolymer PAMPSAA was

used, the surface morphology became fluffy, and less uniform layers were formed, with a maximum z-range of 34.1 nm. As Figure 3 shows, increasing the number of double layers adsorbed on silicon wafer surfaces, the film surface became more uniform, the RMS values decreasing with the increase of the number of layers for both polyion pairs studied. Similar topographic morphology and structures were observed in other studies [8,17], the adsorbed granular structures being related to the physical property of chitosan molecule, which is semi-rigid with a persistence length of the order of 6–11 nm.

#### *Swelling equilibrium of polyelectrolyte multilayer films*

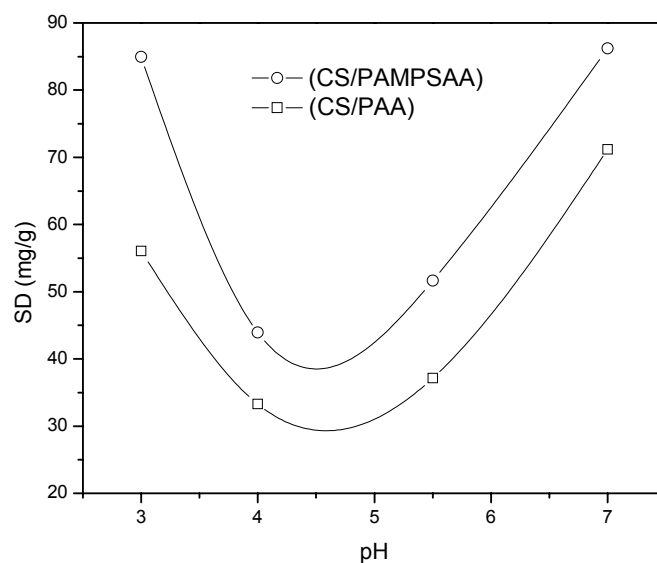
One of the most important properties of multilayer films is their swelling behavior. Figure 4 shows the swelling degree (SD) variation of thin films prepared by layer-by-layer self-assembly, as a function of the number of double layers adsorbed from aqueous solutions.



**Figure 4.** Swelling degree, SD, in water, as a function of the number of polyions double layers; swelling time – 4 days, pH = 5.5, 20 °C.

Figure 4 shows that SD values decreased with the increase of the number of double layers, irrespective of polyanion structure. Moreover, the highest SD values were obtained in the case of PAMPSAA based multilayers, comparative with multilayer films constructed with PAA, irrespective of the number of double layers. The numerous ionic cross-links between layers, in the case of PAA based multilayers, led to smaller water adsorption capacity, comparative with PAMPSAA based multilayers.

The polyions used in this study are weak polyelectrolytes, their ionization degree being dependent on the solution pH. The swelling degree of multilayer thin films, formed with weak polyelectrolytes can be tuned by the pH of swelling medium, which can also influence the dynamics of the ionic bonds holding the layers together. Previous studies on the effect of pH on the swelling of polyelectrolyte multilayer films have shown that specific pH values can lead to irreversible swelling and to the overall loss of material from the films [30]. Figure 5 shows the influence of environmental pH on the SD of multilayer thin films with eight double layers of polyions.



**Figure 5.** Influence of the swelling solution pH on the swelling degree of multilayer thin films with eight double layers adsorbed.

The point of zero charges of CS, determined by potentiometric titration, is at pH 7.4. At lower pH, the primary amino groups of CS can be protonated ( $-\text{NH}_3^+$ ), due to the presence of hydronium ions, CS being completely ionized at pH lower than 4. The polyanions point of zero charges was located at pH 1.8 for PAMPSAA and 2.2 for PAA. Above their point of zero charges, the presence of hydroxyl ions induced the dissociation of carboxyl groups ( $-\text{COO}^-$ ) and thus, a negative streaming potential was obtained. As Figure 5 shows, the lower SD values have been obtained at pH 4 - 5.5 where complementary polyions are ionized. At pH < 4, CS is strongly ionized whereas the number of charges of AA is small, and at pH > 5.5 the ionization degree of CS is very low and AA is completely ionized. Thus, at pH < 4 and > 5.5 the degree of ionic cross-linking between layers was low, reflected in the increase of SD. When PAMPSAA was used as weak polyanion, higher SD values were obtained, on all range of studied pH, without deconstruction of film. The higher resistance of this multilayer may be ascribed to the hydrophobic interactions and the numerous hydrogen bonds ( $>\text{NH}\cdots\text{O}=\text{C}<$ ) formed between layers at pH < 4 and > 5.5.

## Conclusions

This study followed the formation of biocompatible thin films by alternate adsorption of weak polyelectrolytes, of which charge density varied as a function of environment pH. For this study, some biocompatible polymers (CS, PAA and PAMPSAA) have been used. The SD values in water decreased with the increase of polyion layer number and were influenced by the polyanion structure. When PAMPSAA was used as weak polyanion, higher SD values were obtained comparative with PAA based films, on all range of studied pH, without deconstruction of film.

This study shows the possibility to control and tune the swelling behavior of such multilayers by tuning the number of adsorbed layers and the swelling pH, which influence the hydration capacity of the films. These characteristics are very important for applications like controlled release, membrane filtration, and biomaterial coatings. Thanks to biocompatibility properties of CS and the polyanions used in this study, these multilayer films may be potentially useful for many biomedical and environmental protection applications.

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