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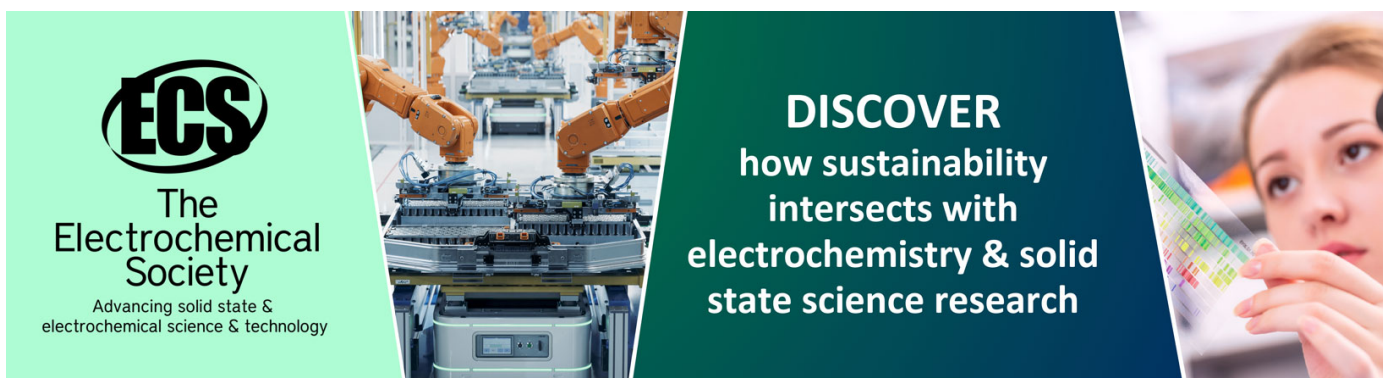
Investigation of multilayered polyelectrolyte thin films by means of refractive index measurements, FT-IR spectroscopy and SEM

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Investigation of multilayered polyelectrolyte thin films by means of refractive index measurements, FT-IR spectroscopy and SEM

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Abstract. Multilayered polyelectrolyte films are promising structures in the biomedical field. In order to meet the demands for biomedical applications, the structures have to be built from biocompatible and/or biodegradable, nontoxic starting materials, possessing some specific functional properties, depending on the particular application. In the present study, the multilayered polyelectrolyte films with potential use as buccal bioadhesive drug delivery systems were investigated. They were prepared via layer-by-layer deposition of successive nanolayers onto substrate. Three different biopolymers were used. The substrate, from poly(lactic acid), was solvent casted. After that, it was subjected to corona treatment, which ensures surface charge excess for the multilayer deposition. The nanolayers were prepared either from 0.01 g/L solutions of chitosan or 0.05 g/L xanthan. Acetate buffer (pH 4.5 and ionic strength 0.1 M) was used as a solvent. The substrate was dipped successively into one of the solutions, allowing formation of polyelectrolyte complexes of chitosan (polycation) and xanthan (polyanion). The substrates were treated in negative corona. The multilayered structures consisted of 8, 9, 14, 15 or 20 nanolayers. Number of techniques, such as refractive index measurements, FT-IR spectroscopy and SEM morphology were employed in order to monitor the properties of the so prepared multilayered polyelectrolyte films.

1. Introduction

In the last decades, polyelectrolyte multilayered thin films (MPTF) prepared by means of layer-by-layer (LbL) self-assembling have been widely used because of their fabrication easiness and great potential for application in biomedicine [1], drug delivery [2, 3], optics [4], food science [5], as biomembranes [6], etc. The technique is based on the consecutive deposition onto solid substrate via electrostatic interaction of polyions [7, 8]. LbL self-assembling process involves only molecular adsorption from solution onto charged surfaces, and it is applicable to a vast class of molecules, polymers and others. During the self-assembling of polyelectrolyte from solution, electrostatically bounded complexes are formed between the oppositely charged polyelectrolyte functional groups. Due to overcompensation during this process, an excess opposite to that of the substrate appears. As a result, irreversibly adsorbed polyelectrolyte multilayers could be deposited one over another. As far as the deposition occurs by



natural adsorption, there are no restriction with respect to substrate material type, size and topology in the application of the technique [7, 8]. Varying the type of adsorbing material, processing conditions (such as polyelectrolyte charge, ionic strength, pH, polymer concentration, and adsorption time) and the method of assembling one can manipulate the molecular interactions and achieve controlled nanostructures with well predictable thicknesses and interpenetration [9].

Self-assembling could be attained by several techniques immersion, spinning, spraying, electromagnetic and fluidic assembling – each of which offers different material and processing advantages [10]. Commonly used dipping-based LbL self-assembling consists of three steps: (1) polyelectrolyte diffusion of from the solution volume to the substrate surface, (2) adsorption of the polyelectrolyte onto the surface via molecular interactions, (3) molecular rearrangement of polymeric chains along the surface and equilibrium phase formation [7].

Although the feasibility of the dipping-based process has been confirmed, it is recognized as time-consuming and to accomplish the process of adsorption equilibrium hours or even up to days could be needed [7]. LbL assembling via spin coating technique (“spinning-assembling”, SA) makes use of spinning a substrate to facilitate the deposition process [11]. The SA is performed by either casting the solution onto a spinning substrate [12] or casting onto stationary substrate, which is then after spun [13]. Spinning quickens the assembling process considerably, due to the various forces governing the process, allowing for layers to be deposited in time limit of about 30 s. SA typically results in more homogenous films in comparison with immersion assembling, thanks to the electrostatic interactions, providing the adsorption and rearrangement of polymers, and the centrifugal forces, air shear, and viscous forces, leading to desorption of weakly bound polymers and dehydration of the films [13].

In the present study, the self-assembling process and its effect on the thickness and the morphology of chitosan/xanthan polyelectrolyte films deposited onto biodegradable poly(lactic) acid substrate is investigated by means of refractive index measurements, FT-IR spectroscopy and SEM.

2. Experimental

2.1. Poly(lactide) substrates formation

Poly(DL-lactide) (PDLA) (inherent viscosity 0.55-0.75 dL/g), purchased from Lactel Absorbable Polymers (USA), was used for the preparation of the biodegradable substrates. The substrates were casted from 2% w/v PDLA chloroform solution and then dried at 35°C for 48 hours. The substrates were then kept in exicator, at room temperature at relative humidity (RH) 54%. Immediately prior to the deposition process, the substrates were treated in corona discharge system, in order to achieve negative surface charge excess. The corona discharge system consisted of corona electrode (needle), grounded plate, and a metal grid placed between them. Substrates were placed onto the grounded plate electrode. A voltage of –5 kV was applied to the corona electrode and –1 kV to the grid. The samples were charged under standard room conditions ($T = 21\div 23$ °C and $RH = 40\text{--}60\%$) for 1 min.

2.2. Layer-by-layer deposition of chitosan/xanthan multilayers

Chitosan (low molecular mass, degree of deacetylation > 75%) and xanthan gum were purchased from Sigma-Aldrich and were used as received. For LbL assembling process 0.1% w/v chitosan and 0.05% w/v xanthan solutions of acetate buffer (pH 4.5 and ionic strength 0.1 M) as solvent were prepared. Either dip-coating or spin-coating was employed for the deposition process. In both cases, the first built-up layer always possesses opposite to the substrate electric charge.

For dip-coating assembling a programmable slide stainer (Poly Stainer IUL, Spain) was used with deposition program: (1) 15 min solution dipping - adsorption of polyelectrolyte molecules; followed by 5 min washing in the acetate buffer (pH 4.5 and ionic strength 0.1 M); (3) 15 min solution dipping - adsorption of polyelectrolyte molecules of opposite charge; (4) 5 min washing in the same acetate buffer. The procedure was repeated and 8, 9, 14, 15 or 20 chitosan/xanthan multilayers were built up. After the MPTF were dried in hot air and then stored in exicator, at RH 54%.

Spin-coating assembling was performed on DDR Labortechnik T54 spin-coater. The polyelectrolyte solutions of chitosan and xanthan were spin coated one over the other (15 s at 1000 rpm) onto the preliminary charged PDLA substrates with an intermediate washing step (acetate buffer) between the deposition of the next layer.

2.3. FTIR spectroscopic measurements

In order to prove the successful self-assembling of the MPTF, FTIR-ATR spectra were collected. Thermo Scientific™ Nicolet™ iS™ 10 FT-IR spectrometer, equipped with a diamond ATR accessory in the range from 650-4000 cm⁻¹, giving a resolution 4 cm⁻¹ and 64 scans was used. The integral intensity was measured using OMNIC software package.

2.4. Surface refractive index measurements

As a crosscheck prove, as well as to estimate the thicknesses of the nanolayers (see below), the refractive indices of MPTF were measured. For all samples, the surface refractive indices (sRI) were measured by mean of disappearing diffraction pattern method [14], using a laser refractometer at two wavelengths (532 nm and 635 nm, each with a power 5 mW). As shown earlier [15], the penetration depth of the evanescent field in this case is less than 1 μm. Hence, the sRI measurements correspond to a surface layer of about 1 μm thickness. When the laser beam reaches the sample at an angle smaller than the critical angle, α_{cr} , the beam may pass through, reaching a reflection grating, placed behind giving rise to a diffraction pattern. When the incidence angle equals or exceeds α_{cr} a single spot of the totally internal reflected beam is observed. Therefore, α_{cr} can be measured as the angle at which the diffraction pattern disappears and one may calculated sRI by the following formula:

$$sRI = N \sin \left(A \pm \arcsin \frac{\sin \alpha_{cr}}{N} \right), \quad (1)$$

where A and N are the refracting angle and refractive index of the prism, used in the laser refractometer, respectively. In the present case, $A = 64.7^\circ$, $N(532 \text{ nm}) = 1.7480$, $N(635 \text{ nm}) = 1.7347$.

2.5. Scanning electron microscopy (SEM)

The general morphology of the obtained MPTF was revealed by means of SEM. A scanning electron microscope Lyra 3 XMU (Tescan) was employed. The working voltage was 30 kV. Prior to the measurements, the samples were covered with a thin film of gold (about 30 nm).

3. Results and discussion

In figure 1, ATR-FTIR spectra of MPTF composed of negatively (-) charged PDLA – substrates with dipping-assembled multilayers are presented.

PDLA substrates spectrum show two strong bands (at 2946 and 2881 cm⁻¹) belonging to the CH stretching region. The same bands are preserved in the MPTF spectra as well but they are broader and have lower intensity. The band at 2996 cm⁻¹ in PDLA substrate appears as a low intensity shoulder, whereas its intensity increases for the dipping-assembled multilayered films. The broad band, positioned between 3000 and 3700 cm⁻¹, corresponds to the superposition of two overlapping vibrations: -OH and amide groups in the carbohydrates structures 1594 cm⁻¹ band appears only in MPTF spectra and should be attributed to the NH₂ group [16]. Its intensity rises with increasing number of layers. Peaks that correspond to asymmetric and symmetric vibrations of NH₃⁺ (at 1632 cm⁻¹ and 1522 cm⁻¹) of chitosan and ionized carboxyl group (COO⁻) of xanthan at 1620 cm⁻¹ were not observed suggesting the formation of complex between both polymers, caused by ionic interactions between afore-mentioned groups [17].

The intensities of all other observed characteristic bands increase with increasing the number of layers.

Figure 2 represent the ATR-FTIR spectra of MPTF obtained by spin-coating assembling. Although, the bands intensities are not as high as in the case of dipping-assembling the general tendency of intensities increase is preserved in these spectra as well.

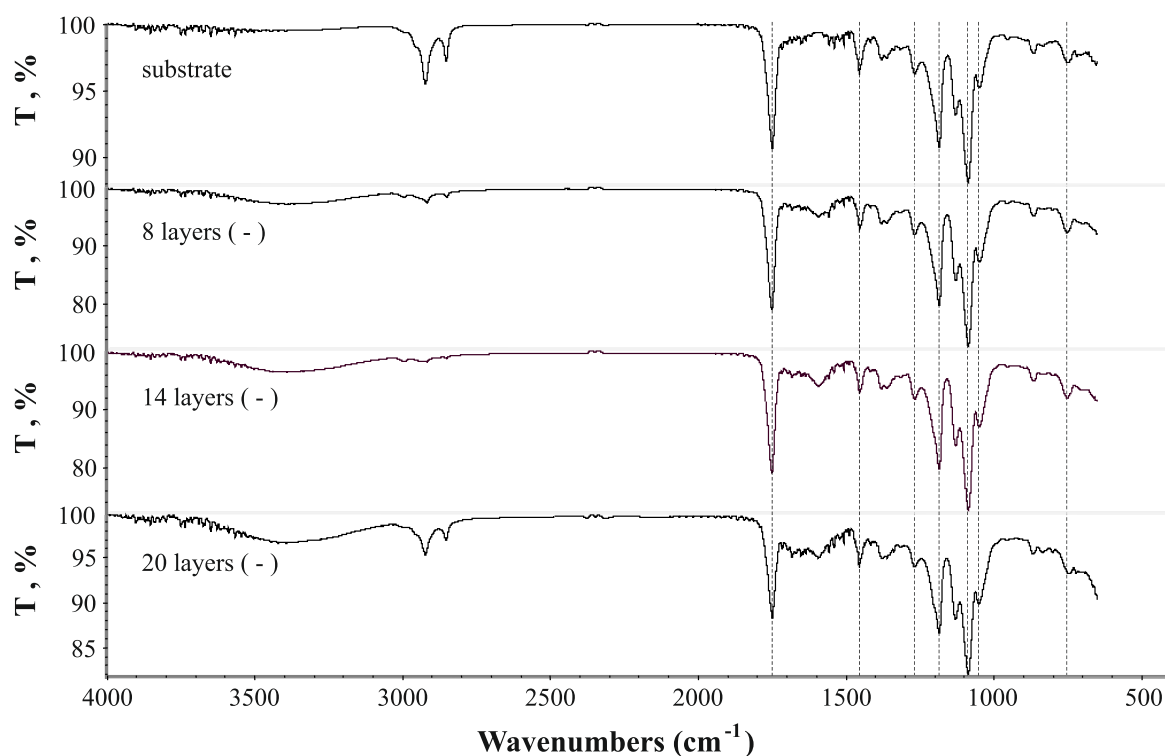


Figure 1. ATR-FTIR spectra of MPTF - negatively charged PDLA with dipping-assembled multilayers (first layer chitosan).

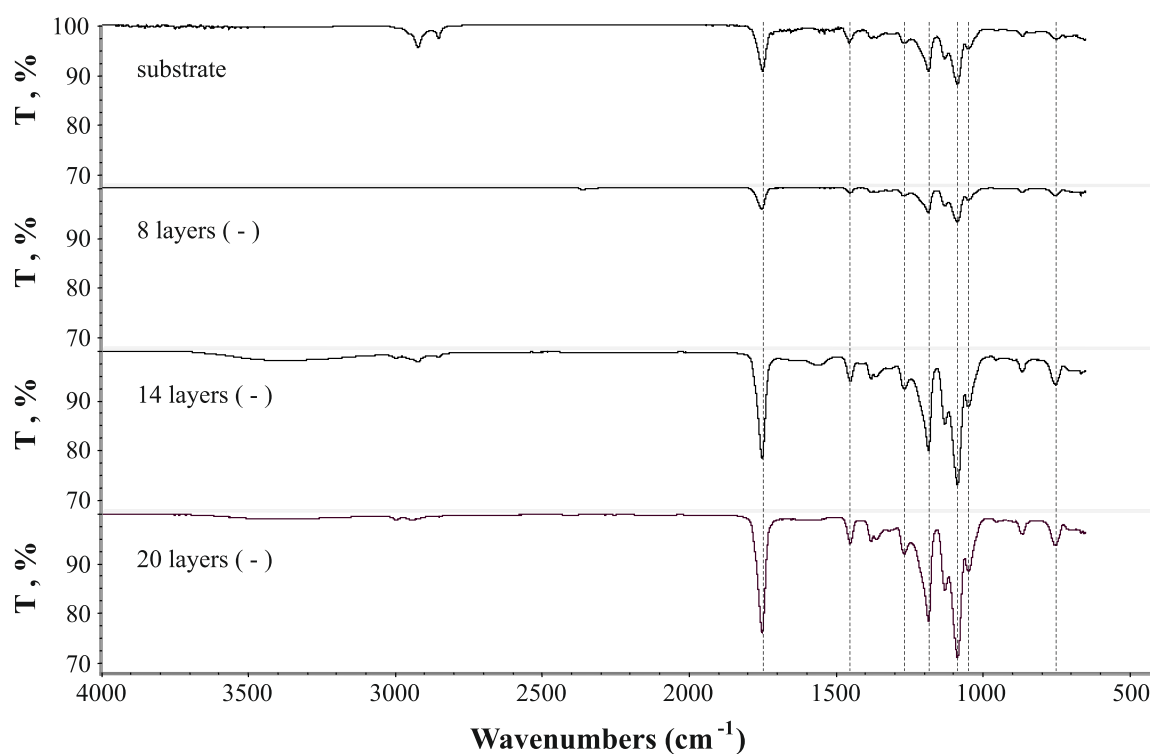


Figure 2. ATR-FTIR spectra of MPTF - negatively charged PDLA with spin-assembled multilayers (first layer chitosan).

Interestingly, the total integral FTIR intensity of MPTF with 20 layers is lower than these of MPTF with 8 and 14 layers. It is plausible that specific physical interactions and/or weak ionic interactions between the functional groups of the carbohydrates could explain the observation [18].

In summary, ATM-FTIR results suggest that:

- Chitosan and xanthan were successfully deposited onto the charged PDLA substrates and the adsorption process is more or less irreversible;
- The increase of number of layers leads to an increase of the amount of deposited hydrocolloids;
- Chitosan and xanthan molecules interact predominantly by ionic interactions, forming polyelectrolyte complexes.

Thicknesses of the MPTF layers were estimated based on measurements of surface refractive index. Figure 3a present sRI of negatively charged PDLA substrates with dipping-assembled multilayers (chitosan-xanthan) depending on the number of layers. Figure 3b represent the same dependences but in the case of spin-assembled MPTF.

Refractive index changes display similar behavior for all samples. The sRIs shift to higher values with increasing number of deposited layers. Hence, the refractive indices confirm once again the presence of multilayers, as it was shown in FTIR experiments. The average sRI over the penetration depth d_p of multilayer films, where Lorentz-Lorenz equation holds, reads:

$$d_p \frac{n_s^2 - 1}{n_s^2 + 2} = d \frac{n^2 - 1}{n^2 + 2} + N d_1 \frac{n_1^2 - 1}{n_1^2 + 2} \quad d_p = d + N(d_1 + d_2), \quad (2)$$

where d_p is the average evanescent wave penetration depth at total internal reflection, ($d_p = 0.82$ nm for 532 nm; $d_p = 0.98$ nm for 635 nm [15]); N is the number of one type of the layers; d_1 , n_1 are the thickness and the refractive index of first type of layers (chitosan); d_2 , n_2 are the thickness and the refractive index of the second type of layers (xanthan); d is the thickness of the PDLA substrate at evanescent wave penetration; n is the refractive index of the charged PDLA substrate. When the number of layers in MPTF increase, the PDLA substrate refractive index impact decreases. The corresponding values for 532 nm are $n_1 = 1.52$ [19], $n_2 = 1.50$ [20], and $n = 1.47$. Solving equation (2) with the particular values shows an increase in the refractive index of the MPTF with increasing number of the layers. By the assumption of equal thickness of all layers, the average layer thickness of single polyelectrolyte layer was estimated to be about $(5 \pm 0.5$ nm) for the spin-assembled MPTF and about $(10 \pm 1.0$ nm) for the dipping-assembled MPTF.

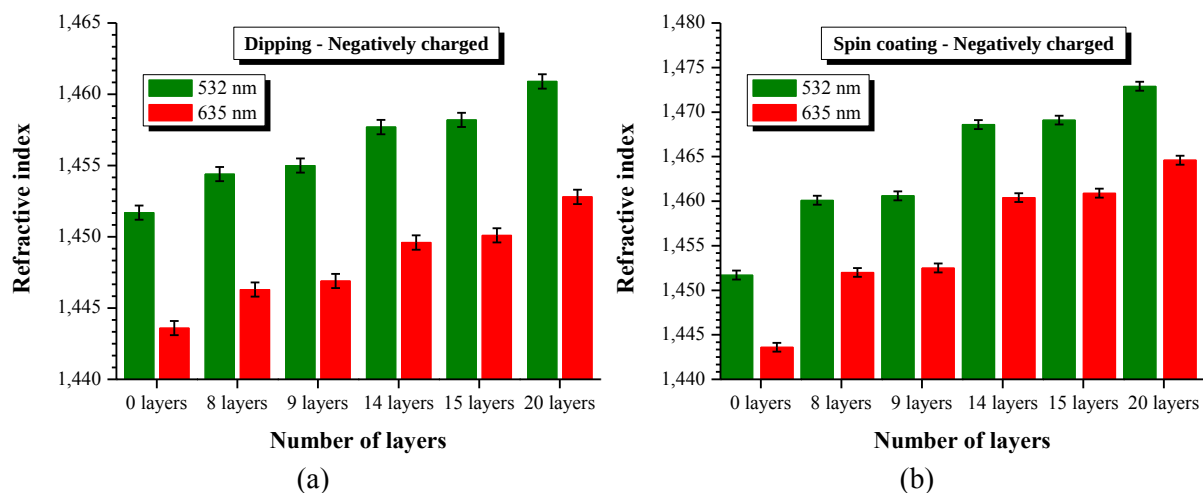


Figure 3. Refractive index changes of negatively charged PDLA with dipping-assembled multilayers (a) and negatively charged PDLA with spinning-assembled multilayers (b).

The results suggest faster thickness growth of the dipping-assembled films. This observation is in agreement with previous investigation on deposition process of weakly charged polyelectrolytes [21] and could be explained by specific interaction such as interdiffusion and interpenetration of the polyelectrolyte molecules. At pH 4.5 of deposition process the molecules of both chitosan and xanthan are partially charged (the pKa value of the chitosan is about 6.5 [22] and the pKa values of xanthan is 3.1 [23]) and part of their chains are free to move in the interior part of the polyelectrolyte complexes. Therefore, an excess amount of polyelectrolyte can be accumulated inside the layer volume and its thickness increases. In contrast, the high centrifugal force causes the film thickness to be lower because most of the unbounded polymer molecules are expelled.

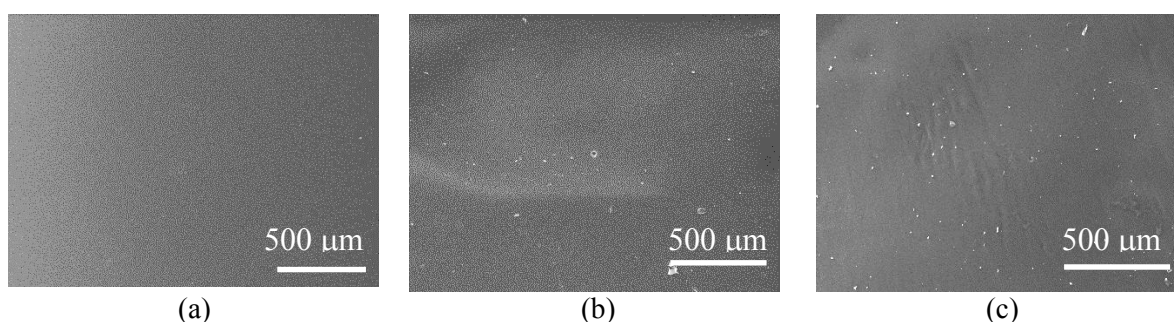


Figure 4. Scanning electron microscopy images of (a) PDLA substrate; (b) MPTF of negatively charged PDLA with 20 dipping-assembled multilayers (first layer chitosan); (c) MPTF of positively charged PDLA with 20 dipping-assembled multilayers (first layer xanthan).

The surface morphology of the bare substrates is quite smooth, consisting of some very fine grain-like structures (figure 4a). The surface morphology does not change with the layer deposition independent of the substrate charge or the deposition method. Representative SEM photograph for MPTF of negatively charged PDLA with 20 dipping-assembled multilayers is shown in figure 4b and photograph for MPTF of positively charged PDLA with 20 dipping-assembled multilayers is shown in figure 4b. The main difference are only the appearance of small impurities or defects onto the surface, which are very common for biopolymers. Their presence confirm once more the successful deposition of multilayers.

4. Conclusions

It is shown that dipping (dipping-assembling) and spin-coating (spinning-assembling) methods are successfully applied for deposition of chitosan and xanthan multilayers on PDLA corona charged substrates. In both cases the binding is irreversible and multilayer growth is predominantly due to ionic interactions and formation of polyelectrolyte complexes. The higher film thickness and surface roughness of dipping-assembled multilayers indicate the presence of inter-polymer diffusion and interpenetration.

Acknowledgements

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