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Simultaneous characterization of physical, chemical, and thermal properties of polymeric multilayers using infrared spectroscopic ellipsometry

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Abstract

In this study, multilayered films of polyethylenimine/poly (sodium-p-styrene sulfonate) (PEI)/(PSS) and type I collagen/heparin sodium (COL)/(HEP) were fabricated using the layer-by-layer technique, and fully characterized using Infrared Variable Angle Spectroscopic Ellipsometry (IRVASE) to simultaneously analyze the chemistry, thickness, and roughness of the multilayers with respect to changes in pH of the washing solution, and changes in temperature. Film topography and Young's modulus were obtained by atomic force microscopy (AFM) and nanoindentation. Our results show that with IRVASE it is possible to analyze the thickness of the multilayers prepared using a washing solution of pH 5, obtaining values of 71.7 nm and 40.3 nm for three bilayers of PEI/PSS and COL/HEP, respectively. Film roughness varies between multilayer systems, obtaining values of 37.76 nm for three bilayers of PEI/PSS and 33.58 nm for three bilayers of COL/HEP. Increasing the pH of the washing solution for PEI/PSS yielded thinner films that were less susceptible to thermal induced changes in film chemistry in the range of 25 – 150 °C. PEI/PSS films decreased in thickness with increasing temperature up to 75 °C, whereas above 75 °C film thickness increased. Through IRVASE, a transition temperature for the PEI/PSS multilayers was observed at 75 °C. Temperatures above 37 °C drastically alter the chemistry and the thickness of the COL/HEP multilayers indicating a possible degradation of the polymers. We obtained, through nanoindentation, a Young's modulus of 15000 kPa and 9000 kPa for 12 bilayers of PEI/PSS and COL/HEP, respectively. These results demonstrate that, using IRVASE, we can simultaneously evaluate the physical, chemical, and thermal properties of synthetic and natural multilayered polymeric films.

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Supplementary Information

Results obtained for MSE, optical constants (n, k) and offset for PEI/PSS and COL/HEP bilayers. In-depth explanation as to what each means and the results that should be obtained. Example of the model fit that the WVASE-IR provides after analyzing the data. High resolution IRVASE spectra of 6 polymeric bilayers of PEI/PSS and COL/HEP. Molecular structure of the polymers. Macroscopic structure of Collagen. Thickness and roughness vs number of bilayers for PEI/PSS system by AFM. Mechanical properties vs number of bilayers of the multilayers of PEI/PSS and COL/HEP by AFM.

Keywords

IRVASE; Layer-by-Layer; polyethylenimine; poly (sodium-p-styrenesulfonate); heparin; collagen type I

1. Introduction

Layer-by-layer (LbL) is a technique used to produce thin layers or coatings on surfaces, taking advantage of the sequence-specific electrostatic interactions between polyelectrolytes [1][2]. It has been widely used for: biomedical applications [3], corrosion control [3], catalysis [4], drug delivery [5], electrochemical sensing [6], protein purification [7], electrochromism [8], ultrastrong materials [9], optics and optoelectronics [10], among others [11][12][13].

Physical and chemical properties of polymeric multilayers fabricated using the LbL technique over different substrates have been characterized through different techniques. Fourier-transform surface Plasmon Resonance (FT-SPR), Quartz Crystal Microbalance with Dissipation (QCM-D), Variable-Angle Spectroscopic Ellipsometry, Scanning Electron Microscopy (SEM), and Atomic Force Microscopy (AFM) have been widely used to evaluate film growth, physical characteristics, morphology, thickness, and roughness [14][15][16]. Other techniques such as UV-VIS spectroscopy, Circular Dichroism Spectrometry, and Zeta Potential Analyzer, have been used to study the formation of multilayers [17][18][19][20][21]. Film chemistry has been evaluated by techniques such as Fourier Transform Infrared Spectroscopy (FTIR), X-ray Photoelectron Spectroscopy (XPS) and Polarization Modulation Infra-Red Reflection Absorption Spectroscopy (PM-IRRAS) [14][15][16][22]. Applying more than one technique and a larger number of samples to fully characterize the physical and chemical characteristics of polymeric LbL films.

Infrared Variable Angle Spectroscopic Ellipsometer (IRVASE) combines the principles of spectroscopic ellipsometry and FTIR that allows the chemical and physical characterization of thin films [23][24]. It is a powerful non-destructive surface technique, that allows the determination of film thickness, optical constants (n , k), and chemical composition of ultrathin multilayered films [24]. IRVASE also provides information of film characteristics such as: anisotropy, roughness, non-uniformity, phonons LO and TO, and doping concentrations [23][24]. The versatility of this technique is also demonstrated with the possibility of coupling other accessories to the equipment, such as a temperature stage, which allows the study of changes in film chemistry and thickness with respect to temperature [25]. Today, literature has reported the use of IRVASE to measure the dielectric function of substrates [26][27], determining the optical constants for lubricants [28] and polymers [23], to measure concentration profiles of doped silicon wafers [29], to evaluate physical-chemical properties of thin spin cast films [24], in studies of thermal stability of protein monolayers and multilayers deposited by drops over silicon substrates [25] and in the detection of inorganic compounds in nanolayers between 1 – 5 nm of thickness, like it was reported in our most recent work [30]. Only one recent report is available in the

literature using IRVASE as a complementary technique to characterize LbL films composed of poly (ethylenimine) and poly (acrylic acid) [31].

The use of IRVASE as a characterization technique for LbL multilayers has remain largely unexplored. Here we present a full study on the formation and IRVASE characterization of synthetic and natural polymeric multilayers. We demonstrate the simultaneous characterization of the physical (thickness and roughness), chemical, and thermal properties of synthetic and natural polymeric multilayers using IRVASE. Two multilayered systems are evaluated: synthetic multilayers composed of polyethylenimine (PEI) and poly (sodium-p-styrene sulfonate) (PSS) and natural multilayers composed of collagen type I (COL) and heparin (HEP). PEI/PSS LbL films have been previously used as sorbents for CO₂ capture [21], adsorption of prussian blue colloids [32], and corrosion control [33]. The natural system of multilayers COL/HEP have been previously used to help promote the blood-compatibility of titanium implants [34][35][36][37], vascularization studies [38] and intravascular endothelialization with the functionalization of antibodies over the multilayers [39][40]. IRVASE was used to study the effect of changes in temperature (25, 37, 50, 75, 100, 125 and 150 °C) and pH (2.7, 4, 5 and 6) of the washing solution used to prepare the multilayers with respect to changes in thickness, roughness and chemical composition. AFM was used to validate thickness results obtained from the IRVASE in addition to obtaining information on the morphology, roughness, and Young's modulus of the multilayers, complementing results obtained from the IRVASE.

2. Materials and Methods

2.1 Materials

Polyethylenimine (PEI) (50% Solution in Water, Mw $\approx$ 750,000) of Sigma-Aldrich, poly (sodium-p-styrenesulfonate) (PSS)(Mw $\approx$ 70,000) of Acros Organics, heparin sodium (HEP) of Celsius Laboratories, Inc and lyophilized type I collagen sponges (COL) derived from cow tendon (generously donated by Integra Lifesciences Holdings Corporation, Añasco, PR), were used to prepare polymeric multilayers over a silicon substrate (resistivity of 5 – 10 Ohm – cm, thickness 356 – 406 μ m, WRS Materials, San Jose, -CA). Sodium acetate anhydrous was purchased from Fisher BioReagents. Acetic acid glacial (ACS Grade) was purchased from Amresco. Ultrapure water at 18 M Ω - cm obtained from an ultrapure water system directly from tap producing RiOsTM and Milli-Q® water (Darmstadt, Germany), was used to prepare polymeric and wash solutions.

2.2 Preparation of the polymeric multilayers

Two systems, one composed of synthetic polymers and another composed of natural polymers, PEI/PSS and COL/HEP respectively, were used in this work to prepare polymeric multilayers using the LbL technique. For each combination of polymers, 3, 6, 9 and 12 bilayers were generated over the silicon substrates. The solution used to the dissolve polymers consisted of acetate buffer (0.1 M sodium acetate anhydrous, 0.1 M acetic acid, pH 5), which was also used as the washing solution. The washing solution was also prepared at a pH 2.7, 4, 5, and 6 to evaluate the effect of changes in pH on the characteristics of the PEI/PSS multilayers. For COL/HEP multilayers, a washing solution of pH 5 was evaluated.

All polymeric solutions were prepared at a concentration of 1 mg/mL. A robotic dipping machine, nanoStrata StratoSequence VI (Tallahassee Florida), was used to develop the LbL films. In this work, the sequence of forming the multilayers consisted in submerging the substrate in a PEI solution for 15 min to establish an anchor layer. Then PSS and PEI were deposited sequentially for 5 min per layer with a 3 min wash in between to remove molecules slightly adhered. The same process was performed for the COL/HEP system. After multilayer preparation, a final wash was performed using ultrapure water at 18 M Ω - cm. The samples were left to dry at room temperature prior characterization.

2.3 Analysis of the chemical composition of polymers and multilayers

FTIR-ATR was used to confirm the presence of functional groups of each polymer dissolved in acetate buffer (0.1 M sodium acetate anhydrous, 0.1 M acetic acid, pH 5) and deposited through drop casting over a silicon substrate. The infrared spectra of the samples were collected using a PerkinElmer Spectrum Two FTIR in ATR mode, with a wavenumber range of 500 to 2000 cm⁻¹ using 500 scans at 4 cm⁻¹ resolution.

Infrared Variable Angle Spectroscopic Ellipsometry (IR-VASE® - Mark II, J.A. Woollam Co., Inc. IR-VASE, Lincoln, Nebraska) was used to study the chemistry of the individual polymers by drop casting and polymeric multilayers constructed over the silicon substrate. The chemical composition analysis for all samples was performed in a wavenumber range from 500 – 2000 cm⁻¹. The measurements were taken using a DTGS detector, an angle of incidence of 65° and 75°, a spectral resolution of 16 cm⁻¹, 15 scans per cycle, 2 cycles, bandwidth of 0.01 μ m, minimum intensity ratio of 2, sample type as isotropic, zone average polarizer, and single position RCE analyzer. A heating stage controlled by an INSTEC – mk2000 coupled to the IRVASE was used to establish the controlled heat ramp. The polymeric multilayers were heated to 25 °C, 37 °C, 50 °C, 75 °C, 100 °C, 125 °C and 150 °C, with an exposition time of 10 min for each temperature, programmed to heat up, maintain the temperature, and then collect ellipsometric data.

2.4 Analysis of the physical characteristics of multilayers

The thickness and roughness of the multilayers formed over the silicon substrate were determined using the IRVASE equipment (IR-VASE® - Mark II, J.A. Woollam Co., Inc. IR-VASE, Lincoln, Nebraska). The WVASE32 software within the IRVASE was used to analyze both properties. Transmission test were done before analyzing the samples to make sure it was less than 0.01%. Collected data were fitted to ellipsometric models that are a function of film thickness and optical constants (n, k). The model consists of default layers of the 1 mm silicon substrate (si_ir) along with its native oxide layer (sio2_ntve_ir_g), followed by an infrared general oscillating layer (genosc_ir) to model the polymeric bilayers. The thickness was displayed in nanometers and the Mean Squared Error (MSE) and the depolarization were observed to be closest to zero. The MSE is the error of the fit to the collected data. The same collected data were fitted to ellipsometric models that are a function of film roughness. The model consists of default layers of the 1 mm silicon substrate (si_ir) along with its native oxide layer (sio2_ntve_ir_g), followed by a surface roughness layer (srough) to model the roughness of the polymeric bilayers. The roughness was observed in nanometers and the MSE was observed to be closest to zero. In addition, the

n and k optical constants and the offset were observed. These should remain constant, or very similar, as the different samples are analyzed. Lastly, the effect on varying temperature and pH over the morphology of the multilayers was also evaluated. Figure S1 and Tables S1, S2, S3 and S4 from the supplemental information provide the results obtained for MSE, optical constants (n, k), and offset, as well as a more in-depth explanation as to what each means and what would be the expected results. It also contains an example of the model fit that the WVASE-IR provides after analyzing the data. Thickness and roughness were calculated as a mean of three measurements and the error bars represent the standard deviation.

An Agilent Atomic Force Microscope (AFM), model 5500 (Keysight Technologies, Santa Rosa, CA) was used for the topographical characterization, to study the changes in roughness, the mechanical properties on the polymeric surfaces and to corroborate the thickness obtained through IRVASE analysis at the different experimental conditions. To analyze the topography and roughness of the multilayers, measurements were performed using a silicon SPM (N9832B) sensor cantilever in contact mode. All measurements were carried out in air, under ambient conditions and each image measurements on a sample correspond to 512×512 pixels. The scanning speed used was 1.96 lines/s. The size of the scanned area was $80 \times 80 \mu\text{m}$. For the topographic analysis, the surfaces were compared by means of the roughness parameters (R_a). Thickness and roughness were calculated as a mean at three different positions of two samples and the error bars represent the standard deviation.

To analyze the mechanical properties and thickness of the sample a silicon SPM (N983A ppp-cont: deflection sensitivity of 58.624 nm/V, and a force constant of 1N/m) sensor cantilever was used. For this analysis, first, the sensitivity of the cantilevers was evaluated by pressing the cantilever over a silicon substrate. Then, the force constant of the cantilever was measured and the elasticity parameter (i.e. tip radius, Poisson ratio) was adjusted. The scan resolution was 512×512 pixels, the area of indentation was $80 \times 80 \mu\text{m}$ with a speed of 1.19 lines/s, the duration was 0.240 s, data point was 2000 with a rate of $12.50 \mu\text{m/s}$. For this analysis 20 indentations in each point of two samples were performed and the error bars represent the standard deviation. The 20-indentation per point was selected to corroborate that the cantilevers were touching the silicon substrate and with this information the thickness of the multilayers were corroborated. Afterwards, the Young's modulus was calculated using the Hertz and Sneddon model (no adhesion contribution).

Statistical analysis

The results were presented as mean $\pm$ standard error of mean. Comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) using Minitab 17 for Windows. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 IRVASE vs FTIR: Chemical composition of polymeric surfaces

To demonstrate the potential of the IRVASE technique and its usefulness as a tool in the characterization of the chemical composition of surfaces, in this study each of the polymers was dissolved in acetate buffer (0.1 M sodium acetate anhydrous, 0.1 M acetic acid, pH 5) and deposited on silicon wafers by drop casting. FTIR-ATR was used to validate the results obtained through IRVASE. To confirm the chemical composition of the polymeric multilayers over the silicon substrate, it was necessary to initially study the molecular structure of each polymer individually to identify their characteristic functional groups and to later associate peaks in IR spectra with vibrations in these groups. The polyelectrolytes used in this work were PSS & HEP as the polyanions, and PEI & COL as the polycations. Their chemical structures are presented in Figure S2 of supplemental information.

The chemical structure of PEI, which is a highly branched cationic polymer, is shown in Figure S2A of supplemental information. This polymer contains in its structure a high density of ionizable groups including: primary amines (RNH_2), secondary amines (R_2NH), and tertiary amines (NR_3) [41]. The amines present in PEI gives its strong positive charge when protonated to NH_3^+ . For this polymer, a pK_a within 7.11 and 8.6 has been reported, allowing it to be considered as a strong polycation at pH 5 [42].

Analyzing the FTIR-ATR and the IRVASE spectra of PEI in Figure 1A, it is possible to identify the functional groups described in the chemical structure [41]. The characteristic peaks for PEI observed are: 1635 cm^{-1} (NH_2 group), 1544 cm^{-1} (N–H bending), 1402 cm^{-1} (CH_2 bending), 1050 cm^{-1} & 1017 cm^{-1} (C–N stretching) [41][43], and other strong absorptions under 1000 cm^{-1} associated to CH_2 [44][45]. Residual acetate buffer may correspond to the COOH peak near 1700 cm^{-1} and to the CO peak near 940 cm^{-1} . Evaluating the spectrum obtained by IRVASE, and comparing it with the one obtained through FTIR-ATR, it is possible to determine that they match, with only some slight displacements between a few bands. The differences in spectra may be due to the limited penetration depth of the FTIR-ATR. IRVASE can penetrate the entire thickness of the sample, over a larger area, generating a stronger signal and reducing signal to noise ratio.

The structure of PSS (Figure S2B from supplemental information) contains a 1,4-substituted benzene ring with a sulfonate group. Since this molecule has a pK_a near 1.0, the sulfonate is deprotonated and negatively charged at pH values larger than 1, resulting in it acting as a strong polyanion [46]. Evaluating both the IRVASE and FTIR-ATR spectra (Figure 1B), the characteristic peaks of PSS are observed including: the SO_3^- asymmetric stretching vibrations at 1186 cm^{-1} and 1135 cm^{-1} , and the SO_3^- symmetric stretching vibrations at 1049 cm^{-1} and 1018 cm^{-1} [47]. Similarly, the C=C group at $1566 - 1404\text{ cm}^{-1}$, which are part of the benzene ring [47].

Heparin is composed of a disaccharide repetitive unit that includes three acidic groups: carboxyl, *N*-sulfonate, and *O*-sulfonate (Figure S2C from supplemental information). The carboxyl group has been found experimentally to have a pK_a ranging from 2.79 to 3.13, and it has been reported that the *O*-sulfo and *N*-sulfo are even more acidic than the carboxyl

group when heparin is dissolved in water [48]. Therefore, in a solution with a pH higher than the pK_a , it is expected that the heparin subunits will have the acidic groups deprotonated, resulting in negative charge and heparin acting as a polyanion [15]. Other notable functional groups in HEP are the hydroxyl and ether groups. Because HEP is a polysaccharide, it is expected that the spectrum will show a peak between $1700-1600\text{ cm}^{-1}$ and another between $1600-1500\text{ cm}^{-1}$, corresponding to the NH_2 group (Amide I) and the N-H, (Amide II), respectively [37]. The spectra obtained by both IRVASE and FTIR-ATR for HEP are presented in Figure 1C. The spectra shows bands at 1627 cm^{-1} and 1558 cm^{-1} , corresponding to the amide I and amide II, respectively [15]. Another peak is observed at 1420 cm^{-1} and is attributed to the CH_2 scissoring, as well as the peaks observed at 1350 cm^{-1} and just below 1000 cm^{-1} [45][44]. The C-N group is shown between 1100 cm^{-1} and 1000 cm^{-1} and at approximately 950 cm^{-1} the C-O-S stretching vibration is observed [15] [49].

Finally, the chemistry of collagen type I was also analyzed. Collagen is known as a fibrous protein (See Figure S2D from supplemental information), which is a succession of amino acids joined together by peptide bonds [50]. IRVASE (Figure 1D), revealed a better-defined spectrum than the one obtained through FTIR-ATR. The characteristic peaks of collagen are seen in both spectra. Figure 1D shows the characteristic peaks of collagen, amide I ($1700-1600\text{ cm}^{-1}$), amide II ($1600-1500\text{ cm}^{-1}$), and amide III ($1300-1180\text{ cm}^{-1}$) in both spectra [51][52]. Thus IRVASE may be used to evaluate the chemical composition of synthetic and natural drop cast polymers as confirmed by FTIR-ATR.

3.2 Multilayered film growth: chemical and physical characteristics of the surface by IRVASE

A robotic dipping machine was used to develop the polymeric multilayers of PEI/PSS and COL/HEP systematically by programming the immersion of the silicon substrate for 5 min for the polymeric solutions and 3 min for the washing solution. The immersion time of 5 min was selected as longer immersion times yielded films of similar thicknesses. IRVASE was used to monitor film growth, determine film composition/chemistry, and determine film thickness and roughness of the surface modified for the polymeric multilayers. AFM was used to analyze the topography, roughness, Young's modulus, and to validate the thickness of the multilayers formed. The thickness was determined by the difference between the contact points of force on the substrate and the multilayers, as has also been reported by Domke and Radmacher, where they measured the elastic properties of gelatin films, with a thickness of $1.4\text{ }\mu\text{m}$ (thick trace) and another of 180 nm (thin trace) using AFM [53]. This was unlike the method employed by Lobo and colleagues, who applied a 'furrow method' to evaluate LbL ultra-thin films with a thickness of at least 60 nm [54][55]. This method consisted in using the AFM to scratch the multilayers formed over the substrate, producing a furrow, allowing to estimate the thickness of the multilayers through a direct measurement of the height of the furrow [54][55]. In our work, 3, 6, 9 and 12 bilayers were developed using a washing solution of pH 5, for both combinations of polymers.

Analyzing our results, Figure 2 shows that the surface of the silicon substrate was effectively modified by the LbL technique as demonstrated by the presence of characteristic peaks for

PEI/PSS. The spectra for all bilayers are different from the neat silicon substrate spectrum, generally showing characteristic absorption bands previously discussed for PEI and PSS. Although slight displacements are observed, due to the interaction among polymers, characteristic PEI peaks are observed at 1628 cm^{-1} for the NH_2 group, 1527 cm^{-1} (N–H bending), 1444 cm^{-1} (CH_2 bending), 1026 cm^{-1} (C–N stretching), and 1000 cm^{-1} (C–N stretching) [41][43]. A higher resolution (increased number of scans and shorter range) of Figure 2A is available in the Figure S3 of supplemental information to better appreciate these peaks. For PSS, the SO_3^- asymmetric stretching vibrations, are identified at 1203 cm^{-1} and 1157 cm^{-1} . The SO_3^- symmetric stretching vibrations are observed at 1110 cm^{-1} and 1056 cm^{-1} . And the C=C vibration at 1519 cm^{-1} and 1404 cm^{-1} were also identified for PSS [47]. Choi and his group [56], developed LbL films of PEI/PSS over a gold substrate. Through FTIR they also confirmed the formation of the PEI/PSS multilayers, validating our results obtained through IRVASE. They reported peaks at 1200 cm^{-1} , 1126 cm^{-1} , 1035 cm^{-1} , and 1008 cm^{-1} which were assigned to the asymmetric vibration of SO_3^- , benzene ring-sulfur vibration, symmetric vibration of SO_3^- , and in-plane bending vibration of para-substituted benzene ring, respectively [56]. Our IRVASE spectra of the multilayers (Figure 2A) revealed these peaks, which have also been reported in previous studies for PSS [57] [58]. When comparing the analysis of FTIR and IRVASE of the drop cast polymers (Figure 1A) it was also possible to identify the characteristic peaks of PEI, as previously mentioned. These observations confirm that the chemistry of these polymeric combinations is present and that the surface was adequately modified compared to the spectrum of the silicon substrate. This confirms that LbL film chemistry may be evaluated using IRVASE. Moreover, since the IR beam penetrates the entire thickness of the sample the chemistry provided by IRVASE represents the chemistry of the LbL film throughout its entire thickness, and not the chemistry of the final layer.

Figure 2A shows that a greater number of bilayers results in spectra with better defined peaks. This may indicate that a larger deposition of polymers is being accumulated over the substrate surface as bilayer quantity increases. This behavior was reported by Richards and his group [21], who fabricated multilayers of PEI/PSS and confirmed their formation and chemistry using energy dispersive X-ray analysis, confocal laser scanning microscopy, and ellipsometry. An increase in PEI deposition was found with increasing PEI/PSS bilayer formation [21]. S. Ravaine and colleagues studied the kinetics of monolayer formation, using infrared spectroscopy to monitor the absorption of the Prussian blue (PB) colloids in PEI/PSS films. They observed an increase in peak intensity in the infrared spectrum of a PEI/PSS film as a function of the number of cycles of immersion in a 10^{-3} M PB solution, followed by rinsing with water [32]. Spectroscopy (either infrared or ultraviolet) has been previously used to monitor LbL multilayer construction. Wang and collaborators [59], fabricated multilayers of 3,4-poly(ethylenedioxythiophene) and PSS using PEI as an anchor layer, where they monitored film growth by UV-VIS.

Considering IRVASE uses the underlying principle of FTIR, we can exploit this technique to monitor film growth by changes in the IR spectrum of the samples as well as obtaining their thickness.

Thickness and roughness values were obtained with the lowest values corresponding to 3 bilayers, of 71.70 nm and 37.76 nm, respectively (Figure 2B–D). In Figure 2B, it is noticeable that during the formation of the 6 bilayers first a significant difference of $p < 0.05$ in thickness compared with 3 bilayers is observed. No significant difference was observed between the 6 and 9 bilayers. But a $p < 0.01$ was obtained between 12 compared with 3, 6 and 9 bilayers. This can be associated to the fact that the formation of the layers has a base stage over the substrate, which helps in the effective formation and adhesion to modify the surface with polymeric multilayers of PEI/PSS.

Kotov and his group fabricated PEI multilayers (branched, $M_w \approx 25,000$ Da)/PSS ($M_w \approx 1 \times 10^6$ Da), using LBL spin-coater [33, 60]. They found that the thickness of the multilayers linearly increases with a correlation coefficient of almost unity. They observed an average thickness per layer pair of 6.7 nm, as measured by ellipsometry, for multilayers constructed using spin-assisted LbL with polymers dissolved in water at pH 3.7, a concentration of 50 mg/mL, and using acetonitrile as rinsing solution [33].

Richards and his group [21], demonstrated that advanced solid sorbents can be fabricated applying the LbL technique and that the absorption capacity is proportional to the number of layers of PEI/PSS formed. They reported in their ellipsometric analysis, that the thickness of the multilayers linearly increases with increasing number of PEI/PSS bilayers, obtaining that each bilayer is approximately 8 nm thick. Therefore, a thickness of approximately 80 nm was obtained for 10 bilayers [21], and in comparison to the results obtained by Kotov, this estimated thickness can be obtained with the fabrication of 12 bilayers. In our work, a 3 bilayered PEI/PSS film yields a thickness of 71.70 nm. This value differs from the work of Kotov and the work of Richards. In our work, PEI and PSS are dissolved at 1 mg/mL a lower concentration as compared to the works of Kotov and Richards for the same polymeric system. The difference may arise in the concentration of the polymers used, the molecular weight of the polymers, the type of LbL methodology (dipping vs spin-coated), or the type of rinsing solution (water or acetonitrile vs acetate buffer) [21, 33]. Our work uses high molecular weight branched PEI ($M_w \approx 750,000$ Da) which may lead to thicker films. Previous work has shown how strongly the molecular weight of the polymers influences the thickness of the multilayers, such that increasing molecular weight yields an increase in film thickness [61].

M. Elzbieciak and collaborators, studied the influence of pH in the polymeric solution over the structure of the multilayers prepared from aqueous solutions using a standard dipping approach [62]. They demonstrated that depending on the pH of the polymeric solutions, the polyelectrolyte combinations that are weakly or strongly charged generated a linear or non-monotonic increase in thickness. In their work using PEI ($MW \approx 70,000$ Da) /PSS ($MW \approx 70,000$ Da) at pH 6 and a concentration of 10^{-3} g/L, they achieved an increase of 2.5 nm per bilayer for the PEI/PSS bilayer film. This value is less than the one reported by Kotov [60] and Richards [21], therefore M. Elzbieciak would have had to elaborate approximately 32 bilayers to obtain a thickness average of 80 nm [62]. These studies reveal the physical characteristics, such as the molecular weight of the polymers used to generate the multilayers, their concentrations, and the pH of the polymeric solutions and washes, are intimately linked to the formation and increase in the number of multilayers.

A similar behavior of having a base stage in the formation of the multilayers has also been reported in works of multilayers fabricated with other combinations of polymers. Wang and collaborators [59], demonstrated by performing an ellipsometric study to evaluate the thickness of PEDOT/PSS bilayers, that an increase in thickness begins around two or three bilayers, corresponding to an interval in thickness of 6–10 nm. This is because the first layers show a support stage, demonstrating a bi-linear tendency in the thickness during an increase in the multilayer films, which shows a thickness behavior that varies linearly with the number of bilayers formed. In their previous work, Schaaf and colleagues prepared multilayers of PSS/polyallylamine (PAH) [63] and associated this bi-linear tendency to the substrate effects that influence the packing density of the first few layers formed. This tendency has also been reported for multilayers prepared of polymeric combinations composed of cationic-modified hyaluronan⁺/hyaluronan [64], poly(L-lysine)/hyaluronan, and poly(L-lysine)/heparin [49]. Our results demonstrate that IRVASE can characterize the thickness of the polymeric multilayers, obtaining the same behavior and magnitudes reported in other works.

To validate our results of the thickness measurements obtained by IRVASE, we decided to use AFM for indentation. To obtain the measurements of each sample, first the mechanical properties of the substrate were measured, to acquire a value in which the indentation of the multilayers over the substrate finalizes. Meaning that once the cantilever in the indentation perceives the properties of the substrate, it will stop. Therefore, the thickness measurement will be calculated by the distance of penetration from the surface of the multilayers to the surface of the substrate. The indentation results (Figure S4A from supplemental information) demonstrated that the thicknesses obtained from the IRVASE is reliable. Obtaining a thickness of 70.12 nm through indentation versus 71.7 nm with IRVASE for 3 bilayers and 217.5 nm versus 253.3 nm for 12 bilayers, respectively. In addition, AFM also confirms that the sample is not damaged after IRVASE analysis, due to the similarity in the data. It is worth highlighting that IRVASE covers a larger area of 176.71 mm² when measured at an angle of 70°, in comparison to AFM which covers 6.4 mm², allowing us to obtain a more precise thickness value. The range of thicknesses that might be obtained through IRVASE is large. Thicknesses as small as ~1 nm may be obtained [30]. The thickness of thick LbL films (more than 500 µm) may be evaluated using IRVASE if the sample is homogenous and with minimal roughness. To ensure adequate values for thickness, the depolarization of the film must be close to zero. Increase in depolarization is observed with non-homogenous LbL films. The non-homogeneity of LbL films may be caused due to impurities in polymer solution, polymers with large polydispersity index, large salt content, or other effects.

IRVASE was also used to estimate the roughness of the multilayers formed over the substrate. The same way AFM was used to validate the results obtained by the IRVASE. The results of the topographic characterization by AFM are presented in (Figure S4B from supplemental information), showing how roughness progressively increases with the number of bilayers. A significant difference of $p < 0.01$ in roughness, as measured by AFM, was obtained on 12 bilayers (130 nm) as compared to 3 bilayers (12 nm) (Figure S4B from supplemental information). This behavior was also observed by IRVASE in Figure 2C, obtaining a roughness of approximately 37 nm versus 240 nm for 3 and 12 bilayers,

respectively, with a significant different $p < 0.01$. This shows a similar scale, but with a magnitude of almost double for the values obtained by the IRVASE versus those obtained by the AFM. This difference could be associated to the fact that the IRVASE does the analysis over a larger area of the surface, adjusting the average. However, these values in roughness are in the range of previously reported polymeric LbL systems using a high-molecular weight PEI [65].

In addition, an analogous behavior over the thickness and roughness of the samples is appreciated in Figure 2B and Figure S4B of the supplemental information which shows the roughness data with respect to Figure 2B of the thickness. We observed two regions of increasing roughness, where the roughness increases slightly between 3 and 9 bilayers. Afterwards, a significant increase in roughness is observed between 9 and 12 bilayers. This confirms the establishment of the base layers. This behavior could be associated to the amount of polymer adhered to the surface of the substrate, which is intimately associated with the number of bilayers formed. This has been reported by M. Elzbieciak and their group [62], where they observed a change in topography and a higher roughness between 6 and 7 bilayers of PEI/PSS, similar to our results.

AFM was also used to measure the mechanical properties of the multilayers. Figure S5A from supplemental information shows the Young's modulus for 3, 6, 9 and 12 bilayers (the same samples used to measure topography and roughness in indentation). These results demonstrated that as the number of bilayers increases, the Young's modulus decreases. We observed a statistical difference ($p < 0.05$) in Young's modulus of 18,929.0 kPa for 3 bilayers and 14,664.8 kPa for 12 bilayers. This can be associated to the fact that the multilayers are softer, also indicating that the surface of the substrate is completely modified, reigning the properties of the multilayers with an increase in the bilayers formed. Therefore, these results demonstrate that it is possible to simultaneously obtain the physical-chemical properties of a surface modified by multilayers using the IRVASE, leaving the sample available for other analysis.

For natural multilayers of COL/HEP, it was also possible to characterize the physical, chemical, and mechanical properties using IRVASE and AFM. Our results over the analysis of the chemistry of the multilayers revealed the presence of the characteristic bands of both polymers. Figure 3A shows the spectrum of 3, 6, 9, and 12 bilayers. Similar to the PEI/PSS system, the intensity of the peaks is more defined as the number of bilayers increases. For 12 bilayers of COL/HEP the spectrum is more defined, observing the characteristic peaks of both polymers. For both collagen and heparin, the amide I or NH_2 peak 1628 cm^{-1} , and the amide II peak (NH) at 1512 cm^{-1} are observed [15][50][51][52]. A higher resolution (increased number of scans and shorter range) of Figure 3A is available in Figure S6 of supplemental information to better appreciate these peaks. In this spectrum, it is also possible to identify other characteristic peaks of HEP such as: CH_2 scissoring at 1442 cm^{-1} , SO_3^- at 1211 cm^{-1} , C-N stretching at 1072 cm^{-1} & 1026 cm^{-1} , and the C-O-S stretching vibration at approximately 987 cm^{-1} [15][49]. Thus the characteristic peaks of collagen and heparin are observed. Similar results are presented by N. Huang and his team when they prepared multilayers of collagen type I and unfractionated heparin over titanium substrates [34][35]. In these studies, they used COL/HEP films as an anticoagulant coating over

titanium cardiovascular implants [34]. They also investigated the effect on coagulation of the degradation of the COL/HEP coating [35]. Of note, we were not able to obtain spectra of any of the multilayered films using the FTIR-ATR equipment.

For COL/HEP films, the behavior of growth and roughness shows a linear tendency with increasing number of layers (Figure 3B and 3C), contrary to PEI/PSS films. Thicknesses of 44.72 nm, 91.26 nm, 128.8 nm and 162.44 nm, were obtained for 3, 6, 9 and 12 bilayers, respectively (Figure 3B). An average of 12.66 nm in increase of thickness per bilayer was observed, in addition to the fact that this system generates thinner multilayers than those obtained in our PEI/PSS system. A significant difference of $p < 0.01$ in the thickness measurements between 6, 9, and 12 multilayers compared with 3 bilayers is observed. Also a significant difference of $p < 0.05$ was obtained between 9 and 12 bilayers. Jian Ji and her team, who also worked with a COL/HEP system [39], found the same linear behavior in the construction of the films. In their work, they used low molecular weight heparin and collagen type I from bovine cartilage. They dissolved the polymers in acetate buffer solution (0.018 M NaAc, 0.082 M HAc, 0.14 M NaCl, pH 4.0) at 1 mg/mL. Their ellipsometric results showed a thickness of approximately 14 nm for one bilayer, which is close to our results.

Analyzing the roughness and the topographic images (Figure 3C and 3D), it is possible to observe that as the number of bilayers increases the roughness also increases. Although not enough to surpass the roughness obtained for the PEI/PSS system, which reaches an average of 242 nm for 12 bilayers. The COL/HEP system has a less rough surface with an average of approximately 208 nm for 12 bilayers. The minimum differences appreciated are due to the physical characteristics of the polymers used. For 3, 6, and 9 bilayers of COL/HEP, our results show roughness of 33.58 nm, 101.66 nm, and 163.06 nm, respectively. A significant difference of $p < 0.01$ in the roughness measurements between 6, 9 and 12 multilayers compared with 3 bilayers is observed. A significant difference of $p < 0.05$ was obtained between 6, 9, and 12 bilayers (Figure 3C). Chau-Chang Chou and his group [36] in one of their work evaluated 5 and 15 bilayers of COL/HEP to study the blood compatibility and adhesion of multilayers coated on cp-Ti substrates. They used heparin sodium salt at 5 mg/mL in DI water and collagen type I derived from bovine Achilles tendon dissolved in 0.2 M acetic acid at 2.5 mg/mL in DI water at pH 4. Their results obtained for roughness by AFM indicated an average of 110 nm for the multilayer system composed of 5 bilayers, which resembles a close relationship to 101.66 nm obtained for our polymeric system of 6 bilayers.

Using the same process used to measure Young's modulus for the PEI/PSS system, an analysis of the COL/HEP multilayers prepared over a silicon substrate, was performed. Figure S5B from supplemental information presents the results, demonstrating that as the multilayers grow, the Young's modulus decreases. These results show that the Young's modulus of the PEI/PSS system is greater than the COL/HEP system, obtaining a value of approximately 15000 kPa for 12 bilayers of the PEI/PSS system, compared to 9000 kPa reported for the natural system of polymers COL/HEP. In comparison to PEI/PSS system, the surface with COL/HEP multilayers showed a significant different $p < 0.01$ in the Young's modulus measurement for 6, 9, and 12 bilayers compared with 3 bilayers (Figure

S5B from supplemental information). This difference can be associated to the chemical structure of the polymers and their bonds. The high value of Young's modulus for the PEI/PSS system can be associated to the structure of PSS, which has a benzene ring and the double bonds of carbon. Therefore, stronger resistance to deformation is exerted.

3.3 Effect of the pH over the physical-chemical characteristics of the polymeric surface

In this study, the effect of changes in the pH of the washing buffer solution over the formation of the layers and their physical-chemical characteristics of each surface was evaluated using the IRVASE. Four pH values, 2.7, 4, 5 and 6, were investigated to study the effects over the chemical and physical characteristics of 12 bilayers of PEI/PSS formed over a silicon substrate. Results are shown in Figure 4. These pH values were selected since they are within the pH range available for acetate buffer, and it has been previously shown that LbL films can undergo significant changes with slight changes in pH [16][62].

Analyzing the IRVASE spectra obtained for the polymeric combinations of PEI/PSS and the variations in the pH, it is noticeable that the chemistry of the polymers that make up the multilayers over the substrate is present as evident by the characteristic peaks previously mentioned (Figure 4A). PEI/PSS films are stable to changes in pH of the washing solution in this range as compared to Figure 2A. Evaluating thickness and roughness values of multilayers of 12 bilayers, an increasing behavior is appreciated, with relation to an increase in pH of the washing solution (Figure 4B). A statistically difference ($p < 0.01$) in thickness is observed in the pH range of 2.7 to 5. At pH 6, a decrease in thickness is obtained. Average thicknesses of 140.82 nm, 189.59 nm, 303.42 nm, and 246.35 nm were obtained for washing solutions of pH 2.7, 4, 5, and 6, respectively. These differences could be associated to the fact that the washing buffer solution at pH 6 exerts a decrease in the charge density of the functional groups of the polymers that were evaluated here (PEI/PSS), which acts over the electrostatic forces of attraction of the molecules, in comparison to the washing buffer solution at pH 5. Generating less conformation of the polymers over the substrate, directly affecting the thickness and roughness of the multilayers.

M. Elzbieciak and their group [62] evaluated the influence of pH of the polymeric solution on the structure of multilayer films of PEI/PSS. They found that when maintaining a pH of 6 for both solutions the thickness of the samples, reported by ellipsometric analysis, presented a linear increase as the number of bilayers increases. For this experiment, their QCM analysis demonstrated that the absorbed mass over the silicon substrate increases with increasing number of layers. An unstable or non-monotonic behavior over the formation and increase in thickness of multilayers was found when the pH of the PEI solution was changed to 10, maintaining a pH of 6 for PSS. The ellipsometric analysis showed that the thickness considerably increased only through the deposition of PSS, due to the fact that at pH 6 it is strongly charged, in comparison to PEI, which at a pH of 10 it is slightly charged. Thus the pH of the polymeric solution plays an important role over the formation and deposition of the multilayers.

Analyzing the roughness of samples, we observe that for the pH range between 2.7 and 6 the measurement show a tendency to increase with increasing pH (Figure 4C), reaching a maximum value of 178.8 nm at pH 6. An increase in surface roughness is noticeable, with a

more heterogeneous surface at pH 6. A significant difference of $p < 0.01$ was observed between multilayers prepared at pH 2.7, pH 5, and pH 6. A $p < 0.05$ was observed between multilayers formed at pH 2.7 vs pH 4, and pH 4 vs pH 5 (Figure 4C). This effect of an increase in roughness can be associated to the fact that a basic pH decreases the charge density of PEI, which has a pK_a interval from 7.11 to 8.6 [42][62]. For PSS, it is possible that at a pH of 5 or 6 the effect is not significant because it has a pK_a of approximately 1 (basic pH increases the charge density of PSS) [46]. Therefore, there is a smaller deposition of PEI due to a collapse of this polymer in the multilayers because of the pH effect in the washing solution, promoting a more heterogeneous morphology, generating a rougher surface.

3.4 Effect of temperature over the physical-chemical characteristics of the polymeric surface

Among other abilities of the IRVASE, it also has the capacity of monitoring changes in structure, chemical composition, and physical properties of the films as a function of changes in temperature. This was performed using a heating stage (INSTECH – mk2000), which was coupled to the IRVASE equipment, allowing a controlled heat ramp. For this study, our experiments were focused on analyzing the effect of exposing 12 bilayers of PEI/PSS prepared at a pH of the washing solution of 2.7, 4, 5 and 6 to changes in temperature. These multilayers were exposed to the following temperatures: 25 °C, 37 °C, 50 °C, 75 °C, 100 °C, 125 °C and 150 °C. For the COL/HEP system, this same controlled temperature ramp was evaluated for a washing solution of pH 5.

Figure 5 shows the results of the effect of changes in temperature of the PEI/PSS system, over the chemistry of the bilayers, their thickness, and roughness. Our results over the chemistry of the bilayers fabricated with different washing pH values, through IRVASE analysis revealed that in this range of temperatures the bilayers preserve their chemical structure with only slight changes in the band under 1200 cm^{-1} , which correspond to the SO_3^- group, the C-N stretching and the C-O-S stretching vibration (Figure 5 A, B, C and D). Peaks corresponding to the NH_2 group (1628 cm^{-1}), the N-H bending (1527 cm^{-1}), and the CH_2 bending (1444 cm^{-1}) in PEI decrease with increasing temperature. Although these slight changes may not be significant, they could be related to possible modifications to the functional groups that are stabilized by the pH of the washing solution. Increasing the pH of the washing solution appears to increase the thermal stability of the PEI/PSS multilayers. At a pH value of 2.7, some changes in the chemistry of the films are observed between $1700 - 1500\text{ cm}^{-1}$ when the temperature is increased. However, these changes are not observed using a washing solution of pH 6. There is little variation in the chemistry of PEI/PSS as a function of temperature when using a washing solution at a pH 6.

Evaluating the changes in thickness and roughness of the PEI/PSS multilayers in function of temperature (Figure 5E), our results indicate that low pH values exert a smaller resistance to changes in physical and chemical properties of the surface.

The multilayers at pH 2.7 and pH 4 revealed the greatest changes in loss of thickness in the range between 25 °C to 75 °C. A decrease of approximately 16 nm for multilayers exposed to temperatures between 25 °C and 75 °C for these two pH values was observed, in

comparison to a decrease of only 8 nm and 14 nm for pH 5 and pH 6, respectively, under the same temperature range. A washing solution of pH 5 appears to be the ideal condition for construction of PEI/PSS multilayers. This is because at this pH a good chemical stability of multilayers is observed and the physical properties report minor alterations to changes in temperature.

Analyzing the influence of changes at a temperature of 75 °C, over the thickness of the multilayers, it is possible to notice that for all the pH values the thickness begins to increase. For pH 5 and pH 6, the final average thickness of multilayers reported a higher value than the one initially obtained at 25 °C. Although the differences in thicknesses fall within the error bars, the trend is observed consistently in all pH values. An increase of approximately 3% for the layers treated with both pH values is observed. This can be related to the glass transition temperature (T_g) of the multilayers, temperature at which it is possible for the polymers within the multilayers to expand thus increasing the thickness of the films. Keddie [66], and Richard [67], studied the effects on the glass-transition temperature of thin films composed of poly(methyl methacrylate) (PMMA) and polystyrene (PS) using spectroscopic ellipsometry. They determined that the glass transition temperature of thin films is related as a function of film thickness. They found that the T_g of PMMA on a gold surface decreases with decreasing film thickness [67], this was also observed in their results when fabricating thin films of polystyrene on silicon [66]. But, in one of their previous works they related the determination of T_g by detecting with ellipsometry the discontinuity in expansivity as the films are heated at a constant rate [68]. They prepared ultra-thin PS films and their ellipsometric results of the change in thickness vs. temperature showed two linear regions of thermal expansivity, which they related to the polymer glass and melt temperatures. They report that the point that divides two regions was associated to the T_g of the polymer [69]. Relying on these results and the behavior registered over the increase and expansion of the thickness of the polymeric multilayers formed by the layer-by-layer technique, we have attributed this behavior to the T_g of the multilayers. Our results for the PEI/PSS system (Figure 5) showed a “point of inflection” at 75 °C, which we have attributed as an approximation to predict the T_g of the polymeric multilayers of PEI/PSS.

The results of the thermal effect over the roughness of the multilayers revealed that this variable decrease approximately at a value of 8.3% at the range of temperatures from 25 °C to 150 °C, regardless of the pH of the washing solution (Figure 5F). We attribute this pattern to the homogenous movement of the polymeric molecules, because of the effect in controlled changes in temperature over the substrate.

Lutkenhaus and her group studied the thermal properties of LbL films of poly(diallyldimethylammonium chloride)/poly(styrene sulfonate) (PDAC/PSS) [69]. They found that different thermal transitions of the multilayers can be observed depending on the properties of the solution used to prepare the multilayers; for example, the ionic force or whether they are hydrated or not. Their results indicated that the hydrated films with an exponential growth reported T_g values around 49–56 °C, while films formed with a linear growth do not have a detectable T_g , in addition to being glassy at room temperature. In the case of multilayers in a dry state, for both types of growth these do not have a detectable T_g up to 250 °C, and are glassy at room temperature. From these results, they could conclude

that the multilayers formed with an exponential growth have a greater segmental mobility than that of linearly growing films. We can associate our results to this hypothesis, observing that the exponential growth of multilayers in our results was also perceived, as shown in Figure 2B. Therefore, we found that the changes in the decrease of thickness and roughness as a function of temperature of our multilayers reported in Figure 5 are due to the kinetic energy (mobility) of the molecules or polymeric chains as well as a potential effect in dehydration, due to the induced heat. It is understood that as the temperature increases, mobility increases, generating a shift in the polymeric chains [67]. Therefore, this mobility allows the polymers to rearrange, modifying the topographic environment and hence the characteristics measured. Lutkenhaus, in another work [69], where 5, 6, and 8 layer pairs of poly (allylamine hydrochloride)/ poly(acrylic acid) were fabricated, monitored through temperature-controlled ellipsometry the changes in thickness of the multilayers. They initially obtained at 25 °C a thickness of the multilayers of 59 ± 3 nm, 119 ± 1 nm, and 183 ± 2 nm, for 5, 6, and 8 layer pairs, respectively. An increase in temperature generated a decreasing effect in the thickness of the multilayers, reporting a final thickness at 275 °C of approximately 49 nm, 104 nm, and 159 nm, for 5, 6, and 8 layer pairs, respectively. They observed that when less multilayers are formed, the decrease in thickness is greater, obtaining a decrease of 14 ± 1 %, 12.18 ± 0.01 %, and 9 ± 4 %, for 5, 6, and 8 layer pairs, respectively, up to a change of 275 °C. They attributed the decrease in thickness of the multilayers to the loss of mass or to an increase in the density of the multilayers [69]. In another work, Lutkenhaus [70], found that for 200 layer pairs of poly(allylamine hydrochloride)/ poly(acrylic acid), the reduction of thickness of the LbL film is the result of the formation of the anhydride and the amidation. This because both are produced when heated [70].

In our work, we also evaluated the changes in chemistry and behavior of the physical characteristics of the COL/HEP multilayers, fabricated at a pH 5 for the washing solution and results are presented in Figure 6. It is possible to observe that significant changes in the stability of the functional groups are perceived when exposed to temperatures above 37 °C (Figure 6A). COL/HEP multilayers exhibit a poor resistance to changes in temperature, in comparison to PEI/PSS multilayers. Analyzing the influence of changes in temperature in each functional group, it is evident that the bands that belong to absorption between 1700 cm^{-1} and 1500 cm^{-1} , corresponding to the amide I and amide II, respectively, are strongly affected by changes in temperature, indicating a possible degradation or decomposition of the polymers. The amide I, amide II, and the CH_2 groups showed a displacement to higher absorption values as well as a decrease in intensity with increasing temperature. After 75 °C, these peaks almost disappear indicating a possible degradation of the polymers or loss in the secondary structure of collagen. Thus, COL/HEP films are more susceptible to changes in temperature, and cannot resist exposure to temperatures above 37 °C.

Analyzing the results obtained for thickness and roughness of COL/HEP films (Figure 6B), these show that an increase in temperature yields a decrease in both thickness and roughness. Comparing this effect with the tendencies observed in the chemical spectra of the multilayers (Figure 6A), we can say that the polymeric chains are not necessarily displacing because of temperature effects, but rather because of the immediate decomposition of the polymers. Contrary to the PEI/PSS system, presented in Figure 5, where an increase in

thickness of the multilayers is attributed to the displacement of their polymeric chains over the T_g of the system. Analyzing the relation between roughness and thickness for both systems, we can observe that for multilayers of COL/HEP the roughness reported values higher than the thickness (Figure 6B), contrary to the PEI/PSS multilayer system (Figure 5). Showing that the synthetic and natural multilayer systems have different behaviors or characteristics, even though the fabrication method is the same. Therefore, these differences can be attributed to the chemistry of each polymer and the interaction of their functional groups between the polymers that compose the multilayers. Our hypothesis over these observed characteristics in the COL/HEP system is associated to the fact that there may be a heterogeneous charge distribution along the polymeric chain. Having specific sites throughout the chain with a bigger charge, which promotes the formation of a heterogeneous surface, which gradually increases with the formation of multilayers.

In summary, the IRVASE technique can also be a tool to evaluate the temperatures of transition or decomposition of polymeric multilayers. This powerful tool allows for the simultaneous physical and chemical characterization of multilayered films. This is because the analysis of both properties complements and exposes the intermolecular phenomenon that is occurring. This indicates the versatility of this technique and how practical it can be to analyze LbL polymeric multilayers.

4. Conclusion

Infrared Variable Angle Spectroscopic Ellipsometry (IRVASE) can simultaneously characterize the physical and chemical properties of polymeric multilayers fabricated by the layer-by-layer technique. Our results show that it is possible to: (1) analyze the formation and growth of the multilayers, showing an exponential increase for the PEI/PSS multilayers and a linear increase for the COL/HEP polymer combination. (2) Study their chemical composition; IRVASE spectra of the multilayers correlated with the characteristic bands analyzed by FTIR-ATR. (3) Study their thickness; IRVASE revealed that it is possible to characterize the formation of multilayers starting with a small amount for both systems, reporting that three multilayers of PEI/PSS prepared using a washing solution of pH 5 had a thickness of 71.7 nm and 40.3 nm for COL/HEP. (4) Study their roughness; the COL/HEP system demonstrated that they have a higher roughness than PEI/PSS, obtaining values of 37.76 nm for three bilayers of PEI/PSS and 33.58 nm for three bilayers of COL/HEP. And (5) study the thermal stability; our results showed that for the PEI/PSS system an increasing temperature over the thickness described a temperature limit (75 °C) that defines the point of reduction or expansion of the space between the polymeric chains in the multilayers. Contrary to the COL/HEP multilayers, which have a chemistry that is drastically altered by temperatures above 37 °C, indicating a possible degradation of the polymers. The effect of the pH of the washing solution over the multilayers of PEI/PSS is intimately related to the formation, thickness and thermal stability of the multilayers. A pH of 5 in the washing solution was effective to obtain thicker multilayers. But, a pH of 6 demonstrated that it was more resistant to changes in temperature. We observed that the synthetic multilayers resist changes in temperature, and in fact they appear to reorganize when the temperature is increased; suggesting a possible transition temperature for the multilayers. Conversely, multilayers composed of the natural polymers collagen and heparin are highly susceptible to

increasing temperature. Our results were validated using atomic force microscopy to confirm values of film thickness and roughness. Finally, 12 bilayers of PEI/PSS showed to have a Young's modulus higher than that of 12 bilayers of COL/HEP. This work adds a complementary technique to the LbL community to simultaneously evaluate physical-chemical and thermal properties of multilayer films.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. IRVASE (Extinction coefficient) and FTIR-ATR (A.U.) spectrum of pure: A) PEI, B) PSS, C) HEP and D) COL.

Figure 2.

Multilayer growth, chemical, and physical characteristics for PEI/PSS system as measured by IRVASE. A) Spectrum of plain substrate, 3, 6, 9, and 12 bilayers, B) Thickness vs. number of bilayers, C) Roughness vs. number of bilayers and D) Surface morphology as measured by AFM of: Substrate, 3 bilayers, 6 bilayers, 9 bilayers, and 12 bilayers. The error bars indicate the standard deviation of the samples as described in the materials and methods section. p-values < 0.01 are represented by #, @, &, ψ , and Θ . p-values < 0.05 is represented by \$. Bars containing the same symbol are statistically different.

Figure 3.

Multilayer growth, chemical, and physical characteristics for COL/HEP system as measured by IRVASE. A) Spectrum of plain substrate, 3, 6, 9, and 12 bilayers, B) Thickness vs. number of bilayers, C) Roughness vs. number of bilayers and D) Surface morphology as measured by AFM of: Substrate, 3 bilayers, 6 bilayers, 9 bilayers, and 12 bilayers. The error bars indicate the standard deviation of the samples as described in the materials and methods section. p-values < 0.01 are represented by #, @, &, ψ and θ . p-values < 0.05 are represented by \$, %, and !. Bars containing the same symbol are statistically different.

Figure 4.

Effects in the variation pH of washing solution to prepare multilayers of PEI/PSS at pH 2.7, 4, 5, and 6 as measured by IRVASE. A) PEI/PSS spectrum, B) Thickness vs. number of bilayers and C) Roughness vs. number of bilayers. All spectra and measurements correspond to 12 bilayers and the error bars indicate the standard deviation of the samples as described in the materials and methods section. p-values < 0.01 are represented by #, @, &, ψ and θ . p-values < 0.05 are represented by \$, %, and !. Bars containing the same symbol are statistically different.

Figure 5.

Effect of PEI/PSS 12 bilayers exposed to different temperatures (25 °C, 37 °C, 50 °C, 75 °C, 100 °C, 125 °C and 150 °C) as measured by IRVASE. A) Bilayer spectrum fabricated at pH 2.7 of the washing solution, B) Bilayer spectrum fabricated at pH 4 of the washing solution, C) Bilayer spectrum fabricated at pH 5 of the washing solution, D) Bilayer spectrum fabricated at pH 6 of the washing solution, E) Measure of surface thickness of multilayers formed at pH 2.7, 4, 5, and 6 and F) Measure of surface roughness at pH 2.7, 4, 5 and 6. The error bars indicate the standard deviation of the samples as described in the materials and methods section.

Figure 6.

Effects of COL/HEP 12 bilayers exposed to different temperatures (25 °C, 37 °C, 50 °C, 75 °C, 100 °C, 125 °C and 150 °C) as measured by IRVASE. A) Bilayer spectrum fabricated at pH 5 of the washing solution and B) Measure of surface thickness and roughness of multilayers formed at pH 5. The error bars indicate the standard deviation of the samples as described in the materials and methods section.