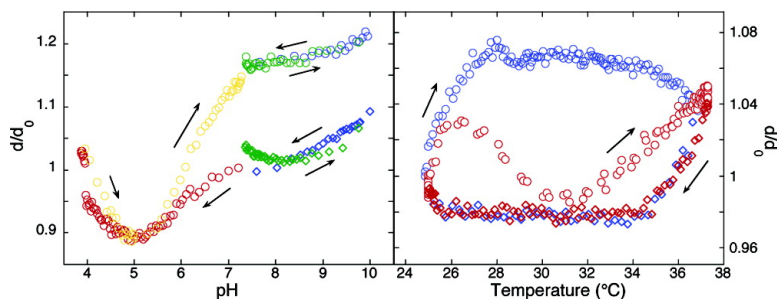Article

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Stability of Polypeptide Multilayers As Studied by in Situ Ellipsometry: Effects of Drying and Post-Buildup Changes in Temperature and pH

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Abstract: Polyelectrolyte multilayers (PEM) of poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) with an initial layer of polyethyleneimine (PEI) were built on silica and titanium surfaces using the layer-by-layer (LbL) technique. The stability of the film during drying/rewetting, temperature cycles, and pH shifts was studied in situ by means of ellipsometry. The film thickness was found to decrease significantly (approximately 70%) upon drying, but the original film thickness was regained upon rewetting, and the buildup could be continued. The thickness in the dry state was found to be extremely sensitive to ambient humidity, needing several hours to equilibrate. Changes in temperature and pH were also found to influence the multilayer thickness, leading to swelling and deswelling of as much as 8% and 10−20% respectively. The film does not necessarily regain its original thickness as the pH is shifted back, but instead shows clear signs of hysteresis.

1. Introduction

The buildup of polyelectrolyte multilayer (PEM) coatings using the layer-by-layer (LbL) deposition technique was first described by Decher et al. in the early 1990s.[1] This is a technique in which a PEM film is created by exposing a charged surface to alternately positively and negatively charged polymers, each leading to surface charge reversal, thereby facilitating adsorption of the next oppositely charged polymer from solution. The overcompensation and charge reversal have been illustrated by ζ-potential measurements,[2] and by direct force measurements.[3]

The LbL process is relatively easy to perform and can be applied to materials of virtually any size and shape.[2] Furthermore, this technique makes it possible to fine-tune and control the thickness and structure of the coating by changing a variety of processing parameters, such as the polymers used, number of layers deposited, the ionic strength at deposition,[4] and in the case of weak polyelectrolytes the deposition pH.[5−8] It has also been shown that it is possible to incorporate and immobilize peptides, proteins, and biomacromolecules[9−11] within the film,

and hence a variety of possible applications within the biotech and biomaterials area, such as tissue engineering,[12] and construction of hollow capsules for drug delivery,[2] have been suggested. PEM buildup of the biocompatible and biodegradable polypeptides PGA and PLL used in this study have been investigated previously[5,9,13,14] and are thought to be of great interest for use as coatings for biomaterials within implant technology.

The buildup of PEM films has been studied both ex situ in air[4,6,15−17] and in situ in solution.[5,9,13,14,18] A number of papers have investigated how the dipping solution pH changes the charge density and charge matching of weak polyelectrolytes, thereby affecting the buildup.[5−8] Some investigations have also been concerned with the effects of temperature on the buildup behavior.[15,19] On the other hand, only a few papers have investigated how the PEM film reacts to post buildup changes in temperature[5,20] and pH.[5,6,21] Hiller et al.[21] proved that it is

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possible to reversibly tune the refractive index of a PAH/PAA system in the range 1.55–1.15 (in dry state). This is accomplished by creating a nanoporous swollen structure by means of a pH cycling treatment, and this was proposed as a technique for creating antireflection coatings. Steitz et al.[20] report that PEM films built using a diblock copolymer with a thermosensitive poly(N-isopropyl-acrylamide) block decreased irreversibly in thickness when the temperature was increased. Burke and Barrett[6] report that dry PLL/hylauronic acid PEM films swell 1–8 times when immersed in solutions of different pH, and Boulmedais et al.[5] have measured small changes in the secondary structure of a PLL/PGA film caused by post buildup changes in both pH and temperature.

Although PEM structures are often systematically dried and rewetted, especially for measurements in air, the number of reports investigating how this treatment affects the PEM structure and its water content are surprisingly small. However, Farhat et al.[22] have reported that a poly(styrenesulfonate)/poly-(diallyldimethylammonium chloride) film contains up to 18% of water when dried at 25 °C and a relative humidity of 30% and that the water content varies with the ambient humidity. In addition, Tanchak and Barrett[23] recently concluded that a poly-(acrylic acid)/poly(allylamine hydrochloride) film that has been dried at a relative humidity of 45% swells by 20–40% (depending on the pH at which they were assembled) when rewetted and that the ambient humidity, when dried, strongly influences the rate of the swelling, needing a minimum of 3 s (low humidity) up to 30 min (high humidity) to equilibrate in solution.

In this paper, we have investigated how the biomedically interesting PLL/PGA polypeptide multilayer film reacts to ambient changes such as drying/rewetting, and temperature and pH cycling. This was done by continuously measuring the thickness, refractive index, and adsorbed mass of the PEM film using in situ ellipsometry. The stability of the PEM film is of importance not only in terms of construction and storage, but also for understanding how the PEM film reacts when in contact with various environments in vivo.

2. Materials and Methods

2.1. Materials. Poly(L-glutamic acid) (PGA), Mw = 50 000–100 000 g/mol (cat no. P-4886); poly(L-lysine) (PLL), Mw = 30 000–70 000 g/mol (cat no. P-2636); polyethyleneimine (PEI), Mw ≈ 750 000 g/mol (cat no. 18 197–8); and buffer salts tris[hydroxymethyl]-aminomethane (TRIS; cat no. T-1503) and 2-[N-morpholino]ethane-sulfonic acid (MES; cat no. M-8250) were all purchased from Sigma-Aldrich. Sodium chloride (pro analysis grade) was purchased from Merck. All chemicals of commercial origin were used as received, and solutions were prepared using ultrapure water (Milli-Q) (Milli-Q plus system, Millipore). Polyelectrolytes were dissolved in a MES/TRIS buffer (25 mM MES, 25 mM TRIS, and 100 mM NaCl), pH 7.4. Polyelectrolyte solutions and buffers were stored at 4 °C and used within 24 h.

Ellipsometry measurements were performed both on silicon surfaces with a silica layer of approximately 300 Å (specified by manufacturer and determined by ellipsometry) and on silicon surfaces coated with

approximately 1500 Å of titanium. The surfaces were kindly provided by Dr. Stefan Welin-Klintström and Bo Thuner (Linköping University, Sweden), respectively. The silica surfaces were cleaned by first boiling them for 5 min in Milli-Q/NH$_3$(25%)/H$_2$O$_2$(30%) (5:1:1), followed by rinsing in Milli-Q, and then boiling for 5 min in Milli-Q/HCl(25%)/H$_2$O$_2$(30%) (5:1:1). The silica surfaces were then thoroughly rinsed in Milli-Q and ethanol (99.7%) and finally stored in ethanol (99.7%) at room temperature. The titanium-coated silicon surfaces were passivated for at least 20 h in HNO$_3$ (50%)[24] and were stored in ethanol (99.7%). The passivated titanium-coated surfaces had a spontaneously formed oxide layer of approximately 40 Å (as determined by ellipsometry). All substrate surfaces were rinsed with ethanol and Milli-Q and then treated in a plasma cleaner (Harrick Scientific Corp., model PDC-3XG, Ossining, NY) in low-pressure at 30 W for 5 min immediately before use.

2.2. Ellipsometry. Ellipsometry is an optical method that measures the changes in polarization of light upon reflection at a planar surface.[25] The instrument used in this study was a Rudolph thin film ellipsometer, type 436 (Rudolph Research, Fairfield, NJ), equipped with a xenon arc lamp and high-precision step motors, controlled by a personal computer. Measurements were performed at a wavelength of 4015 Å and an angle of incidence of 67.7°. A more detailed description of the setup of the instrument is given by Landgren and Jönsson.[26] Prior to multilayer adsorption, four-zone measurements were performed in air and in buffer solution, to determine the complex refractive index ($N = n - ik$) of the substrate bulk material as well as the refractive index (n_o) and thickness (d_o) of the outermost oxide layer. Polyelectrolytes were then injected into the cuvette, and the ellipsometric angles ψ and Δ were recorded in situ every 10 s. When the optical properties of the substrate and the ambient media are known, the mean thickness (d_f) and refractive index (n_f) of the growing film can be solved numerically from the change in the optical angles ψ and Δ.[27] The thickness and the refractive index were then used to calculate the adsorbed amount, Γ (mg/m^2), according to the de Feijter formula:[28]

$$\Gamma = d_f \frac{n_f - n_{\text{buffer}}}{dn/dc} \qquad (1)$$

A dn/dc value of 0.15 mL/g for PGA and PLL was obtained from refractometer measurements (Wyatt Technology Optilab DSP interferometric refractometer). The dn/dc value was found to be constant, within experimental errors, over the whole pH range. Errors in the calculated thickness and refractive index co-variate, and, as a result, the adsorbed mass can be calculated with a much higher accuracy.[29] The measurement cell system, where the substrate surface is emerged vertically in a 5 mL thermostated quartz cuvette, is a noncontinuous flow system with continuous stirring, allowing the substrate to be rinsed and the cuvette solution to be exchanged between subsequent polyelectrolyte additions.

2.3. PEM Preparation and Measurements. Polyelectrolytes were deposited directly in the ellipsometry measurement cell at the following concentrations: PEI 5 mg/mL, PGA 1 mg/mL, and PLL 1 mg/mL in a MES/TRIS buffer at pH 7.4. The polyelectrolytes were added directly into the continuously stirred cuvette, and rinsing was performed between additions by continuously exchanging the medium for pure buffer. A more detailed description of the buildup procedure can be found elsewhere.[14]

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The PEM film was dried by first building the desired number of layers while measuring in situ in liquid in the ellipsometer. The PEM film was then rinsed by exchanging the liquid in the cuvette with water to drain the film of excess salt ions. The measurements were then stopped, the cuvette was emptied, and the surface was gently blown dry with a stream of nitrogen gas. The surface was re-aligned to compensate for the lower index of refraction in air before in situ measurements were restarted in air. After completed air measurements (2−16 h), the surface was rewetted and re-aligned in buffer solution, and in situ measurements and buildup were commenced. Drying was performed either after 4, 8, and 12, or 6 and 10 layers of buildup.

A temperature cycle was achieved by increasing or decreasing the temperature of the water bath controlling the cuvette temperature, while measuring in situ in the ellipsometer. Different rates of temperature elevation were tested (from ∼0.12 to ∼2 °C/min). The temperature was measured with a small thermo element inserted in the cuvette as close to the surface as possible.

The bulk refractive index of the ambient media changes with temperature $\Delta n_{H_2O}(25-37\ °C) = 0.00158$ (value interpolated for a wavelength of 401.5 nm).[30] To compensate for this change, the ellipsometry result file was calculated separately for two different bulk refractive indices. A modified thickness, refractive index, and adsorbed mass were then calculated by giving the two files a weight relative to the measured temperature.

A pH shift was created by continuously (∼315 μL/min) exchanging the MES/TRIS buffer pH 7.4 in the cuvette for either pure MES pH 3.9 or TRIS pH 10.1, with the same ionic strength, while measuring in situ in the ellipsometer. Because the pH in the cuvette is changed by a pumping dilution process, the rate of change is much faster at the beginning of the pumping with an average speed of 0.25 pH units/min during the first pH unit. However, the rate rapidly slows and has an average speed of 0.035 pH units/min for the remainder of the pH change. With these relatively low rates, the highly hydrated PEM film should at least be close to equilibrium during most of the pH cycling. The pH was measured and logged every 2 s on a personal computer. No differences in the bulk refractive index of the buffers MES, TRIS, and MES/TRIS were found.

3. Results

3.1. Drying and Rewetting. Each addition of polyelectrolytes to the cuvette could be followed as a clear change in thickness, refractive index, and adsorbed mass (Figure 1).

The adsorbed mass increases more than linearly with the number of deposited layers, which is characteristic for buildup of this PEM system at physiological pH and ionic strength and has been explained by a net diffusion of adsorbing polypeptides into underlying layers.[14] It is evident that the PEM film thickness decreases somewhat when rinsed with water, as indicated in the figure by the arrows. Furthermore, a considerable decrease in the PEM film thickness occurred when the surface was dried in air, also reflected in a significantly higher refractive index (Figures 1 and 2).

However, upon rewetting of the film, it swelled back to its original thickness and refractive index, and the buildup could be continued as before (Figure 1).

The thickness measured in air increased with increasing number of layers; however, the relative difference between the wet and the dry thickness decreased slightly for each new layer, which is also indicated by the decreasing refractive index in air (Figure 2).
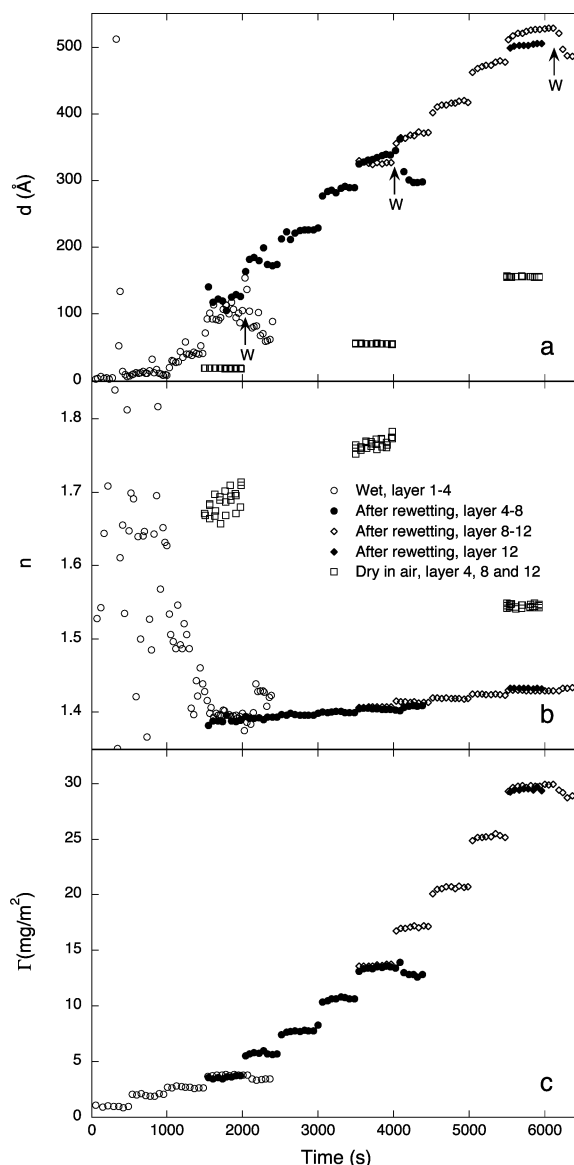
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Figure 1. Time evolution of the (a) calculated thickness *d*, (b) refractive index *n*, and (c) adsorbed mass Γ during the PEI-(PGA-PLL)-PGA-water-(W)-air-(PLL-PGA)$_2$-water(W)-air-(PLL-PGA)$_2$-water(W)-air buildup on a titanium surface. The buildup was performed in three cycles (○ ● ◇), each consisting of 4 layers with intermediate rinsing with water (W) prior to each drying sequence. The film was subsequently rewetted in buffer; the final rewetting after 12 layers of buildup (◆) was performed after overnight measurements in air. Dry measurements in air are represented in the figure by □ (the actual time for the air measurements has been omitted to give a more illustrative presentation of the rewetting and buildup behavior).

It was also found that the PEM film measured in air collapsed even further by "jumping" from thicker, more scattered thickness to thinner, more stable values (Figures 3 and 4); the thickness and refractive index given in Figures 1 and 2 are the final values after equilibration.

The time it took for the film to collapse to its minimum thickness varied from 1 to 8 h. The thickness of the thickest films was often seen to "jump" back and forth between two values before finally stabilizing on the thinner thickness (Figures 3 and 4). These "jumps" are reflected not only in the calculated film thickness, but also in the primary data ψ and Δ, and occur on a time-scale of 20 min (Figure 3) to 1 h (Figure 4).
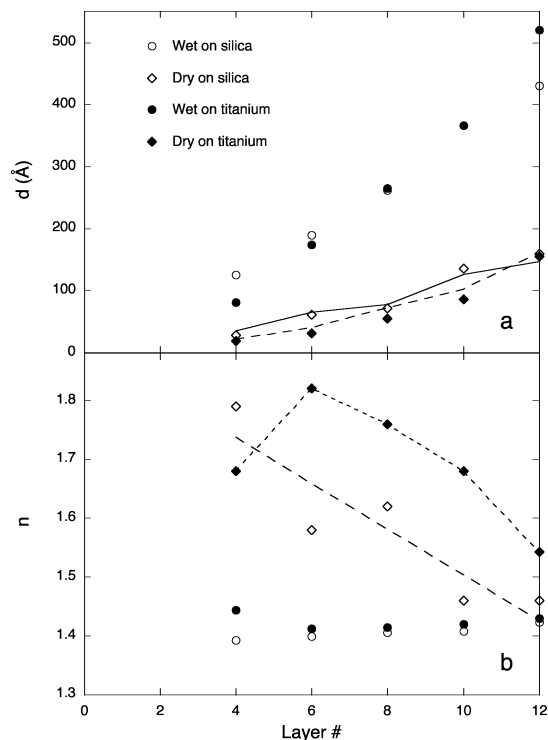
Figure 2. PEM film in the wet (circles) and dry (diamonds) state. Thickness (a) and refractive index (b) of films, built on silica and titanium surfaces (unfilled and filled symbols, respectively). The lines in (a) are the thickness calculated with a constant refractive index (1.52). The lines associated with the refractive index in air do not represent any data, but are only intended as guides for the eye.
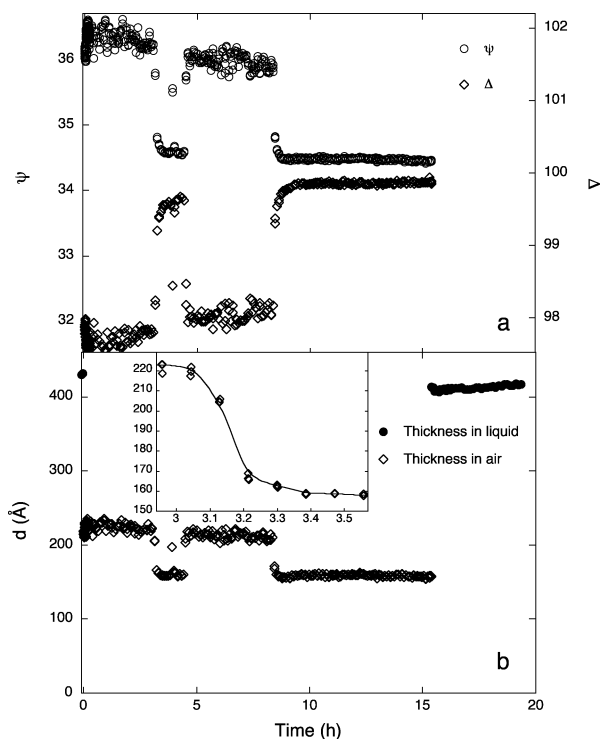

Figure 3. In situ measurements on a PEI-(PGA-PLL)₅-PGA (12 layers) multilayer film built on a silica surface. (a) Changes of the ellipsometric angles ψ (○) and Δ (◇) measured in air. (b) Changes in the film thickness calculated from ψ and Δ in liquid (●), and during overnight drying in air (◇). (b, inset) Close-up of the first "jump".

3.2. Temperature Cycling. The film lost as much as 25% of its mass and decreased even more in thickness when heated
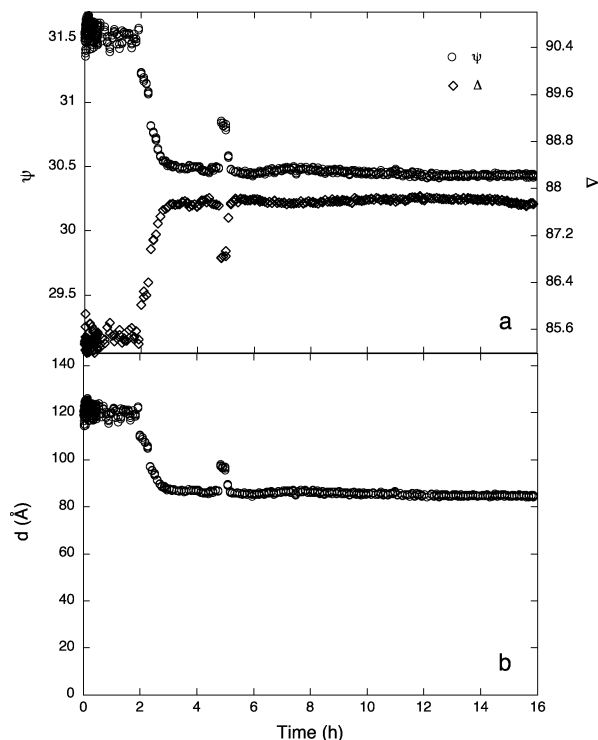

Figure 4. In situ measurements on a PEI-(PGA-PLL)₄-PGA (10 layers) multilayer film built on a titanium surface. (a) Changes of the ellipsometric angles ψ (○) and Δ (◇) measured in air. (b) Changes in the film thickness calculated from ψ during overnight drying in air (○).

rapidly (2 °C/min) directly from 25 to 37 °C and cooled back down to 25 °C. By heating rapidly but in small intervals of 3−4 °C at a time, this mass loss could be diminished to below 10% (data for these two runs are not shown). On the other hand, as can be seen in Figure 5, heating the film slowly (0.12 °C/min) from 25 to 37 °C, the adsorbed mass stays constant (Figure 5a), while the thickness and refractive index changes in a way suggesting that it first swells by approximately 8% at 28 °C, and then deswells slowly during the remaining heating period, ending up at a swelling of approximately 4% of its original thickness at 37 °C (Figure 5b and c). In Figure 6, it can be seen that once the highest temperature (37 °C) is reached, the thickness and refractive index stay constant.

The film collapsed considerably (~6%) to values just below its original thickness when cooled from 37 to 34 °C. The thickness was then more or less constant, finally ending up at approximately the original thickness. When a second faster temperature cycle was commenced directly after the slower cycle, the film swelled in a somewhat sinusoidal manner, ending up at approximately the same swelling of 4% at 37 °C, without showing any significant changes in adsorbed mass. During the somewhat slower cooling period that followed (0.4 °C/min), the thickness and refractive index retraced the data from the previous cooling.

3.3. pH Shifts. When lowering the pH of the ambient solution, the PEM film thickness decreased to a minimum of approximately 80−90% of its original thickness at pH 5 (representative data can be seen in Figure 7b).

When continuing to lower the pH, the film typically swelled again and approached its original thickness at pH 4. Upon increasing the pH, the thickness once again went through a minima at pH 5, before it swelled to 110−120% of its original thickness back at pH 7.4. Raising the pH from 7.4 to 10.1
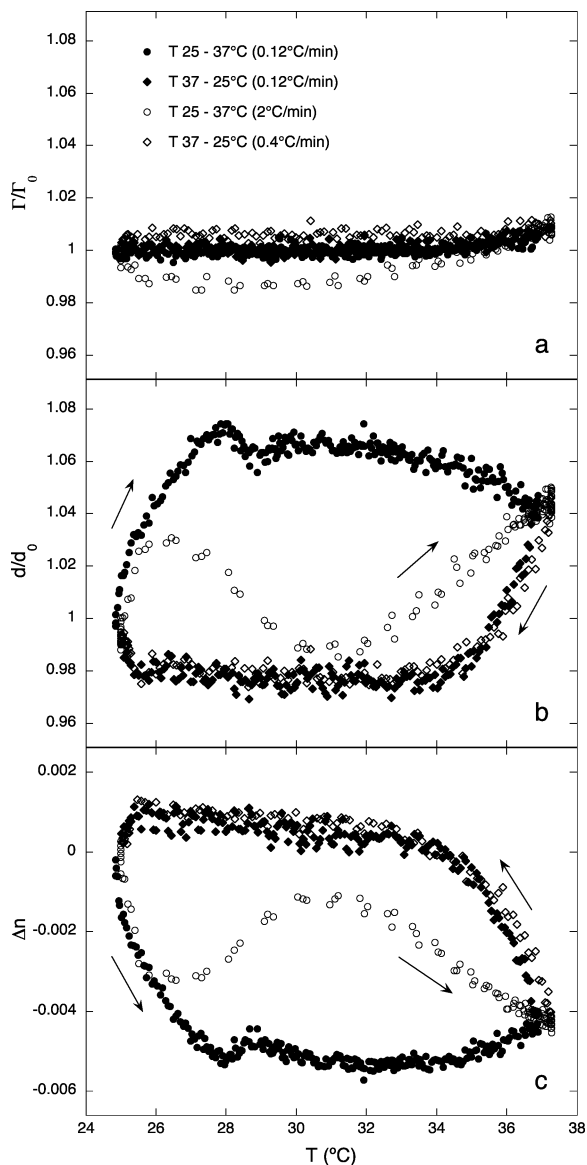
Figure 5. Changes in the relative adsorbed mass (a), relative thickness (b), and refractive index (c) during temperature cycling from 25 to 37 to 25 °C, first slowly (filled symbols) and then rapidly (open symbols), of a PEI-(PGA-PLL)$_5$-PGA (12 layers) multilayer film built on a silica surface.

Figure 6. Evolution of the relative thickness (○) of a PEI-(PGA-PLL)$_5$-PGA (12 layers) multilayer film built on a silica surface and the temperature in the cuvette (line) with time, during the last degree of temperature elevation (36−36.9 °C, negative time) and at the constant temperature of 36.9 °C (0−36 min).

Figure 7. Relative changes in the adsorbed mass (a) and thickness (b) with pH of a PEI-(PGA-PLL)$_5$-PGA (12 layers) multilayer film built on a silica surface. The pH was lowered from 7.4 to 3.9 (○), raised to 10.1 (●), and lowered back to 7.4 (○). In a second experiment, the pH was first raised from 7.4 to 10.1 (◆) and then lowered back to 7.4 (◇).

swelled the film by approximately 10% regardless of whether the PEM film had first been exposed to lower pH values or not (exp. 1 and 2, respectively, Figure 7b). In both cases, the thickness collapses back to values just above those measured before the pH was raised. The adsorbed mass varied by as much as ±5% during the pH cycling (Figure 7a).

In one experiment, a slow-temperature cycle was performed after the pH cycling had finished. In this run, it was found that the 15% swelling resulting from the pH cycling disappeared when cycling the temperature and the film once again regained its original thickness as measured prior to changing the pH (data not shown).

4. Discussion

4.1. Drying. As mentioned in the Introduction, there are several studies that have measured the PEM buildup in the dry state,[4,6,15−17] some that have followed the buildup in situ in the wet state,[5,9,13,14,18] but in this paper we focus on what actually happens to the film when it is dried, and how this affects the
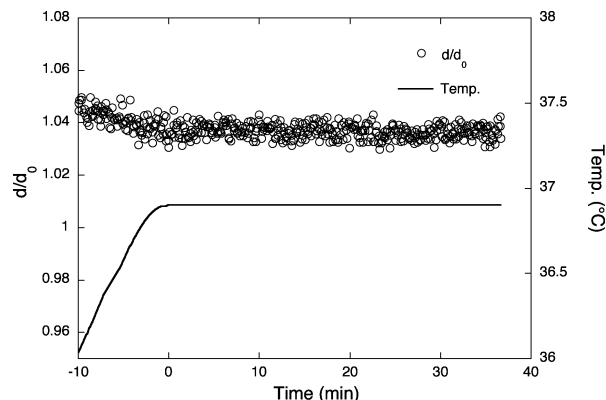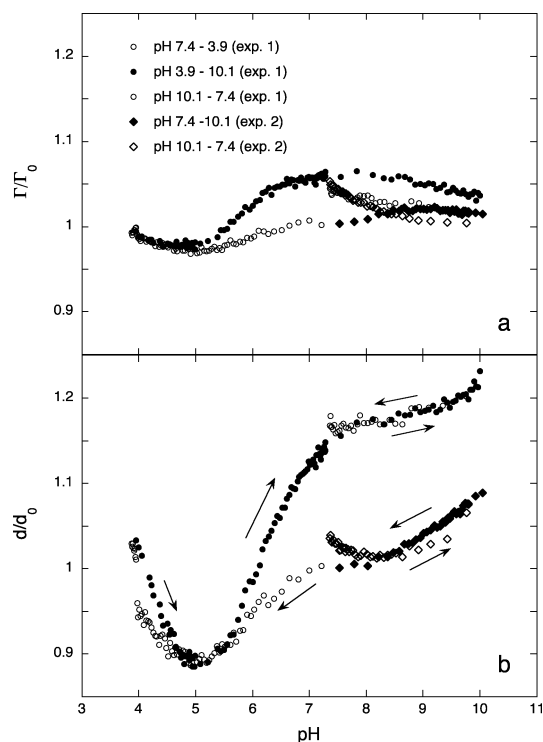
continuous buildup. This should be of practical interest in terms of both buildup procedure and measurements, as well as for storage purposes.

The multilayer buildup presented in Figure 1 is more or less identical to those published previously for the same polyelectrolyte system measured with in situ ellipsometry.[14] It therefore seems reasonable to assume that the intermediate drying and measurements in air does not affect the continuation of the buildup. This is clearly indicated in Figure 1c, where no mass loss can be seen, and even more so by the fact that both the thickness and the refractive index resumed its original values, measured prior to drying, when rewetted (Figure 1a and b). Hence, drying of the film does not result in any irreversible changes in the layer structure. Noteworthy is that this holds

true even when the film has been left in air overnight (~16 h) (Figures 1 and 3). It should be pointed out that the PEM film needs to be drained from excess salt ions prior to drying to avoid precipitation of salt crystals which otherwise would disturb the measurements in air as well as the ability of the film to regain its original values when rewetted. The deswelling and the small decrease in mass (8% and 3%, respectively, Figure 1) that occurred when the buffer solution in the cuvette was exchanged for water might be related to the fact that the pH of the deionized water is very easily influenced and will probably, at least locally, be dominated by the top layer of PGA (pK_a = 5.1[31]). It is likely that the pK_a of the PEM film is influenced also by the underlaying layers, and is therefore somewhat higher than for the pure PGA, as has been shown for a PLL/hyaluronic acid PEM.[6] The deswelling and mass decrease correspond with the changes seen at around pH values of 5.5−6 in the pH cycling experiment, which would be a reasonable local pH for the film. Hence, the changes can probably be explained by the loss of counterions at lower pH, which is further discussed in section 4.3.

The substantial decrease in thickness in combination with the increase in refractive index upon drying indicate that the PEM film definitely undergoes a reversible collapse when dried (these data are corrected for the lower refractive index of ambient air) (Figures 1−4). The dry thicknesses presented in Figure 2 are somewhat lower than what has previously been published for the same PEM system by Cheng et al.,[17] 135 and 85 Å on silica and titanium, respectively, as compared to 200 Å on a modified gold surface published by Cheng et al. after 10 layers of buildup. On the other hand, as can be seen in Figures 3 and 4, the initial thickness measured in air starts at relatively high values that later collapse to its final much thinner value after as much as 8 h in air. After 10 layers of buildup, the initial thickness was ~120 Å on titanium (Figure 4) and 230 Å on silica (data not shown) before the collapse, which in the case of the silica surface is close to what was published by Cheng et al.[17] The reason for this delayed final collapse and for the fact that the film thickness seems to be able to "jump" back and forth between two thicknesses is probably that the film is sensitive to the ambient humidity. This has been established by Kügler et al.,[16] stating that the PEM thickness increases with increasing humidity, and that the equilibration of the swelling and deswelling occurs over a time-scale of hours. In other words, it is possible that the data previously reported were measured on films that had not been dried long enough to equilibrate and hence still contained a rather large amount of water. There was substantial difference in outdoor humidity at the times that the two experiments presented in Figures 3 and 4 were conducted (90% and 45%, respectively, data received from the Swedish Meteorological and Hydrological Institute, SMHI). This might explain why the former needed 8 h to equilibrate as compared to 2 h for the latter.

In Figure 2a, it can be seen that the final dry thickness does not change much when calculated with a constant refractive index of 1.52 (the same as was used by Cheng et al.[17] for the same PEM system) as compared to when the thickness and effective film refractive index were both generated from ψ and Δ, which is the more accurate way to evaluate the data. In the most extreme case (6 layers on titanium), the calculated

refractive index is 1.82 with a corresponding thickness of 30.4 Å as compared to 39.7 Å calculated with the lower index of refraction 1.52. This indicates that the thickness for the thin dry film can be measured with reasonably good accuracy, even though the refractive index calculated, presented in Figure 2, is probably unreasonably high for the thinnest films.

The fact that the relative difference between the dry and the wet film appears to be decreasing as more layers are added (~80% for four layers and ~70% for 12 layers) correlates well with the changes in water content (~75% for four layers and ~60% for 12 layers)[14] and is suspected to be related to the hypothesis that there may be a net diffusion of polypeptides into the film during the buildup, rendering the film more dense as more layers are added[14] (indicated by the increasing refractive index seen in Figure 1b). Thus, the layers are expected to collapse to a smaller extent when the number of deposited layers increases.

4.2. Temperature Cycling. The PEM film also proved to be remarkably stable to slow temperature changes in terms of adsorbed mass (once the ellipsometrical result file was corrected for the changing ambient bulk refractive index, no real change could be seen in the adsorbed mass) (Figure 5a). On the other hand, in terms of thickness and refractive index, the film seems to be sensitive even to small changes in temperature, responding within seconds with a swelling/deswelling behavior (Figure 5b and c).

However, ellipsometry measurements are very sensitive to small temperature gradients and tension in the cuvette walls, which may lead to errors in the values of ψ and Δ. For instance, similar changes in ψ and Δ could also be seen when continuously changing the temperature while measuring on a silica/silicon surface, thereby indicating that it is not possible to correctly calculate the thickness and refractive index of a PEM film while the temperature is continuously changing. This would also explain the sinusoidal changes during the fast temperature elevation, which could also be repeated on a silica/silicon surface. On the other hand, the final thicknesses measured at 37 °C should be correct because the gradients disappear once the temperature is constant, which is probably why no further changes can be seen once the final temperature is reached (Figure 6). In other words, the in situ changes in thickness and refractive index during the temperature elevation and cooling cannot be trusted; however, it can still be concluded that the PEM thickness swells and collapses by as much as approximately 4% between 25 and 37 °C, while the mass of the film stays constant.

It has been established that PEM buildup at elevated temperatures yields thicker films due to changes in polymer−solution interactions[19] and probably even more so due to the fact that polyelectorlytes have a higher internal mobility at higher temperatures, which enables them to swell into conformations that are unfavorable at lower temperature.[15] However, due to the nature of the polypeptides used for this PEM film, there are two more temperature-dependent factors to consider, cross-linking between amine and carboxylic groups[5,32] and changes in the secondary structure.[5] Cross-linking is favored at elevated temperatures and should not occur to a large extent when raising the temperature to only 37 °C. On the other hand,

(31) Venyaminov, S. Y.; Kalnin, N. N. *Biopolymers* **1990**, *30*, 1259−1271.

(32) Harris, J. J.; DeRose, P. M.; Bruening, M. L. *J. Am. Chem. Soc.* **1999**, *121*, 1978−1979.

it is likely that the thickness is influenced by temperature-induced changes in the secondary structure. In the temperature range 26−89 °C, Boulmedais et al.[5] reported increasing secondary structure (increasing α-helix and decreasing β-sheet) with increasing temperature.

When the PEM film is cooled back to 25 °C, it almost resumes its original thickness, indicating that the temperature-induced conformational swelling and changes in secondary structure are more or less reversible. Boulmedais et al.[5] reported that the changes in secondary structure were mostly reversible, although there was a small increase in the β-sheet content after a completed temperature cycle. They also reported that β-sheets have a stabilizing effect on the multilayer structure, which might explain why no mass is lost during the second much faster temperature cycle, contrary to the 25% mass loss measured when the temperature was rapidly elevated to 37 °C without the initial slower temperature cycle (data not shown). It should be noted that the multilayer structure could also be stabilized by cross-links formed during the first temperature cycle, Johansson et al. report that the pH-sensitive PEM collagen/hyaluronic acid could be stabilized to restrain physiological pH by a chemical cross-linking treatment.[12] On the other hand, as mentioned earlier, it seems unlikely that cross-linking would occur at ordinary body temperature.

4.3. pH Cycling. Some papers have investigated the effect of pH on the buildup of weak polyelectrolytes,[5−8] whereas only a few papers have investigated how the PEM film reacts to changes in pH after the film has been built.[5,6,21]

We found that the PLL/PGA PEM film was sensitive to pH changes and that it undergoes a nonreversible swelling/deswelling behavior when cycling the pH of the ambient solution. The swelling/deswelling behavior is probably governed mainly by two factors: the loss and gain of charge density, and by changes in the secondary structure, both of which might lead to structural changes in the film. PGA loses some of its charge when lowering the pH ($pK_a = 5.1$ for PGA[31]). This might lead to a higher degree of α-helix content, which has been reported for PGA in solution,[31] and less internal repulsion within the PEM film. These phenomena might be the explanation for why the film deswells by 10−20% at pH 5. Small changes in both α-helix and β-sheet content related to variations in pH have been reported for the same PEM system by Boulmedais et al.;[5] however, only pH changes of films built at pH 4.4 were studied. By further decreasing the pH from 5 to 3.9, the PEM film swelled somewhat. The further decrease in pH leads to a continued decrease in the PGA charge density, and, thus, a higher degree of freedom could be expected due to fewer ionic bonds with PLL. When the pH is increased back to 7.4, the PEM film swelled to 110−120% of its original thickness, indicating that not all of the regained PGA charges are associated with PLL, but some are now instead compensated by salt ions.

At higher pH values, PLL can form α-helix structures,[31] and there is also a possibility of changes in the β-sheet content;[5] however, none of these changes seems to have a deswelling effect on the PEM film when the pH is raised above 7.4. Instead, the PEM film swelled by approximately 10% when the pH was increased from 7.4 to 10.1, both with and without a prior acidic pH cycling. It would seem that the main factor governing the swelling behavior in this case is the increased freedom gained when some of the interpolymer links are broken ($pK_a = 9.4$ for

PLL[6]). The thickness of the film after pH cycling 7.4−10−7.4 did not attain its original value, further indicating that not all interpolymer links were regained.

During the pH cycling, the adsorbed mass changed by as much as ±5%. Some of that mass change might be explained considering that removing the charge of one PGA or PLL monomer (removing one sodium counterion and adding a hydrogen ion or removing both one chloride counterion and a hydrogen ion) would decrease the mass of that monomer by as much as 15% and 22%, respectively. A change in the counterion concentration within the PEM film would in the ellipsometry measurements be sensed as a change in the film refractive index, which in turn affects the calculated adsorbed mass. This reasoning is in line with the assumption that some of the charges regained during pH cycling are no longer bound to other polymer charges, but are instead compensated by counterions, and thereby the mass of the adsorbed film increases.

On an additional note, we can report that when performing a temperature cycling (as discussed in the previous section) after a pH cycle has been conducted, the thickness deswelled back to the original value measured prior to the pH cycling. This may indicate that most of the charges that become compensated by counterions during pH cycling might once again have connected to its copolymer during the temperature-induced higher energy state.

5. Conclusions

Upon drying, the PGA/PLL PEM film thickness collapsed by as much as 70%, but proved to be stable enough to swell back to its original thickness when rewetted without any loss in mass, and the buildup could be continued with additional layers. On the other hand, this highly hydrated PEM system was found to be very sensitive to the ambient humidity during drying, needing several hours to equilibrate in air.

The PEM film was found to be stable to slow changes in temperature in the range 25−37 °C, showing a constant mass and relatively small, mainly reversible changes in thickness. The fact that a PEM film that had undergone a slow temperature cycling was able to withstand a second much faster raise in temperature without losing any mass indicated that a slow temperature cycling has a somewhat stabilizing effect on the film, possibly by an accumulation of the β-sheet content.

During pH cycling, changes could be seen both in the film thickness and in the mass, showing a clear hysteresis when the pH was lowered and then raised again. These changes are suggested to be related to changes in the charge density within the film, affecting the secondary structure, the intramolecular repulsions, and the intermolecular connections, as well as the concentration of counterions within the film. The original thickness could be regained by performing a slow-temperature cycling after the pH cycling.

Clearly, there are substantial possibilities of modifying the structure, and thus the properties, of polyelectrolyte multilayer films by changing external stimuli (e.g., pH and temperature). A proper understanding of these effects is of great importance for tuning multilayer properties to applications.