

pH-Responsive Properties of Multilayered Poly(L-lysine)/Hyaluronic Acid Surfaces

Susan E. Burke and Christopher J. Barrett*

Department of Chemistry, McGill University, 801 Sherbrooke Street West,
Montréal, Quebec, Canada H3A 2K6

Received June 11, 2003; Revised Manuscript Received August 22, 2003

Multilayer films have been prepared by the sequential electrostatic adsorption of poly(L-lysine) and hyaluronic acid onto charged silicon surfaces from dilute aqueous solutions under various pH conditions. Microelectrophoresis was used to examine the local acid–base equilibria of the polyelectrolytes within the films as a function of the total number of layers in the film and the assembly solution pH. The acid–base dissociation constants were observed to deviate significantly from dilute solution values upon adsorption, to be layer dependent only within the first 3–4 layers, and to show sensitivity to the assembly solution pH. As a result, some of the physicochemical properties of the films have also been found to exhibit pH-responsive behavior. For example, the thickest films result when at least one of the polyelectrolytes is only partially dissociated in solution. As well, the pH-dependent degree of dissociation of the surface functional groups can be used to vary the contact angle of a water droplet by as much as 25° and the coefficient of friction by up to an order of magnitude. In addition, the extent to which PLL/HA films can be made to swell in solution can be varied by a factor of 7 depending on the assembly solution and swelling solution pH. The anomalies found in the dissociation constants account for the trends in these pH-dependent properties. Here, we demonstrate that knowledge of the acid–base dissociation behavior in multilayer films is key to understanding and controlling the physical properties of the films, particularly surface friction and wettability, which are fundamentally important factors for many biomaterials applications. For example, we outline a mechanism whereby biopolymer thin films can be electrostatically adsorbed under highly charged “sticky” conditions and then quickly transformed into stable low-friction films simply by altering the pH environment.

Introduction

The layer-by-layer electrostatic self-assembly technique has evolved over the past decade as a viable means of preparing controlled multilayer films of biopolymers.^{1,2} The basic principle behind this method of film preparation is the sequential and repeated electrostatic adsorption of positively and negatively charged polymers onto a charged substrate from dilute solution. The resulting films are stable and uniform, but have highly interpenetrated architectures. The successful build-up of polymer layers is achieved only when the addition of a new layer results in charge over-compensation on the film surface. One of the many attractive features of layer-by-layer self-assembly is the remarkable nanoscale control that can be exercised over the properties of the films (i.e., thickness, roughness, wettability, and swelling behavior), by varying the conditions used to assemble the films such as pH, ionic strength, polymer functionality, and polymer concentration.² In addition, recent studies have shown that multilayer films can be assembled onto a wide variety of substrates differing in size, composition, and geometry.³ The relative ease of multilayer film assembly has led to the extension of the layer-by-layer concept in the preparation of interesting hybrid multilayer systems such as

those incorporating nanoparticles, enzymes, dendimers, and carbon nanotubes.^{4–7} Consequently, the versatility of the layer-by-layer method makes it an attractive technique for a number of areas including electrooptics, coatings, sensors, drug delivery, and catalysis.^{2,8} In particular, polyelectrolyte multilayer films containing biopolymers have many potential biomaterial applications.^{9,10} Efforts are currently focused on evaluating the potential of such films as novel applied biomaterials by characterizing their interactions with proteins and cells. However, to understand and to tailor such interactions, it is essential to know about the fundamental physicochemical properties of the multilayer films.

One example of an interesting biopolymer combination that has been successfully assembled into a multilayer film is hyaluronic acid and poly(L-lysine).^{11,12} It was found that this combination of polyelectrolytes yielded multilayer films that grow in an exponential fashion due to the unique ability of poly(L-lysine) to migrate in and out of the film.¹² Poly(L-lysine) (PLL) is a linear polyisopeptide, which in dilute aqueous solution adopts either a statistical coil, α -helix, or β -sheet conformation depending on the solution pH.¹³ It has many materials applications in such areas as the promotion of cellular adhesion, microcapsule coatings, and drug delivery.^{14,15} Hyaluronic acid (HA) is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine.¹⁶ In dilute solution, the

* To whom correspondence should be addressed. E-mail: christopher.barrett@mcgill.ca.

conformation of the HA chains can be converted from a random coil to single helical structure with a change in pH or ionic strength.¹⁷ It is found in the vitreous body, extracellular matrix, and synovial fluid, and participates in many crucial functions in vivo including joint lubrication, tissue hydration, and wound healing.¹⁶ In addition, the rich suite of properties of hyaluronic acid has led to its incorporation in a wide variety of cosmetic and pharmaceutical applications.¹⁶ Polyelectrolyte complexes have also been made from PLL and HA because their combined properties are useful in such application as tissue engineering and gene carrier technology.^{18,19}

Because hyaluronic acid and poly(L-lysine) are both classified as weak polyelectrolytes (only partially charged at moderate pH near their pK), the pH and ionic strength environments greatly influence the physicochemical properties of these polymers in solution, and in turn, it is believed such changes will impact the properties of PLL/HA multilayer films. Over the past few years, it has been demonstrated that many of the physicochemical properties (i.e., surface wettability, surface roughness, film morphology, dielectric properties, and layer thickness) of multilayer films composed of simple model weak polyelectrolytes are strongly dependent on slight changes in the solution pH and ionic strength conditions used in the assembly process.^{20–25} As an attempt to uncover a chemically based explanation for these observed physical changes, our group has recently shown with multilayer films composed of the simple polyelectrolytes poly(acrylic acid) and poly(allylamine hydrochloride) that such dramatic changes in the properties of the films can be accounted for by the shifts in the acid–base equilibria of the polyelectrolytes upon adsorption from dilute solution.²⁶

In this paper, we attempt to answer the question of whether the shifts of the dissociation constants of the model polyelectrolytes upon adsorption from dilute solution can be generalized to more complex real systems, and whether such changes in acid–base equilibria have a comparable impact on the physicochemical properties of such films. Specifically, we examine by microelectrophoresis the acid–base equilibria of PLL/HA multilayer films as a function of the total number of layers in the film and the solution pH used in the assembly process. In addition, the pH and ionic strength dependence of some of the surface and bulk properties of the film such as film thickness, surface wettability, surface friction, and swelling behavior are investigated and the results are discussed in terms of the acid–base equilibria of the polyelectrolytes in the films.

Materials and Methods

Materials. Sodium hyaluronate ($M_w = 150\,000$ – $250\,000$ g/mol) was purchased from Sigma and converted to the acid form via hydrolysis with HCl. Poly(L-lysine hydrobromide) ($M_w = 70\,000$ g/mol) was obtained from Fluka and treated with excess NaCl to yield the chloride salt of PLL. Aqueous solutions (Millipore, Milli-Q 18.2 M Ω cm) of each polyelectrolyte were prepared using concentrations of 2.0 mM polymer and 0.15 M NaCl. The salt was added to create sufficient electrostatic screening to promote polymer adsorp-

tion onto the substrate.²⁴ The pH of the solutions was adjusted to the same value with either HCl or NaOH. The polyelectrolytes were adsorbed on both colloidal silica (70–100 nm, Nissan Chemical Industries) and polished (100) silicon wafers (WaferNet, Inc.). The colloidal silica was obtained as a 40 wt % aqueous solution (pH = 9 ~ 10), which was diluted and adjusted to an appropriate pH value. The wafers were cleaned in a concentrated chromium(III) oxide/sulfuric acid solution for 24 h and then rinsed thoroughly with purified water prior to polymer adsorption.

Film Preparation. Multilayers were assembled on colloidal silica by repeated adsorption, washing, and centrifuging steps, using the same protocol as previously outlined.²⁶ In the adsorption steps, solutions of 2.0 mM polyelectrolyte (based on repeat unit molecular weight) and 0.15 M NaCl were used. The wash solutions (Millipore, Milli-Q 18.2 M Ω ·cm) were adjusted to the same pH as the polyelectrolyte solutions. No salt was added to the wash baths. The procedure was carried out until a maximum of 10 layers (5 bilayers (PLL/HA)₅) were deposited.

Multilayers were also assembled on planar wafers using a dipping protocol that involved a 10 min immersion of the wafer in a 250 mL PLL bath (2.0 mM PLL/0.15 M NaCl) adjusted to the desired pH, followed by an immersion of the wafer in three consecutive 250 mL wash baths of water (Millipore, Milli-Q 18.2 M Ω cm) adjusted to the same pH as the polyelectrolyte solution for 1.0, 2.0, and 2.0 min respectively. No salt was added to the wash baths. The wafer was then exposed to a HA solution (2.0 mM HA/0.15 M NaCl) for 10 min. The films were then washed in the same manner as described above for the deposition of the PLL layer. This cyclic process was carried out until a desired number of layers were deposited. The films were then dried with a stream of compressed air and further dried in a vacuum desiccator for 24 h prior to use. This method of film preparation was used to make films for the thickness and contact angle studies.

The films containing 150 layers (used for swelling experiments) were prepared on silicon wafers (1.5 × 4.5 cm) by a variation of the dipping method using a commercial spin-coater, to minimize the amount of polymer solution required.²⁷ In this case, the polyelectrolyte solution (1.0 mL) was poured on a stationary substrate, which was then spun for 20 s at 5000 rpm (Spin Processor, Laurell Technologies). Subsequently, 1.0 mL of the wash water (same pH as polyelectrolyte solution) was poured on the substrate in the rest state and then the wafer was spun again for 20 s at 5000 rpm. The wash was repeated three times. The thickness of films containing 10, 25, and 50 layers prepared using the spin method were found to be in good agreement with the thickness values obtained for those assembled using the stationary dip protocol (Supporting Information).

Microelectrophoresis. Solutions of 30 mg of dry colloidal particles in 15 mL of 1.0 mM NaCl were prepared for the electrophoretic mobility measurements, which were determined using a Microelectrophoresis Apparatus Mk II (Rank Brothers, Bottingham). We calculated the zeta potential of our assemblies from the Smoluchowski equation using an average mobility value based on 20 measurements.²⁶

Ellipsometry. The thickness of the films was determined using a Gaertner Ellipsometer (with a HeNe laser at 633 nm). The ellipsometric determinations of total layer thickness are reported as the statistical mean of 15 measurements after subtraction of surface oxide layer thickness (measured average of 27 ± 3 Å). Because of the thin nature of the films, the refractive index (n_f) was fixed at 1.55 as reported for PLL/HA films.¹¹ For the swelling experiments, the films were placed in the bottom of a home-built solution chamber containing plexiglass windows set at 70°. The thickness of the films was first determined in air and then water was added to the chamber.²² The system was allowed to equilibrate prior to determining the swollen thickness of the film. The equilibrium degree of swelling was determined by dividing the average swollen thickness by the average dry thickness ($\times 100\%$). The film was then rinsed with pure Milli-Q H₂O and dried under vacuum. The same film was cycled through the various solution pH values for swelling using the same rinse and dry steps between experiments. The dry film thickness was determined before each individual swelling experiment. The swelling experiments required the use of sufficiently thick 150-layer PLL/HA films so that the instrument could independently determine the refractive index before and after swelling ($n_f = 1.54\text{--}1.56$ dry, $n_f = 1.35\text{--}1.41$ wet).

Contact Angle Measurements. The CCD camera and goniometer of an Optrel GBR Multiskop was used to measure the contact angle of water on the surface of the films. An advancing contact angle was measured using the standard sessile drop technique.²⁰ A water droplet was suspended from the tip of the syringe, and the platform on which the film was placed was raised until the film surface touched the droplet. The subsequent addition of small amount of water to the droplet on the surface produced the static advancing contact angle within a few seconds, and a measurement was taken after 5 s. The average droplet volume was 1.5 ± 0.2 μL . An image of the droplet was obtained with a CCD camera, and Contactangle software was used to calculate the contact angle of both the left and right-hand side of the droplet. The droplet profile is fitted using the Young–La Place equation to obtain the surface energy, which is used to compute the contact angle using Young's equation. Prior to measurement, the films were rinsed with the specific water solution (pH and ionic strength adjusted) for which the contact angle was to be measured and dried under vacuum for 5–6 h. The reported contact angles are an average of twenty measurements and have an uncertainty of $\pm 3\text{--}4^\circ$.

Lateral Force Microscopy. The measurements were performed using a Nanoscope III (Digital Instruments) fitted with a liquid cell. A Si₃N₄ cantilever (Nanoprobes, Digital Instruments) with a spring constant of 0.06 N/m was used. The scan size was set at a 100 nm square. The scan speed was 1.00 Hz, which was within the range where lateral force is independent of the scan velocity (0.4–5.0 Hz). The applied load was varied between 0 and 35 nN. The deflection sensitivity was determined in force calibration mode using a bare silicon wafer in solution of 1.0 mM NaCl (Milli-Q H₂O). The lateral deflection voltage was converted to a force

using the lateral stiffness value calculated from the dimensions of the cantilever following the analysis of Neumeister et al.²⁸ The lateral force was determined from the friction loop where the difference between the trace and retrace curves is twice the lateral force. The coefficient of friction is taken as the ratio of the friction force to the applied load.²⁹ The solution pH used for the in situ lateral force studies was varied from pH = 3.0 to 10.0, whereas the NaCl concentration was maintained at 1.0 mM.

Fourier Transform Infrared Spectroscopy in the Attenuated Total Reflection Mode (FTIR-ATR). The films were assembled on the $\langle 100 \rangle$ surface of polished silicon wafers. A KRS crystal was sandwiched between two wafers coated with the film. The multilayer films were prepared using the dipping protocol, whereas the single layer films of PLL and HA were spin cast on the wafers. A Bruker IFS-48 spectrometer was used to obtain the spectra. The angle of nonpolarized light was set at 45°. A total of 3000 scans were taken with a resolution of 4 cm^{-1} . A background correction was made using the spectrum obtained for a bare silicon wafer measured under the same conditions.

Results and Discussion

1. Multilayer Film Formation. Microelectrophoresis and ellipsometry were used to follow the build-up of the multilayer films on colloidal particles and planar wafers, respectively. Although electrophoretic mobility measurements are an indirect method of studying film growth, they are useful for providing an indication of whether the electrical charge on the surface has changed with each additional layer. Figure 1A illustrates the zigzag pattern in the zeta potential from negative to positive for the colloidal particles after the addition of each new polymer layer. However, a change in the sign of the zeta potential does not indicate whether a new layer was added or simply that desorption of polymer has occurred. In addition, the acid–base equilibria of the surface layer also influences the sign of the zeta potential. Therefore, ¹³C cross-polarization/magic angle spinning (CP-MAS) and elemental analysis studies were used to confirm the sequential addition of each layer on the colloidal particles.³⁰ Ellipsometry also provides a direct measure of the film thickness on planar substrates. Figure 1B contains thickness profiles for two 10-layer PLL/HA film assembled at pH = 5.0 and 9.0 respectively on a silicon wafer, which confirms the build-up of the polymer on the surface. Notably, the pH conditions used to assemble the films influences the thickness of the films. This is just a small example of the impact that pH changes can have on the film properties as will be discussed. However, to understand this phenomenon, it is important to first explore the dissociation behavior of the polyelectrolytes in the films.

2. Acid–Base Equilibria Shifts. A number of recent studies have been devoted to investigating the acid–base equilibria of multilayer films composed of weak polyelectrolytes.^{26,31,32} The interest in this area stems from the unprecedented control that can be exercised over the properties of these films (i.e., thickness, wettability, swelling behavior, etc.) by very slight variations in the pH and ionic

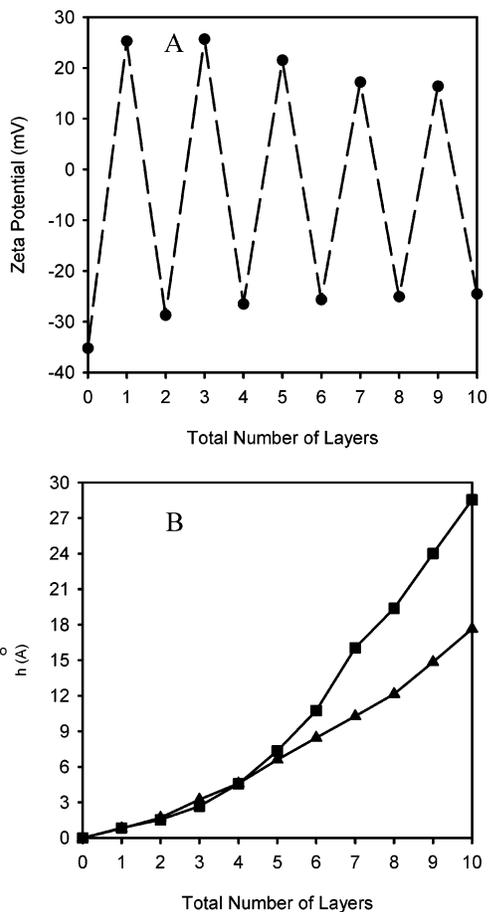


Figure 1. Illustrations of multilayer film build-up. (A) PLL/HA films assembled on colloidal silicon at pH = 7.0, electrophoretic mobility measured at pH = 6.0 (●). (B) Thickness of PLL/HA films assembled at pH = 5.0 (▲) and 9.0 (■) on silica wafers.

strength conditions used during film assembly.^{21,22} Because such dependence on assembly pH is only observed for weak polyelectrolytes, it is believed that learning about the changes in the acid–base equilibria of these polymers is essential in trying to rationalize the unusual trends in the pH and ionic strength dependent physical properties of such films.²⁶ In a similar manner, we examined the influence of the total number of layers in the films and the assembly solution pH on the apparent dissociation constant of the outer surface layer of the films. For this study, the multilayer films were assembled at a fixed pH on colloidal silica and microelectrophoresis was used to follow the changes in the electrical properties of the films with variations in the pH of the solution in which the polymer-coated particles were suspended. The plots of zeta potential versus solution pH represent titration curves, with the inflection point corresponding to the apparent dissociation constant of the film surface. An example of this is shown in Figure 2 for a (PLL/HA)₄ film assembled at pH = 9.0. Reversibility was confirmed by increasing the pH back up to pH = 7.0 and recovering the original strongly charged film. From this plot, a $pK_{a(\text{app})} = 4.89 \pm 0.05$ can be determined as the inflection point. As a reference, the zeta potential versus solution pH curve of bare colloidal silica ($pK_a = 5.84 \pm 0.09$) is also shown. This set of zeta potential versus pH measurements and subsequent $pK_{a(\text{app})}$ determinations was made for all layers 1–10.

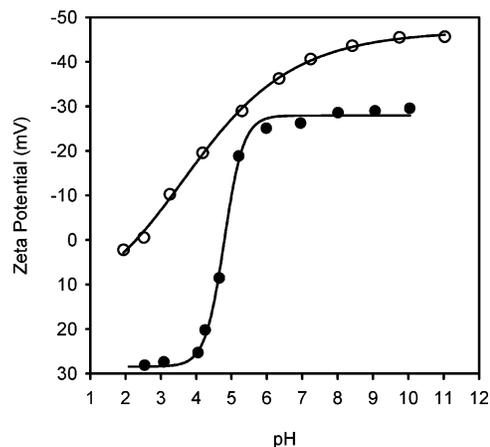


Figure 2. Titration curves for bare colloidal silica (○) and a (PLL/HA)₄ film assembled at pH = 9.0 (●). The solid lines are visual guides.

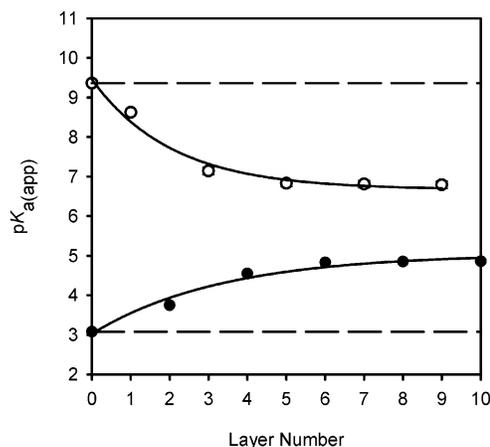


Figure 3. Dependence of the apparent dissociation constant of PLL (○) and HA (●) on the total number of layers in PLL/HA multilayer films assembled at pH = 7.0. The dashed line indicates the dilute solution $pK_{a(\text{app})}$ values. The solid lines are visual guides.

Figure 3 summarizes the layer number dependence of the $pK_{a(\text{app})}$ of multilayer films assembled at pH = 7.0 terminated in PLL and HA for 1–10 layers. All results clearly demonstrate that both PLL and HA experience a significant shift in their $pK_{a(\text{app})}$ values upon adsorption, compared to the accepted values (in dilute solution) of 9.36 ± 0.08 and 3.08 ± 0.03 respectively,³³ in the presence of 1.0 mM NaCl. It is also clear that the total number of layers in the film appears to influence the dissociation behavior of the polyelectrolyte chains on the surface. The dependence of the acid–base equilibria on the layer number appears to level off as the total number of layers increases. In fact, the $pK_{a(\text{app})}$ values of both PLL and HA remain relatively constant after the first 3–4 layers are deposited. We speculate that the variable shifts in the dissociation constants in the first few layers of the film are due to inhomogeneous coverage of the polyelectrolyte on the surface within these layers, and the resulting electrostatic influences of the charged silica substrate ($pK_a = 5.84 \pm 0.09$, IEP = 2.61).²⁶ However, the undeniable decrease in the acid strength of HA and base strength of PLL is quite surprising. It has been previously speculated and experimentally shown that the charge on the multilayer film surface strongly influences the acid–base equilibria of adsorbing polyelectrolyte chains.^{21,26} The opposite charge on the surface promotes an increase in the

charge density of the adsorbing chains therefore, lowering the $pK_{a(\text{app})}$ of polyacids and increasing the $pK_{a(\text{app})}$ of polybases.

For PLL/HA multilayer films, the overall trends in the $pK_{a(\text{app})}$ shifts upon adsorption in comparison to the dilute solution values we believe are influenced by the ability of both of these polymers to adopt some degree of secondary conformational order with changes in the local pH and ionic strength environment.^{13,17,34} In the intermediate pH range, HA is known to have some degree of chain stiffening in solution due to local hydrogen bonded helical regions, whereas PLL chains are reported to experience a random coil to α -helix transition at pH \sim 10.5.¹³ Both PLL and HA also adopt secondary conformational order in the solid state.^{35,36} In addition, it has been reported that PLL chains undergo a surface induced transition from a random coil to α -helix and β -sheet conformations upon adsorption in a multilayer film of poly(L-lysine) and poly(L-glutamic acid) assembled at pH = 8.4.³⁵ Secondary conformations in the polymer chains involve some degree of intermolecular H bonding, leaving the participating functional groups unavailable for proton exchange. Hence, the degree of secondary conformational ordering would have to increase upon adsorption to account for the reduction in the acid strength of HA and base strength of PLL from dilute solution.

The secondary structure of these polymers can be analyzed using FTIR-ATR. The amide I band in the FTIR spectra is a sensitive marker of secondary structure because it is mainly a reflection of the carbonyl stretching vibrations, the frequency of which depends on H-bonding and interactions with amide units, which are the interactions involved in forming the secondary structure.³⁷ Typically, for PLL in solution, at intermediate pH values, the amide I region contains a single narrow peak centered at 1643 cm^{-1} , which is indicative of the random coil conformation of the chains.³⁵ At low to moderate pH values, the solution spectrum of HA has an amide I band centered at 1650 cm^{-1} indicating the presence of helical content.³⁸ The amide I region of the spectra of the PLL/HA films (Supporting Information) has a strong, broad peak between \sim 1635–1655 cm^{-1} , which suggests that the chains retain a similar structure upon adsorption as they had in solution. However, the peak centered around 1625 cm^{-1} represents α -helix content in the PLL chains, whereas those present at \sim 1610 and 1680 cm^{-1} are contributions from β -sheet structures.³⁵ In addition, the helical content of the HA chains is still fairly high at pH = 9.0 despite the fact that HA is strongly charged in solution at this pH value.¹⁶

In addition to the shifts in the $pK_{a(\text{app})}$ values upon adsorption in comparison to the dilute solution, the acid–base equilibria of these polyelectrolytes do show a slight dependence on the assembly solution pH conditions in the range of pH = 5.0–9.0. Notably, it was not possible to successfully assemble films at pH = 3.0. It is speculated that film assembly is hindered at this pH by a combination of the low charge density on the substrate surface and HA chains.^{21,39} The results in Table 1 indicate that the films terminated in HA experience a slight reduction in acid strength with an increase in the assembly solution pH,

Table 1. Summary of $pK_{a(\text{app})}$ for PLL/HA Multilayer Films

polymer	layer #	assembly pH	$pK_{a(\text{app})}$
HA	2	5.0	3.68
HA	2	7.0	3.75
HA	2	8.0	3.82
HA	2	9.0	3.86
HA	8	5.0	3.83
HA	8	7.0	4.85
HA	8	8.0	4.88
HA	8	9.0	4.89
PLL	3	5.0	7.02
PLL	3	7.0	7.14
PLL	3	8.0	7.75
PLL	3	9.0	8.13
PLL	7	5.0	6.74
PLL	7	7.0	6.81
PLL	7	8.0	7.36
PLL	7	9.0	7.84

whereas the base strength of PLL-terminated films increases with increasing assembly solution pH. Titration curves are shown in Figures 4. Such trends in the acid–base equilibria shifts can be rationalized by considering the influence of the charge density of the surface (silica or polyelectrolyte) on which the polymer chains adsorb, the degree of dissociation of the polyelectrolytes in solution, and the ability of the polymer chains to adopt secondary conformations. For example, at lower assembly pH values (i.e., pH = 5.0–7.0), the amino functional groups on a PLL terminated film are \geq 50% protonated (NH_3^+). Any approaching HA chains would experience an increase in the degree of dissociation to compensate for this charge on the surface and, therefore, would be able to undergo less secondary conformational order upon adsorption in comparison to HA chains approaching a PLL-terminated surface from solutions of pH = 8–9 where the amino groups are \leq 50% protonated. The same principle applies in the case of PLL when a PLL layer is assembled at lower pH, the carboxyl groups on the existing HA-terminated surface have a lower degree of dissociation than at higher pH values; therefore, approaching PLL chains must undergo more protonation at higher solution pH values to neutralize the existing charge on the surface. As a result of this increase in PLL protonation, the PLL chains can undergo less secondary ordering as they approach the surface at higher solution pH values compared to at lower pH values.

3. Film Thickness. It has previously been shown that changes in the local pH condition both during and after multilayer film assembly strongly influence the physico-chemical properties of films composed of weak polyelectrolytes. In fact, Granick et al. found that even a single layer of weak polyelectrolytes embedded at the bottom of a 10-layer film is greatly influenced by the local environment at the surface layer.⁴⁰ In one of the earliest studies on weak polyelectrolyte multilayer films, Shiratori and Rubner showed that they could vary the bilayer thickness in PAH/PAA multilayer films from less than 10 Å to more than 120 Å by changing the pH of the assembly solutions.²¹ Figure 5 summarizes the dependence of the dry film thickness on layer number and assembly solution pH for PLL/HA multilayer films ranging from 1 to 50 layers. The results indicate that

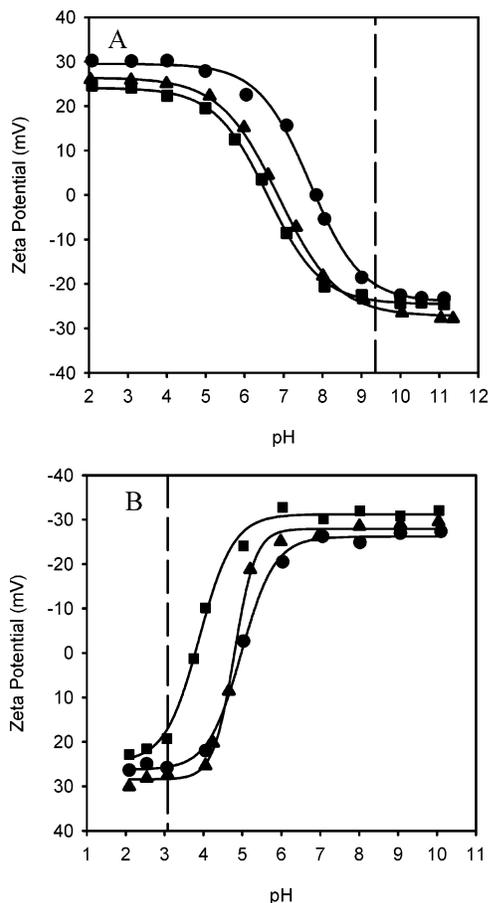


Figure 4. Acid–base equilibria curves for PLL surface layers in PLL/HA multilayer films assembled at different pH values. (A) 7-layer film and (B) 8-layer film assembled at pH = 5.0 (■), 7.0 (▲), and 9.0 (●). The dotted lines indicate the $pK_{a(app)}$ (defined as the inflection point) of PLL and HA in dilute solution. Uncertainty in $pK_{a(app)} \pm 0.02$ –0.08.

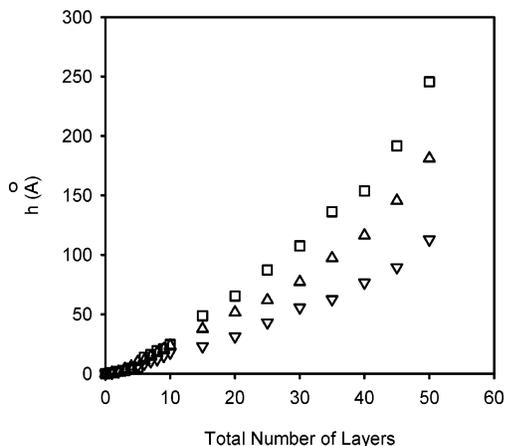


Figure 5. Dry thickness of films assembled at different pH values pH = 5.0 (Δ), 7.0 (∇), and 9.0 (\square). Uncertainty in thickness values of ± 3 –4 Å.

the dry film thickness is characterized by nonlinear growth, which is influenced by assembly solution pH. This nonlinear growth in film thickness with layer number is not surprising; recent reports have appeared in the literature about nonlinear growth of multilayer films resulting from either or both the chemical nature of the polyelectrolytes involved or the influence of solution environment on the adsorption kinetics.^{11,41,42} Notably, Picart et al. reported their observation of such behavior for 24-layer PLL/HA multilayer films as-

sembled from Tris-HCl buffer solution at pH = 7.40.¹¹ However, they report PLL/HA film thickness values that are much larger than those found in this study for films with a comparable number of layers and assembly pH values. This discrepancy is attributed to differences in the assembly conditions employed and the fact that the film thickness measurements were done while the films were swollen in buffer solution.

For all assembly conditions investigated, the films experience a similar exponential build-up, but the dry film thickness does vary with changes in the assembly solution pH. The films were assembled at pH = 5.0, 7.0, and 9.0. The thickest films were obtained when the assembly and wash solutions were fixed at pH = 9.0, whereas the thinnest films resulted from assembly at pH = 7.0. To understand such trends in film thickness, Shiratori and Rubner indicated that it is important to consider the dissociation behavior of the polyelectrolytes at different solution pH values.²¹ In the case of PLL/HA films, PLL is weakly charged in solution at pH = 9.0 and becomes progressively more protonated as the pH is decreased, whereas the dissociation of HA is incomplete at pH = 5.0, but increases with increasing pH. Polyelectrolytes that are only weakly charged tend to adsorb onto surfaces in a more loopy conformation and in larger quantities than when they are fully charged.²¹ At pH = 5.0, the films are not as thick as those assembled at pH = 9.0 because HA is not as weakly charged in solution at this pH in comparison to PLL in solution at pH = 9.0. This also explains why the PLL/HA films assembled at pH = 7.0 are thinner than those assembled at pH = 5.0 or 9.0 considering that HA and PLL are fully or almost fully charged in solution at pH = 7.0.

4. Surface Wettability. Contact angle measurement provides a qualitative means of looking at the differences in the density of functional groups and degree of ionization of these groups with variations in the solution conditions used in film assembly, as well as the pH and ionic strength of the sessile droplet.^{43,44} We studied the contact angle of sessile droplets covering the pH range of 2.0–11.0 and NaCl concentration of 10^{-3} – 10^{-1} M on PLL/HA multilayer films assembled using different pH conditions. The results indicate that the surface wettability of the films has little dependence on the pH conditions used in film assembly (Supporting Information). However, there is an undeniable difference in the contact angle as the droplet pH is varied, which is a reflection of the influence of pH on the ionization of the functional groups on the surface (Figure 6). For example, at low pH, the contact angle of water on films terminated in PLL is low because the amino groups almost all in the NH_3^+ form, but as the pH of the droplet is increased, the amino groups begin to lose their charge leaving the film with a more hydrophobic character, which causes an increase in the contact angle. In the case of HA-terminated films, the contact angle decreases with increasing pH because the COOH groups become more dissociated as the pH of the water droplet is increased.

In addition to the strong influence of droplet pH on the contact angle of water on PLL/HA films, it was found that the salt concentration in the droplet also causes changes in

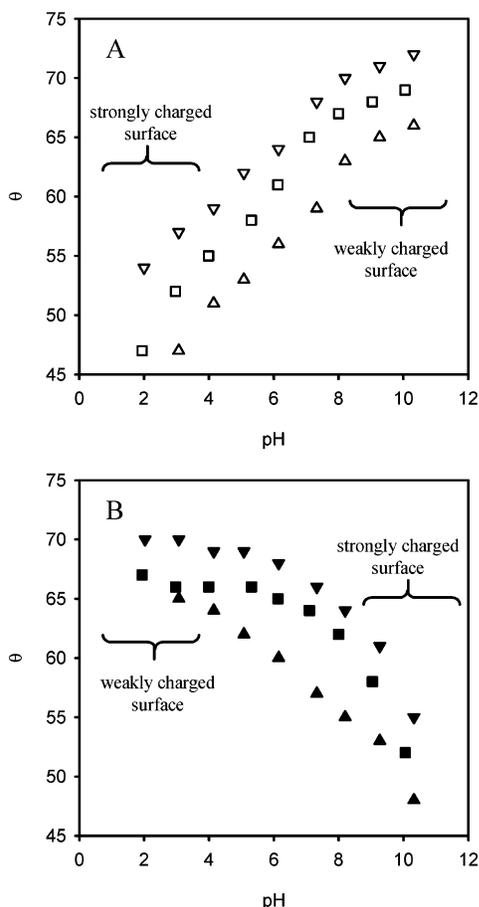


Figure 6. Influence of salt concentration in the sessile droplet on the contact angle for (A) (PLL/HA)₂₂-PLL film assembled at pH = 9.0. Sessile droplet [NaCl] = 1.0 (Δ), 10.0 (\square), and 100.0 mM (∇); (B) (PLL/HA)₂₅ films assembled at pH = 9.0. Sessile droplet [NaCl] = 1.0 (Δ), 10.0 (\square), and 100.0 mM (∇).

the contact angle. Figure 6 contains plots of the contact angle of water at different pH values and fixed concentrations of NaCl on PLL/HA films of 45 and 50 layers respectively assembled at pH = 9.0. The contact angle of the sessile droplet increases, as the salt concentration in the droplet is increased for both films over the entire droplet pH range. The implications of increasing the salt concentration is an increased screening of the surface charges, which makes the surface appear more hydrophobic leading to a decrease in the surface wettability.

Notably, the plots of contact angle versus droplet pH are not titration curves. Whitesides et al. showed that for a film of linear polyethylene functionalized with carboxylic acid groups the density of COOH groups on the surface under a droplet is much higher than the hydroxide ion concentration in an unbuffered aqueous droplet of base.⁴³ The COOH groups themselves effectively buffer the system. Calculations of the approximate density of functional groups on the surface of PLL/HA multilayer films indicates that the same excess of surface functional groups also exists for the contact angle versus pH curves for these films. For example, the number of COOH groups (HA surface layer) under a 2.0 μ L droplet at pH = 7.0 is estimated to be approximately 2.0×10^{12} based on repeat unit size determined from usual bond lengths, whereas the number of H⁺ ions in the droplet is 1.2×10^{11} . This could explain why the pH at the inflection

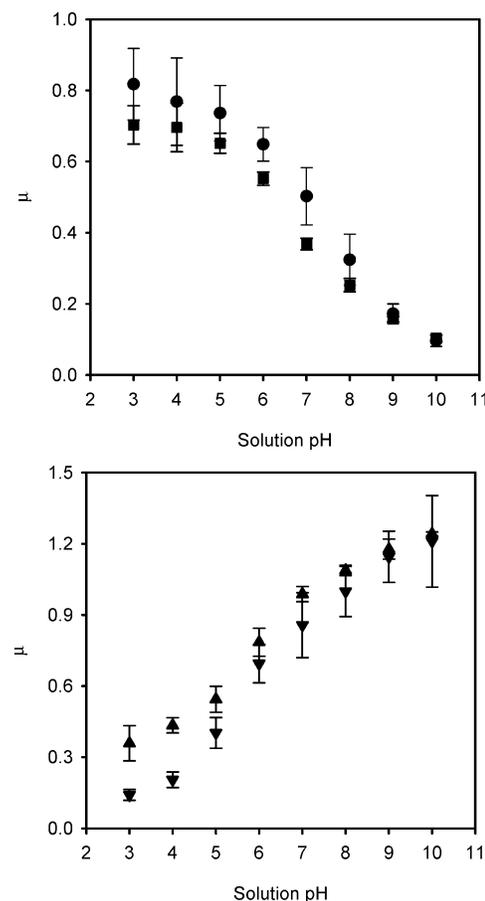


Figure 7. Influence of solution pH on the coefficient of friction of a (A) (PLL/HA)₂₅ film assembled at pH = 5.0 (\blacksquare) and 9.0 (\bullet). (B) (PLL/HA)₂₂-PLL film assembled at pH = 5.0 (\blacktriangledown) and 9.0 (\blacktriangle).

point on the curves is different than the $pK_{a(\text{app})}$ obtained from the zeta potential studies. In addition, this may also account for the fact that the contact angle shows little dependence on the assembly pH conditions. In this case, the contact angle studies were not carried out in buffer to obtain $pK_{a(\text{app})}$ values because of the higher error associated with this method of characterization in comparison to electrophoretic mobility and also because of the need to introduce different buffers for different pH values, which may negatively effect the films.

5. Surface Friction. In addition to studying the wetting properties of the surface of PLL/HA films, the surface friction was investigated for its dependence on the assembly conditions and local pH environment. In all cases, the friction force was found to be linearly dependent on the applied load in the range investigated (Supporting Information). Similar results were obtained for increasing and decreasing applied load. Notably, the conditions used for the friction scans were nondestructive to the films; however, no region of the film was scanned more than once. The friction studies were carried out in an aqueous environment because of the ability of adsorbed moisture on the film surface to distort the measurements.⁴⁵ In addition, other factors that affect the friction measurements such as the applied load, contact area, and temperature were roughly the same for each experiment.²⁹

Figure 7 summarizes the coefficient of friction (μ) obtained for PLL/HA films terminated with a layer of either PLL or

HA over the solution pH range of 3.0–10.0. Dependence of the surface friction on the solution pH were previously observed for an SiO₂ surface.⁴⁶ It appears that remarkable control can be exercised over the surface friction by simply altering the solution pH conditions. In fact, in some cases, the coefficient of friction of PLL/HA multilayer films approaches the in situ value of 0.034 for a silicon nitride tip sliding across a poly(tetrafluoroethylene) (Teflon) surface.⁴⁷ The interpretation of the results requires consideration of the charge density of both the tip and sample, the sign of the charge relative to one another, and the surface structure (roughness) of the film. For example, at solution pH values below the isoelectric point of Si₃N₄ (IEP = 6.0),⁴⁸ the tip has a net positive charge, whereas the film terminated with a layer of HA has a moderate to low net negative charge (i.e., $pK_{a(\text{app})} = 3.83\text{--}4.89$). This results in electrostatic attraction between the tip and film surface, leading to strong adhesion forces.⁴⁹ This has recently been experimentally demonstrated for polyelectrolyte multilayer films.⁴⁹ In contrast, at pH values above pH = 6.0, the film surface and tip are both negatively charged, with the charge density (repulsive interactions) of both increasing with increasing pH. The friction force is directly related to the adhesion force, and therefore, the coefficient of friction decreases with increasing solution pH.⁴⁶ Similar rationale is used to understand the trends in the μ dependence with solution pH for films terminated in PLL. The adhesion force is higher at high solution pH values when the tip and the film are oppositely charged in comparison to the low pH range where both surfaces have a net repulsive interaction.

The coefficient of friction is also dependent on the surface roughness of the films, which accounts for the slight differences in the range of μ values for films assembled using different pH conditions. The lower the charge density of the polyelectrolyte in solution, the more loopy and globular the conformation of the chains upon adsorption.²¹ Also, when one layer adsorbs in a loopy conformation, the subsequent layers also adopt conformations high in loops and tails.²¹ The films assembled at pH = 9.0 have rougher surfaces than those assembled at pH = 5.0 (RMS = 19 and 7 nm respectively)⁵⁰ resulting in higher values of μ at comparable solution pH values. When the films are assembled at pH = 9.0, the PLL chains ($pK_a = 9.36$) are more weakly charged than the HA chains ($pK_a = 3.08$) in solution and visa versa for assembly at pH = 5.0. However, at pH = 9.0, the PLL chains are ~50% charged in solution in comparison to the >50% charge density of HA chains in solution at pH = 5.0. The differences in the charge density and hence chain conformation of the polyelectrolytes at different assembly pH values is also reflected in the film thickness studies. Notably, because the acid–base chemistry of both the tip and the film play an integral role in determining the coefficient of friction the plots of μ verses the solution pH do not represent an acid–base titration of the film surface.

This knowledge of the coefficient of friction in combination with the acid–base equilibria and surface wettability data offers insight into the mechanism of how the charge density of biopolymer chains such as HA can be manipulated to stick to surfaces, but once anchored by ionic cross-links

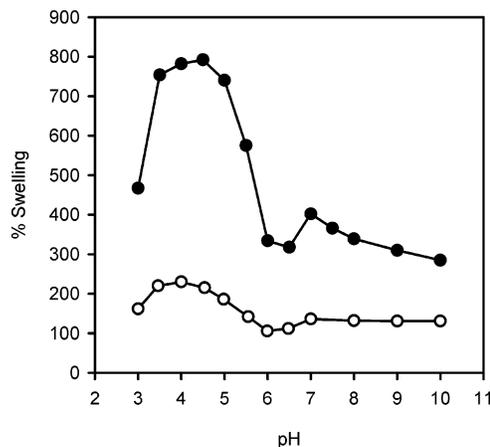


Figure 8. Dependence of the equilibrium degree of swelling for (PLL/HA)₇₅ films assembled at pH = 5.0 (○) and 9.0 (●).

can be switched from “sticky” charged surfaces to “slick” yet stable low friction surfaces. For example, at a biological pH of ~ 7.4, HA chains are fully or nearly fully charged ($pK_{a(\text{app})} = 3.08$),³³ but when they are adsorbed onto a charged surface, they begin to lose their charge at this pH. In fact, under the right assembly conditions, a film terminated in HA can be made to be essentially neutral at biological pH (Figure 4) and, therefore, a very effective low friction surface coating. Here, we have demonstrated that controlling the acid–base chemistry is likely the key component of the remarkable mechanism whereby biopolymers can be anchored to surfaces in a strongly charged state and then transformed into stable low friction surface coatings by drastic pK_a shifts.

6. Swelling Behavior. In addition to studying the dependence of the surface properties of PLL/HA multilayer films on pH, we investigated the bulk swelling behavior of these films using solutions differing in pH and ionic strength. It is postulated that, similar to pH-sensitive hydrogels, the equilibrium swelling of weak polyelectrolyte multilayer films will be dependent on such factors as the pK_a of the ionizable groups, the concentration of ionizable units present in the film, the cross-link density of the films, the hydrophilicity/hydrophobicity of the films, as well as the pH and ionic strength of the swelling solution.⁵¹ The equilibrium degree of swelling of PLL/HA films is greatly influenced by the solution pH used in film assembly, as well as the swelling solution pH, but only shows a slight dependence on the ionic strength.

The curves in Figure 8 illustrate the variation in the degree of swelling of 150-layer PLL/HA films assembled at pH = 5.0 and 9.0 with changes in the swelling solution pH at a fixed salt concentration of 1.0 mM. In this study, 150-layer films were used to ensure that the thickness and refractive index of the films could be resolved by the ellipsometer in both the dry and swollen state. It is apparent that the degree of equilibrium swelling is significantly higher for the film assembled at pH = 9.0 compared to the one assembled at pH = 5.0 over the entire range of swelling solution pH values. This result is rationalized by considering the details of polyelectrolyte adsorption during film assembly and the proposed build-up mechanism for the films. At pH = 5.0, PLL is fully charged in solution and HA is near fully

dissociation, whereas at pH = 9.0, PLL is weakly charged and HA is now fully dissociated. Strongly charged polymers have more of a wormlike structure in solution, and adsorb to surfaces in a fairly flat conformation with a high population of ionic cross-links to the surface. On the other hand, weakly charged polymers tend to take more of a globular shape in solution and adsorb to surfaces in a conformation rich in loops and tails with much fewer ionic links to the surface per chain.³¹ Therefore, PLL/HA films assembled at pH = 9.0 can undergo a greater degree of chain extension and segment mobility upon swelling than films assembled at pH = 5.0. In addition, it has been hypothesized that the exponential growth of PLL/HA multilayer films results from diffusion of PLL in and out of the film, leading to a greater incremental thickness contribution from the PLL layers in comparison to the HA layers.¹² It is speculated that the weaker the charge on the PLL chains the greater the amount of polymer that can diffuse within the films, which implies that the films assembled at pH = 9.0 contain a higher concentration of PLL than HA in comparison to a PLL/HA film with a similar dry thickness assembled at pH = 5.0. The above-mentioned differences in the PLL/HA films assembled at pH = 5.0 and 9.0 explain why those assembled at pH = 9.0 experience a higher degree of equilibrium swelling.

The charge density of the polyelectrolytes upon adsorption and the proposed build-up mechanism are also important in understanding the effect of swelling solution pH on the swelling behavior of the films. For both types of films, the pH range for maximum swelling is pH ~ 3–6. In this pH range, the HA chains are not fully dissociated, whereas the PLL chains are in a fully charged state and, therefore, prefer to be in the most extended conformation. As previously mentioned, the films are suspected to have a larger incremental thickness contribution from PLL than HA because of the ability of the PLL chains to diffuse in and out of the film, and thus, the films adopt a larger equilibrium thickness in the pH region where PLL experiences the greatest extension. The maximum swelling is more pronounced in the film assembled at pH = 9.0 because the chains adsorb with a more loopy conformation with a lower ionic cross-link density per chain than the one assembled at pH = 5.0. The swelling curves do not appear to experience a significant change in the degree of swelling in the pH range (pH ~ 7–10) where the HA units are fully charged because HA has a lower incremental thickness contribution in the film and also because at both assembly solution pH values HA is significantly charged and therefore adsorbs in a fairly flat conformation with a large ionic cross-link density per chain.

Table 2 summarizes the dependence of the degree of equilibrium swelling on the ionic strength of the swelling solution at pH = 3.0, 6.0, and 9.0. There is no significant difference in the reversible degree of swelling when the concentration of NaCl in the swelling solution is less than 10⁻¹ M (i.e., it is possible to swell the films, dry them back to the original dry thickness, and then swell them again to the same degree). However, the films undergo a substantial increase in swelling as the salt concentration is increased. In comparison, polyelectrolyte hydrogels typically experience

Table 2. Influence of Ionic Strength on the Swelling of a (PLL/HA)₇₅ Film Assembled at pH = 9.0

solution pH	[ion] M	h _{dry}	n _f dry	h _{swell}	n _f swell	% swelling
3.0	10 ⁻³	1015	1.55	4744	1.38	467
3.0	10 ⁻²	1013	1.56	6533	1.36	645
3.0	10 ⁻¹	1013	1.55	9747	1.35	962
6.0	10 ⁻⁶	1009	1.55	3423	1.39	339
6.0	10 ⁻³	1014	1.55	3393	1.39	334
6.0	10 ⁻²	1010	1.56	3928	1.38	389
6.0	10 ⁻¹	1012	1.56	8420	1.35	832
9.0	10 ⁻⁵	1013	1.56	3072	1.40	303
9.0	10 ⁻³	1009	1.55	3129	1.40	310
9.0	10 ⁻²	1013	1.55	3416	1.39	337
9.0	10 ⁻¹	1013	1.55	7985	1.36	788

a decrease in swelling as the salt concentration is increased because of electrostatic screening.⁵⁰ However, it must be noted that hydrogels are covalently cross-linked, which, unlike ionic cross-links, are unaffected by changes in the local ionic strength environment. In the case of multilayer films, the ionic bonds that hold the layers together are known to break upon increase in the local ionic strength.²² At low NaCl concentrations, it is conceivable that the flux of ions into the film from the bulk solution is too low to cause significant electrostatic screening of the charged groups on the polyelectrolyte chains and significant ionic bond breaking. As the salt concentration is increased, it is believed that the equilibrium swelling behavior of these films results from a balance between electrostatic screening and ionic cross-link breaking in the film. Notably, the swelling process was found to be completely reversible under all conditions investigated, therefore, there is no detectable loss of polyelectrolyte chains from the film. In fact, it has been shown that much higher ionic strength conditions are required for complete film destruction.^{22,52} As one final observation, it appears from these swelling results that the strong friction dependence on pH described in the previous section is not related to the swelling of the layers but only to the acid–base equilibria. If the degree of swelling played a strong role in determining friction, then the films would be most frictionless at lower pH for both the PLL and the HA, the opposite of what is observed in Figure 7.

Throughout this discussion, it has become clear that the pH-dependent trends in the physicochemical properties of PLL/HA multilayer films are a reflection of the trends in the acid–base equilibria in the films. Although some previous studies have focused on studying the dissociation behavior in multilayer films of weak polyelectrolytes,^{26,31,32} this is the first study to clearly link how the acid–base equilibria trends are manifested in the physicochemical properties of the films. Hence, controlling the dissociation behavior of weak polyelectrolyte multilayer films is a valuable tool for tailor-making films with desired properties. This phenomenon is a particularly important consideration for the many potential biomaterials applications for multilayer films, which have specific requirements for the fundamental physical properties such as wettability, surface friction, and degree of swelling. These properties dictate how the material will interact with biological structures such as proteins and cells and, therefore, determine the suitability of the material to a particular application. In particular, we have shown that

the surface wettability can be varied by as much as 25°, whereas the surface friction can be altered by a factor of 10 and the degree of swelling by a factor of 8 for films composed of the same two polyelectrolytes, simply by varying the pH. Dramatic changes in the character of the films can be created, readily and reversibly making these films promising for a diversity of applications.

Conclusion

We have observed significant shifts in the acid–base equilibria dissociation constants of poly(L-lysine) and hyaluronic acid upon incorporation into a multilayer film, from 1 to 3 pH units different from the accepted dilute solution values. This is consistent with the results from a previous study on acid–base equilibria in a model polyelectrolyte multilayer film;²⁶ however, here the polyacid becomes a weaker acid and the polybase a weaker base upon adsorption. Such behavior stems from the ability of the chains of these polyelectrolytes to adopt secondary conformations and to increase the degree of conformational order upon adsorption. Hence, the trends in the shifts of acid–base equilibria are strongly dependent upon the types of polyelectrolytes in the multilayer film. We have also found that the acid–base equilibria properties of the polyelectrolytes in the film can be used to tailor the physicochemical properties of the film by varying the assembly solution pH and ionic strength environment. In particular, the dry film thickness can be varied by as much as 100 Å by changing the assembly solution pH. In addition, the contact angle of a water droplet on the films can be varied by 25° depending on the droplet pH, the polymer terminating the film, and the ionic strength of the droplet. Furthermore, the bulk swelling ability of the films depends not only on the pH and ionic strength of the swelling solution but also on the structure of the film (i.e., ionic-cross-link density), which is determined by the assembly solution conditions. The acid–base properties of the films also significantly influence their surface friction properties. The coefficient of friction can be manipulated by as much as a factor of 10 for any given film. This leads to a mechanism we propose whereby biopolymer thin films can be electrostatically adsorbed under highly charged “sticky” conditions but then quickly transformed into stable low-friction films simply by altering their $pK_{a(\text{app})}$ on adsorption, at the same pH environment.

Acknowledgment. The authors thank NSERC Canada and McGill University for financial support in the form of operating grants (CB) and scholarships (SB). Professors Derek Gray, Ashok Kakkar, and Linda Reven are gratefully acknowledged for use of equipment. Many thanks are also extended to Ozzy Mermut and Vicki Meli for assistance with the LFM experiments and to Rashida Smith for CP-MAS NMR assistance.

Supporting Information Available. Plots of the amide I region of the FTIR spectrum of a (PLL/HA)₁₀ film, the dependence of contact angle on the pH of the sessile droplet for films assembled at different pH values, and friction force

verses applied load for a (PLL/HA)-PLL film assembled at pH = 9.0. A table of data comparing PLL/HA films prepared using the dip and spin methods is also provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Decher, G.; Hong, J. D. *Makromol. Chem., Macromol. Symp.* **1991**, *46*, 321. Decher, G. *Science* **1997**, *277*, 1232.
- (2) *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials*; Decher, G., Schlenoff, J. B., Eds.; Wiley-VCH: Weinheim, Germany, 2002.
- (3) Caruso, F. *Chem. Eur. J.* **2000**, *6*, 413.
- (4) Caruso, F.; Kichtenfeld, H.; Giersig, M.; Möhwald, H. *J. Am. Chem. Soc.* **1998**, *120*, 8523.
- (5) Caruso, F.; Trau, D.; Möhwald, H.; Renneberg, R. *Langmuir* **2000**, *16*, 1485.
- (6) Watanabe, S.; Regen, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 8855.
- (7) Rouse, J.; Lillehei, P. T. *Nano Lett.* **2003**, *3*, 59.
- (8) Caruso, F.; Schüler, C. *Langmuir* **2000**, *16*, 9595.
- (9) Chluba, J.; Voegel, J.-C.; Decher, G.; Erbacher, P.; Schaaf, P.; Ogier, J. *Biomacromolecules* **2001**, *2*, 800.
- (10) Zhou, L.; Yang, J.; Estavillo, C.; Stuart, J. D.; Schenkman, J. B.; Rusling, H. F. *J. Am. Chem. Soc.* **2003**, *125*, 1421.
- (11) Picart, C.; Lavalle, P.; Hubert, P.; Cuisinier, F. J. G.; Decher, G.; Schaaf, P.; Voegel, J.-C. *Langmuir* **2001**, *17*, 7414.
- (12) Picart, C.; Mutterer, J.; Richert, L.; Luo, Y.; Prestwich, G. D.; Schaaf, P.; Voegel, J.-C.; Lavalle, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12531.
- (13) Yasui, S. C.; Keiderling, T. A. *J. Am. Chem. Soc.* **1986**, *108*, 5576.
- (14) Kono, K.; Simura, S.; Imanishi, Y. *Biochemistry* **1990**, *29*, 3631.
- (15) Chu, B. C.; Howell, S. B. *Biochem. Pharmacol.* **1981**, *30*, 2545.
- (16) Lapčák, L.; Lapčák, L.; De Smedt, S.; Deseester, J.; Chabreček P. *Chem. Rev.* **1998**, *98*, 2663.
- (17) Ghosh, S.; Kobal, I.; Zanette, D.; Reed, W. F. *Macromolecules* **1993**, *26*, 4685.
- (18) Hu, M.; Sabelman, E. E.; Lai, S.; Timek, E. K.; Zhang, F.; Hertz, V. R.; Lineaweaver, W. C. *J. Biomed. Mater. Res.* **1999**, *47*, 79.
- (19) Asayama, S.; Bigawa, M.; Takei, Y.; Akaike, T.; Maruyama, A. *Bioconjugate Chem.* **1998**, *9*, 476.
- (20) Yoo, D.; Shiratori, S. S.; Rubner, M. F. *Macromolecules* **1998**, *31*, 4309.
- (21) Shiratori, S. S.; Rubner, M. F. *Macromolecules* **2000**, *33*, 4213.
- (22) Mendelsohn, J. D.; Barrett, C. J.; Chan, V. V.; Pal, A. J.; Mayes, A. M.; Rubner, M. F. *Langmuir* **2000**, *16*, 5017.
- (23) Harris, J. J.; Bruening, M. L. *Langmuir* **2000**, *16*, 2006.
- (24) Fery, A.; Schöler, B.; Cassagneau, T.; Caruso, T. *Langmuir* **2001**, *17*, 3780.
- (25) Hiller, J.; Mendelsohn, J. D.; Rubner, M. F. *Nature Mater.* **2002**, *1*, 59.
- (26) Burke, S. E.; Barrett, C. J. *Langmuir* **2003**, *19*, 3297.
- (27) Lee, S.-S.; Hong, J.-D.; Kim, C.-H.; Kim, K.; Koo, J. P.; Lee, K.-B. *Macromolecules* **2001**, *34*, 5358.
- (28) Neumeister, J. M.; Ducker, W. A. *Rev. Sci. Instrum.* **1994**, *65*, 2527.
- (29) David, D. J.; Misra, A. *Viscoelastic and Mechanical Properties. Relating Materials Properties to Structure*; Technomic Publishing CO., Inc.: Lancaster, PA, 1999; pp 469–471.
- (30) Smith, R. N.; Reven, L.; Barrett, C. J. *Macromolecules* **2003**, *36*, 1876.
- (31) Ramile, H. H.; Schlenoff, J. B. *Langmuir* **2002**, *18*, 2863.
- (32) Kato, N.; Schuetz, P.; Fery, A.; Caruso, F. *Macromolecules* **2002**, *35*, 9780.
- (33) The polyelectrolyte solutions used for the titration experiments contained 2.0 mM polymer and 1.00 mM NaCl. 0.398 M NaOH was used as the titrant. The solutions were allowed to equilibrate for 10 min before each measurement.
- (34) Turner, R. E.; Kin, P.; Cowman, M. K. *Arch. Biochem. Biophys.* **1988**, *265*, 484.
- (35) Boulmedais, F.; Schwinté, P.; Gergely, C.; Voegel, J.-C.; Schaaf, P. *Langmuir* **2002**, *18*, 4523.
- (36) Arnott, S.; Mitra, A. K.; Raghunathan, S. *J. Mol. Biol.* **1983**, *169*, 861.
- (37) Urban, M. W. *Attenuated Total Reflectance Spectroscopy of Polymers: Theory and Practice*; American Chemical Society: Washington, DC, 1996.

- (38) Gilli, R.; Kacuráková, M.; Mathlouthi, M.; Navarini, L.; Paoletti, S. *Carbohydr. Res.* **1994**, *263*, 315.
- (39) Mikelsaar, R.; Scott, J. E. *Glycoconjugate J.* **1994**, *11*, 65.
- (40) Xie, A. F.; Granick, S. *Macromolecules* **2002**, *35*, 1805.
- (41) Ruths, J.; Essler, F.; Decher, G.; Riegler, H. *Langmuir* **2000**, *16*, 8871.
- (42) Lavallo, P.; Gergely, C.; Cuisinier, F. J.; Decher, G.; Schaaf, P.; Voegel, J. C.; Picart, C. *Macromolecules* **2002**, *35*, 4458.
- (43) Homes-Farley, S. R.; Reamey, R. H.; McCarthy, T. J.; Deutch, J.; Whitesides, G. M. *Langmuir* **1985**, *1*, 725.
- (44) Thomas, R. R.; Stephans, L. E. J. *Colloid Interface Sci.* **2002**, *251*, 339.
- (45) Piner, R. D.; Mirkin, C. A. *Langmuir* **1997**, *13*, 6864.
- (46) Marti, A.; Hähner, G.; Spencer, N. D. *Langmuir* **1995**, *11*, 4632.
- (47) Podestà, A.; Fantoni, G.; Milani, P.; Guida, C.; Volponi, S. *Thin Solid Films* **2002**, *419*, 154.
- (48) Lin, X.-Y.; Creuzet, F.; Arribart, H. *J. Phys. Chem.* **1993**, *97*, 358.
- (49) Mermut, O.; Barrett, C. J. *Polym. Mater. Sci. Eng.* **2003**, *88*, 441.
- (50) The root-mean-square roughness values were obtained from dry atomic force microscope (Nanoscope III, Digital Instruments) $10 \times 10 \mu\text{m}$ scans of 50-layer PLL/HA films. The scan rate was 1.50 Hz.
- (51) *Polyelectrolyte Gels: Properties, Preparation, and Application*; Harland, R. S., Prud'homme, R. K., Eds.; ACS Symposium Series 480; American Chemical Society: Washington, DC, 1992.
- (52) Mermut, O.; Barrett, C. J. *Analyst* **2001**, *26*, 1861.

BM034184W