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Swelling Behavior of Hyaluronic Acid/Polyallylamine Hydrochloride Multilayer Films

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The reversible swelling behavior of multilayer films containing hyaluronic acid and polyallylamine hydrochloride was investigated using in situ ellipsometry, since many of the natural functions and applied uses of hyaluronic acid are related to the extraordinary ability of this biopolymer to swell, and to respond conformationally to the local solution environment. This swelling was observed to be substantial, and depended strongly on the film thickness, the pH conditions used to prepare the films, and the swelling solution pH and ionic strength. The swelling results were also rationalized in terms of the dissociation behavior of the polyelectrolytes in the multilayer assemblies, measured by the ζ potential, on colloidal particles. The films were found to swell by as much as 8 times their dry thickness, and the extent of film hydration was observed to depend on the thickness of the films in a nonlinear fashion. This was related to the internal structure of the films, which is dictated by the assembly pH conditions. In addition, the swelling solution pH and ionic strength influence the electrostatic environment in the films and, in turn, have a substantial effect on the overall swelling behavior.

Introduction

The sequential adsorption of oppositely charged polyelectrolytes onto a charged surface from dilute aqueous solutions has become a very attractive means of preparing versatile polymer films and coatings.[1,2] The success of this layer-by-layer assembly of polyelectrolyte multilayer thin films is based upon the fact that the adsorption of the polyelectrolyte chains leads to charge overcompensation on the film surface. The resulting films have highly interpenetrated architectures held together with ionic cross-links, and contain both cationic and anionic functional groups intermixed throughout the matrix. The multilayering process is not limited to simple linear polyelectrolytes,[2] but instead almost any charged species such as colloidal particles, proteins, DNA, enzymes, and inorganic material can be multilayered with polyelectrolytes to produce multicomposite materials.[3−8] One of the many attractive features of multilayer systems is the magnitude to which their properties can be tailored by varying the conditions used to assemble the films (i.e., polymer concentration, polymer functionality, solution pH, and ionic strength).[2,9−11] In particular, films prepared from weak polyelectrolytes can exhibit highly variable properties such as thickness, surface roughness, wettability, swelling behavior, and surface friction because of the influence of pH and ionic strength on the dissociation of the weakly acidic and basic functional groups on the chains.[12−16] The resulting ease of preparation and versatility of multilayer assemblies has led to their use in such varied applications as contact lens coatings and antifouling covers.[17,18] In addition, recent studies have shown that there are many other promising applications for multilayered materials in such areas as drug delivery, sensors, wound healing, and membrane filtration.[19−22]

Hyaluronic acid (HA) is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine.[23] In dilute solution the conformation of the HA chains can be converted from a random coil to a single helical structure with a change in pH or ionic strength. 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. In addition, the rich suite of properties of hyaluronic acid has led to its incorporation in a wide variety of cosmetic and pharmaceutical applications. Many of the functions and applications of hyaluronic acid are highly dependent on the swelling behavior of this biopolymer.[23]

In this study, hyaluronic acid is incorporated into multilayer films with polyallylamine hydrochloride (PAH). Polyallylamine hydrochloride was chosen as the polycation in this study because much is know about its behavior in multilayer films with other weak polyelectrolyes, and also because biomaterial applications of multilayer films containing PAH have previously been established.[24] One of the most important properties of hyaluronic acid/polyallylamine hydrochloride multilayer films is their swelling behavior. The extent to which these films can swell and the swelling dynamics influence such factors as the hydration of the material, the refractive index, and the loading and release behavior of small molecules in the films.[19,25,26] The swelling of ionic polymeric networks is known to be most strongly influenced by the ionization of the network. The factors that

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influence the ionization upon exposure to solution include the concentration of ionizable functional groups within the polymer network, the degree of ionization, the dissociation equilibrium of the functional groups, the pH and ionic strength of the swelling medium, the nature of the counterions, and the polarity of the swelling solution.[27,28] Other material properties that are known to influence the swelling include the degree of cross-linking and the hydrophobic/hydrophilic balance of the material.[27]

Although much is known about the swelling behavior of polyelectrolyte hydrogels, a class of ionic polymeric networks, the characteristic properties of these materials are not directly comparable to those of polyelectrolyte multilayer films because of the differences in the nature of the bonds holding the two systems together.[28] Hydrogels are stitched together with covalent cross-links that are not influenced by the properties of the swelling solution. On the other hand, the ionic cross-links holding multilayer assemblies together are more dynamic and can easily be broken and re-formed depending on their local solution environment.[29] However, the ionization properties of the material play a major role in the swelling behavior of both classes of ionic networks, but unlike hydrogels, the swelling of polyelectrolyte multilayer films has received only limited attention in recent years.

The first studies to report on the swelling behavior of multilayer assemblies focused on the influence of added salt on the hydration of multilayer films containing a strong polyelectrolyte in partnership with either a weak or another strong polyelectrolyte. The degree of film swelling was found to increase with an increase in the concentration of salt used in both the swelling solution and during the assembly process.[29,30] Multilayer films of weak/strong polyelectrolyte combinations are also found to exhibit swelling behavior that is sensitive to both the pH and humidity of the environment to which they are exposed.[31,32] Subsequent studies have been devoted to exploring the swelling behavior of multilayer films containing only weak polyelectrolytes because their ionization is highly sensitive to the pH of the local environment.[14,15,24] Mendelsohn et al. reported that PAH/poly(acrylic acid) (PAA) multilayer films swell to a larger extent when assembled at lower pH values in comparison to films containing the same polymers, but assembled under neutral pH conditions.[14,24] However, they reported that the degree of film swelling was independent of the solution pH in the acidic range.[14] Recently, Tanchak and Barrett studied the kinetics and mechanism of the swelling process in PAH/PAA multilayer films as a function of the assembly pH conditions and relative humidity.[33] They found that both the rate and mechanism of film swelling were influenced by the assembly pH conditions and the ambient humidity. In addition, as part of a recent controlled-release study involving HA, we have also reported preliminary results that the degree of swelling in multilayer films containing other weak polyelectrolyte combinations, hyaluronic acid and either poly(L-lysine) or polyallylamine hydrochloride, is strongly dependent on the pH of the swelling medium.[15,34]

This paper focuses on studying the swelling behavior of weak polyelectrolyte multilayer films to acquire a comprehensive understanding of how the assembly conditions and the swelling solution environment can be used to control the swelling of hyaluronic acid/polyallylamine hydrochloride multilayer films. We used a home-built liquid cell for in situ ellipsometry under various solution conditions for multilayers on silicon surfaces, and we also prepared analogous biopolymer films on colloidal silica to obtain the ζ potential surface charge in situ, again under various solution conditions. This swelling is an important parameter for many of the potential biomaterial applications for such films. We explore the influence of the assembly solution pH, the thickness of the films, and both the pH and ionic strength of the swelling solution on the degree of film swelling. Outlining the extent to which each factor influences the swelling behavior of the films is fundamental to effectively utilizing the potential of the assemblies for particular biomaterial applications.

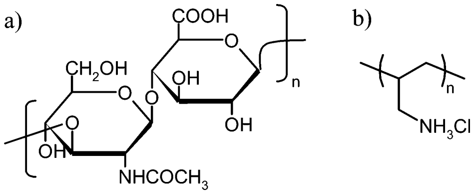

Figure 1. Polyelectrolyte structures of (a) hyaluronic acid and (b) polyallylamine hydrochloride.

Materials and Methods

Materials. PAH (MW = 60000) was purchased from Polymer Sciences, Inc., and sodium hyaluronate (MW = 150000−250000) was received from Sigma and converted to the acid form (HA) via hydrolysis with HCl (Figure 1). Both polymers were used without further purification. The films were assembled on colloidal silica with an average diameter of 70−100 nm (Nissan Chemical Industries). The colloidal silica was obtained as a 40 wt % aqueous solution (pH 9−10). Multilayer assembly was also carried out on polished ⟨100⟩ silicon wafers purchased from WaferNet, Inc. The wafers were pretreated by soaking them in a bath of 25% H_2SO_4 and 75% CrO_4 followed by a thorough rinsing with purified water prior to use. All solutions were made with water (resistivity of 18.2 MΩ·cm) purified using a Millipore, Milli-Q water purification system.

Multilayer Thin Film Preparation. The films were prepared from aqueous solutions of 2.0 mM polyelectrolyte (on the basis of the repeat unit molecular weight) and 0.01 M NaCl. The salt was added to promote polymer adsorption. The solutions were stirred for ~12 h to ensure complete dissolution of the polymers. The solution pH was adjusted with either HCl or NaOH. For assembly on colloidal silica, a solution of the colloid stock was diluted to 0.5 g/L and adjusted to the appropriate pH value.[35] A 30 mL sample of the colloidal solution was mixed with 30 mL of the polycation solution (final polymer concentration of 2.0 mM). The suspension was gently agitated for 30 min to allow for polymer adsorption onto the surface. The suspension was then centrifuged followed by removal of the supernatant. The polyelectrolyte-coated particles were then resuspended in purified water with the same pH as the adsorption solutions via sonication for ~5 min. The suspension was allowed to stand for 30 min before centrifugation. The wash procedure was completed two additional times to remove any unadsorbed polymer. The particles were then suspended in the polyanion solution to adsorb the second layer. The assembly and washing of the second layer were carried out in the same way as described above. The deposition and wash steps were carried out until a maximum of 12 layers (6 bilayers (PAH/HA)$_6$) were deposited. A small sample of particles was removed after the addition of each new layer for characterization. The resulting colloidal slurries were dried under vacuum at 25 °C for 48 h.[35]

Polyelectrolyte multilayer films were assembled on the planar wafers using an automatic slide stainer (Varistain 24-4, Shandon). The substrate was first immersed in a 500 mL solution of PAH for 10 min followed by three consecutive salt-free wash baths of 500 mL of purified water adjusted to the same pH as the polyelectrolyte solution for 1.0, 2.0, and 2.0 min, respectively.[12] The substrate was then dipped into 500 mL of the HA solution for 10 min, which was adjusted to the same pH as the PAH solution. This was followed by three wash cycles as described above. Each dipping step was accompanied by gentle agitation. This process was carried out in a cyclic process until the desired numbers of layers were deposited. The wash baths were replenished after the addition of every 10 layers (5 bilayers). The films were then dried with a stream of compressed air and further dried in a vacuum desiccator at 25 °C for 24 h prior to use.

Microelectrophoresis. The buildup of the polyelectrolyte layers and acid−base dissociation behavior of the multilayer films were monitored using a ZetaPlus instrument (Brookhaven Instruments Corp.). Solutions of 30 mg of dry colloidal particles in 15 mL of 1.0 mM NaCl were prepared for the electrophoretic mobility measurements. The ζ potential was calculated from the Smoluchowski equation using an average mobility value based on 20 measurements. As an indirect means of following the multilayer buildup process, the ζ potential of a series of colloidal suspensions of the same pH value was determined as a function of the total number of polyelectrolyte layers on the particles. This was done to follow the change in the sign of the mobility from positive to negative as the surface layers were switched from PAH to HA.[35] The acid−base dissociation of each polyelectrolyte layer was investigated by following the change in the ζ potential of the colloidal suspension at different pH values. A plot of the ζ potential verses solution pH of the suspension yields a titration curve from which the p$K_{a(app)}$ value of the surface polyelectrolyte layer is taken as the inflection point on the curve.[15,35]

Ellipsometry. The thickness and refractive index of the films were determined in both the dry and swollen states using a Gaertner Ellipsometer (with a HeNe laser at 633 nm). The ellipsometric determinations of total layer thickness and refractive index are reported as the statistical mean of 15 measurements after subtraction of the surface oxide layer thickness (measured average of 26 ± 4 Å). The swelling experiments involved the use of a home-built solution chamber containing Plexiglas windows set normal to the incident and reflected beams at 70°.[15] The film was fixed to the bottom of the chamber with Teflon brackets. The film thickness was first determined in air, and then water was added to the chamber. The swelling process was monitored with time to ensure equilibrium was reached. The equilibrium swelling ratio was determined by dividing the average swollen thickness by the average dry thickness.[33] The film was then rinsed with pure Milli-Q water 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 to check for dryness and material loss. All measurements were carried out at 22 ± 3 °C with an ambient relative humidity of 70−73%. The refractive index of the films was measured before and after swelling. The refractive index of the dry films was 1.56 ± 0.01, while the value ranged from 1.36 ± 0.02 to 1.45 ± 0.02 for swollen films.

Atomic Force Microscopy. The surface roughness of the films was determined using atomic force microscopy in contact mode (Nanoscope IIIa, Digital Instruments). The dry films were scanned using a Si_3N_4 cantilever (Nanoprobes, Digital Instruments) with a spring constant of 0.06 N/m at a scan speed of 1.00 Hz with a resolution of 512 × 512. The surface roughness was determined from the RMS value obtained from the Nanoscope software. Surface images of the films were also obtained in contact mode to check for homogeneity and complete film coverage.

Results and Discussion

Multilayer Growth. Multilayer films were prepared by the sequential adsorption of polyallylamine hydrochloride and hyaluronic acid onto planar wafers. Figure 2A illustrates the increase in the dry film thickness as the total number of layers increases for films prepared as a function of the assembly solution pH. Complete surface coverage is dependent on the assembly pH conditions, but was achieved in all cases with less than 10 total layers. The plots indicate that the film thickness is linearly dependent on the number of layers in the film under all assembly pH conditions investigated, indicating that each bilayer deposited is of similar thickness. This was previously observed to be the case for PAH/HA films containing up to 30 layers assembled at pH 4.0, 6.0, 8.0, and 10.0 as reported in our earlier study.[34] However, this behavior is in contrast to that of other multilayer films containing hyaluronic acid. In particular, multilayer films containing polylysine and hyaluronic acid exhibit exponential layer growth.[15,36,37]

Figure 2B shows how the thickness of the films varies with the assembly pH for a fixed total number of layers in the film. The profiles indicate that the maximum film thickness is achieved when the films are assembled under conditions in the low pH regime. The PAH/HA films experience a minimum in their thickness in the mid assembly pH range, but increase again when the assembly conditions become more basic. The trends in the thickness profiles are a result of the influence of the solution pH on the conformation of the polymer chains in solution and ultimately their conformation when adsorbed to the surface,[12,13,15] since the charge density of weak polyelectrolytes is sensitive to the

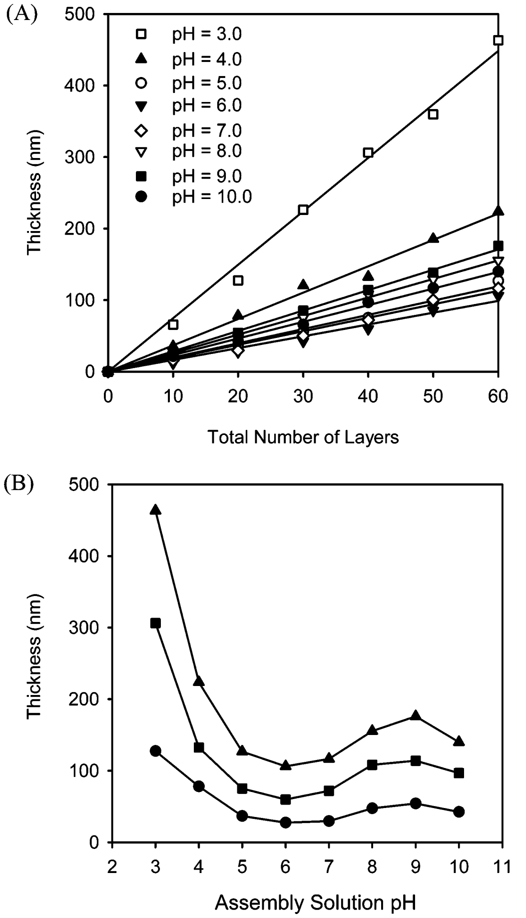

Figure 2. (A) Profile of the dry thickness of PAH/HA films assembled at different pH values as a function of the total number of layers in the film. (B) Dry film thickness of $(PAH/HA)_{10}$ (●), $(PAH/HA)_{20}$ (■), and $(PAH/HA)_{30}$ (▲) films versus assembly solution pH. The uncertainty in the thickness is ±1.2−2.5%, and for the swelling ratio it is ±0.8−1.4%.

local pH environment. When fully charged, the chains are stretched into rodlike conformations because of the electrostatic repulsions of the neighboring functional groups,[38] and the chains tend to adsorb to the surface with this stretched conformation. However, when the solution pH causes a reduction in the charge density of the polymer chains, they adopt more collapsed globular structures and adsorb to a surface in a conformation rich in loops and tails.[39] This leads to an overall increase in the thickness of the polyelectrolyte films. The thickest films are formed when either the HA chains are assembled in a low charge density state under acidic conditions or the PAH chains are only partially charged in solution at high pH values.[15,35] The thinnest films result when both polyelectrolytes are in their fully charged state. Hence, the interplay between the charge density of the HA and PAH chains in solution most strongly influences the thickness profile of the multilayer assemblies as a function of the assembly solution pH.

Acid−Base Equilibria. The sensitivity of weak polyelectrolytes to the solution pH is defined by the acid−base equilibrium of the chains. The dissociation constant associated with this equilibrium is described as an apparent value ($pK_{a(app)}$) and is influenced by a number of factors such as the nature of the functional groups, the proximity of these groups to one another, the nature of the solvent, and the ionic strength of the solution.[40] The charge density of the chains affects their physical properties in solution, but has also been found to greatly influence the overall physicochemical properties of the resulting multilayer assemblies such as the degree of film swelling.[12,13,33,34,41] Hence, it is of importance to learn about the acid−base equilibria of the polyelectrolytes in the film to understand the swelling behavior of these assemblies from a fundamental point of view.

We have previously shown that, in PAH/HA films assembled at pH 3.0, PAH behaves as a weaker base and HA a weaker acid in comparison to their dilute solution $pK_{a(app)}$ values.[34] Here, we extend this study to determine the influence of the assembly solution pH conditions on the acid−base equilibria of the films. Microelectrophoresis was used to study the acid−base equilibria, from which an apparent dissociation constant of the film surface was determined. This technique does not allow us to evaluate the dissociation behavior in the interior of the film. The $pK_{a(app)}$ for films terminated in PAH and those terminated in HA was determined as a function of the assembly solution pH conditions over the range of pH 3.0−10.0. Films containing 11 and 12 layers in total were used for the study. We have previously established that the $pK_{a(app)}$ of the polyelectrolyte terminating the surface is independent of the total number of layers in the film after the first three to four layers are deposited.[15,34,35] The films were assembled on colloidal silica, and the $pK_{a(app)}$ of the surface was determined from the inflection point of the titration curves obtained from plotting the ζ potential of the film as a function of the solution pH (Figures 3 and 4).[35]

The plots indicate that the dissociation constants for both PAH and HA significantly deviate from their dilute solution value upon incorporation into the multilayer films. For all assembly pH conditions investigated, the $pK_{a(app)}$ of PAH decreases from the dilute solution value of 8.56 ± 0.08, indicating that the polycation behaves as a weaker base in the film.[34] This finding is in contrast to previous observations of an increase in the base strength of PAH when assembled in multilayer films with poly(acrylic acid).[35] Hyaluronic acid exhibits weaker acid strength under all conditions investigated, indicated by the increase in its $pK_{a(app)}$ upon incorporation into the multilayer assembly from the dilute solution value of 3.08 ± 0.03.[15] The reduction in the acid strength of HA has previously been observed in multilayer films of HA and poly(L-lysine).[15] The HA chains can adopt secondary ordering in the form of an α-helix in both the solution and solid state.[40] This conformational ordering is dependent upon the local pH environment and is accompanied by stiffening of the chains. The extent of the H-bonded helical content of the HA chains has been found to increase upon incorporation into multilayer films.[15] This hinders the participating protons of the carboxylic acid groups from acid−base exchange, therefore lowering the acid strength of HA in the multilayer assembly. Furthermore, the formation of the helical turns has been found to leave the more hydrophobic regions of the HA chains on the outer surface of the organized structure.[42] Therefore, it is believed that an increase in the degree of conformational ordering of the HA chains contributes to the decrease in the base strength of PAH when

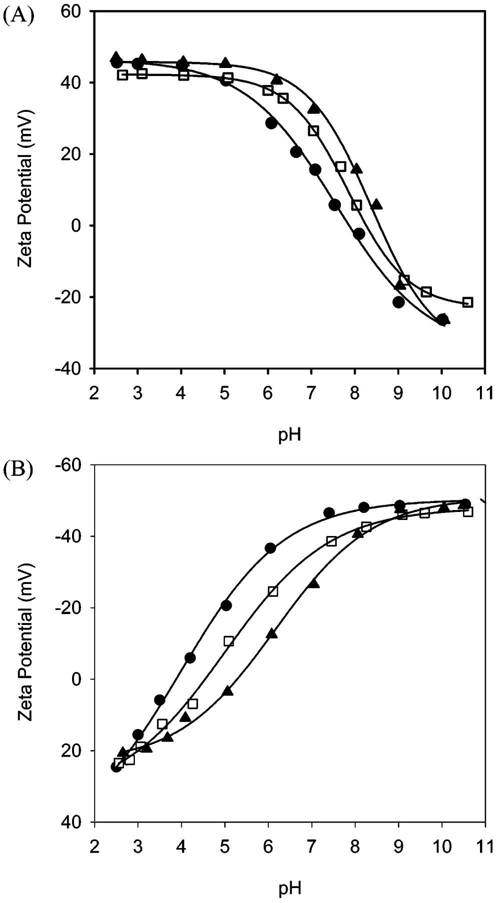

Figure 3. Some example titration curves for (A) $(PAH/HA)_{5.5}$ and (B) $(PAH/HA)_6$ assembled at pH 4.0 (●), pH 7.0 (□), and pH 10.0 (▲). The uncertainty in $pK_{a(app)}$ is ±0.03−0.09.

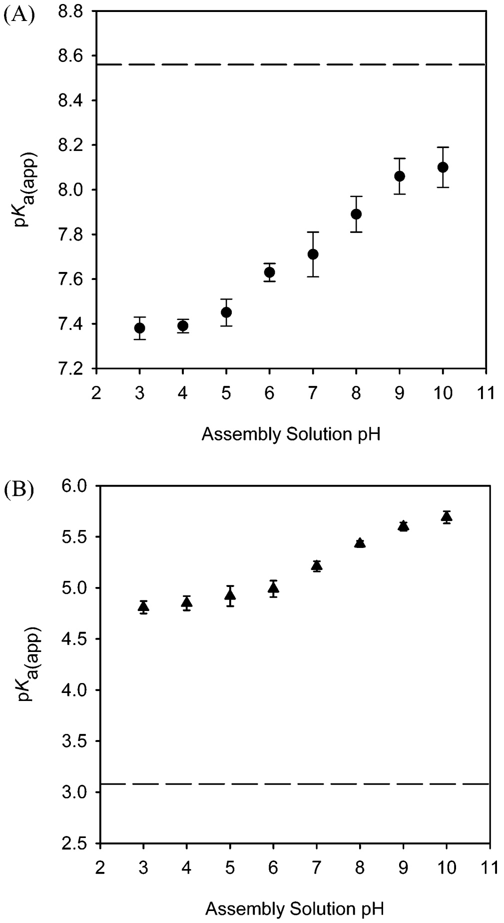

Figure 4. Dependence of the $pK_{a(app)}$ of (A) $(PAH/HA)_{5.5}$ and (B) $(PAH/HA)_6$ on the assembly solution pH. The dashed lines indicate the dilute solution $pK_{a(app)}$.

assembled with HA in a multilayer film. As highly charged PAH chains with a rodlike conformation approach the surface of the film terminated in HA, they begin to experience a change in the local environment caused by the low charge density of the HA surface. This most likely leads to a reduction in the charge density of the PAH chains to lower the energy of the system.

Figure 4 also indicates that the acid−base dissociation behavior of both polyelectrolytes shows some dependence on the assembly solution pH. The acid strength of HA in the films increases when the films are assembled under more acidic conditions, while the base strength of the PAH chains in the film increases when the assembly baths become more basic. These particular trends are also general to other multilayered polyelectrolyte combinations, and can be rationalized by considering the charge density and hydrophilic/hydrophobic character of the surface on which the polymer adsorbs.[15,35] For example, when the assembly conditions become more acidic, films terminated in PAH are highly charged because the amine functional groups of the chains are mostly in the NH_3^+ state. Hence, the approaching HA chains must increase the number of dissociated carboxylic acid groups to compensate for the high population of NH_3^+ groups. This leads to a reduction in the degree of secondary helical formation, in turn liberating the hydrogen-bonded carboxylic acid groups, making their protons more liable for proton exchange. However, when the assembly conditions become more basic, the charge density of the amine groups of the PAH chains on the surface decreases, and the approaching HA chains experience a more hydrophobic environment near the film surface. This promotes secondary conformational ordering of the adsorbing HA chains, hindering the dissociation of their carboxylic acid groups. In a similar manner, the charge density and hydrophilic/hydrophobic balance of the HA surface influence the dissociation behavior of the amine groups on the adsorbing PAH chains. Ultimately, the acid−base dissociation behavior of each individual layer collectively impacts the properties of the film and therefore is a key factor in understanding the overall swelling behavior of these assemblies.

Multilayer Film Swelling. The bulk properties of multilayer films such as the degree of swelling are strongly influenced by the internal characteristics of the material such as the extent of ionic cross-linking, the $pK_{a(app)}$ of the acid−base functional groups, the conformation of the chains, and the hydrophilic/hydrophobic balance.[2] Because these parameters are influenced by many factors involved in the assembly process, and also the environmental conditions to which the films are inevitably exposed, it is important to learn about the extent to which each factor affects the overall swelling behavior of the films. Specifically, the assembly solution pH, the thickness of the films, and the pH and ionic strength of the solution environment. All of the swelling experiments were reversible in nature, tested by swelling the film in question and drying it back to the original dry thickness,

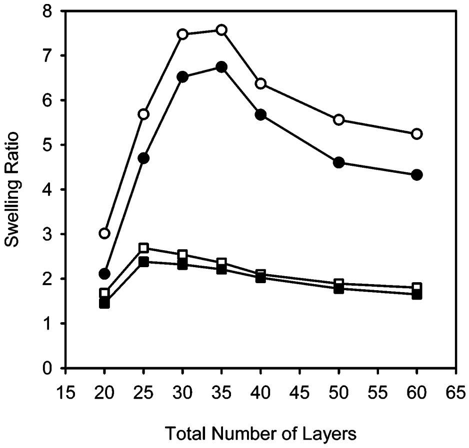

Figure 5. Influence of the film thickness on the swelling ratio for a film assembled at pH 4.0 (circles) and pH 7.0 (squares): swelling solution pH 3.0 (open symbols) and pH 9.0 (closed symbols). The dry film thickness is indicated numerically for films with a given total number of layers. The uncertainty in the thickness is ±1.2−1.8%, and that for the swelling ratio is ±0.4−1.5%.

followed by another swelling cycle to obtain the same swelling ratio.

Effect of Film Thickness on Swelling. The effect of film thickness on the extent to which the assemblies can be hydrated was explored by swelling a series of PAH/HA films assembled at a fixed pH, but with different total numbers of layers. Figure 5 outlines the trends in the swelling behavior of films ranging from 20 to 60 layers. All of the films investigated were homogeneous and exhibited complete substrate coverage as determined by AFM. The multilayering process was carried out at pH 4.0 and 7.0, and the films were exposed to solutions of both pH 3.0 and pH 9.0 to determine whether the results were specific to a particular assembly pH value or to a solution pH. The multilayering process was carried out at pH 4.0 and 7.0, and the films were exposed to solutions of both pH 3.0 and pH 9.0 to ensure the results were specific to neither a particular assembly pH value nor a solution pH. In all cases, the swelling ratio reached a maximum and then began to decrease as the thickness of the films increased. For films assembled at pH 7.0, this maximum is reached when the film contains approximately 25−27 layers, corresponding to a dry film thickness of ~40 nm. This maximum shifts to a dry thickness of ~130 nm (35−37 layers) for films assembled at pH 4.0.

The existence of such a maximum may be a result of a number of different events occurring in the system. For example, it is conceivable that changes in the local environment within the films strongly influence the degree of swelling. As the swelling medium begins to penetrate the film, proton exchange occurs between the solvent and the functional groups of the polyelectrolytes to satisfy the acid−base equilibria of the system. This changes the pH of the penetrating solvent, therefore resulting in a pH gradient within the film.[43] In turn, the pH gradient produces a variation in the degree of dissociation of the amine and carboxylic acid moieties as a function of the depth normal to the solvent front. This leads to differences in the degree of chain extension resulting from electrostatic repulsions between like charges on the chains and therefore impacts the degree of swelling. Since the pH gradient is dependent on the thickness of the film, so too is the swelling ratio.

Another factor that may lead to thickness-dependent swelling is the mechanical forces operating in the system.[26,44] The polyelectrolyte multilayer film acts like a semipermeable membrane, absorbing water and causing the swelling of the system. The movement of water from the solvent phase to the polymer phase leads to the creation of an osmotic pressure in the film.[23] However, the limitations on the extent to which the polymer chains can stretch during swelling produces a retraction force.[26] A balance between the two forces exists when the film swelling reaches an equilibrium state. The concentration of solvent within the matrix of the film influences the balance between extension and retraction forces. In addition, the thickness of the films and the ionic cross-link density determine the concentration and depth of penetration of the water molecules in the polymer matrix, and therefore also the overall swelling of the films.

The thickness-dependent swelling profiles of the PAH/HA films exhibit a shift in the maximum swelling ratio depending on the solution pH conditions used during film assembly. The maximum swelling ratio is reached at a dry film thickness of ~40 nm (25−27 layers) when the assembly pH is 7.0, but does not occur until the film is ~130 nm thick in the dry state (35−37 layers) for films assembled at pH 4.0. The shift in the maximum is attributed to the differences in the internal structure and characteristics of the film. Both PAH and HA are highly charged in solution at pH 7.0. Therefore, the chains adsorb onto a surface with a rodlike conformation. The high charge density results in a high population of ionic cross-links stitching the films together. However, at pH 4.0 the HA chains are only partially charged in solution and adsorb in a globular conformation with some helical content. The resulting films contain fewer cross-links because of the lower charge density of the HA chains, yielding more free volume in the films. Consequently, the films assembled at pH 4.0 contain more loops and tails that can stretch to a greater extent than the tightly stitched film assembled at pH 7.0.[45] This also means there are more free functional groups available for proton exchange when the films are assembled at pH 4.0 leading to greater repulsive interactions. All of these factors influence the balance between the expansion and retraction forces operating in the films. In addition, the concentration of free functional groups also influences the pH gradient that is created within the polymer matrix. The effect that the assembly pH conditions have on the internal structure of the films and their resulting swelling behavior is discussed in further detail in the following section. Although the extent of swelling is also dependent on the pH of the swelling medium, the overall trends in the swelling with variable film thickness are independent of the pH of the solution.

Effect of Assembly Solution pH on Swelling. A series of $(PAH/HA)_{15}$ films were assembled from solutions at different pH values and then exposed to an aqueous environment at a fixed pH to determine the influence of the assembly pH conditions on the extent of film swelling. This study was done using films of 30 layers because this thickness range

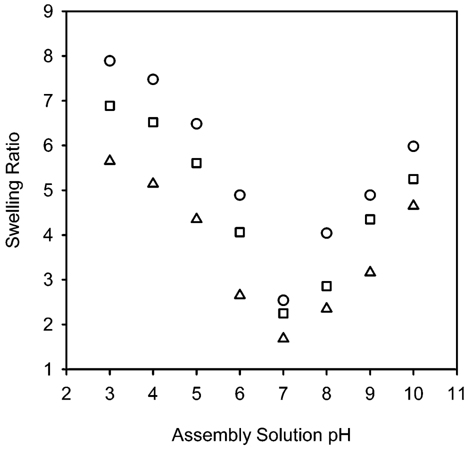

Figure 6. Dependence of the swelling ratio on the assembly pH conditions for films exposed to solutions of pH 3.0 (○), pH 7.0 (△), and pH 9.0 (□). The uncertainty in the swelling ratio is ±0.7−1.1%.

yields the maximum degree of swelling as previously shown. Figure 6 summarizes the degree to which the films swell when exposed to water at neutral pH and also to acidic (pH 3.0) and basic (pH 9.0) aqueous solutions for comparison. The swelling ratio is minimal for films assembled under conditions in the neutral pH range, but increases in comparison when the assembly is done in either acidic or basic conditions.

As previously discussed, these trends are related to the internal structure of the films. When the solution conditions are neutral, the polyelectrolyte chains are strongly charged with rodlike conformations in solution. The resulting films have layers that are tightly stitched together with ionic cross-links. The film matrix has little free volume, a low population of loops and tails, and a limited number of free functional groups. Consequently, upon exposure to aqueous solution, these films are hindered from swelling. Since there are very few functional groups available for proton exchange, there will be only minimal stretching from weak repulsive interactions. Even when these groups do become charged in solution, chain stretching is limited because of the very short segments of the chains between cross-links. The reduced free volume in the films also hinders the influx of water into the matrix, which further constrains the swelling process. However, the ionic cross-links holding these films together are dynamic and are sensitive to the local solution environment. They can break and re-form during the swelling process.

The reorganization in the films that results from the breaking and re-forming of ionic bonds is indirectly observed by monitoring their surface roughness before and after exposure to the swelling solution.[34] Table 1 summarizes the root-mean-square roughness of the PAH/HA films after exposure to the swelling solutions studied using AFM surface analysis. Subjecting the films to acidic or basic conditions produces an increase in roughness, which indicates that a number of ionic bonds are broken, increasing the size and population of loops and tails in the film. However, when the films are exposed to a neutral pH solution, the roughness slightly decreases because the increase in charge density of both the amine and carboxylic acid functional groups leads to the formation of ionic cross-links in the films, further hindering the swelling process.

Table 1. Root-Mean-Square (RMS) Roughness of PAH/HA Films before and after Swelling

	RMS roughness[a] (nm)			
(PAH/HA)$_{15}$	before	after exposure to swelling solution		
assembly pH	swelling	pH 3.0	pH 7.0	pH 9.0
4.0	12.6	14.6	12.0	13.7
7.0	3.26	5.64	3.41	4.31
10.0	10.2	13.1	9.8	11.3

[a] Uncertainty in the root-mean-square roughness of ±0.01−0.04 nm.

Swelling is much less restricted for PAH/HA films when the multilayering is done with at least one of the polymers in a reduced charge state because this yields films rich in loops and tails, functional groups with exchangeable protons, and free volume stemming from the more compact conformation of the low charge density chains. The highly dissociated polyelectrolyte chains of opposite charge are forced to reduce their charge fraction to lower the energy of the system as they approach the film surface of low charge density, causing these chains to adsorb in more of a globular conformation. Films with such structures are formed when the assembly is performed under acid conditions with the HA chains with a reduced charge fraction and PAH chains highly charged in solution. Similar results are also observed when the films are prepared from basic solutions because, in this case, the PAH chains have a lower charge density in solution and the HA chains are fully dissociated. This rationale explains the increase in the swelling ratio as the assembly solution pH becomes more acidic and also more basic.

It is speculated that the swelling ratio is larger for PAH/HA films prepared under highly acidic conditions (pH ≈ 3−5) relative to those assembled from highly basic solutions (pH ≈ 9−10), regardless of the swelling solution pH, primarily because of the conformational differences between the PAH and HA chains when assembled under reduced charge density conditions. Unlike the PAH, the HA chains have the ability to adopt hydrogen-bonded helical conformations, accompanied by chain stiffening, when their charge fraction is low, making it difficult for the participating carboxylic acid groups to undergo proton exchange.[23] In contrast, the PAH chains are more flexible and liable for proton exchange as they approach the film surface, even when they are in a globular conformation. Hence, films prepared with weakly charged HA chains are thicker than those assembled with PAH in the reduced charge state because much more HA must adsorb to neutralize the surface charge and still maintain charge overcompensation. Consequently, the films prepared with HA with a reduced charge fraction contain more free volume, a higher population of loops and tails, and more free functional groups. All of these factors lead to an increase in the extent to which the films swell. Although the trends in the film swelling with assembly pH are the same for each swelling medium investigated, there is some dependence of swelling on the pH of the medium. The details of this phenomenon are outlined in the following discussion.

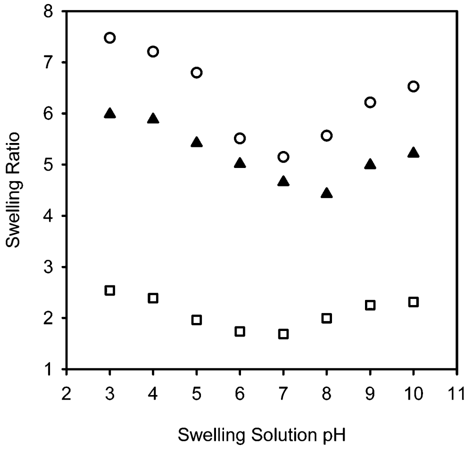

Figure 7. Influence of the swelling solution pH on the degree of $(PAH/HA)_{15}$ film swelling for films assembled at pH 4.0 (○), pH 7.0 (□), and pH 10.0 (▲). The uncertainty in the swelling ratio is ±0.6−1.0%.

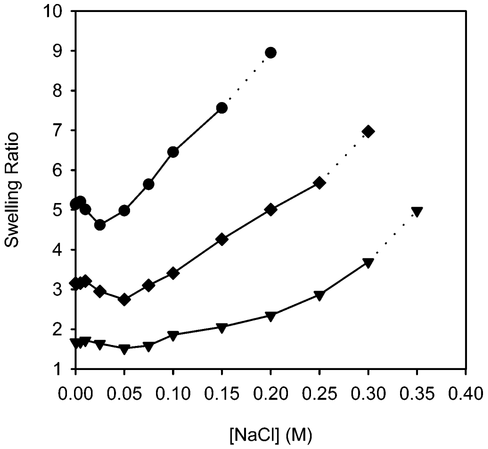

Figure 8. Effect of the NaCl concentration in the swelling medium (pH 7.0) on the ratio of film swelling for films assembled at pH 4.0 (●), pH 7.0 (▼), and pH 10.0 (◆). The dotted line indicates the onset of film decomposition. The uncertainty in the swelling ratio is ±0.4−1.2%.

Swelling Medium pH. The hydration capability of the PAH/HA films is not simply a product of the physical properties of the films, but is also related to how the swelling medium interacts with both polyelectrolytes in the assembly. Since varying the solution properties is known to have a strong influence on the swelling of polyelectrolyte multilayer films,[15,31,32] we examined the effect that the solution pH has on PAH/HA film swelling. Figure 7 illustrates the variation in the swelling ratio for films containing 30 layers, assembled at both pH 4.0 and pH 7.0 on the pH, with a change in the swelling solution pH in the range of pH 3.0−10.0. Notably, decomposition of the assemblies was observed when the films were exposed to solution environments more acidic than pH ≈ 2.5−3.0 and more basic than pH ≈ 10.0−10.5. The profiles for both films exhibit a high level of swelling in an acidic environment, with a gradual decrease in swelling as the solution pH approaches neutrality. It is followed by an increase in the swelling ratio as the swelling medium becomes more basic. The trends in the swelling ratio with variable swelling solution pH are consistent with those previously observed for the pH-dependent swelling of a $(PAH/HA)_{10}$ film assembled at pH 3.0.[34]

The influence of the swelling solution pH is essentially a product of the acid−base chemistry of the amine and carboxylic acid functional groups in the film. When the solution pH is at or near neutrality, the $pK_{a(app)}$ values of PAH and HA dictate that the majority of free functional groups of each polymer will be charged. In films containing only positively or negatively charged species, this would lead to extension of the chains due to electrostatic repulsions between the charged moieties and ultimately to an increase in equilibrium swelling of the system.[27,28] However, in polyampholytic films such as the PAH/HA assemblies, under these conditions, the close proximity of the positive and negative charges effectively screens the electrostatic repulsive forces, resulting in minimal swelling of the system.[27] Because of the differences in the dissociation constant of PAH and HA, in acidic and basic environments one of the polyelectrolytes will always have a lower charge density than the other. This imbalance leads to electrostatic repulsion between the charged species in excess and consequently to an increase in the overall swelling of the film. The maximum in the swelling behavior at low pH values suggests that, when fully charged, the PAH chains undergo more chain extension than HA chains when they are fully charged. This is probably due to reduced flexibility of the chains and a hindering of the chain extension by the secondary helical structure of the HA molecules.

Notably, the solution pH corresponding to minimum swelling shifts depending on the pH conditions used for film preparation. This phenomenon stems from the different pH conditions required to balance the concentration of positive and negative charges in the film, which is a product of the dependence of the $pK_{a(app)}$ values on the pH of the assembly solutions. The pronounced difference in the magnitude of film swelling at any given solution pH for films prepared under different pH conditions is attributed to a combination of the differences in their thickness, and internal structures as previously discussed.

Effect of Swelling Medium Ionic Strength on Swelling. The charge density and resulting behavior of the weak polyelectrolytes in the multilayer assembly are influenced not only by the pH of the swelling medium, but also by the ionic strength. The presence of salt in the solution can create electrostatic screening for the charge on the free functional groups, but can also influence both the dynamics of the ionic bonds holding the layers together and the forces operating in the system.[27,29,30] In fact, previous studies on the effect of salt on the swelling of polyelectrolyte multilayer thin films have shown that high salt concentration can lead to irreversible swelling and overall material loss from the films.[29] To determine the effect of the ionic strength of the solution on the swelling of the PAH/HA films, the salt concentration of the swelling medium was varied while fixed neutral conditions (pH 7.0) were maintained. The multilayer systems used in this study contained 30 layers and were assembled at pH 4.0, 7.0, and 9.0. The plot of the swelling ratio versus the salt concentration in the swelling solution is shown in Figure 8.

The swelling profile indicates that the films are relatively unaffected by the presence of added salt at very low concentration of NaCl. This behavior has previously been observed in other multilayer systems and is attributed to the inability of a significant concentration of the ions to penetrate into the film.[29,30] As the NaCl concentration is increased, the equilibrium swelling thickness begins to decrease. At this point, the electrostatic screening of the charge on the free functional groups in the film by the salt ions causes the stretched chain segments to collapse by reducing the repulsive forces between like charges.[27] The salt concentration at which this occurs depends on the pH conditions used to assemble the films because this dictates the concentration of free functional groups and the ratio between the amine and the carboxylic acid species. However, this decrease in swelling continues only within a limited salt concentration range. Further increase in the added NaCl content causes the swelling ratio to increase. This phenomenon is in contrast to what is typically observed for polyelectrolyte hydrogels, but is known to occur in both polyelectrolyte multilayer films and polyampholytic hydrogels.[27−30,46,47] The two latter systems contain both positively and negatively charged moieties within the same matrix. It is believed that, in both cases, the increase in the ion concentration leads to an increase in the osmotic pressure within the matrix, consequently resulting in a higher degree of swelling.[46,47] However, for polyelectrolyte multilayer films an additional factor promotes film swelling. The ionic bonds that hold these films together are dynamic and are strongly affected by the presence of the salt ions.[29] The bonds begin to break when the salt concentration is increased, which lengthens the chain segments and increases the free volume available to accommodate the influx of water into the system. However, because of the dynamic nature of these bonds, the structural integrity of the films can withstand only a certain ionic strength, after which point the films begin to deconstruct and reversible swelling is no longer possible.[29] As indicated by the swelling profiles, the salt concentration at which this occurs depends on the pH conditions used to assemble the films. This stems from the fact that the concentration of free functional groups within the film and the density of ionic cross-links are highly dependent on the assembly pH conditions. Decomposition occurs at lower salt concentration for films with fewer ionic cross-links and higher concentrations of free functional groups.

The magnitude of the change in film swelling induced by salt added to the swelling solution is related to the pH conditions used to assemble the films. This occurs because the overall effect that the ionic strength of the swelling solution has on the hydration properties of the films is related to the internal structure of the films. This includes such factors as the thickness of the films, the concentration of free functional groups, and the extent of ionic cross-linking between layers. The added salt concentration had the greatest effect on films assembled at pH 4.0 because such films have the highest population of free functional groups with the lowest ionic cross-link density. In comparison, the degree of swelling of films assembled at pH 7.0 is affected the least because the high population of ionic cross-links hold the layers together tightly and also because the effect of electrostatic screening by the salt ions is lower since there are fewer free functional groups within the film matrix.

Conclusion

In this paper, we have shown that the swelling behavior of multilayer films containing the biopolymer hyaluronic acid is significantly dependent on both the properties of the film and the swelling medium, with substantial swelling observed under certain conditions. Both PAH and HA are weak polyelectrolytes, and as such their properties in solution are highly sensitive to pH and ionic strength. In fact, we found that the $\mathrm{p}K_{\mathrm{a(app)}}$ of both polymers significantly deviates from that in dilute solution when they are assembled into multilayer films. The base strength of PAH and the acid strength of HA decrease upon incorporation into the films, and are dependent on the solution pH conditions used during the assembly process. Their dissociation behavior in solution essentially dictates the internal structure of the films such as thickness, ionic cross-link density, free volume, and population of free functional groups. The internal structure, in combination with the dissociation behavior of the free functional groups within the film, determines the overall swelling behavior of the films. We have found that the degree of film swelling is related to the total number of layers and assembly solution pH, because both of these factors contribute to the structure of the film matrix. In addition, the pH and ionic strength of the swelling medium were also found to influence the swelling behavior of the films by affecting the electrostatic forces operating in the hydrated film and also the dynamics of the ionic cross-links holding the layers together. Our studies suggest that it is possible to control and tune the swelling behavior of such multilayer assemblies by learning how each parameter of the system influences the hydration capacity of the films. This knowledge is important to gain to apply these materials in many applications such as controlled release, membrane filtration, and biomaterial coatings.

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