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pH-Dependent Loading and Release Behavior of Small Hydrophilic Molecules in Weak Polyelectrolyte Multilayer Films

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ABSTRACT: The pH-dependent loading and release behavior of small hydrophilic molecular probes in polyallylamine and hyaluronic acid multilayer films was investigated. These studies were carried out using the water-soluble dyes Indoine Blue and Chromotrope 2R, and their loading and release profiles were followed with UV−vis spectroscopy. The results of these studies suggest that the incorporation and release of material from such films depend on the degree of film swelling, the ability of the dye molecules to aggregate in the film, as well as the attractive and repulsive interactions occurring between the probe molecules and the acid−base functional groups in the films. Both the degree of dissociation of the acid−base functional groups and the extent of film swelling are pH-sensitive. As a result, the amount of each dye incorporated in the film also depends on the pH conditions, with a maximum loading capacity of 3.8 $\times$ 10^{-3} mg/cm^3 of Chromotrope 2R and 5.7 $\times$ 10^{-2} mg/cm^{-3} of Indoine Blue. In turn, we found that by manipulating the pH of the release solution it is possible not only to control the rate of the release of the molecular probes but also to regulate the amount of probe material released. In fact, completely trapping the incorporated probes in the multilayer films was also successfully demonstrated. These findings can be rationalized by examining the interactions of the dye molecules with the polyelectrolytes, the ability of molecules to form J-aggregates, and pH-dependent physicochemical properties of the multilayer system, such as the acid−base equilibria of the polyelectrolytes and the swelling behavior of the films. It is believed that this information is fundamentally important to understanding of how to effectively manipulate the films for potential controlled release applications.

Introduction

The layer-by-layer self-assembly technique of preparing polyelectrolyte multilayer thin films has proven to be a viable technique that can yield novel multifunctional materials.[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.[3] The buildup of material is achieved through charge overcompensation after the deposition of each layer. The simplicity of this film fabrication approach has led to the extension of this concept to the preparation of multilayer systems incorporating more than just simple polyelectrolytes. Examples of other charged species used to create hybrid materials include small molecule dyes, colloidal particles, enzymes, and carbon nanotubes.[4−7] 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 assembly conditions such as pH, ionic strength, polymer functionality, and polymer concentration.[8−10] Current efforts in the field are focused on utilizing the rich suite of properties of multilayer films in a variety of applications including ion-selective membranes, antifouling coatings, sensors, catalysis, and small molecule delivery systems.[1,11−15]

In recent years, much effort has been devoted to attempting to control the properties and function of multilayer films through the use of pH-responsive polymers. Many of the physicochemical properties (i.e., acid−base dissociation, surface wettability, surface roughness, film morphology, surface friction, and layer thickness) of multilayer films composed of weak polyelectrolytes undergo dramatic variations with only slight changes in the solution pH and ionic strength conditions used in the assembly process.[8,16−21] In fact, Xia and Granick recently showed that the charge density of a single weak polyelectrolyte layer embedded at the bottom of a 10-layer film is greatly influenced by the local environment at the surface layer.[22] The flexible nature of such films has intrigued many to attempt to harness these properties for a variety of applications.[1,23−25]

One of the most promising potential uses for pH-responsive multilayer films is in the area of controlled release, with implications in drug delivery, personal care products, sensors, and filtration.[26−33] Although the use of multilayer films in controlled release applications has received considerable attention,[34−36] only a few studies have reported using weak polyelectrolyte multilayer films for pH-dependent release.[26−28] One such study by Sukhorukov et al. involved the use of hollow microcapsules formed by multilayering on colloidal particles followed by the removal of the colloidal core.[26] They reported that microcapsules composed of at least one weak polyelectrolyte (polyallylamine/polystyrenesulfonate (PAH/PSS) multilayer films) can be made to be porous in nature depending on the local pH environment. A change in pH can be used to “open” and “close” the pores, which was used to load and release macromolecules from the capsule core. Hiller and Rubner also showed that they could exercise some control over the loading and release of small anionic molecules from PAH/PSS multilayer films using a combination of pH-dependent film swelling and local hydrophobicity in the films.[27] In a related study, Chung and Rubner successfully dem-

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onstrated control over the release rate of anionic dye molecules from weak polycation/weak polyanion films of polyallylamine and poly(acrylic acid) using release solution pH, ionic strength, and highly cross-linked capping layers.[28] However, these studies demonstrated neither pH dependence on the amount of dye released nor the ability to trap the material in the films indefinitely under one set of conditions and release under another set of conditions. In addition, the results were not placed in the context of the changes in the acid–base equilibria of the polyelectrolytes upon incorporation into multilayer assemblies. Notably, one study has shown that it is possible to complex positively charged Rhodamine 6G with poly(methacrylic acid) prior to the formation of hydrogen-bonded multilayer assemblies with poly(ethylene oxide) and to retain the dye in the film at the pH it was assembled.[37] However, increasing the solution pH leads to the instability and deconstruction of the films.

From the extensive research over the past few years on multilayer thin films, it is clear that unlike single layer films or polymer gels there is no general description of the physicochemical properties of different multilayer systems, especially for those assembled from weak polyelectrolytes.[1,38,39] One of the simplest examples of this is found in the buildup mechanism, which in some cases is linear in nature, whereas other polyelectrolyte combinations exhibit exponential growth.[8,20,40] This can even be the case for the buildup of films using the same two polymers but different assembly conditions.[41] For weak polyelectrolyte multilayer systems, the acid–base dissociation behavior of the polymer films is found to be a major factor controlling the overall properties of the films. A few studies have reported significant shifts in the charge density of weak polyelectrolytes in multilayer films.[8,17,22,42] However, recent studies have shown that the deviations in the acid–base equilibria from the dilute solution values can be in the direction of increasing or decreasing acid/base strength depending on the particular polyelectrolyte combination in the film.[20,43,44] For example, poly(acrylic acid) behaves as a stronger acid and poly(allylamine hydrochloride) a stonger base when assembled together in a multilayer film in comparison to their acid–base equilibria in solution.[43] In contrast, hyaluronic acid and poly(L-lysine) exhibit reduced acid and base strength, respectively, upon assembly into a multilayer film.[20] The $pK_{a(app)}$ shifts range from 1 to 4 pH units upon incorporation into a multilayer assembly. The complex nature of such films obviously possess a challenge when one tries to choose a polyelectrolyte multilayer system for a particular application. Thus, one must first try to learn more about the fundamental physicochemical properties of PAH/HA multilayer films before it is possible to understand how to use these films in the context of controlled release. In particular, it is intuitive that the thickness of the film, the dissociation behavior of the free acid/base functional groups of the polyelectrolytes in the film, and the degree of film swelling in an aqueous environment will strongly influence the transport of hydrophilic material in the films. In addition, one must also try to understand how the dye molecules, which can be viewed as models for any small water-soluble organic compound containing a charged moiety, behave within the confines of the polyelectrolyte multilayer film.

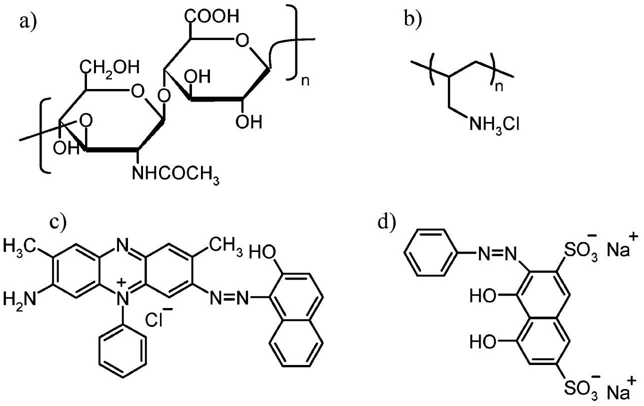

Figure 1. Chemical structures of polyelectrolyte repeat units and dye molecules: (a) hyaluronic acid, (b) poly(allylamine hydrochloride), (c) Indoine Blue, and (d) Chromotrope 2R.

In this paper, we attempt to extend the use and understanding of weak polycation/weak polyanion multilayer films for the application of controlled loading and release of small hydrophilic molecules. We show that the loading and release of both a cationic and an anionic dye species from polyallylamine/hyaluronic acid (PAH/HA) multilayer films are dependent on the swelling-controlled diffusion of the small molecules through the film, the ability of the dye molecules to aggregate, and the balance between electrostatic attractive and repulsive interactions between the dye molecules and the acid/base functional groups in the film. It is possible to use pH shifts to control not only the rate at which the dye molecules escape the film but also the amount of dye that is released. In addition, we demonstrate the first successful attempt at trapping the water-soluble dye molecules in polyelectrolyte multilayer films at one pH and achieving complete release at another pH. These findings are discussed in terms of the acid–base dissociation of the polyelectrolytes in the films and the pH-dependent film swelling.

Experimental Section

Materials. Poly(allylamine hydrochloride) (PAH) (MW = 60 000 g/mol) was purchased from Polymer Sciences, Inc., and used as received. Sodium hyaluronate (MW = 150 000–250 000 g/mol) was obtained from Sigma and converted to the acid form (HA) via hydrolysis with HCl. For film preparation, aqueous polymer solutions (Millipore, Milli-Q, resistivity of 18.2 MΩ·cm) were made using a concentration of 2.0 mM of polyelectrolyte (based on repeat unit molecular weight) and 0.01 M NaCl. The salt was added to promote polymer adsorption. The solution pH was adjusted with either HCl or NaOH. The films were deposited on colloidal silica (70–100 nm, Nissan Chemical Industries), polished ⟨100⟩ silicon wafers (WaferNet, Inc.), and Fisher brand glass microscope slides. The colloidal silica was obtained as a 40 wt % aqueous solution (pH = 9–10), which was diluted and adjusted to an appropriate pH value. Both the wafers and glass slides were cleaned in a bath of 25% H_2SO_4 and 75% CrO_4 followed by a thorough rinsing with purified water prior to use. Chromotrope 2R (dye content ~ 75%) and Indoine Blue (dye content ~ 70%) were purchased from Aldrich and were used as received. The chemical structures of the polyelectrolyte repeat units and the dye molecules are shown in Figure 1.

Thin Film Preparation. Polyelectrolyte multilayer films were assembled on solid supports with the aid of 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.[45] 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 number of layers was 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.

Multilayer films were assembled on colloidal silica by repeated adsorption, washing, and centrifuging steps, using the same protocol as previously outlined.[43] In the adsorption steps, solutions of 2.0 mM polyelectrolyte (based on repeat unit molecular weight), 0.01 M NaCl, and 0.5 g/L of colloidal silica were used. After 30 min of adsorption time, the suspension was centrifuged and the supernatant was removed. The polymer-coated particles were washed with purified water. The wash solutions were adjusted to the same pH as the polyelectrolyte solutions. No salt was added to the wash baths. The particles were redispersed by sonication for 5 min, and the suspension was allowed to stand for 30 min followed by centrifugation. This wash procedure was completed three times to remove unabsorbed polyelectrolyte to prevent the formation of polyelectrolyte complexes in solution. The deposition and wash steps were carried out until a maximum of 12 layers (six 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.

Film Thickness and Swelling. 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 and refractive index are reported as the statistical mean of 15 measurements after subtraction of surface oxide layer thickness (measured average of 28 ± 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°.[20] The film is fixed to the bottom of the chamber with Teflon brackets. The thickness of the films was first determined in air, and then water was added to the chamber. The system was allowed to equilibrate for approximately 4 h prior to determining the swollen thickness of the film. The equilibrium swelling ratio was determined by dividing the average swollen thickness by the average dry thickness. The film was then rinsed with pure Milli-Q H_2O 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. In a similar manner, the film thickness was measured after the loading and release experiments to check for material loss.

Acid−Base Titration. The acid−base titration curves were obtained by determining the zeta potential of the films layered on colloidal silica as a function of the pH of the colloidal suspension. 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 ZetaPlus instrument (Brookhaven Instruments Corp.). The zeta potential was calculated from the Smoluchowski equation using an average mobility value based on 20 measurements. The inflection point on the zeta potential vs pH curves was taken as the $pK_{a(app)}$ value of the surface of the films as previously established.[20,43]

Surface Imaging. The film morphology and surface roughness were determined using atomic force microscopy in contact mode (Nanoscope IIIa, Digital Instruments). The 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.

Dye Loading. Stock solutions of 0.15 g/mL of Chromotrope 2R and 0.15 mg/mL of Indoine Blue were prepared using Milli-Q H_2O and were pH adjusted using either NaOH or HCl. These dye concentrations were chosen because they were determined to be high enough to ensure maximum dye loading. The absorbance of the dye solutions was not sensitive to the pH. The films were immersed in the dye solutions at room temperature for 24 h to allow for equilibrium. The films were then rinsed with a stream of Milli-Q H_2O for 5 min to remove excess dye. The films were then dried under vacuum for 24 h prior to use. The amount of dye loaded into the films was determined using a Varian Cary 300 spectrophotometer (scan rate of 100 nm/min). The absorbance values obeyed Beer's law and were converted to dye concentrations using calibration curves (Supporting Information). Indoine Blue has a maximum absorbance at 550 nm, while $\lambda_{max} = 508$ nm for Chromotrope 2R in solution. The λ_{max} for both Indonine Blue and Chromotrope 2R in solution was independent of the dye concentration.

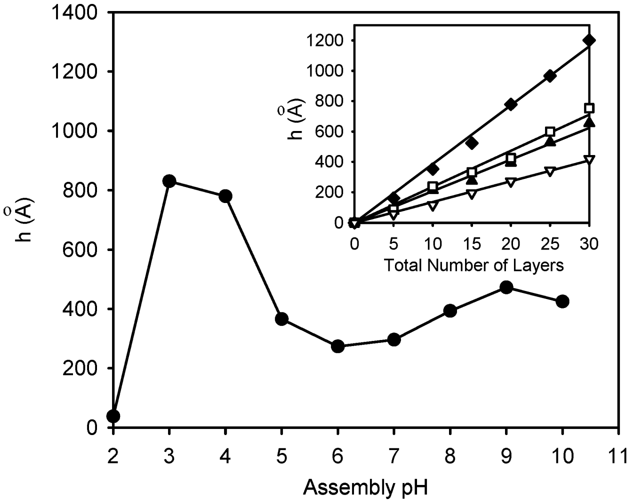

Figure 2. Dependence of $(PAH/HA)_{10}$ film thickness on the assembly solution pH. Insert plot shows the linear growth of PAH/HA films as the total number of layers is increased for films assembled at pH = 4.0 (◆), pH = 6.0 (▽), pH = 8.0 (▲), and pH = 10.0 (□). Error in thickness of ±5−16 Å.

Dye Release. The release of the dye from the films was also followed using UV−vis spectroscopy. The maximum absorbance of the dye in the film was first measured. The film was then immersed into a beaker containing 500 mL of purified water (adjusted to the desired pH), which was mechanically stirred during the release process. The film was removed at periodic intervals and dried with a stream of compressed air, and the absorbance was measured. The solution was replaced with fresh water every 20 min.

Results and Discussion

Film Thickness. The thickness and architecture of polyelectrolyte multilayer films have previously been reported to have a major influence on the incorporation and release of material from such films.[26,28] Figure 2 contains a profile of the thickness of $(PAH/HA)_{10}$ multilayer films assembled using different pH conditions. The film thickness varies from ∼300 to 800 Å. The films are thickest when they are assembled under acidic conditions, the pH range where hyaluronic acid is only partially charged in solution. Notably, the inset figure indicates that PAH/HA multilayer films grow in a linear fashion, unlike multilayer films of hyaluronic acid and poly(L-lysine), which exhibit exponential growth.[20,46] This indicates that the PAH/HA film architecture is composed of bilayer repeat units that are relatively equivalent in thickness throughout the entire film profile.

Precedence has previously been established for the fact that multilayer thin films composed of weak polyelectrolytes can exhibit a wide range of thickness values when assembled under different pH and ionic strength conditions, but with the same total number of layers.[8,16] This phenomenon manifests itself because the charge density of such polyelectrolytes is strongly dependent on solution pH and ionic strength, which in turn influences the conformation of the polymer chains in solution and ultimately their conformational state upon adsorption on the substrate. When one or both of the polyelectrolyte species are assembled in the film under reduced charge density, the films tend to be thicker with fewer ionic cross-link pairs because of the globular nature with which the polymer chains adsorbed onto the surface.[16] In the case of PAH/HA multilayer films assembled using different solution pH values, the thickness profile exhibits two maxima. One of these occurs in the acid region of the pH scale, where HA is weakly charged in solution. The other maximum appears at higher solution pH values, in which case the PAH chains have a reduced charge density. This suggests that the interplay between the charge density on the polycation and polyanion chains strongly influences the resulting thickness of weak polyelectrolyte multilayer films. Notably, it is not possible to assemble stable, homogeneous films at pH values below pH $\sim$ 2.5 and above pH $\sim$ 10.0 because the low charge density of the polyanion and polycation chains, respectively, prevent multilayer growth.

The previous studies on controlling the loading and release of material from multilayer films reported that that the highest loading capacity was achieved with the films that were thickest and had a low ionic or covalent cross-link density.[28] On the basis of this, and all of the above-mentioned thickness properties of PAH/HA films, the loading and release studies reported in this paper were carried out using films assembled at pH = 3.0 because this pH regime produced the thickest films, which are held together with the fewest ionic cross-links.

Acid−Base Equilibria. The variable charge density of weak polyelectrolytes not only influences the overall thickness of the multilayer assemblies but also is known to affect many of the other physicochemical properties of the films.[8,16,20] Therefore, it is of great interest to learn about the acid−base equilibria occurring in the films in order to understand their behavior from the fundamental view of the internal electrostatic environment in the films. To determine the dissociation constants of the polyelectrolytes in the films, similar to previous studies,[20,43] multilayers were assembled on colloidal silica at pH = 3.0, and the zeta potential of a series of aqueous suspensions of the particles was determined as a function of the solution pH. The resulting plots represent titration curves from which the dissociation constant of the surface of the film is determined from the inflection point (Supporting Information). The assembly of weak polyelectrolyte multilayer films on colloidal silica is only possible for a limited total number of layers before flocculation becomes an issue.[20,43] In this study, 12 total polyelectrolyte layers ($(\mathrm{PAH/HA})_6$) were deposited on 70−100 nm diameter particles at pH = 3.0. Although it was not possible to determine the dissociation behavior of $(\mathrm{PAH/HA})_{10}$ films, previous studies indicate that the p$K_{\mathrm{a(app)}}$ of the surface of weak polyelectrolyte films is only dependent on the total number of layers in the films for films containing fewer than $\sim$5 layers, after which point the p$K_{\mathrm{a(app)}}$ remains relatively constant for films terminated with the same polyelectrolyte species because the film surface is homogeneous.[20,43] The dissociation constants of PAH/HA films containing 7−12 layers were determined, and the results are summarized in Table 1.

Table 1. Dissociation Constants for PAH/HA Films Assembled at pH = 3.0

no. of layers	surface polyelectrolyte	p$K_{\mathrm{a(app)}}$[a]
7	PAH	7.42
9	PAH	7.48
11	PAH	7.38
8	HA	4.58
10	HA	4.74
12	HA	4.81

[a] Uncertainty in p$K_{\mathrm{a(app)}}$ of ±0.03−0.08.

The p$K_{\mathrm{a(app)}}$ values for PAH and HA in solution are 8.56 ± 0.08 and 3.08 ± 0.03, respectively.[20,43] Upon incorporation into the multilayer films, poly(allylamine hydrochloride) becomes a weaker base and hyaluronic acid a weaker acid in comparison to their dilute solution behavior. This result is not surprising for hyaluronic acid since a similar reduction in its acid strength was observed in multilayer films with poly(L-lysine).[20] However, it has previously been found that PAH becomes a stronger base when assembled with poly(acrylic acid) in a multilayer film.[43] It is well-known that in aqueous solutions of pH = 3.0 HA is only partially dissociated, and as such the chains adopt some degree secondary conformational ordering in the form of a helical structure.[47] The formation of such ordered structures requires intramolecular hydrogen bonding, involving the protons on the carboxylic acid moiety. Hence, this secondary ordering process hinders the possibility of acid−base dissociation of the participating protons. In addition, the intra- and intermolecular interactions of the HA chains increase the viscosity of the HA solution, which makes it more difficult for the chains to reorganize themselves.[47] The chain mobility is further hindered when they adsorb onto the surface of the substrate. This makes it even more difficult for the removal of the carboxylic acid protons involved in the hydrogen-bonded secondary conformational structure. There has also been some evidence in the literature that indicates that HA has a significant hydrophobic character at lower pH values when the chains have a higher helical content.[48] We propose that this hydrophobic character and the lower acid strength of HA contribute to the reduction in the base strength of PAH. In solution at pH = 3.0, the PAH chains are fully charged and are in a more rodlike conformation. However, as they approach the surface of the film terminated in HA, the PAH chains begin to experience a different local environment created by the low charge density and hydrophobic character of HA. This may lead to a reduction in charge density of the PAH chains to lower the energy of the system because the local environment at the surface has a low charge density and is more hydrophobic in nature in comparison to the highly charged approaching PAH chains. Since the p$K_{\mathrm{a(app)}}$ values for films terminated in the same polymer are so similar for films with different total number of polyelectrolyte layers (i.e., $(\mathrm{PAH/HA})_5$ and $(\mathrm{PAH/HA})_6$ films), it is assumed that the general trends also apply for films with more layers such as the $(\mathrm{PAH/HA})_{10}$ films under investigation.

Film Swelling. Another factor that plays a pivotal role in the mechanism of small molecule incorporation and release from polymer films, including polyelectrolyte multilayer films, is the extent to which the films swell upon exposure to aqueous solution.[26−28,39] The ability of the films to swell is related to the ionic cross-

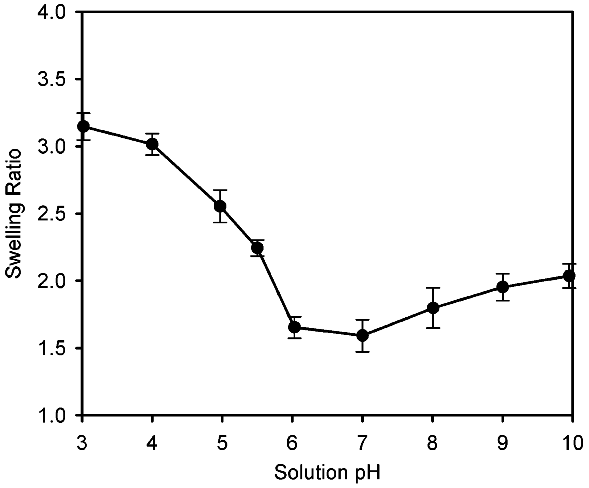

Figure 3. Ratio of the equilibrium swelling thickness to the dry film thickness for $(PAH/HA)_{10}$ films assembled at pH = 3.0 exposed to different solution pH values.

link density holding the layers together, the dynamic nature of these bonds, the degree of hydrophilicity, and the electrostatic forces operating in the hydrated films.[31] In turn, all of these parameters are directly related to the acid−base equilibria of the free functional groups in the films. The swelling ratio for $(PAH/HA)_{10}$ multilayer films (assembly pH = 3.0) as a function of solution pH is shown in Figure 3. This ratio is defined as the thickness of the swollen film divided by the dry film thickness. The swelling profile indicates that the films are able to swell from ~1.5 to a maximum of ~3 times the dry thickness depending on the solution pH. The equilibrium swelling of the films goes through a minimum in the mid-pH range but increases when the solution becomes more acidic and also more basic.

Notably, above pH ~ 5.0, the free carboxylic acid groups on the HA chains in the film are more than 50% dissociated, while more than half of the free amine groups of the PAH chains are in the NH_3^+ state when the local solution pH is less than ~7.5. Hence, in this mid-pH regime between pH = 5.0 and 7.0, the majority of the free carboxylic acid and amino groups in the film are charged, which would normally cause the chains to extend due to electrostatic repulsions. However, in this case, the close proximity of the positive and negative charged moieties effectively screen the electrostatic forces leading to minimum swelling of the film. An increase or decrease in the swelling solution pH leads to an imbalance in the ratio of the charge density of the free functional groups of the polycation chains to that of the polyanion chains. Electrostatic repulsions develop between the charged moieties of the more highly dissociated species. For example, at low pH stronger repulsive forces exist between the amine groups. The repulsive forces cause the chains to extend, leading to an increase in the swelling ratio.[39] 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.

In a comparable manner to the covalent cross-link density of polyelectrolyte gels, the extent to which the multilayer films are held together with ionic cross-links also influences the degree to which the films can swell.[17,39] The higher the cross-link density, the more resistant the film becomes to expansion caused by osmotic forces operating in the system.[49] However, unlike covalent bonds, the ionic cross-links of multilayer films are dynamic and are sensitive to pH and electrostatic screening. This allows the chains to undergo some reorganization during the swelling process due to breaking and re-forming of the ionic cross-links that hold the films together, which can also lead to further swelling of the films.[17] This reorganization processes has been found to lead to reversible and irreversible pore formation in certain weak polyelectrolyte mutlilayer films.[17,28] This effect tends to manifest itself in the pH ranges (low and high) that lead to the greatest extent of film swelling. However, in the case of PAH/HA multilayer assemblies, exposure of the films to solutions within the range investigated (pH = 3.0−10.0) did not produce irreversible damage such as pores. However, reorganization of the surface topography and increased roughness occurred as a result of exposure to solutions of different pH values. An example of this is shown in Figure 4, which displays the AFM images, taken in the dry state, of the surface of a $(PAH/HA)_{10}$ film before and after exposure to solution of the pH extremes over the range investigated. Notably, these changes are reversible. For example, exposure of the film assembled at pH = 3.0 to a solution of pH = 10.0 results in an increase in surface roughness, which is reduced upon reexposure to a solution of pH = 3.0.

Dye Loading. The loading and release of small molecules from polyelectrolyte gels and other types of pH-sensitive polymer films are effectively governed by a number of properties of the system such as the degree of swelling, hydrophilic/hydrophobic balance, $pK_{a(app)}$, charge density, pore size, and the properties of the small molecules such as size, charge, and hydrophilicity.[39,42,50] Thus, with our knowledge of the pH-dependent physicochemical behavior of PAH/HA multilayer films, it is now possible to explore the loading and release behavior of small hydrophilic molecules in these films with the aim of understanding these processes from a fundamental point of view.

The small molecule species used in this study are Chromotrope 2R and Indoine Blue. Both dyes are azobenzene derivatives with charged moieties and are of similar size and hydrophilic/hydrophobic character. Figure 5 illustrates the equilibrium concentration of dye molecules incorporated in the films as a function of the solution pH. In both cases, the loading process was observed to be pH-dependent. Although each dye contains moieties that remain fully charged over the entire pH range, the presence of the weakly acidic and basic groups in the multilayer films greatly influences the ideal loading conditions. Chromotrope 2R molecules contain two sulfate groups and prefer to be within the polymer matrix when the pH is low because of the existence of strong electrostatic attractive forces associated with the fully charged free amine groups on the PAH chains and weak repulsive forces from the marginally dissociated carboxylic acid groups of the HA chains. The concentration of this dye in the film is lower when the solution pH is more basic because the molecules experience repulsive forces in the film under these conditions due to the increasing charge density of the free carboxylic acid groups of the HA chains. In contrast, Indoine Blue molecules have a tertiary amine group that is positively charged in solution at all pH values and a

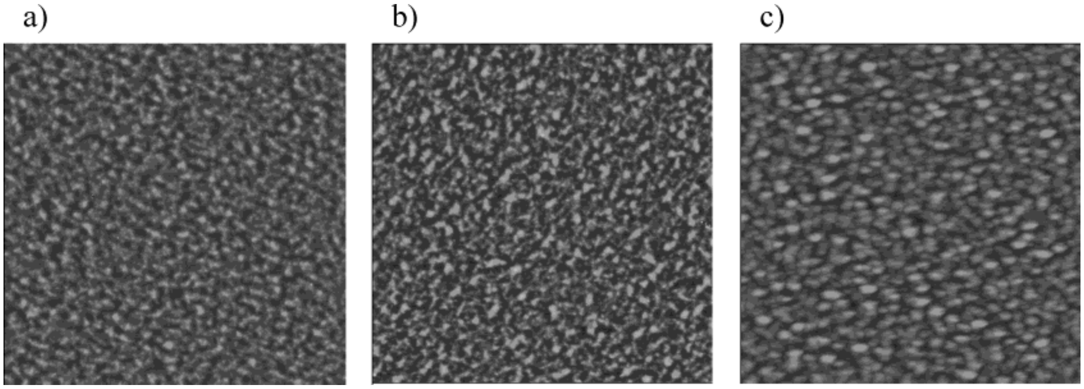

Figure 4. AFM (25 × 25 μm) height images of $(PAH/HA)_{10}$ multilayer films assembled at pH = 3.0: (a) before exposure to solution (rms = 5.3 nm), (b) after exposure to pH = 3.0 (rms = 7.2 nm), (c) after exposure to pH = 10.0 (rms = 11.6 nm).

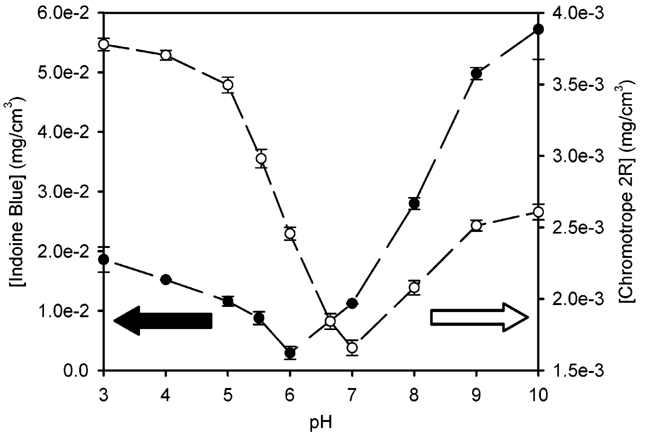

Figure 5. Concentration of dye molecules incorporated into $(PAH/HA)_{10}$ multilayer films assembled at pH = 3.0 as a function of the solution pH: Indoine Blue (●) and Chromotrope 2R (○).

weak primary amino group with a pK_a values of ~10–11, which will be uncharged in solution only in extreme basic conditions.[51] The loading profile indicates that this dye species experiences a maximum incorporation in the PAH/HA films when the solution pH is in the basic range. This is because they experience the greatest attraction from the free carboxylic acid groups in the film and minimum repulsive forces from the free amine groups of the PAH chains. The minimum in dye loading in both cases occurs between pH ~ 5–7. From the acid–base equilibria studies, we learned that in this range both the free carboxylic acid and amine groups in the film experience the greatest charge density in an aqueous environment, and as a result, the electrostatic forces in the film are essentially balanced, minimizing the electrostatic interactions between the polymer chains and the dye molecules.

Interestingly, the amount of both of the dyes that enters the films unexpectedly increases in the pH range when the electrostatic repulsive forces between the dye molecules and the film increase and the attractive forces decrease. For Indoine Blue this occurs at low pH values and for Chromotrope 2R at high pH values. Notably, a similar trend is observed in the equilibrium swelling profile for PAH/HA films. As shown in Figure 3, the swelling of a $(PAH/HA)_{10}$ film also experiences a minimum in the mid-pH range. This perhaps suggests that the degree of film swelling also has some influence on the loading of the dye molecules. The increase in the swelling of the film at low and high pH values facilitates the incorporation of the dye molecules by creating more free volume for the dye molecules to enter. The increasing free volume in the films with swelling provides more mobility for the molecules to avoid undesirable electrostatic forces and find sites for more favorable van der Waals and hydrogen-bonding interactions. However, it should be mentioned that the kinetics of swelling is not the rate-limiting step in the incorporation process because it occurs much faster (seconds to a few minutes) than the time allowed for the incorporation of the dyes. The kinetics of weak polyelectrolyte multilayer film swelling has been extensively investigated by Tanchak and Barrett.[52]

Another factor that must be considered when discussing the properties of the system that promote dye loading is the potential for favorable associations between the dye molecules. Many small molecules that have both a significant hydrophilic and hydrophobic character have the ability to aggregate when in close proximity to each other as in highly concentration solutions or the confines of a polymer matrix.[53,54] The aggregation of chromophore species is characterized by a shift in the wavelength of absorption. In the case of Indoine Blue and Chromotrope 2R, the absorption wavelengths are insensitive to the concentration of dye in solution. However, the absorption curves for both dyes experience a significant red shift upon incorporation into the PAH/HA multilayer films (Figure 6). The shift toward higher wavelengths indicates the formation of J-aggregates, in which case the dye molecules are arranged in one dimension to achieve a parallel orientation of their transition moments with an angle of zero between the transition moments and the line joining the center of the molecules.[53] The J-aggregation of aromatic dyes, such as the ones used in this study, results in a side-by-side alignment of the aromatic rings on neighboring molecules.[54] This type of aggregation is favorable for these dyes because of the potential for hydrogen bonding between the substituents of the aromatics rings such as the hydroxyl groups of Chromotrope 2R and the hydroxyl and amino group of Indoine Blue. J-aggregation has previously been observed in polyelectrolyte multilayer systems containing a polyelectrolyte with azo chromophores attached to the polymer backbone.[55]

Typically, J-aggregates produce narrower line widths in the spectrum compared to the single chromophore molecules.[53,54] The broader line widths observed for both dye species upon incorporation in the PAH/HA films are attributed to a distribution of the aggregation numbers, which has previously been observed to occur in nanocrystal and mixed Langmuir–Blodgett films containing

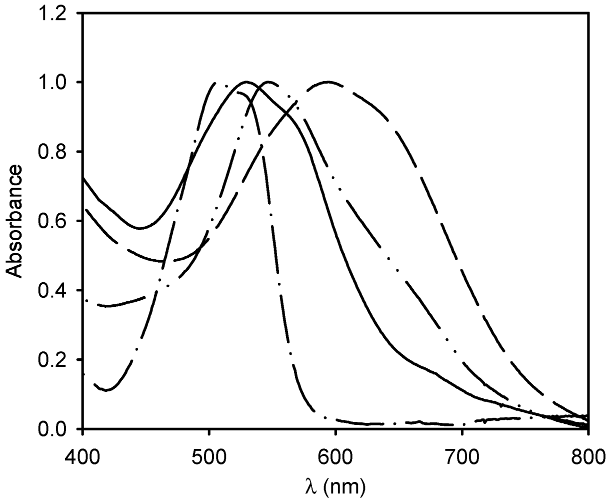

Figure 6. Normalized absorbance spectra of Chromotrope 2R in solution (– · –), Chromtrope 2R in a $(PAH/HA)_{10}$ film (—), Indonine Blue in solution (– ·· –), and Indonine Blue in a $(PAH/HA)_{10}$ film (– –).

aggregated chromophores.[56,57] In this study, it was found that the extent of the red shift is dependent on the concentration of dye in the films with a maximum λ_{max} shift of ~23 nm for Chromotrope 2R and ~52 nm for Indoine Blue (Supporting Information). This suggests that wavelength shift is actually due to aggregate formation and not just a result of the dyes being incorporated into the film. The above discussion indicates that there are a host of factors that must be considered in order to optimize the loading of small hydrophilic molecules in PAH/HA films. However, it remains unclear as the extent each factor contributes to the incorporation process. As will become clear in the next section, any possible aggregation does not appear, however, to hinder the release process.

Release of Dye. We have learned that a complex interplay between the pH-sensitive physicochemical characteristics of the PAH/HA films and the properties of the dye molecules controls the overall loading process. Intuitively, the same factors must also be considered when addressing the release of these molecules from the films. Although there have been several models developed to describe the release of small molecules from polymer films, there are none that can completely model the complexity associated with the release of charged molecules from polyelectrolyte multilayer films.[50]

For this study, the films were loaded with Chromotrope 2R and Indoine Blue at pH = 3.0 and 10.0, respectively, because these pH conditions result in maximum dye loading. Figure 7 shows the time-dependent release profiles of Chromotrope 2R and Indoine Blue from $(PAH/HA)_{10}$ films in solutions of different pH values. Not surprisingly, the release of both dye species is dependent on the solution pH. In the case of Chromotrope 2R, the release rate increases with increasing solution pH. As the release solution becomes more basic, the electrostatic attractive forces between the free amine groups in the film and the Chromotrope 2R molecules decrease because the amine groups begin to lose their charge. At the same time, the repulsive forces created by the increasing charge density of the free carboxylic acid groups of the HA chains promotes the release of the dye molecules. A similar argument applies to the pH-sensitive release of Indoine Blue molecules. However, in this case, the positively charged molecules escape from the films to a greater extent at low pH values when the free carboxylic acid groups lose their charge and the free amine groups increase their charge density, creating a more repulsive electrostatic environment. The change in the electrostatic environment also influences the extent of J-aggregation of the incorporated dye molecules. The higher the concentration of dye in the films, the greater the percentage that is involved in aggregation.[53,54] The concentration of dye in the film is pH-dependent. Thus, under pH conditions that promote electrostatic attractions between the dye and the free functional groups in the film, the dye molecules are closer to each other because of their high concentration, and thus they are more likely to aggregate. A reduction in the attractive forces and accompanying increase in repulsive interactions between the dye molecules and the film leads to a disruption of the aggregates and promotes the release of dye molecules. In turn, the concentration of dye in the film is reduced, causing the system to establish a new equilibrium with fewer aggregating molecules. This is observed as a reduction in the red shift of the absorption maximum as more dye escapes the film.

Although electrostatic forces have a major influence on controlling the release of the charged dye molecules from the films, the extent to which the films swell in solution also plays a role in the release process. During the course of film swelling, there are many dynamic events happening concurrently that influence the equilibrium in the film. For example, the influx of water brings with it a certain concentration of counterions that serve in the capacity of electrostatic screening, therefore reducing the attractive and repulsive forces acting on the dye molecules. Hence, to some extent, the movement of these small molecules is controlled by diffusion. In turn, diffusion through the film is influenced by hydrodynamic and steric factors.[49] In other words, to escape from the films, the molecules must have other forces acting on them such as viscous drag, and they must also have an unrestricted path on which to travel. Film swelling likely opens up voids and channels in the polymer matrix to facilitate small molecule movement. Also, the larger the free volume in the film, the more likely small aggregates of dye molecules will escape in addition to the single molecules. Notably, the swelling of the film reaches an equilibrium state much faster than the release of the dye molecules.[52] Therefore, the release process is not governed by the swelling kinetics. In addition, the dependence of release rate on the solution pH does not mimic that of the degree of film swelling. This indicates that although the loading process is clearly affected by both electrostatics and film swelling, the release process is mostly driven by electrostatic factors.

Another interesting characteristic of the release behavior of Chromotrope 2R and Indione Blue from $(PAH/HA)_{10}$ multilayer films is that the percentage of dye released is also strongly dependent on the solution pH (Figure 8). The trends in the percentage of dye released as a function of solution pH mimics those of the release rates in both cases. This behavior is attributed largely to the dynamics of the electrostatic interactions between the dye molecules and the functional groups in the film with variation in the solution pH. The acid–base equilibria of the free functional groups on the polyelectrolyte chains dictate the fraction of the amine and carboxylic acid groups that exist in the dissociated form

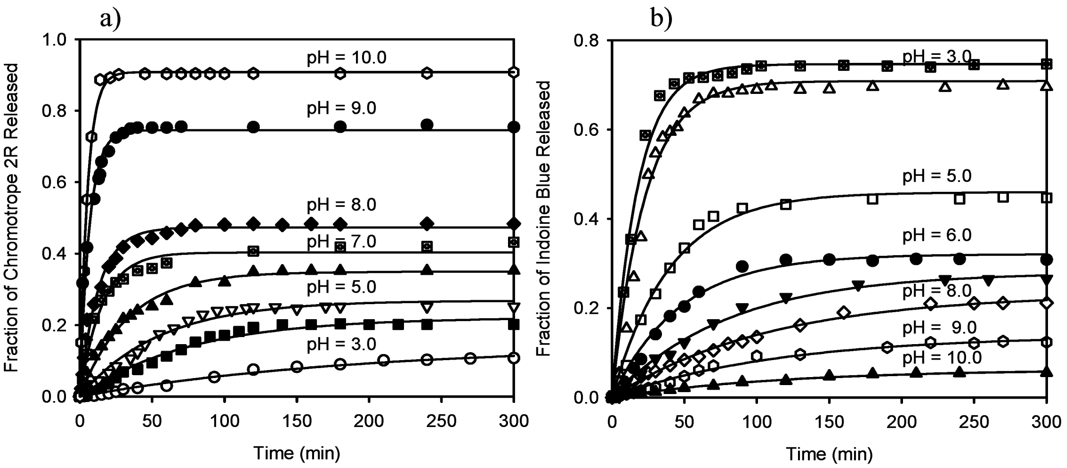

Figure 7. Release profiles for (a) Chromotrope 2R and (b) Indoine Blue as a function of solution pH from $(PAH/HA)_{10}$ multilayer films assembled at pH = 3.0. Loading pH = 9.0 for Indoine Blue and pH = 3.0 for Chromotrope 2R.

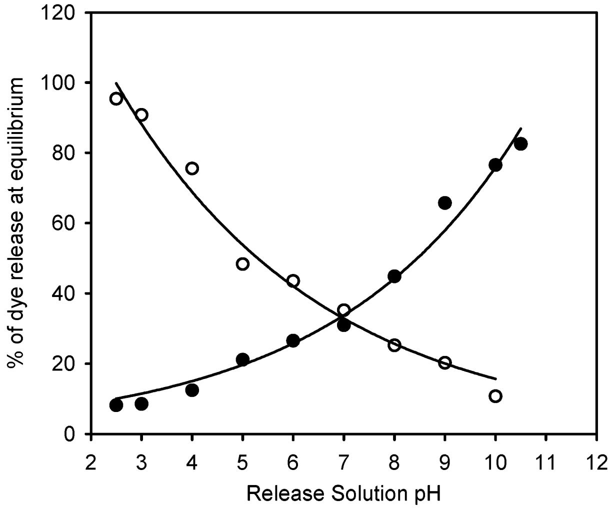

Figure 8. Total percentage of incorporated Chromotrope 2R (●) and Indoine Blue (○) released under equilibrium conditions from the $(PAH/HA)_{10}$ films at different solution pH values.

at any particular pH value. For example, given that the $pK_{a(app)}$ of the amine groups of the PAH chains is ~7.4, more than half of the free amine groups available for electrostatic associations with the Chromotrope 2R molecules are dissociated at pH values below 7.4. In fact, in the low-pH regime there is effectively complete dissociation of the free amine groups and a minimal fraction of carboxylic acid groups in the charged state, allowing for strong attractions between the Chromotrope 2R molecules and the film. The greater the electrostatic attraction, the less likely the dye molecules will escape the film. Moreover, at high concentration of dye molecules in the films the additional factor of J-aggregation of the dye molecules also hinders the release of the dye from the film. Similar principles also apply to the Indoine Blue molecules, but in this case the greatest electrostatic attractions occur at high pH values. Interestingly, the plots of the percentage of dye released vs the solution pH for Chromotrope 2R and Indoine Blue cross each other in the mid-pH range. Under these pH conditions, the percentage of the incorporated dye that escapes the film is independent of the charge on the small molecules. This is the pH range where the film swelling is minimal and the electrostatic repulsive and attractive forces in the film are essentially balanced. Hence, at this point, the incorporation of dye is mostly driven by hydrophobic factors.

The most significant observation from this study is that the release profiles indicate that under certain pH conditions it is possible to essentially trap ~90% the dye molecules in the films. This trapping occurs at pH ~ 10.0 for Indoine Blue and pH ~ 3.0 for Chromotrope 2R. The ~10% that is released is believed to be from the first few layers closest to the surface. The validity of this trapping phenomenon has been tested by exposure of the films to solutions at the appropriate pH values for a period of 3 weeks, with little to no change (<1%) in the amount of dye retained in the films. This behavior is particularly surprising considering the high to moderate degree of film swelling at pH = 3.0 and 10.0, respectively, which would lead to increased diffusion and electrostatic screening. However, the high concentration of dye molecules in the films and the strong electrostatic attractions existing between the small molecules species and the functional groups in the film under these pH conditions promote a high degree of J-aggregation of the molecules. It is believed that the combination of strong attractive forces, reduced repulsive interactions, and large extent of dye aggregation contribute to the retention of the dye molecules. Interestingly, the results suggest that it is possible to trap and protect small molecules in the PAH/HA films under both acidic and basic conditions and to release a significant amount of the material under neutral pH conditions. It is important to point out the observed behavior of these dye molecules in the multilayer films is not necessarily specific to these particular dye molecules. Both dyes are small organic molecules that have a significant extent of hydrophilic and hydrophobic character, which is general to many important categories of molecules such as those relevant for drug design. Hence, these studies serve as a basis for understanding how to effectively use polyelectrolyte multilayer films in controlled release applications.

Conclusion

In this report we have shown that the loading and release behavior of small hydrophilic molecules in weak polyelectrolyte multilayer films of PAH and HA is controlled by a complex interplay between the physicochemical properties of the films and the small molecule species. Electrostatic interactions and the degree of swelling of the films were found to provide a great means of control over the loading and release process. Both of these properties are governed by the acid−base equilibria in the films. The $pK_{a(app)}$ values of PAH and HA in the multilayer films deviated from the dilute solution values. HA becomes a weaker acid by almost 2 pH units and PAH a weaker base by ~1.5 units. This resulted in pH-dependent film swelling, with a swelling ratio ranging from ~1.5 to 3.0. The incorporation of the cationic dye Indoine Blue and the anionic dye Chromotrope 2R into $(PAH/HA)_{10}$ multilayer films showed strong dependence on the solution pH. The maximum loading was achieved under pH conditions that resulted in the greatest electrostatic attraction between the dye molecules and the film, minimum repulsive interactions between the two, and greatest extent of J-aggregation of the small molecules in the film. The loading profiles experience a local minimum in the mid-pH range, which mimics that of the pH-sensitive swelling behavior of the films. The release rate and the percentage of dye that escapes the films also exhibit pH dependence and are directly proportional to one another. The optimum solution conditions for release are achieved when repulsive rather than attractive forces are dominant between the dye molecules and the films. The swelling of the film also helps to facilitate the release of the dye molecules by transporting counterions into the film to screen the electrostatic interactions and also by creating voids and pores through which the molecules can travel. One of the most interesting findings in this study is that it is possible to trap the small molecules in the film at a certain solution pH and to release the trapped molecules simply by changing the pH. This ability to trap the small molecules is attributed to favorable electrostatic interactions and the formation of dye aggregates in the film. Overall, this study has shown that PAH/HA multilayer films are viable systems for the controlled release of small molecules and that key to effectively using the films in this manner is to first understand the fundamental physicochemical properties of the multilayer assemblies.

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Supporting Information Available: Calibration curves for Chromotrope 2R and Indonine Blue, titration curves for PAH/HA films assembled at pH = 3.0, but with different total numbers of polymer layers, and a plot of $\Delta\lambda_{max}$ as a function of dye concentration in PAH/HA films assembled at pH = 3.0. This material is available free of charge via the Internet at http://pubs.acs.org.

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