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Polyelectrolyte Multilayers Fabricated from ‘Charge-Shifting’ Anionic Polymers: A New Approach to Controlled Film Disruption and the Release of Cationic Agents from Surfaces

Xianghui Liu, Jingtao Zhang, and David M. Lynn

Department of Chemical and Biological Engineering, University of Wisconsin – Madison, 1415 Engineering Drive, Madison, Wisconsin 53706. E-mail: dlynn@engr.wisc.edu

Abstract

We report an approach to the design of ‘charge-shifting’ anionic polymers that provides control over the disruption of ultrathin polyelectrolyte multilayers in aqueous media. We demonstrate that the addition of citraconic anhydride to poly(allylamine) yields an anionic, carboxylate-functionalized polymer (polymer **2**) that can be converted readily back to cationic poly(allylamine) in acidic environments. The incorporation of polymer **2** into polyelectrolyte multilayers thus provides an approach to the fabrication of films that are stable at neutral pH but that erode over a period of several days in acidic media (e.g., pH ~5). Experiments using a structural analog of polymer **2** with carboxylate side chains that do not hydrolyze readily provided support for the view that the disruption of these films occurred as a result of polymer side chain hydrolysis and a resulting change in the net charge of the polymers. Because this approach is based upon the use of anionic polymers to induce film instability, it provides a platform for the design of multilayers that can be used to provide control over the release of cationic film components. As proof of concept, we demonstrated that ultrathin films ~100 nm thick fabricated using polymer **2** sustain the release of fluorescently labeled PAH for up to four days when incubated at pH 5.0. The synthetic approach used here is modular and tunable and can be used to introduce anionic ‘charge-shifting’ character to a broad range of other polyamines. With further development, this approach could expand significantly the range of different cationic agents (e.g., cationic proteins, peptides, polymers, nanoparticles, etc.) that can be released or delivered from surfaces using polyelectrolyte multilayers.

Introduction

Methods for the layer-by-layer assembly of multilayered polyelectrolyte films (or ‘polyelectrolyte multilayers’) provide nanometer-scale control over the structures and compositions of thin films fabricated from a broad range of cationic and anionic polymers.^{1–4} An evolving understanding of the structures and properties of these multilayered materials – and the ways that they can (or cannot) be manipulated at various length scales – has contributed to the design of functional thin films of interest in a growing number of fundamental and applied contexts.^{5–10}

Owing to the polyvalent nature of the electrostatic interactions in polyelectrolyte multilayers, these assemblies are frequently regarded to be ‘stable’ in physiologically relevant environments (that is, they often do not dissolve readily or undergo large changes in macroscopic film properties when incubated in physiological media).^{3,5,7,9} This general stability confers several potential practical advantages, and has contributed significantly to the development of

polyelectrolyte multilayers in biomedical and biotechnological contexts. The work reported here arose from our interest in developing methods that can be used to disrupt electrostatic interactions and promote the controlled disassembly of polyelectrolyte multilayers in aqueous media.^{8,10} Methods that provide such control could contribute to the development of thin films and coatings that permit the release of precise and well-defined quantities of chemical or biological agents from the surfaces of macroscopic, microscopic, or nanoscopic objects. Here, we report a new approach to the design of polyelectrolyte multilayers that provides control over film erosion and the release of cationic agents from film-coated surfaces.

This investigation builds upon the results of several past studies demonstrating that it is possible to design polyelectrolyte multilayers that erode, degrade, or disassemble in aqueous environments by fabricating them using polyelectrolytes with functionality that can be cleaved or degraded (for example, either hydrolytically,¹¹⁻¹⁶ enzymatically,¹⁷⁻¹⁹ or reductively^{20, 21}). The incorporation of degradable polyelectrolytes permits tunable and/or triggered control over film disassembly and can be used to design films that provide control over the release of a variety of different agents.¹¹⁻²¹ Additional details on these and other past approaches to promoting film disruption can be found in several recent reviews.^{8,10} We note here, however, that the majority of past reports on the incorporation of degradable polymers into multilayered films have focused on the use of degradable *cationic* polymers and thus, in general, on the design of films designed to erode and control the release anionic polyelectrolytes (e.g., DNA).^{8,10}

In principle, the fabrication of polyelectrolyte multilayers using degradable *anionic* polymers would provide a platform for the design of thin films that permit control over the release of cationic agents (e.g., cationic proteins, peptides, polymers, nanoparticles, etc.). Several past reports have demonstrated that enzymatically degradable anionic polymers can be used to fabricate multilayers that degrade in the presence of specific enzymes^{17,22} or upon contact with cells.^{17,23} Progress toward the fabrication of films using hydrolytically degradable anionic polymers, however, has been limited by the dearth of commercially available synthetic polymers that are both anionic and degradable, and, more generally, by the challenges associated with the synthesis of such polymers. We report here an alternative approach to the fabrication of multilayers that erode and release cationic film components. This approach is not based upon the incorporation of degradable anionic polymers, but on the design and incorporation of synthetic ‘charge-shifting’ anionic polymers.

Our group²⁴ and others²⁵⁻²⁹ demonstrated recently that it is possible to design ‘charge-shifting’ cationic polymers (that is, cationic polymers that undergo dynamic reductions in their net charge) by designing polymers with amine-functionalized side chains that can be cleaved hydrolytically. Polymer **1** (Eq 1) presents an example of this approach;²⁴ this polymer undergoes a gradual reduction in net charge (e.g., from cationic to anionic) upon side chain hydrolysis. We demonstrated recently that polymer **1** could be used to fabricate multilayers that release plasmid DNA for up to three months, and that both film erosion and the release of DNA could be understood in terms of disruptions in the ionic interactions in these assemblies that occur upon polymer side chain hydrolysis.²⁴

Eq (1)

The investigation reported here sought to determine whether the ‘charge-shifting’ concept described above for the design of cationic polymers could be applied to the design of ‘charge-shifting’ anionic polymers (i.e., anionic polymers that undergo gradual and time-dependent *increases* in net charge). We also sought to determine whether such polymers could be used to exert control over the disruption of polyelectrolyte multilayers and fabricate films that provide control over the release of cationic agents. Here, we demonstrate that the addition of citraconic anhydride to poly(allylamine hydrochloride) (PAH), a weak polyelectrolyte used widely for the fabrication of polyelectrolyte multilayers, yields an anionic, carboxylate-functionalized polymer (polymer **2**) that can be used to fabricate multilayers that erode and release cationic polymers in aqueous environments. This synthetic approach exploits methods developed for the reversible addition of carboxylate functionality to amines,³⁰⁻³⁶ and results in an anionic polymer that can be converted readily back to cationic poly(allylamine) by amide hydrolysis upon exposure to acidic environments (Scheme 1, top).

We demonstrate that the incorporation of polymer **2** into polyelectrolyte multilayers provides an approach to the fabrication of multilayers that remain stable at near-neutral pH (e.g., pH ~ 7) but erode and release their cationic components when exposed to low pH environments (e.g., pH ~ 5; Scheme 1, bottom). We demonstrate further that this approach can be exploited to fabricate ultrathin multilayers (e.g., ~100 nm thick) that sustain the release of a fluorescently labeled cationic polymer from film-coated surfaces for up to four days. The approach here is modular and can be used to introduce anionic ‘charge-shifting’ character to a broad range of other amine-functionalized polymers.³²⁻³⁶ These methods thus suggest the basis of a general approach to the fabrication of multilayered thin films and coatings designed to provide control over the release of a broad range of different cationic agents from surfaces.

Experimental Section

General Considerations

¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC+ 300 (300.135 MHz) and Varian UNITY 500 (499.896 MHz) spectrometers. Chemical shift values are given in ppm and are referenced with respect to residual protons from solvent. Silicon substrates (e.g., 0.5 × 3.5 cm²) used for the fabrication of multilayered films were cleaned with methylene chloride, ethanol, methanol, and deionized water, and dried under a stream of filtered compressed air. Surfaces were then activated by etching with oxygen plasma for 5 min (Plasma Etch, Carson City, NV) prior to film deposition. The optical thicknesses of films deposited on silicon substrates were determined using a Gaertner LSE ellipsometer (632.8 nm, incident angle = 70°). Data were processed using the Gaertner Ellipsometer Measurement Program. Relative thicknesses were calculated assuming an average refractive index of 1.577 for the multilayered films. Thicknesses were determined in at least five different standardized locations on each substrate and are presented as an average (with standard deviation) for each film. All films were dried under a stream of filtered compressed air prior to measurement. The pH of buffers used for erosion and hydrolysis experiments was recorded using a pH meter and, for the preparation of deuterated buffers, is reported as pH. Fluorescence measurements of

solutions used to erode multilayered films fabricated from fluorescently labeled polymers were made using a Fluoromax-3 fluorimeter (Jobin Yvon, Edison, NJ). Film topography and surface roughness were obtained from height data imaged in tapping mode on a Nanoscope Multimode atomic force microscope (Digital Instruments, Santa Barbara, CA), using scan rates of 10-20 $\mu\text{m/s}$ to obtain 256×256 pixel images. Silicon cantilevers with a spring constant of 40 N/m and a radius of curvature of <10 nm were used (model NSC15/NoAl, MikroMasch USA, Inc., Portland, OR). For each sample, at least two different $10 \mu\text{m} \times 10 \mu\text{m}$ scans were obtained at randomly chosen points near the center of the film at each time point. Height data were flattened using a 2nd-order fit. Root-mean squared surface roughness (R_{rms}) was calculated over the scan area using the NanoScope® software package.

Materials

Test grade n-type silicon wafers were purchased from Si-Tech, Inc. (Topsfield, MA). Poly (allylamine hydrochloride) (PAH, MW $\approx$ 60,000) was obtained from Alfa Aesar Organics (Ward Hill, PA). Citraconic anhydride and succinic anhydride were purchased from Aldrich Chemical Co. (Milwaukee, WI). 7-dimethylaminocoumarin-4-acetic acid, succinimidyl ester (NHS-activated coumarin) was purchased from Invitrogen (Carlsbad, CA). All other materials were used as received without further purification unless otherwise noted. Deionized water (18 M Ω) was used for washing steps and to prepare all polymer solutions. Solutions of cationic polymers used for dipping (20 mM with respect to the molecular weight of the polymer repeat unit) were prepared in 18 M Ω water and pH was adjusted to pH $\sim$ 7 by using 1N NaOH. Solutions of anionic polymers (20 mM with respect to the molecular weight of the polymer repeat unit) were prepared in 18 M Ω water and pH was adjusted to pH $\sim$ 8. All buffers and polymer solutions were filtered through a 0.2- μm membrane syringe filter prior to use unless otherwise noted.

Synthesis of Citraconic Amide-Substituted Polymer 2

PAH (100 mg) was dissolved in 1.0 N NaOH (3 mL) and stirred overnight. Citraconic anhydride (400 μL , $\sim$ 2 equivalents relative to amine functionality in PAH) was added dropwise to the PAH solution, and the reaction mixture was stirred overnight at room temperature. During the reaction, aqueous NaOH (6.0 N) was added as necessary to maintain the pH of the reaction solution above pH 8. The resulting reaction mixture was dialyzed (SpectraPor, MWCO = 3500) against water (adjusted to pH $>$ 7 using NaOH) for 24 hours and lyophilized to yield the final product as a white powdery solid in near quantitative yield. The addition of citraconic anhydride to a primary amine leads to two isomers, one with a methyl group proximal to the newly generated amide bond, and one with the methyl group distal to the newly generated amide bond (see structure in text). The ratio of distal to proximal isomers formed under the conditions outlined above was determined to be $\sim$ 7:3 using ^1H NMR spectroscopy. ^1H NMR data for the final product: (D_2O) δ (ppm) = 1.2 (2H, br, $\text{CH}_2\text{CHCH}_2\text{NH}$), 1.7 (1H, br, $\text{CH}_2\text{CHCH}_2\text{NH}$), 1.93 (3H, s, $\text{COCHCCH}_3\text{COONa}$), 3.1 (2H, br, $\text{CH}_2\text{CHCH}_2\text{NH}$), 5.6 (proximal isomer, 0.3H, s, $\text{COCCH}_3\text{CHCOONa}$), 5.8 (distal isomer, 0.7H, s, $\text{COCHCCH}_3\text{COONa}$).

Synthesis of Succinic Amide-Substituted Polymer 3

PAH (100 mg) was dissolved in 1.0 N NaOH (3 mL) and stirred overnight. Succinic anhydride (400 μg , $\sim$ 2 equivalents relative to amine functionality in PAH) was added dropwise to the PAH solution, and the reaction mixture was stirred overnight at room temperature. During the reaction, aqueous NaOH (6.0 N) was added as necessary to maintain the pH of the reaction solution above pH 8. The resulting reaction mixture was dialyzed (SpectraPor, MWCO = 3500) against water (adjusted to pH $>$ 7 using NaOH) for 24 hours and lyophilized to yield the final product as a white powdery solid in near quantitative yield. ^1H NMR data for the final product:

(D₂O) δ (ppm) = 1.2 (2H, br, CH₂CHCH₂NH), 1.7 (1H, br, CH₂CHCH₂NH), 2.5 (4H, s, COCH₂CH₂COONa), 3.1 (2H, br, CH₂CHCH₂NH).

Synthesis of Coumarin-Labeled Poly(allylamine hydrochloride)

PAH (550 mg) was dissolved in methanol (~5 wt% in methanol) and 1 mL of a sodium methoxide solution (35 wt% in methanol) was added. The resulting reaction mixture was stirred for 4 hr at 45 °C, precipitated NaCl was removed by filtration, and NHS-activated coumarin (9.5 mg, 0.5 mol% relative to the amine repeat units in PAH) was added and the reaction mixture was stirred overnight at room temperature. One equivalent of HCl was added to the reaction mixture, and the resulting reaction product was concentrated by rotary evaporation. The crude product was dissolved in water and purified by dialysis (SpectraPor, MWCO = 3500) against deionized water at ambient temperature for three days. The resulting solution was lyophilized to yield a yellow solid that was used without further purification.

Characterization of Side-Chain Amide Hydrolysis

¹H NMR experiments used to characterize the kinetics of side chain hydrolysis for functionalized polymers were conducted in the following general manner. Polymer (~10 mg) was dissolved in either deuterated phosphate buffer (0.6 mL, 0.5 M, pH = 7.4) or deuterated acetate buffer (0.6 mL, 0.5 M, pH = 5). 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (~3 mg) was added as an internal standard, and the resulting solution was placed in a glass NMR tube. The NMR tube was kept in a 37 °C incubator and removed periodically for analysis by ¹H NMR spectroscopy. The disappearance of the α -proton of the side chains at 5.6 and 5.8 ppm was monitored and integrated versus the trimethylsilyl protons of the internal standard.

Fabrication of Multilayered Films

Multilayered films were fabricated on planar silicon substrates using an alternate dipping procedure according to the following general protocol: (1) Substrates were submerged in a solution of polycation for 5 min, (2) substrates were removed and immersed in an initial water bath for 1 min followed by a second water bath for 1 min, (3) substrates were submerged in a solution of polyanion for 5 min, and (4) substrates were rinsed in the manner described above. This cycle was repeated until the desired number of polycation/polyanion bilayers (typically 20) had been deposited. For experiments designed to characterize film growth profiles by ellipsometry, films were dried after every five cycles of the above procedure using filtered compressed air. Films to be used in erosion and release experiments were either used immediately after fabrication or dried under a stream of filtered compressed air and stored in a vacuum desiccator until use. All films were fabricated at ambient room temperature.

Characterization of Film Erosion and Release Kinetics

Experiments designed to investigate film erosion and release kinetics were performed in the following general manner: Film-coated substrates were placed in a plastic UV-transparent cuvette, and either 1.0 mL of HEPES buffer (pH = 7.4) or 1.0 mL of acetate buffer (pH = 5.0) was added to cover completely the film-coated portion of the substrates. These samples were incubated at 37 °C and removed at predetermined intervals for characterization by ellipsometry. Films were rinsed under deionized water and dried under a stream of filtered compressed air prior to measurement. Values of optical film thickness were determined in at least five different predetermined locations on the substrate by ellipsometry and the samples were returned immediately to the buffer solution. For experiments designed to monitor the concentrations of fluorescently labeled PAH released into solution, fluorescence measurements were made using the solution used to incubate the sample (excitation wavelength = 376 nm; emission wavelength = 470 nm).

Results and Discussion

The investigation reported here makes use of methods developed for the reversible conversion of amine functionality to carboxylic acid functionality by treatment with α -methyl derivatives of maleic anhydride.³⁰⁻³⁶ Past studies have demonstrated, for example, that the addition of citraconic anhydride to primary amines results in the formation of a citraconic amide, and that citraconic amides can be hydrolyzed readily under acidic conditions to regenerate primary amine functionality (Eq 2).³⁰

(Eq 2)

While the hydrolysis of amide bonds in aqueous media generally occurs very slowly, the hydrolysis of citraconic amides occurs rapidly under acidic conditions owing to intramolecular catalysis facilitated by the neighboring carboxylic acid group, which is maintained in close proximity to the amide bond by the conformational rigidity of the nearby double bond.^{30,31} The kinetics and mechanism of this reaction have been investigated using small-molecule model systems,^{30,31} and this general approach has been applied to the chemical modification of proteins (e.g., by reaction with surface-accessible lysine residues) as a means to reversibly manipulate protein charge.³⁷⁻³⁹

The addition of small-molecule anhydrides to polymers with primary amine functionality presents a straightforward and modular approach to the generation of ‘charge-shifting’ anionic polymers, or anionic polymers capable of undergoing changes in net charge (i.e., from net anionic to net cationic) upon side chain amide hydrolysis. Several recent reports have demonstrated the application of this approach to the design of polymer-based carrier systems for the delivery of nucleic acids,^{32,34,35} proteins,³³ or small molecules³⁶ that create disruptive electrostatic interactions,^{33,36} unmask other disruptive functionality,^{32,34,35} or shed functionality that is no longer needed³⁵ upon exposure to low pH environments (e.g., such as those found in cell endosomes or lysosomes or other extracellular environments).

Our past studies demonstrate that ‘charge-shifting’ cationic polymer **1** can be used to destabilize DNA-containing multilayers and promote the release of DNA (an anionic polyelectrolyte) from surfaces.²⁴ On the basis of these past results, we reasoned that the approach described above could be exploited to design ‘charge-shifting’ anionic polymers capable of promoting the disassembly of polyelectrolyte multilayers – and the release of cationic agents – in aqueous media. If successful, such an approach could expand significantly the range of different agents that can be delivered or administered from surfaces using polyelectrolyte multilayers. To explore the potential of this approach and demonstrate proof of concept, we focused our initial studies on the fabrication of polyelectrolyte multilayers fabricated using polymer **2**.

Synthesis and Characterization of Citraconic Amide-Functionalized Polymer **2**

We synthesized citraconic amide-functionalized polymer **2** by the ring-opening addition of citraconic anhydride to PAH in analogy to methods described previously for the functionalization of small-molecule amines and polyamines.³⁰⁻³⁶ Treatment of PAH with an excess of citraconic anhydride in an aqueous NaOH solution at room temperature resulted in exhaustive functionalization with citraconic amide-functionalized side chains, as determined

by ^1H NMR spectroscopy (Eq 3). We note here that the addition of citraconic anhydride to a primary amine can lead to the generation of two isomers, one with a methyl group distal to the newly generated amide bond, and one with the methyl group proximal to the newly generated amide bond (e.g., see Eq 3). The ratio of distal to proximal side chain isomers in samples of polymer **2** used in this study was determined to be $\sim 7:3$ by integration of the α -protons of the side chains using ^1H NMR spectroscopy. Polymer **2** was isolated as a solid in near quantitative yield and was soluble in aqueous buffers at all concentrations required for subsequent experiments described below.

(Eq 3)

As described above, past studies report that citraconic amides are relatively stable in alkaline media, but they hydrolyze readily in acidic media.³⁰⁻³⁵ Characterization of the kinetics of hydrolysis of the side chains of polymer **2** upon incubation in deuterated phosphate buffer (pH = 7.4) at 37 °C using ^1H NMR spectroscopy demonstrated that side chain hydrolysis occurred slowly at near-neutral pH. As shown in Figure 1 (closed squares), only $\sim 25\%$ of the side chains of polymer **2** were hydrolyzed after incubation in phosphate buffer for eight days. However, the side chains of polymer **2** were hydrolyzed rapidly when the polymer was incubated at lower pH. For example, as shown in Figure 1 (closed triangles), $\sim 55\%$ of side chains were hydrolyzed within the first five hours when polymer **2** was incubated in deuterated acetate buffer (pH = 5.0). These pH-dependent results are consistent with the results of past studies.³⁰⁻³⁵

We note here again that the addition of citraconic anhydride to poly(allylamine) results in the formation of two side chain isomers (see Eq 2). Further inspection of the data in Figure 1 reveals that the rate of hydrolysis of the side chains of polymer **2** that have the methyl group distal to the amide bond is significantly greater than the rate of hydrolysis of the side chains having the methyl group proximal to the amide bond (Figure 1, dashed lines; individual data for hydrolysis at pH 7.4 not shown). Hydrolysis of the distal isomer occurs very rapidly (e.g., $\sim 70\%$ conversion; open diamonds) within the first five hours and is $>95\%$ complete after 70 hours. By contrast, hydrolysis of the proximal isomer occurs much more slowly, and is only $\sim 20\%$ complete after incubation for eight days. These large differences in rate are consistent with the results of past studies reporting the significant influence of substituent effects on the hydrolysis of amides formed using α -methyl derivatives of maleic anhydride. In the context of this current investigation, these results, when combined, demonstrate that polymer **2** is converted over a period of several days to a polymer that contains a majority of side chains that are cationic (i.e., primary amines, with the remainder being citraconic amide proximal isomers that continue to hydrolyze more slowly) upon incubation in acidic media. We note that, in general, the actual net charge of polymer **2** would depend upon a number of different factors, including the extent of side chain hydrolysis as well as environmental factors such as solution pH and ionic strength (the relative net charges and extents of reaction depicted in Scheme 1 are indicated for illustrative purposes only). We return to these considerations again in the discussion below.

Fabrication and Characterization of Polyelectrolyte Multilayers Fabricated Using Polymer 2

We performed a series of experiments to determine whether polymer **2** could be used to fabricate polyelectrolyte multilayers using PAH and an alternate dipping procedure similar to that used in numerous past studies.¹⁻⁴ For these and all subsequent experiments described below, multilayered films were fabricated layer-by-layer directly on the surfaces of planar silicon substrates to facilitate the characterization of film growth and increases or decreases in film thickness using ellipsometry.

Figure 2 shows a plot of optical film thickness versus the number of PAH/polymer **2** layer pairs (referred to hereafter as 'bilayers') deposited. Inspection of these data (filled squares) reveals an average final film thickness of ~90 nm after the deposition of 20 bilayers. We note that film thickness increased in a manner that was supra-linear, rather than linear, with respect to the number of bilayers deposited. This film growth behavior is similar to that reported recently for the 'exponential' growth of polyelectrolyte multilayers fabricated from a variety of different weak polycation/polyanion pairs,⁴⁰⁻⁴⁵ and could result from the ability of one or both polyelectrolytes to diffuse within these films during assembly.⁴⁰ The particular mechanism and molecular-level origin of the 'exponential' growth behavior observed here is the subject of ongoing research. Characterization of the surface of a film 20 bilayers thick using atomic force microscopy revealed these films to be continuous with a root-mean squared (R_{rms}) surface roughness of ~13 nm.

The central hypothesis guiding our approach is that time-dependent hydrolysis of the amide functionality in the side chains of polymer **2** [which, ultimately, results in the conversion of polymer **2** to cationic poly(allylamine)] can be used to (i) change the nature of the electrostatic interactions in multilayered assemblies, and (ii) promote film disruption and disassembly in ways that can be used to provide control over the release of cationic film components. To explore the potential of this approach and demonstrate proof of concept, we next performed a series of experiments using films fabricated from polymer **2** and PAH labeled with a fluorescent coumarin derivative (PAH_{FL}). PAH_{FL} was used in these experiments to facilitate characterization of the time-dependent release of PAH from films incubated in aqueous environments (described below). The growth profiles of films fabricated using polymer **2** and PAH_{FL} did not vary significantly from the growth profile shown in Figure 2 using polymer **2** and unlabeled PAH (as determined by ellipsometry, data not shown).

The stability (or instability) of films fabricated from polymer **2** and PAH_{FL} in near-neutral or acidic media was investigated by incubating these assemblies in HEPES buffer (pH = 7.4) or acetate buffer (pH = 5.0) at 37 °C. Figure 3 shows a plot of decreases in optical film thickness as a function of time measured during the incubation of films fabricated from 20 bilayers of polymer **2** and PAH_{FL} (~90 nm thick). Inspection of these data reveals these films to be stable (that is, they do not decrease significantly in optical thickness) upon incubation at pH 7.4 for approximately seven days (closed squares), but that these films decrease in thickness completely, and with a profile that is essentially linear, over a period of 48 hours when incubated at pH 5.0 (open squares).

The large, pH-dependent differences in the stability and erosion profiles of the PAH_{FL}/polymer **2** films shown in Figure 3 are consistent with the large and pH-dependent differences in the relative rates of solution-phase amide side chain hydrolysis shown in Figure 1. These observations, when combined, provide general support for the view that polymer **2** could serve to destabilize multilayers and promote film disassembly through a time-dependent 'charge-shifting' mechanism that involves polymer side-chain hydrolysis. We considered, however, that the erosion of these materials at pH 5.0 could also arise from other factors, such as changes in the percent ionization of polymer **2** that could occur when these films are incubated in acidic environments. Several past studies have demonstrated, for example, that polyelectrolyte

multilayers fabricated from weak polyacids [e.g., poly(acrylic acid), poly(methacrylic acid), etc.] can be dissolved and ‘erased’⁴⁶⁻⁵⁰ or transformed physically in other ways^{51,52} when exposed to changes in pH that change the ionic character of the polymers and disrupt interpolyelectrolyte interactions (e.g., by protonation or deprotonation of anionic carboxylate functionality). We note that, in general, past reports describing film dissolution in multilayers fabricated using weak polyelectrolytes describe transformations that often occur very rapidly (e.g., on the order of minutes),^{46,49-52} rather than over the period of ~2 days as observed in our experiments.

To provide additional support for our charge-shifting hypothesis, we conducted an additional series of experiments using films fabricated from PAH and succinic amide-functionalized polymer **3**. Polymer **3** is an analog of polymer **2** synthesized by the ring-opening addition of succinic anhydride to PAH (Eq 4). Polymer **3** is particularly useful as a control in these experiments for several reasons: (i) because succinic anhydride does not contain the double bond functionality present in citraconic anhydride, succinic amides are more conformationally flexible than citraconic amides, (ii) the terminal carboxylate functionality of succinic amides is thus not maintained in close proximity to the amide group, and, as a result (iii) rates of hydrolysis of succinic amides are much slower than the corresponding rates of hydrolysis of citraconic amides.³³

(Eq 4)

Figure 2 (dashed line) shows a plot of film thickness for a PAH/polymer **3** film versus the number of bilayers of polymer **3** and PAH deposited, and demonstrates that the conformational flexibility of the polymer side chain does not influence film growth significantly relative to films fabricated from polymer **2**. However, we observed striking differences in the stability of films fabricated from polymer **3** when these films were incubated in aqueous media. Inspection of the data in Figure 3 (dashed lines) reveals that films fabricated from polymer **3** are stable and do not decrease in optical thickness for up to seven days upon incubation at either pH 7.4 or pH 5.0. These results provide additional strong support for the view that the erosion of films fabricated from polymer **2** occurs as a result of the hydrolysis of the side chains in polymer **2** (and a concomitant change in the net charge of the polymer), and not as a result of other factors (such as changes in pH, ionic strength, or the percent ionization of the polymer) that could occur during the incubation of these films in acidic environments.

The gradual erosion of films fabricated from polymer **2** and PAH_{FL} in acidic environments results in the gradual and controlled release of PAH_{FL} into solution. Figure 4 shows a plot of solution fluorescence intensity versus time measured during the film incubation and erosion experiments described above in Figure 3. Inspection of these data reveals that PAH_{FL} was released gradually into solution for up to ~100 hours (open squares) when films fabricated from polymer **2** were incubated at pH 5.0. By contrast, levels of solution fluorescence observed during the incubation of these films at pH 7.4 (closed squares) were very low over the entire course of the experiment.

These data are consistent with the pH-dependent results of film erosion discussed above and demonstrate that polymer **2** can be used to design polyelectrolyte multilayers that are stable at neutral or near-neutral pH, but permit control over the surface-mediated release of a cationic agent for approximately four days under acidic conditions. On the basis of these solution fluorescence measurements, we estimate that films 20 bilayers thick contained approximately 20 μg of PAH_{FL} per cm^2 . Because layer-by-layer assembly can be used to exert precise control over film thickness and, thus, the amount of PAH incorporated (e.g., by controlling the number of layers of polymer deposited), this general approach can be used to increase (or decrease) the amounts of PAH or other functional cationic agents incorporated into and released from these materials. Finally, we note that the remaining data in Figure 4 (dashed lines) correspond to cumulative amounts of solution fluorescence measured during the incubation of control films fabricated from PAH_{FL} and polymer **3**. These data demonstrate that these films do not release significant amounts of PAH_{FL} and are consistent with the results of the erosion experiments described above.

Summary and Conclusions

We have reported an approach to the design of ‘charge-shifting’ anionic polymers that provides control over the disruption of ultrathin multilayered polyelectrolyte films in aqueous environments. We demonstrated that the addition of citraconic anhydride to poly(allylamine), a commercially-available polyamine used widely to fabricate polyelectrolyte multilayers, yields an anionic, carboxylate-functionalized polymer (polymer **2**) that can be converted readily back to cationic poly(allylamine) in acidic environments (e.g., pH 5). The incorporation of polymer **2** as an anionic component in polyelectrolyte multilayers provides an approach to the fabrication of films that are relatively stable at neutral pH (e.g., pH $\sim$ 7) but that erode over a period of several days when exposed to low pH environments (e.g., pH $\sim$ 5). Control experiments using a structural analog of polymer **2** functionalized with carboxylate side chains that do not hydrolyze readily provided support for the view that the disruption of these films occurred as a result of polymer side chain hydrolysis (and a resulting change in the net charge of the polymer) and not as a result of other factors, such as changes in pH or ionic strength, that could occur upon the incubation of these assemblies.

Because this approach is based upon the use of anionic polymers to induce film instability, it provides a platform for the design of polyelectrolyte multilayers that can be used to provide control over the release of cationic film components. To explore the potential of this approach and demonstrate proof of concept, we demonstrated that ultrathin films (e.g., $\sim$ 100 nm thick) fabricated using polymer **2** sustain the release of fluorescently labeled PAH for up to four days when incubated at pH 5.0.

The work described here differs fundamentally from approaches that have been reported previously for the disruption of polyelectrolyte multilayers using degradable cationic polymers, and could, with further development, expand significantly the range of different cationic agents (e.g., cationic proteins, peptides, polymers, nanoparticles, etc.) that can be released or delivered from surfaces using polyelectrolyte multilayers. In addition, the synthetic approach used here is modular and can be used to introduce anionic ‘charge-shifting’ character to a broad range of other primary amine-functionalized polymers. It should also prove possible to use this general approach to tune the ‘charge-shifting’ character of these polymers over a broad range of times, and for a broad range of potential applications, by varying either the numbers or the structures of the ‘charge-shifting’ anionic side chains incorporated. The relative instability of these materials at pH values representative of those found in the endosomes and lysosomes of cells also suggests opportunities to design film-coated particles or hollow multilayer microcapsules designed for the intracellular delivery of therapeutic macromolecules.

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Figure 1.

Kinetics of side chain amide hydrolysis at 37 °C for polymer **2** in deuterated phosphate buffer (500 mM, pH = 7.4, ■) and acetate buffer (500 mM, pH = 5.0, ▲), respectively, as determined by ^1H NMR spectroscopy. Data depicted with dashed lines correspond to the kinetics of the proximal (△) and distal (◇) side chain isomers of polymer **2** at pH 5.0.

Figure 2. Plot of ellipsometric thickness versus the number of PAH/polymer **2** bilayers (■, solid lines) or PAH/polymer **3** bilayers (▲, dashed lines) deposited on bare silicon substrates.

Figure 3.

Plot of film erosion versus time for multilayered films fabricated from fluorescently labeled PAH and polymer **2** (solid lines) incubated at 37 °C at pH 7.4 (■) and pH 5.0 (□). This plot also includes erosion profiles for films fabricated from polymer **3** (dashed lines) incubated at 37 °C at pH 7.4 (▲) and pH 5.0 (△). Film thicknesses were determined using ellipsometry at each time point and are expressed as percentages of the original thicknesses of each film.

Figure 4.

Plot of coumarin fluorescence versus time showing the release of coumarin labeled PAH from multilayered films fabricated from using polymer **2** (solid lines) incubated at 37 °C at pH 7.4 (■) and pH 5.0 (□). This plot also includes release profiles for films fabricated from polymer **3** (dashed lines) incubated at 37 °C at pH 7.4 (▲) and pH 5.0 (△).

Scheme 1.

Top: Hydrolysis of the citraconic amide side chains of anionic ‘charge-shifting’ polymer **2** under acidic conditions yields cationic poly(allylamine). Bottom: Polymer **2** (light/textured gray) is anionic and can be used to fabricate polyelectrolyte multilayers. Time-dependent changes in the net charge of polymer **2** result in changes in the nature of the ionic interactions in the multilayers and promote film disruption and the release of cationic film components (dark gray). Polymer **2** and PAH are shown as being completely ionized for illustrative purposes (see text).