Hydrogen-Bonded Multilayers of a Neutral Polymer and a Polyphenol

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ABSTRACT: We report on association of tannic acid (TA) with neutral or charged polymers in solution and at surfaces and contrast hydrogen-bonded and electrostatically associated polymer/TA complexes and TA/polymer layer-by-layer (LbL) films as per their stability in the pH scale. The neutral polymers used for hydrogen bonding with TA were poly(*N*-vinylcaprolactam) (PVCL), poly(*N*-vinylpyrrolidone) (PVPON), poly(ethylene oxide) (PEO), or poly(*N*-isopropylacrylamide) (PNIPAM), and the polymer used to explore electrostatic binding with TA was 90% quaternized poly(4-vinylpyridine) (Q90). Association of TA with polymers in solution was explored by measuring the turbidity of solutions. At surfaces, LbL film deposition and pH stability were followed by phase-modulated ellipsometry and in-situ Fourier transform infrared spectroscopy in attenuated total reflection mode (ATR-FTIR). While electrostatically stabilized films of TA with Q90 could not be deposited at low pH values (pH = 2), hydrogen-bonded films of TA with PVCL, PVPON, PEO, and PNIPAM could be constructed at pH 2 and did not dissolve until a critical dissolution pH of 9.5, 9, 8.5, and 8 (measured in 0.01 M buffer solutions) for PVCL/TA, PVPON/TA, PEO/TA, and PNIPAM/TA, respectively. In addition, all multilayers could be also constructed at pH 7.5 in solutions with low ionic strength. The high pH stability of these systems as compared to multilayers of the same neutral polymers with polyacrylic (PAA) or polymethacrylic (PMAA) acids is due to higher pK_a value of TA of ∼8.5 as estimated in this paper. We also show that multilayers of TA with a copolymer of *N*-vinylpyrrolidone containing 20 mol % of primary amino groups, PVPON-NH_2-20, were highly stable in a wide pH range from 1.3 to 11.7 because of combined stabilization through both electrostatic and hydrogen-bonding interactions. For all systems, pH windows for deposition and stability of LbL films at surfaces correlated with the phase behavior of TA complexes in solution. High pH stability of hydrogen-bonded films of TA as well as the capability of tuning the critical pH value for film dissolution in the range close to physiological pH values makes such multilayer systems promising candidates for biomedical applications.

Introduction

Layer-by-layer (LbL) adsorption of polymers at surfaces has been recently widely used as one of the most powerful techniques to modify and functionalize surfaces or to prepare free-standing polymer films with novel and advanced properties. Starting from its first description by Decher,[1,2] the LbL technique has been most widely applied to charged polymers, whose deposition at surface is largely controlled by charge compensation mechanisms.[3] However, the role of nonelectrostatic forces, such as hydrophobic ones, has been recognized as a significant contribution to film growth,[4,5] and pairs of neutral polymers were deposited within LbL based on hydrophobic interactions.[6] Hydrogen bonding has been also earlier explored as a driving force for the LbL film formation. In the case of deposition from aqueous solutions, Stockton and Rubner were first to show that polyaniline can be co-self-assembled with a number of nonionic polymers such as poly(vinylpyrrolidone) (PVPON), poly(vinyl alchohol) (PVA), poly(acrylamide) (PAAM), and poly(ethylene oxide) (PEO).[7] Multilayers were also constructed from a luminescent conjugated copolymer of the poly(phenylenevinylene) type which contained hydroxyl groups capable of hydrogen bonding with amine groups of a co-self-assembled poly(ethylenimine).[8] Simulteniously with the work by Rubner's and Neumann's groups, the use of organic solvents for construction of polymeric hydrogen-bonded films was explored by Wang et al.[9,10] When a combination of poly(vinylpyridine) (PVP) with poly(acrylic acid) (PAA)[11,12] or PVP with carboxylated dendrimers[13] was self-assembled in this way, microporosity could be introduced within the film by exposing the film to an aqueous solution at basic pH where PAA or dendrimer components selectively dissolved. The role of composition of an organic solvent mixture (ethanol/DMF)[14] on the hydrogen-bonding self-assembly of PVP and poly(4-vinylphenol) (PVPh) has also been explored by Zhang et al., and PVP/PVPh multilayers were shown to dissolve in highly polar organic solvents, such as DMF.

Our group has been exploring pH-responsive multilayers produced by hydrogen-bonded LbL self-assembly of a neutral polymer and weak polyacids in aqueous solution. This approach was initiated by one of us[15,16] and leads to construction of films which are erasable by a pH increase, when poly(carboxylic acid)s become ionized. Such multilayers are capable of absorbing functional molecules such as dyes or drugs, which can then be released at pH values higher than film's dissolution pH. While this controlled delivery option might be useful for some biomedical applications, stable films or films dissolving at physiological pH values are required in other applications. For all previously reported hydrogen-bonded multilayers based on polycarboxylic acids, however, films disintegrated at physiological conditions. One way to achieve multilayers stable at physicological pH is to introduce cross-linking between hydrogen-bonded components.[17–19] Here, we explore another approach to impart higher pH stability to hydrogen-bonded multilayers. The approach is based on a decrease in charge density of the ionizable polymer component of the film at neutral pH values, achieved through selection of a polymer with pK_a values significantly higher than those for poly(carboxylic acid)s. To that end, we chose a water-soluble natural polyphenol—tannic acid—a weak polyacid whose phenolic groups act as excellent H donors and form strong hydrogen bonds with compounds containing carbonyl groups. Because of the high pK_a value of TA of ∼8.5, its association through hydrogen bonding is expected to persist at neutral pH values. In addition, we show that by varying the nature of a neutral polymer component, disintegration pH range of polymer/TA films can be easily

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manipulated, covering the range of pH from physiological to more basic values.

TA is an attractive molecule for inclusion within surface films because of its known biofunctional properties, such as antitumor, antienzymatic, antibacterial, antimutagenic, and antioxidant activities.[20] High content of TA and other plant polyphenols in food has been correlated with reduced cardiovascular diseases and cancer.[21] Despite its unique and interesting properties, self-assembly of TA at surface is not widely explored. Adsorption of TA within monolayers was studied by Gimenez-Martin and and co-workers, with the purpose of modulation of surface properties of leacril (a copolymer of 90% acrylonitryle and 9% vinyl acetate) fibers.[22] The only example of LbL assembly of TA come from Lvov's group, who demonstrated inclusion of TA within LbL films with amine-based polyelectrolytes through primarily electrostatic interactions. Apart from deposition of TA-containing films onto planar substrates, this group also constructed microcapsules with TA-containing walls and explored permeability of labeled dextranes into such capsules.[23–25]

In this paper, we focus on hydrogen-bonded self-assembly of TA and contrast hydrogen-bonded and electrostatic self-assembly of TA at surfaces and in solution. We show that hydrogen-bonded and electrostatic self-assembly of TA occur at low and neutral pH values, respectively, and that corresponding LbL films have opposite pH stability/dissolution profiles. In all cases, multilayer deposition and pH response was controlled by phase behavior of interpolymer complexes in solution. By varying the nature of hydrogen-bonding neutral polymers, we have modulated the critical film dissolution pH in the range $7 \leq pH \leq 10$. Hydrogen-bonded multilayers fabricated from TA and several hydrogen-bonding polymers revealed correlation of the results with the chemical nature and hydrophobicity of the neutral polymers. High pH stability of as-deposited non-cross-linked LBL films at physiological pH as well as controlled dissolution of polymer films in the range from neutral to slightly basic pH values makes such coatings attractive for future biomedical applications.

Experimental Section

Materials. Poly(*N*-isopropylacrylamide) (PNIPAM; M_w 300 000), poly(ethylene oxide) (PEO; M_w 200 000), and poly(*N*-vinylpyrrolidone) (PVPON; M_w 360 000) were purchased from Scientific Polymer Products, Inc. Poly(*N*-vinylcaprolactam) (PVCL; M_w 1800) was purchased from Polymer Source, Inc. Poly(4-vinylpyridine) (PVP) with a M_w of 200 000 (Scientific Polymer Products, Inc.) was quaternized with ethyl bromide in ethanol solution using established methods[26] to obtain a polymer of 90% pyridinium units (Q90) as determined by IR spectroscopy. Branched poly(ethylenimine) (BPEI; M_w 70 000) was purchased from Polysciences as 30% aqueous solution.

Copolymer of *N*-vinylpyrrolidone and glicidyl methacrylate, poly(*N*-vinylpyrrolidone-*co*-GMA-20) (20% of GMA, M_w 19 000), was synthesized as described previously.[27,28] The produced copolymer was reacted with 100-fold excess of ethylenediamine with respect to GMA content in the copolymer at room temperature for 24 h. The resulting product denoted as PVPON-NH_2 contained 20% of amino-group-containing units.

Tannic acid (TA; M_w 1701.20), hydrochloric acid, sodium hydroxide, sodium chloride, and monobasic sodium phosphate were purchased from Sigma-Aldrich Chemical Co. D_2O with 99.9% isotope content was purchased from Cambridge Isotope Laboratories. All chemicals were used as received. The deionized (DI) H_2O was purified by passage through a Milli-Q system (Millipore).

Turbidimetric Analysis. Turbidity was measured using UV–vis spectrophotometer U-300 Hitachi (Hitachi International Inc.). Measurements were performed at 450 nm where individual TA, PVPON, or Q90 solutions have no absorption bands. The increased absorbance upon mixing of TA and PVPON or Q90 solutions reflected the formation of water-insoluble complexes between TA and the polymers.

Deposition of Multilayers for Ellipsometry and AFM. TA multilayers with PNIPAM, PEO, PVPON, or PVCL were deposited at the surface of oxidized Si wafers from 0.5 mg/mL polymer solutions in 0.01 M phosphate buffer at pH 2, pH 4, and pH 7.5. Prior to film depositon, silicon wafers were irradiated with UV light for 2 h to remove organic impurities from the surface of supplied wafers. UV-irradiated wafers were then washed with DI water and treated with concentrated sulfuric acid for 10 min. After rinsing with DI water, silicon wafers were immersed in 0.25 M NaOH solution for 10 min, rinsed with DI water, and dried under a flow of nitrogen. To enhance the adhesion of multilayer films to the substrate, BPEI layer was allowed to adsorb from 0.5 mg/mL solution in 10^{-2} M phosphate buffer at pH 7.5 as a precursor layer. The precursor layer was used for depositions of all polymer systems at pH 7.5 as well as for the deposition of PNIPAM/TA films at pH 2. Note that polymer solutions were cooled to 10 °C prior to deposition for PNIPAM/TA system at pH 2. Q90/TA and PVPON-NH_2-20/TA multilayers were deposited from 0.5 mg/mL polymer solutions in 0.01 M phosphate buffer at pH 7.5. All film depositions were performed by alternatingly immersing the silicon wafers in polymer solutions for 5 min followed by two intermediate rinsing steps with 0.01 M phosphate buffer using Robot Arm Catalyst 3 (CRS Robotics Corp.) operated with homemade software. The thickness of the dry films was measured using a home-built phase-modulated ellipsometer.[29] pH stability of the films were investigated by exposing the films to buffer solutions at different pH values. pH of the buffer solutions was adjusted with either 0.1 M HCl or NaOH solutions.

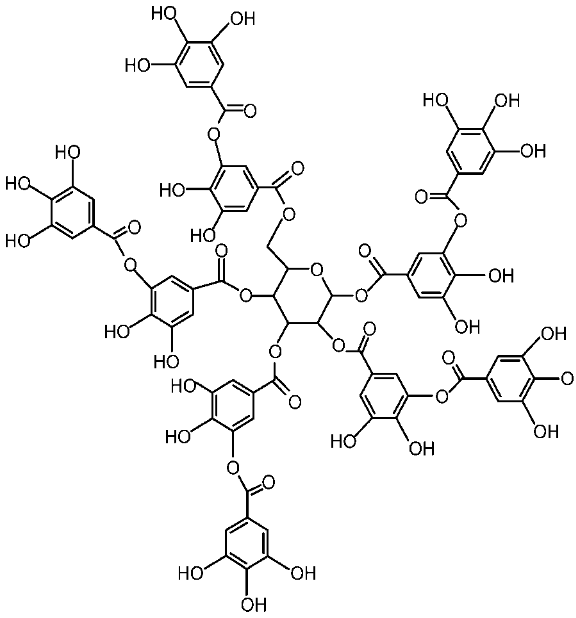
ATR-FTIR. A Bruker Equinox-55 FTIR spectrometer equipped with a narrow-band mercury cadmium telluride detector and a home-built flow-through cell was used for in-situ ATR-FTIR experiments. Multilayers were deposited on the bare surface of oxidized ATR Si crystal installed within the flow-through stainless steel cell. PVCL and TA were allowed to adsorb from 0.5 mg/mL buffered solutions of D_2O at pH 2. Adsorption of each polymer layer was followed by a rinsing step with 0.01 M phosphate buffer at the same pH. pH-triggered destruction experiments were performed by sequentially replacing the cell volume with buffered D_2O at a specific pH. At each pH, the system was allowed to come to equilibrium where no significant decrease in the intensity of absorption bands was recorded. The peak integration was performed using Galactic Grams/32 software.

Atomic Force Microscopy (AFM). AFM measurements were performed in air at room temperature using a NSCRIPTOR Dip Pen Nanolithography system (Nanoink). AFM images are presented in the Supporting Information.

Results and Discussion

I. Tannic Acid in Solution. Scheme 1 shows structure of TA, which includes five digalloyl ester groups esterified to a glucose core.[30] Figure 1 shows that at acidic and neutral conditions the UV–vis absorption spectrum of TA exhibited two peaks: one centered at ~223 nm and the other at 283 nm. With an increase in pH to basic values, two additional peaks at longer wavelengths, i.e., at ~245 and ~323 nm, emerged, and their intensity increased at expense of a decrease in the intensity of 223 and 283 nm bands. The longer wavelength 245 and 323 nm peaks in the TA spectra were assigned to ionized form of TA molecules,[23] resulting from ionization of the phenolic hydroxyl groups of galloyl groups of TA. The inset in Figure 1 illustrates that the ratio of the absorbance at 323 nm to that at 283 nm, A_{323}/A_{283}, sharply increased in the interval of pH from 7.5 to 10. Importantly, a change in the relative peak intensities was reversible in the pH scale. Assuming that extinction coefficients of nonionized and ionized forms of TA at 283 and 323 nm are equal, a pK_a value of TA can be estimated from the dependence of A_{323}/A_{283} on pH in Figure 1. The estimated pK_a value of 8.5 for TA is in good agreement with pK_a of 8.45 for

Scheme 1. Chemical Structure of Tannic Acid



ionization of phenolic hydroxyl groups in gallic acid reported by Bykova et al.[31] Phenols are known to exhibit lower pK_a values as compared to aliphatic alcohols because the electrical charge distribution on the aromatic ring results in reduction of negative charge on oxygen atoms. In TA, which contains 2 or 3 $-$OH groups per phenol ring, this phenomenon is enhanced, and the pK_a is shifted to lower values compared to monophenols.

Our data also showed that while TA is stable in solutions at pH lower than 7, special care needs to be taken when working with TA solutions at pH $>$ 7, where TA undergoes oxidation due to reaction with atmospheric oxygen. When such oxidation occurred, TA solution became colored, and two additional absorbance bands, probably associated with the oxidation products, emerged at 263 and 360 nm. Controlling solution temperature and exposure to light successfully inhibited oxidation of TA solutions at 7 $<$ pH $<$ 10.

II. Interpolymer Complexes of TA in Solution. Several groups including ours[32–35] have established and studied correlation between deposition of LbL films at surfaces with phase behavior of interpolymer complexes (IPCs) in solution. Therefore, prior to depositing polymer/TA multilayers at solid substrates, we sought to investigate the phase behavior of hydrogen-bonding mixtures of TA and neutral polymers and to contrast these results with interaction of TA and a positively charged polymer, Q90. Polymer structures involved in formation of interpolymer complexes in solution and self-assembly at surfaces are shown in Scheme 2.

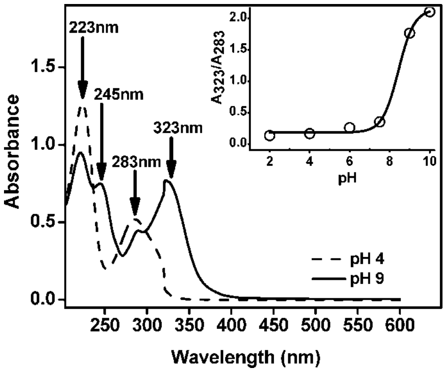
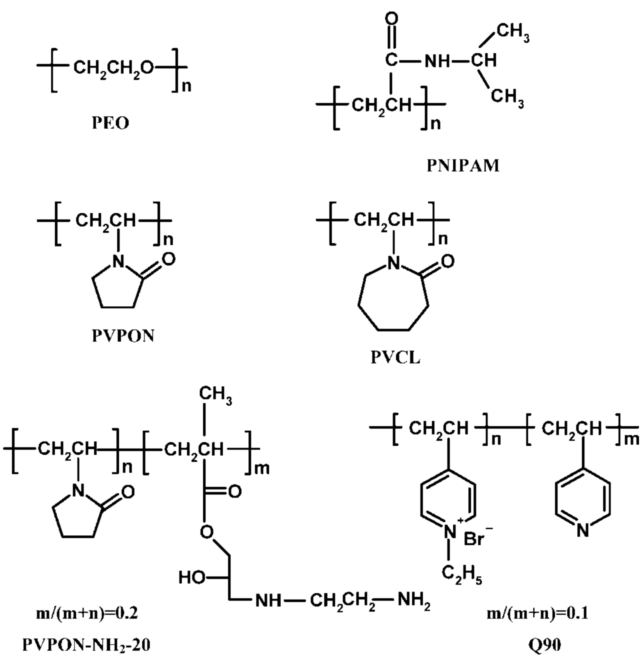


Figure 1. UV−vis absorption spectrum of TA at pH 4 (dashed line) and pH 9 (solid line). pH was adjusted by adding either HCl or NaOH to the TA solution. Inset shows the ratio of absorbances of phenolate (323 nm) to neutral form (283 nm) of TA plotted as a function of pH.

Scheme 2. Polymers Used for Binding with TA in Solution and/or at a Solid Surface



The turbidimetric titration technique was used for monitoring the formation of water-insoluble complexes between TA and the polymers. Figures 2A and 3A show absorbance at 450 nm plotted as a function of molar fraction of TA phenol groups (f_{TA}) in the mixture, determined as the ratio of molar concentration of TA phenol groups to the sum of molar concentration of TA phenol groups and Q90 or PVPON monomeric units in Q90/TA or PVPON/TA aqueous solutions, respectively. Q90/TA (Figure 2A) and PVPON/TA (Figure 3A) systems show drastic differences in their phase behavior as a function of pH.

In the case of Q90, which carries 90% of permanent charge, electrostatic interactions between Q90 and TA are expected to drive Q90/TA association (see Scheme 3). Indeed, at pH 2 where TA is not ionized, Q90/TA solutions remained transparent at all compositions of Q90/TA mixtures, indicating no binding between Q90 and TA. However, at a higher pH of 7.5, Q90/TA IPCs formed. The maximum turbidity at f_{TA} = 0.7−0.85 corresponded to formation of water-insoluble IPCs. In excess of Q90, Q90/TA complexes were solubilized by the excess positive charge in Q90 loops. Solubilization in excess of TA was less efficient because of higher rigidity of TA and its incomplete ionization at pH 7.5. Assuming that at the maximum turbidity ($f_{TA} \approx 0.7$) charges are mutually compensated within IPCs, we inferred ionization of phenolic groups of TA as ~35%. This value is higher than that expected for TA solutions at pH 7.5 based on estimated pK_a value of TA of ~8.5. A similar conclusion of increased ionization of TA within IPC follows from data in Figure 2B. This figure shows that upon exposure of Q90/TA complex to pH $<$ 7.5 significant association between Q90 and TA chains persisted to pH values as low as 3.5−4. Higher ionization of phenol groups of TA when it resides in vicinity of or forms ionic pairs with positive charges in Q90 chains is consistent with earlier reports of ionization of polycation-bound weak polyacids.[36–38]

While TA did not bind with Q90 at pH 2, it was able to efficiently associate with PVPON at this pH value. The turbidity

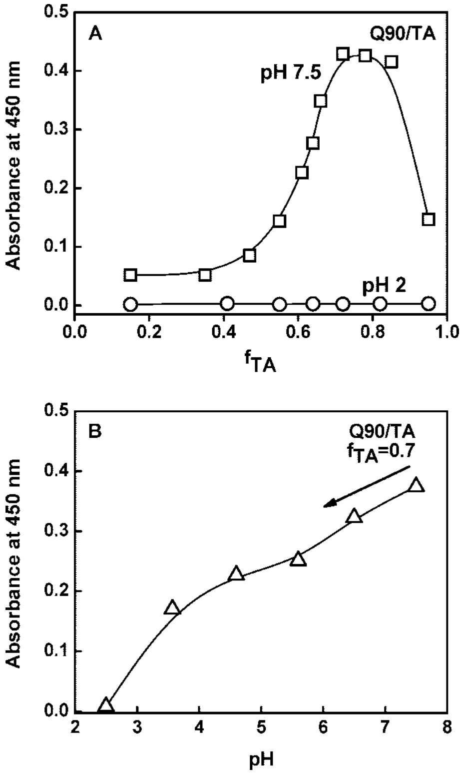


Figure 2. (A) Turbidity of Q90/TA mixtures measured as absorbance at pH 2 (open circles) and pH 7.5 (open squares) as a function of the mole fraction of TA units (f_{TA}). Mole fraction was achieved by mixing 2.2 mM Q90 (in repeating units) and 3.9 mM TA (in moles of phenol groups) solutions at various ratios. (B) Turbidity of Q90/TA IPCs measured as absorbance of a mixture of 2.2 mM Q90 and 3.9 mM TA (f_{TA} = 0.7) formed at pH 7.5 and exposed to decreasing pH values with 20 min intervals. The pH was supported by 0.01 M phosphate buffer.

data in Figure 3A illustrate that PVPON and TA form water-insoluble IPCs at acidic pH values. As a nonionic polymer with strong hydrogen-accepting carbonyl groups, PVPON associates with TA through hydrogen bonding (see Scheme 4). Twenty-five phenolic hydroxyl groups in TA molecule, which are protonated at pH 2, are excellent hydrogen donors for binding with carbonyl groups of pyrrolidone rings. The multiplicity of hydrogen bonding between TA and PVPON assures the overall strength of PVPON/TA association. From the maximum turbidity of PVPON/TA mixtures at $f_{TA} \sim 0.45-0.5$, the molar ratio of TA to PVPON units within PVPON/TA water-insoluble IPC was calculated as ~0.8−1.0. At pH 7.5, where ionization of TA increased, PVPON/TA mixtures had lower turbidity at all compositions (Figure 3A), since increased negative charge in TA molecules solubilized PVPON/TA IPCs at this pH.

Figure 3B shows time evolution of turbidity of PVPON/TA mixtures when PVPON/TA IPCs with $f_{TA} = 0.45-0.5$ was preformed at pH 2 and then exposed to solutions with pH 6, 7.5, 8, or 8.5. Solubilization kinetics of the PVPON/TA IPCs was faster at higher pH values, when ionization of TA was higher. The inset in Figure 3B summarizes turbidity values for such experiments, when the PVPON/TA mixture was exposed to increasing pH values with 20 min intervals. Completely transparent solutions formed only at pH 8.5, which is close to the estimated pK_a value of TA in solution. Comparison of data in Figures 3A,B shows that absorbance values of PVPON/TA IPCs were always lower when such IPCs were directly prepared at a certain pH value. Such hysteresis is explained by the fact that in the case when PVPON/TA IPCs were formed at pH 2, ionization of phenol groups of TA was suppressed due to hydrogen bonding with PVPON units. Since dissociation of the PVPON/TA complex occurs at a specific critical ionization of TA, preformed PVPON/TA IPCs should be exposed to higher pH values to reach this critical ionization degree. Similar hysteresis in pH stability was observed for other hydrogen-bonded polymer systems deposited at surfaces using the LbL technique.[39]

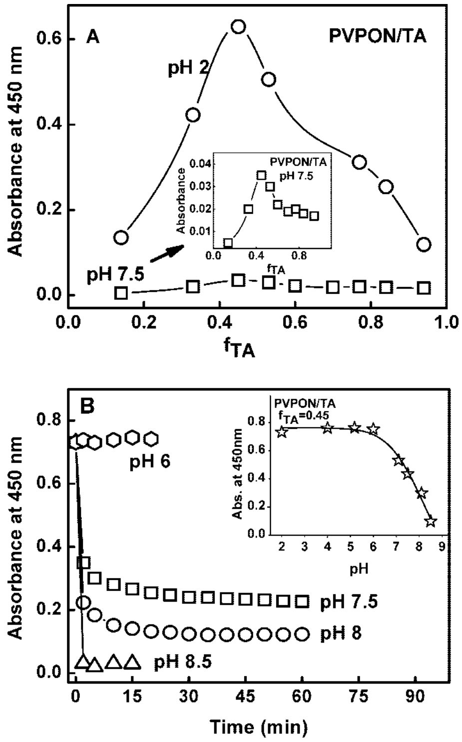
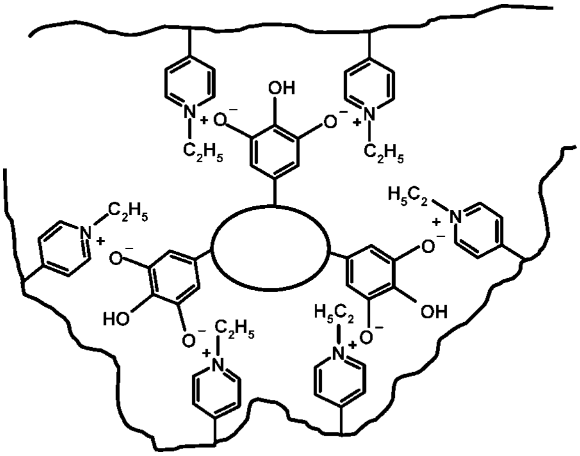


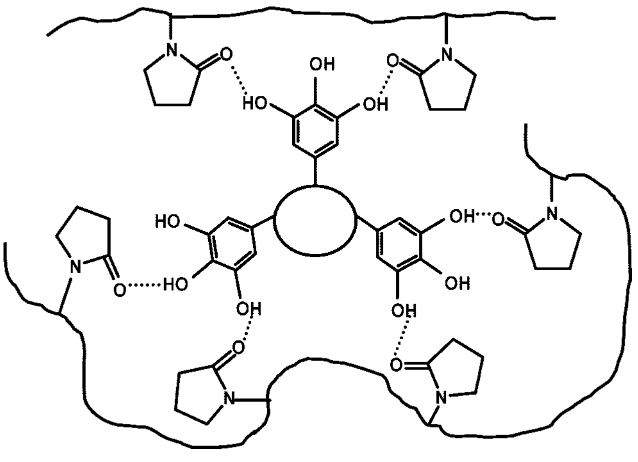
Figure 3. (A) Turbidity of PVPON/TA solutions measured as absorbance at pH 2 (open circles) and pH 7.5 (open squares) as a function of the mole fraction of TA units (f_{TA}). Mole fraction was achieved by mixing 4.5 mM PVPON (in repeating units) and 7.35 mM TA (in moles of phenol groups) solutions at various ratios. (B) Time evolution of turbidity of PVPON/TA solutions at several pH values. IPCs with f_{TA} = 0.45 were formed by mixing 4.5 mM PVPON and 7.35 mM TA solutions at pH 2. Inset shows absorbance values of PVPON/TA with f_{TA} = 0.45 after 20 min exposure to increasing pH values. pH was supported by 0.01 M phosphate buffer (main panel) or adjusted to higher pH values by adding diluted NaOH.

Scheme 3. Schematic Representation of Electrostatic Pairing between Quaternized Pyridinium Groups of Q90 and Phenolate Groups of TA



Note that in Schemes 3 and 4 we assume that binding of TA with polymers occurs either through ionized or nonionized

Scheme 4. Schematic Presentation of Hydrogen-Bonding Interactions between Carbonyl Groups of PVPON and Phenolic Hydroxyl Groups of TA



phenol groups of TA, respectively. Earlier reported aromatic stacking interactions between TA rings are not considered.[40]

We have also observed that hydrogen-bonded water-insoluble IPCs were formed in acidic solutions between TA and other neutral hydrogen-bonding polymers, such as PEO, poly(*N*-vinyl methyl ether) (PVME), poly(acrylamide) (PAAM), PVCL, and PNIPAM with M_w 200 000, 200 000, 10 000, 1800, and 300 000 (data not shown). Interestingly, when aliphatic polyphenol– poly(vinyl alcohol) (PVA) with M_w 125 000 was used in place of TA, no turbidity was observed at either molar compositions of solutions. Considering that PVA molecules with M_w 125 000 have a larger number of hydroxyl groups per chain as compared to the number of phenol groups in TA molecule, this result might seem surprising. Greater acidity of H atoms of phenol rings (compared to aliphatic alcohols) provides TA with a higher capacity to form hydrogen-bonded IPCs in aqueous solutions. The additional role of high density of hydrogen-bonding phenol groups in TA molecule which enhances cooperativity of binding is another contribution to strong association of TA with a variety of neutral hydrogen-bonding synthetic polymers.

III. TA Multilayers. *III.1. Electrostatically Assembled Q90/TA Multilayers.* Guided by previous work on electrostatic self-assembly of TA with polycations,[23] and our data on association of Q90 with TA in solutions at neutral pH values, we pursued fabrication of Q90/TA LbL films. At pH 7.5, multilayers could be constructed (with an average bilayer thickness of 3.5 nm as measured by ellipsometry) due to electrostatic pairing between the quaternized pyridinium units of Q90 and phenolate form of TA molecules.

Figure 4 shows that Q90/TA films demonstrated superior pH stability when built at pH 7.5 and then exposed to lower pH values. Such high stability of Q90/TA films might seem surprising, considering the high pK_a value of TA (pK_a ~ 8.5) and the fact that deconstruction of the layers at acidic conditions is generally expected because of pH-induced misbalance between positive and negative charges within the film. However, Q90/TA films were more stable at low pH values than earlier studied by our group Q90/poly(methacrylic acid) (PMAA) films,[36] in spite of lower pK_a value for PMAA (pK_a ~ 6).[16,41] As a comparison, i.e. ~80% of the Q90/TA film retained on the surface at pH 1.3 as seen in Figure 4, while Q90/poly(methacrylic acid)(PMAA) film was completely removed from the surface at this pH value.[36] The higher stability of TA self-assembly might be a result of higher rigidity of TA molecule, resulting in a higher cooperativity of binding of phenolate oxyanions with positively charged groups in Q90 chains. Addition of 0.15 M NaCl decreased stability of Q90/TA multilayers at low pH values (Figure 4), as salt screened ionic pairing between Q90 and TA, resulting in weakened Q90/TA interactions and partial dissolution of the film.

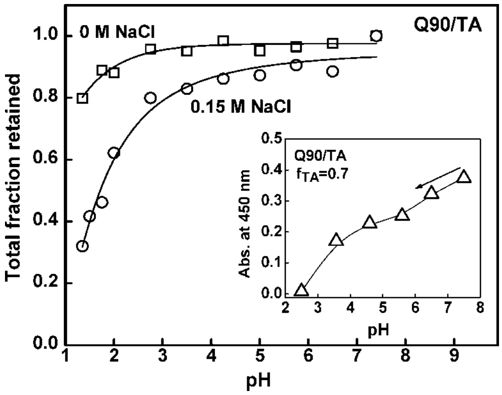


Figure 4. pH-triggered disintegration of six-bilayer Q90/TA film deposited at pH 7.5 and exposed to lower pH values without (squares) or with additional 0.15 M NaCl (circles) as monitored by ellipsometry. Inset shows turbidity of Q90/TA IPCs measured as absorbance of a mixture of 2.2 mM Q90 and 3.9 mM TA (f_{TA} = 0.7) formed at pH 7.5 and exposed to decreasing pH values with 20 min intervals.

Significantly, in spite of higher stability of Q90/TA multilayers at low pHs, the films could not be deposited at pH 2. The observed history dependence of pH stability of LbL films constructed from a weak polyelectrolyte is consistent with previously reported data[39] and is explained by differences in ionization of weak polyelectrolytes when they are included into electrostatically assembled films as compared to their ionization in solution. Unlike Q90/TA system, films of TA and neutral hydrogen-bonding polymers could be deposited at acidic pH values, as this is discussed in the next section.

III.2. Hydrogen-Bonded Multilayers of TA. Multilayers Deposited at pH 2. We then focused on self-assembly of polymers which are neutral in a wide pH range and have hydrogen accepting either carbonyl groups (such as in PNIPAM, PVPON, or PVCL) or ether oxygen (such as in PEO) which are capable of binding to hydrogen-donating phenolic hydroxyl groups of TA. The LbL deposition of PNIPAM/TA, PEO/TA, PVPON/TA, and PVCL/TA at the surface of silicon wafers was explored at various pH values using the ellipsometry technique.

All systems showed linear growth mode at pH 2 (with average thickness per bilayer 4.5, 4.6, and 4.2 nm for PVPON/TA, PVCL/TA, and PNIPAM/TA films), except the PEO/TA system, which exhibited “exponential growth” mode with layer thickness first increasing and then showing film fouling when number of layers exceeds 14 (Figure 5).

PEO/TA films were rough and appeared visibly opaque. Irregular hydrogen-bonded films of PEO were reported earlier by one of us for the case of its binding with PMAA;[16] rough PEO/poly(acrylic acid) films were also reported by Hammond’s group.[42] Exponential growth, high roughness, and large bilayer thickness of PEO-containing films are explained by weak binding between PEO and hydrogen-donating polymer units. Note that the dependence of the strength of hydrogen-bonding interactions on the nature of hydrogen-bonding groups has been earlier demonstrated for polymer blends and mixtures in solution.[43–45]

Among PVPON/TA, PVCL/TA, and PNIPAM/TA systems, PVCL/TA films were the most robust, showing systematic increase in film thickness at both steps of PVCL and TA deposition. The facts that the PVCL/TA system did not exhibit adsoption/desorption behavior and that TA was deposited at the film surface with monolayer increments are explained by higher

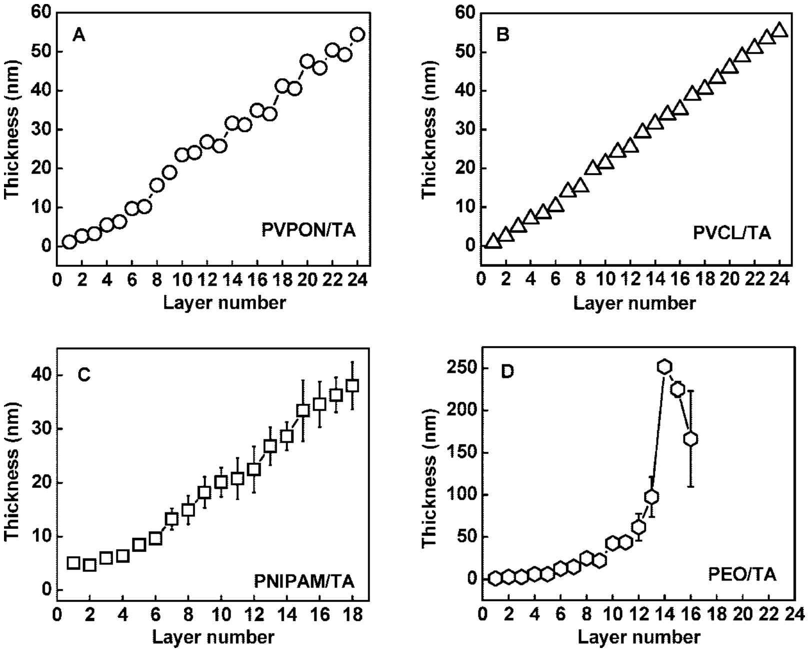


Figure 5. Ellipsometry data on LbL deposition of polymers with TA at pH 2, when films were dried after each deposition step. Error bars are within symbol size if not shown.

hydrophobicity of PVCL chains. Although PVCL and PVPON are homologues polymers, two more methylene groups in the caprolactam ring contribute significantly to the hydrophobicity for stabilization of hydrogen-bonded layers. The significance of contribution of hydrophobic interactions in hydrogen-bonded LbL self-assembly has been recognized earlier and results in higher stability of PVCL/PMAA films as compared to films of PMAA and other neutral polymers.[46,47]

The PVPON/TA system showed obvious zigzag-type growth, i.e., an increase in thickness at the TA deposition step, followed by some loss in thickness during deposition of PVPON (Figure 5A). Note that the thickness of TA layers deposited at the top of the film was 5 ± 2 nm, i.e., higher than molecular dimensions of TA molecule (1.85 × 1.65 × 1.01 nm).[23] Self-association of the polyphenols when bound to a peptide was earlier reported.[40] Also, deposition of TA layer in aggregated state was shown by Lvov and co-workers for the PAH/TA system.[23] At the step of PVPON deposition, the excess amount of TA was removed from the surface, leaving behind a monolayer of TA. Solubilization of TA molecules by excess amount of PVPON chains is not surprising as PVPON is highly soluble in water and is commonly used as a stabilization/dispersion agent for colloidal and nanoparticle suspensions. Desorption always occurred at the step of deposition of linear polymer chains, while highly rigid TA molecules were not capable to solubilize adsorbed polymer chains.

Multilayer Deposition at Various pH Values. Multilayer growth profiles for all polymer systems are presented in Figure 6 where thickness is plotted as a function of bilayer number at pH 4 and pH 7.5. A decrease in amounts adsorbed within the film at higher pH is related to the extent of association between the polymer pairs. Increased ionization of TA molecules prohibits the association with its neutral partners, resulting in thinner films. It is also evident from the AFM phase images shown in the Supporting Information, Figure 1S, that smoother films were formed at higher pH values due to increased ionization of TA and decreased aggregation of TA. $(PVCL/TA)_6$ films deposited at pH 2 and 7.5 had rms roughness of 9 and 2 nm, respectively (Figure 1S).

For all systems, bilayer thickness decreased at higher pH values. As seen in Figure 3B, turbidity of PVPON/TA solutions is significantly decreased at higher pH values, as this is reflected in data in Figure 6 obtained for surfaces.

The effect of pH on the deposition of hydrogen-bonded films was earlier reported by us for PVPON/PMAA and PEO/PMAA systems[48] and demonstrated by Hammond and co-workers for the PEO/PAA system. A decrease in the adsorbed amount with increasing deposition pH was found, followed by complete loss of film deposition due to increased ionization of the polyacid and dissociation of interpolymer hydrogen bonds.[5] Results in Figure 6 are similar to those reported earlier, with one important difference that in the case of TA film construction was also possible at neutral values of pH. The shift in pH window for deposition of hydrogen-bonded multilayers to higher values is caused by higher pK_a of TA acid as compared to earlier used PAA and PMAA.

pH-Induced Dissolution of Multilayers Deposited at pH 2. It was shown earlier that an increase in pH results in destruction of hydrogen-bonded multilayer films, and the critical pH for film dissolution correlates with pK_a of the polyacid and the strength of hydrogen bonding determined by the nature of hydrogen-bond donor and acceptor groups.[16,46] Using the same approach, we here investigated the pH-induced dissolution of PVPON/TA films. Importantly, PVPON/TA films were stable at pH values up to 8.75. Figure 7 contrasts the time evolution of PVPON/TA film thickness at pH 8.5, 8.75, 9, and 9.25. Similarly to results obtained for PVPON/TA complex in solution (Figure 3B), PVPON/TA films showed faster equilibration time with increasing pH. However, the pH stability interval of the PVPON/TA system was wider at surfaces than that in solution. For example, at pH 8.5, only 10% of the film dissolved which can be attributed to the loosely bound outer layers, while complete dissolution of PVPON/TA complexes occurred momentarily in solution at this value of pH. A shift of the pH stability profile of about 1 pH unit to higher values for the films as compared to PVPON/TA complexes in solution is due to surface proximity effects. We hypothesize that lower local dielectric environment within the film results in suppressed ionization of TA and therefore higher critical dissolution pH of PVPON/TA multilayers.

Figure 8 summarizes the destruction profiles of six-bilayer PVPON/TA, PVCL/TA, PNIPAM/TA, and PEO/TA films

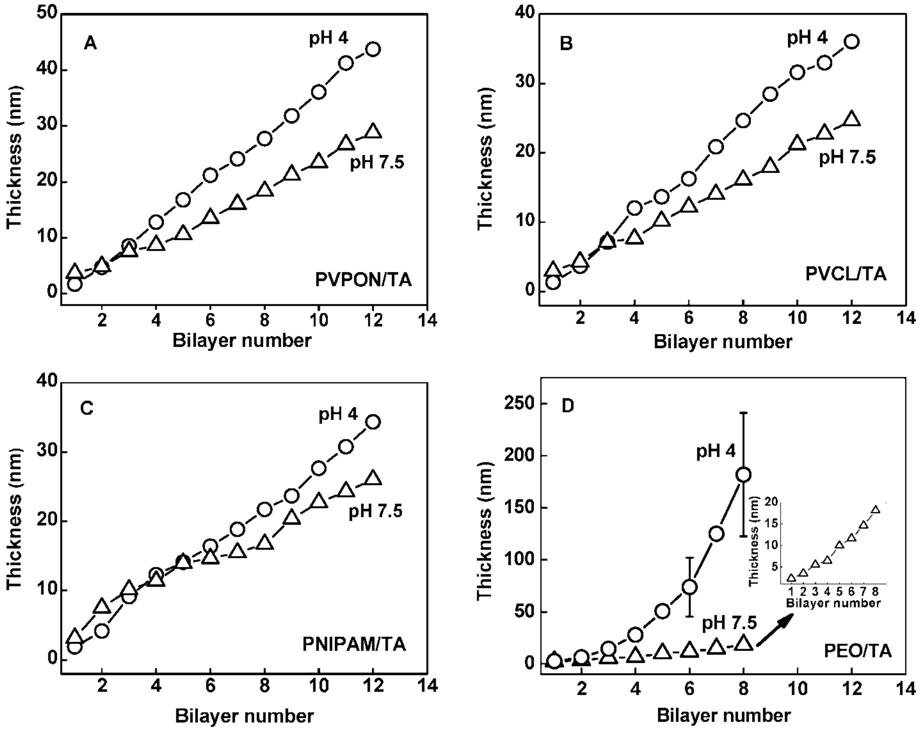


Figure 6. LbL deposition of PVPON/TA, PVCL/TA, PNIPAM/TA, and PEO/TA films at pH 4 and pH 7.5 as monitored by ellipsometry. Films were dried after deposition of each bilayer. Error bars are within symbol size if not shown.

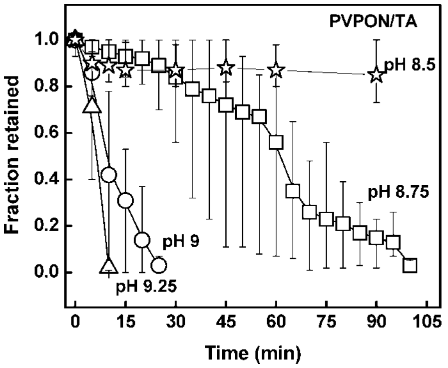


Figure 7. Time evolution of the film erosion for six-bilayer PVPON/TA film deposited at pH 2 and exposed to pH 8.5, 8.75, 9, or 9.25. Films immersed in buffer solutions were removed from solutions at certain time intervals, dried for thickness measurements by ellipsometry, and then returned to the buffer solution. The thickness values were averaged from the data determined at 5−6 different spots. Big error bars in the film dissolution region reflect inhomogeneous peeling of the films from the substrate. The upper and lower limit of the error bars correspond to the highest and lowest thickness recorded by ellipsometry at different spots.

which were deposited at pH 2 and then exposed to buffer solutions at higher pH for 20 min. The critical disintegration pH was defined as pH at which ∼50% of the film dissolved in 20 min after immersion in buffer solution. In buffer solutions with low concentrations of salt, the critical pH values for film disintegration were 9, 9.5, 8, and 8.5 for PVPON/TA, PVCL/TA, PNIPAM/TA, and PEO/TA films, respectively. Importantly, PVPON/TA and PVCL/TA systems were stable at pH values higher than pK_a of TA. Earlier, we found that multilayers of poly(carboxylic acid)s with PVPON and PVCL also showed stronger pH stability compared to that those with poly(2-hydroxyethyl acrylate) (PHEA), PEO, PAAM, PVME, or PNIPAM and attributed this to strong hydrogen bonding and large number of hydrogen-bonded units between PVPON (PVCL) and the polyacid. In the case of multilayers of TA, a similar trend was observed. The ∼0.5 pH higher critical dissolution pH for PVCL/TA as compared to that of PVPON/TA systems correlated with increased hydrophobicity of VCL units due to the presence of two more methylene groups in VCL ring as compared to the VPON ring.

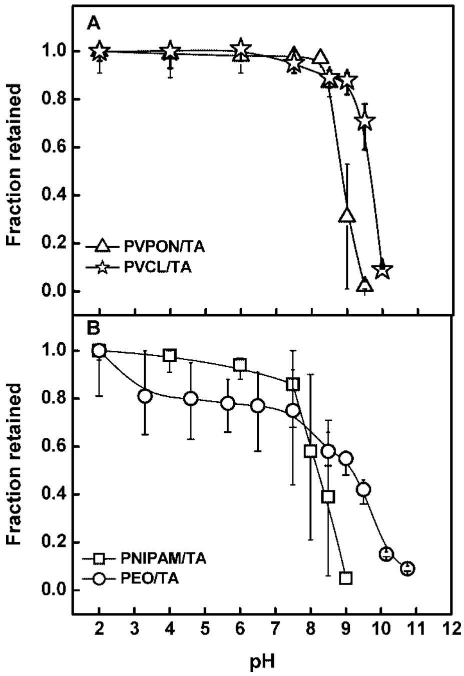


Figure 8. pH-triggered disintegration of six-bilayer PVPON/TA, PVCL/TA (panel A) and PNIPAM/TA, PEO/TA (panel B) films deposited at pH 2 and exposed to 10^{-2} M phosphate buffer solutions of increasing pH. Fractions retained on the surface after exposure to buffer solutions for 20 min are plotted versus pH.

Also seen in Figure 8B is the unexpected stability of the PEO/TA system at high pH values (note that PEO/polycarboxylic acid films dissolved at pH > 4.6,[46] lower than all other

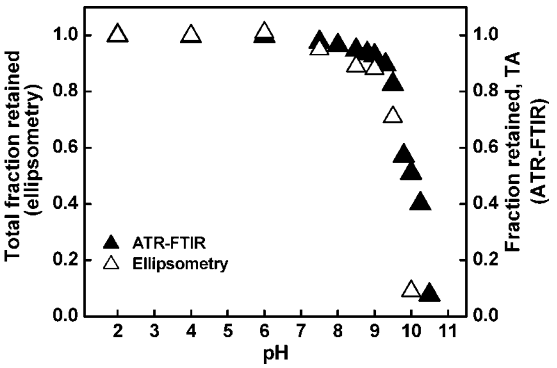


Figure 9. pH-triggered disintegration of six-bilayer PVCL/TA (open triangles) and twelve-bilayer PVCL/TA (filled triangles) monitored by ellipsometry and ATR-FTIR, respectively. Films were deposited at pH 2 and exposed to 10^{-2} M phosphate buffer solutions of increasing pH. ATR-FTIR measurements were obtained in buffered D_2O solutions.

hydrogen-bonded polymer systems). In contrast, the critical dissolution pH observed for the PEO/TA system lies in between PNIPAM/TA and PVPON/TA systems.

The addition of 0.15 M NaCl resulted in a ~0.5 pH shift of the film dissolution transition to lower values for all systems (data not shown). The destabilizing effect of the added salt is explained by enhanced ionization (decreased pK_a value) of TA and increased osmotic pressure within the film, resulting in disintegration of hydrogen-bonded polymer layers at the lower pH value. Considering that the film disintegration rate could be fine-tuned in the range of critical dissolution pH (Figures 7 and 8), ionic strength, and the variation of the nature of neutral hydrogen-bonding polymer, desired film disintegration rate in the desired pH range can be achieved. Such approach might be useful for designing films capable to dissolve (and deliver loaded functional molecules) with a controlled rate.

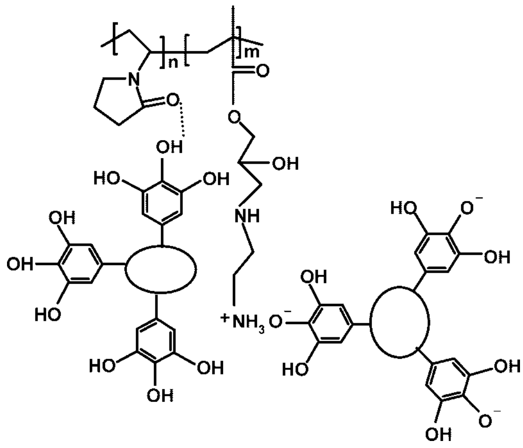
Figure 9 shows that ellipsometric and in-situ ATR-FTIR techniques gave consistent results when applied to measurements of film destruction profiles of PVCL/TA films as a representative system. The ATR-FTIR spectrum of a 12-bilayer PVCL/TA film with corresponding peak assignment is given in the Supporting Information (Figure 2S). In Figure 9, the data are presented as absorbances of the 1720 cm^{-1} band (associated with stretch C=O vibrations of the ester groups of TA) normalized to the initial absorbance of the band at pH 2.

III.3. PVPON-NH$_2$-20/TA Multilayers. We were then interested in exploring the ways to further improve pH stability of TA-containing multilayers. This approach was based on designing a multilayer system that is capable of switching from hydrogen bonding to electrostatic association between the polymer pairs upon pH variations. For this purpose, we used PVPON copolymer, PVPON-NH$_2$-20, which contained 20% of primary amino groups.[28,39] PVPON-NH$_2$-20/TA mutilayers were self-assembled with TA at pH 7.5. The driving force for film deposition was a combination of electrostatic interactions between the protonated amino groups of PVPON-NH$_2$ and phenolate form of TA as well as hydrogen-bonding interactions between carbonyl groups of PVCL and phenolic hydroxyl groups of TA (see Scheme 5).

Because of the combination of these interactions, PVPON-NH$_2$-20/TA films could be constructed at pH 7.5 with the average bilayer thickness of 6.5 nm (as measured by ellipsometry). Note that the PVPON/TA system gave a significantly smaller thickness of 2.4 nm at the same pH value.

The pH-triggered disintegration of the multilayers was then performed by exposing the films from neutral to extreme acidic (Figure 10A) or extreme basic (Figure 10B) values. Interestingly, PVPON-NH$_2$-20/TA films were stable in a wide pH range from 1.3 to 11.7 at low ionic strength. At highly acidic environment where electrostatic interaction between the amino groups of PVPON-NH$_2$ and TA weakened, hydrogen-bonding interactions between the carbonyl group of PVPON and phenolic hydroxyl group of TA were capable of retaining 90% of the film at the surface. Addition of salt resulted in only slightly increased film dissolution of ~3−7% at pH 1.3.

Scheme 5. Schematic Presentation of Hydrogen-Bonding and Electrostatic Interactions between PVPON-NH$_2$-20 Copolymer and TA



On the other hand, PVPON-NH$_2$-20/TA films also did not dissolve in basic solutions at pH up to 11.7. Addition of salt slightly lowered the critical dissolution pH by ~0.5 pH units. Such extreme stability at extremely basic pHs is due to

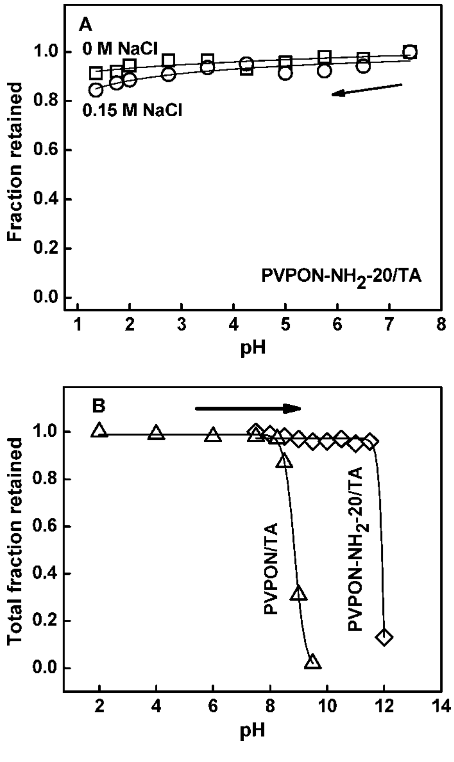


Figure 10. (A) pH-triggered disintegration of six-bilayer PVPON-NH$_2$-20/TA film deposited at pH 7.5 and exposed to 10^{-2} M phosphate buffer solutions with decreasing pH in the absence of additional salt (squares) or with additional 0.15 M NaCl (circles). (B) pH-triggered disintegration of six-bilayer PVPON/TA (triangles) and PVPON-NH$_2$-20/TA (diamonds) films deposited at pH 2 and pH 7.5, respectively, and exposed to 10^{-2} M phosphate buffer solutions with increasing pH. The fraction remaining was calculated from film thicknesses measured by ellipsometry.

electrostatic pairing between protonated primary amino groups of PVPON-NH_2 and phenolate form of TA. Further increase in pH to pH >11.7 resulted in film dissolution due to deprotonation of primary amino groups in the PVPON-NH_2-20 copolymer and dissociation of electrostatic pairs between TA and PVPON-NH_2-20. Note that PVPON/TA films lacked additional electrostatic interactions and dissolved at pH ~9, as this is shown in Figure 10B. Comparing dissolution profiles of PVPON-NH_2-20/TA and PVPON/TA systems, it was remarkable to observe that the presence of 20% of primary amino groups in PVPON-NH_2-20 caused such a dramatic improvement in the pH stability of PVPON-NH_2-20/TA films.

Conclusions

We have presented a comparative study of self-assembly of a water-soluble biologically active polyphenol molecule—TA—within layer-by-layer polymer films based on hydrogen-bonding interactions, electrostatic pairing, or combination of the two. Dissolution of polymer/TA multilayers had reverse pH profiles in cases of hydrogen-bonded and electrostatically associated films, i.e., hydrogen-bonded multilayers of TA with neutral polymers dissolved at high pH values, while electrostatically stabilized films of TA with cationic polymer dissolved upon pH lowering. The results were in good correlation with phase behavior of IPCs of TA and corresponding polymers in solution, with slightly higher pH stability of TA-containing films at surfaces. A combination of centers of hydrogen bonding and electrostatic pairing within the polymer chain allowed construction of copolymer/TA films with high pH stability. In the case of hydrogen-bonded films of TA with neutral water-soluble polymers, multilayer films dissolved at basic pH values, which were higher than those for earlier studied films of the neutral polymers with poly(acrylic acid) and poly(methacrylic acid). The possibility of tuning the pH range for polymer/TA film dissolution to neutral/basic values might allow future use of such hydrogen-bonded films in biomedical applications as bioactive films which are stable at a physiological pH values and/or as films which dissolve with a controlled rate and deliver functional molecules from surfaces.

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Supporting Information Available: AFM images of hydrogen-bonded $(PVCL/TA)_6$ films and representative ATR-FTIR spectrum of a $(PVCL/TA)_{12}$ film. This material is available free of charge via the Internet at http://pubs.acs.org.

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