

Polyelectrolyte Multilayers of Poly(vinylamine hydrochloride-co-N-vinylformamide) with Variable Primary Amine Content and a Weak Polyacid Poly(acrylic acid)

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ABSTRACT: Poly(vinylamine hydrochloride-co-N-vinylformamide) [poly(VAm-co-NVF)] with variable VAm content, and a weak polyacid poly(acrylic acid) were assembled on a quartz crystal microbalance (QCM) substrate from their aqueous solutions, thus forming polyelectrolyte multilayers. The effects of varying the VAm content on assembly profiles and film structures were analyzed by quantitative QCM analysis, attenuated total reflection spectra techniques, and atomic force microscopic observation. At a VAm content of less than 40% of the total number of poly(VAm-co-NVF) units, the layer-by-layer assembly did not proceed, and the frequency shift of the QCM zigzagged with the step number. Above 40% VAm content, the frequency increased continu-

ously with an increasing number of steps. The assembly amount was also maximal at 40% VAm content. The films obtained swelled with water molecules, and this swelling was followed by an increase in thickness in the aqueous phase. The results obtained were compared to those obtained from a combination of poly(VAm-co-NVF) and a strong polyacid, poly(sodium styrenesulfonate). © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 102: 3927–3933, 2006

Key words: polyelectrolyte multilayer; layer-by-layer assembly; charge density; electrostatic interaction; quartz crystal microbalance

INTRODUCTION

Fabrication of ultrathin polymer films on material surfaces enables functional modification of the surface characteristics without changes in mechanical properties. Analysis of film fabrication permits the investigation of surface and bulk properties of the films for understanding novel polymeric surface behaviors. Although conventional solution- and spin-casting methods can be readily used to prepare polymer films, it is occasionally difficult to apply these methods, especially to water-soluble polyelectrolytes such as charged synthetic polymers and biomacromolecules. It is also difficult to regulate the assembly nanostructures of films using conventional methods.

Simple alternate immersion of solid materials into oppositely charged polyelectrolyte solutions, a method known as layer-by-layer (LbL) assembly, produces polyelectrolyte multilayers on the material surfaces, based on sequentially formed polyion complexes.^{1–6} The film thickness can be regulated at a macromolecular level and various types of polyelectrolytes can be incorporated into a single film in an LbL manner. Since this method involves an immersion process, variously shaped materials can be applied. Not only fundamental investigations of the assembly processes and the physicochemical properties of the resulting films, but also potential applications of the films have been demonstrated by using suitable polymer combinations.

The effects of charge density on the assembly process between polyamines and a weak polyacid poly(acrylic acid) (PAA), the density of which was changed by altering the solution pH, have been reported.^{7,8} Furthermore, assemblies between amine-containing copolymers, in which the unit number of amines changed the density, and strong or weak polyacids have also been investigated in the presence or absence of NaCl.^{9–15} The thickness was not increased simply by increasing the density. In some cases, the thickness was maximal at a particular charge

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density,^{9,11–13} although the threshold density was dependent on the chemical structure of the polymers and the assembly conditions. The change in charge density also affected the swelling properties of the films as well as the cell interactions with the films.¹⁶ Not only the physicochemical properties of resulting films but also potential functions of the films may be strongly affected by the charge density of the polymers used.

Our research group has investigated assemblies between poly(vinylamine hydrochloride-co-*N*-vinylformamide) [poly(VAm-co-NVF)] with various amounts of VAm and PSS from their aqueous solutions in the absence of salt, and revealed that the thickness of the assembly was maximal at ~30% VAm content.¹³ This observation was derived from the difference in the conformation of copolymers in the aqueous phase. A decrease in the density reduced intramolecular electrostatic repulsion of poly(VAm-co-NVF), resulting in a more globular conformation. The charges of poly(VAm-co-NVF) were suitable for ionic interactions, and then the single layer of poly(VAm-co-NVF) with a greater thickness was formed, resulting in maximal total thickness at the most suitable charge density. Although various assembly systems have been previously discussed in terms of the effect of the charge density on LbL assembly,^{9–13} the potential applications of other related combinations of poly(VAm-co-NVF) and other polyacids remains significant.

In the present study, poly(VAm-co-NVF)s with variable VAm content and a weak PAA were assembled from their aqueous solutions. The assembly process was quantitatively monitored by the quartz crystal microbalance (QCM) technique, and the films obtained were characterized. The results were compared to those obtained from a strong polyacid PSS under the same assembly conditions.¹³ Since the anionic charges of PAA, which are used in the interaction with the VAm units, should be decreased compared to PSS, a different assembly behavior was expected. The threshold charge density of the present combination was shifted to the polymers with greater VAm content, and the physicochemical properties were changed significantly. The chemical structures of the polymers used are shown in Figure 1.

EXPERIMENTAL

Materials

PolyNVF was prepared by free radical polymerization of NVF (M_n 15,000, M_w/M_n 3.2), as described in our previous paper.^{17,18} PolyNVF was partially hydrolyzed in aqueous 2*N*-NaOH solution at 60°C for an adequate amount of time, followed by neutralization at ~pH 7 × 2*N* HCl.¹³ Varying the reaction time regulated the VAm content. PAA (M_w 2000) was pur-

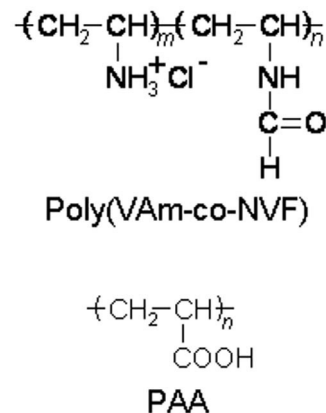


Figure 1 Chemical structures of poly(VAm-co-NVF) and PAA used in the present study.

chased from Aldrich and used without further purification.

Quartz crystal microbalance

The quantitative analysis of assembly followed methods described in previous studies.^{12,13} An AT-cut quartz crystal with a parent frequency of 9 MHz (9 mm in diameter) was coated on both sides with polished gold electrodes (4.5 mm in diameter), of which the mean roughness was 1.5 nm. The amount of polymer assembled, Δm , was measured by the frequency shift of the quartz crystal microbalance (QCM), ΔF , using the Sauerbrey's equation as follows¹⁹:

$$\Delta F = \frac{2F_0^2}{A\sqrt{\rho_q\mu_q}} \times \Delta m$$

where F_0 is the parent frequency of QCM (9×10^6 Hz), A is the area of the electrode (0.159 cm²), ρ_q is the density of the quartz (2.65 g cm⁻³), and μ_q is the shear modulus (2.95×10^{11} dyne cm⁻²). Before LbL assembly, the QCM electrodes were treated three times with a piranha solution [conc. H₂SO₄/H₂O₂ (30 wt % in water) = 3/1, v/v] for 1 min each time, followed by rinsing with pure water and drying with N₂ gas to clean the electrode surface.

Assembly

The QCM was immersed in an aqueous poly(VAm-co-NVF) solution (0.02*M*) (pH 5.8) for 20 min at 25°C, removed, washed with pure water, and dried with N₂ gas. The frequency shift was then measured in air. The QCM was immersed again in an aqueous PAA solution (0.02*M*) (pH 3.5) for 20 min at 25°C, and the same procedure was repeated. This alternating cycle was repeated for the assembly. Although assembly could be similarly obtained by starting with the PAA step,

all assemblies were performed with the copolymers as the starting polymer. Accordingly, odd and even steps indicate copolymer and PAA immersion, respectively. The pHs of aqueous solutions of poly(VAm-co-NVF)s and PAA were 5.8 and 3.9, respectively. Since PAA was dissolved in pure water, the carboxyl groups were partially ionized as a weak polyacid, and the carboxyl ions were used for electrostatic interactions with protonated primary amines of poly(VAm-co-NVF)s.

Characterization

Attenuated total reflection (ATR) spectra were obtained with a PerkinElmer Spectrum One in air at ambient temperature. One side of a poly(ethylene terephthalate) film was coated with gold to obtain a reflective surface. The polymers were then assembled for 20 steps similar to the method used in QCM measurements. The interferograms were coadded 32 times, and Fourier transformed at a resolution of 4 cm^{-1} . Atomic force microscopic (AFM) observation was performed with a Digital Instruments NanoScope III that was operated in noncontact mode in air and water at ambient temperature. We did not perform any image processing other than flat leveling. The mean roughness (R_a) in a given observed area was estimated from the following equation:

$$R_a = \frac{1}{L_x L_y} \int_0^{L_y} \int_0^{L_x} |F(x,y)| dx dy$$

where $F(x,y)$ is the surface relative to the center plane, which is a flat plane parallel to the mean plane, and L_x and L_y are the dimensions of the surface. Scratching of assembled polymers for thickness measurements using the AFM tip was performed under conditions which did not scratch the gold QCM substrate, as described in our previous study.¹³

RESULTS AND DISCUSSION

QCM analysis of LbL assembly at each deposition step quantifies the assembled amounts of polymer from the frequency shift of the QCM, and facilitates monitoring of adsorption or desorption of polymers at each step, as well as enabling the detection of total assembly profiles.^{12,13} Figure 2(a) shows the frequency shifts against the assembly step when a QCM was alternately immersed in poly(VAm-co-NVF) solutions with different VAm contents and PAA at 0.02M for 20 min at 25°C. Ten types of poly(VAm-co-NVF)s were prepared with the VAm content ranging from 0 to 100% (polyVAm). The shifts were reproducible in all cases, and the experimental errors were within 10% deviation.

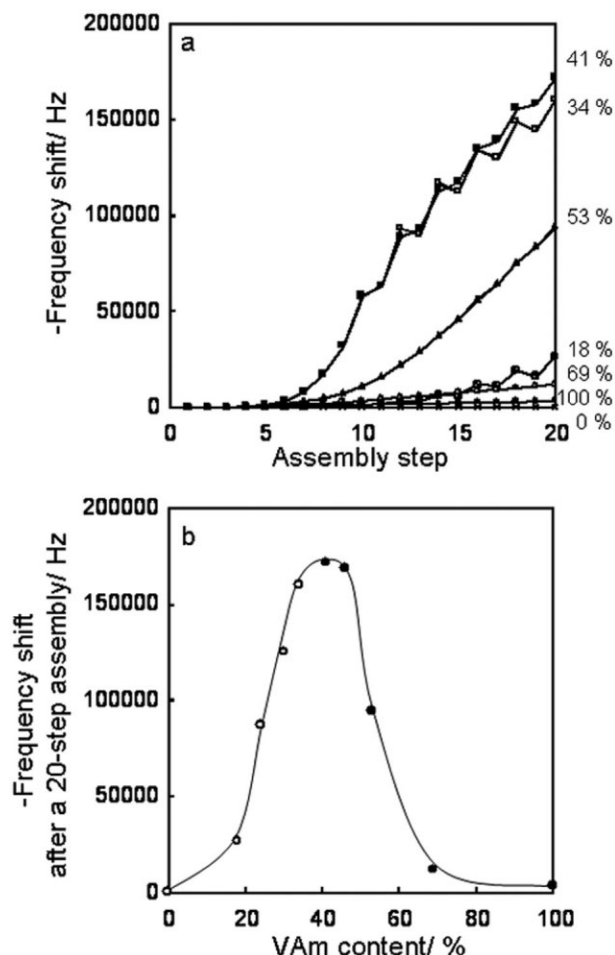


Figure 2 (a) Frequency shifts against assembly step. Poly(VAm-co-NVF) with variable VAm content and PAA were assembled at odd and even steps, respectively. (b) The frequency shifts after a 20-step assembly against the VAm content of poly(VAm-co-NVF). Open and closed symbols indicate zigzag and continuous assemblies, respectively.

The shifts should be converted to the thickness of the assemblies. However, since the swelling of each assembly was dependent on the VAm content (see Fig. 5), the assembly was discussed only in terms of the frequency shift. Assembly was observed from poly(VAm-co-NVF)s with all of the VAm contents analyzed, except for assembly from polyNVF without VAm.

To evaluate total assembly amounts, the frequency shift after a 20-step assembly was plotted against the VAm content of poly(VAm-co-NVF), as shown in Figure 2(b). The shift increased steeply from 18 to 34% and reached a maximum at 41%, and plateaued until 46% VAm. The VAm content at the maximum shift was consistent with the threshold content for zigzag and stepwise assembly. The shift was subsequently decreased until 69% VAm and then became almost constant at VAm contents exceeding 69%. This decrease is explained similar to that observed for assem-

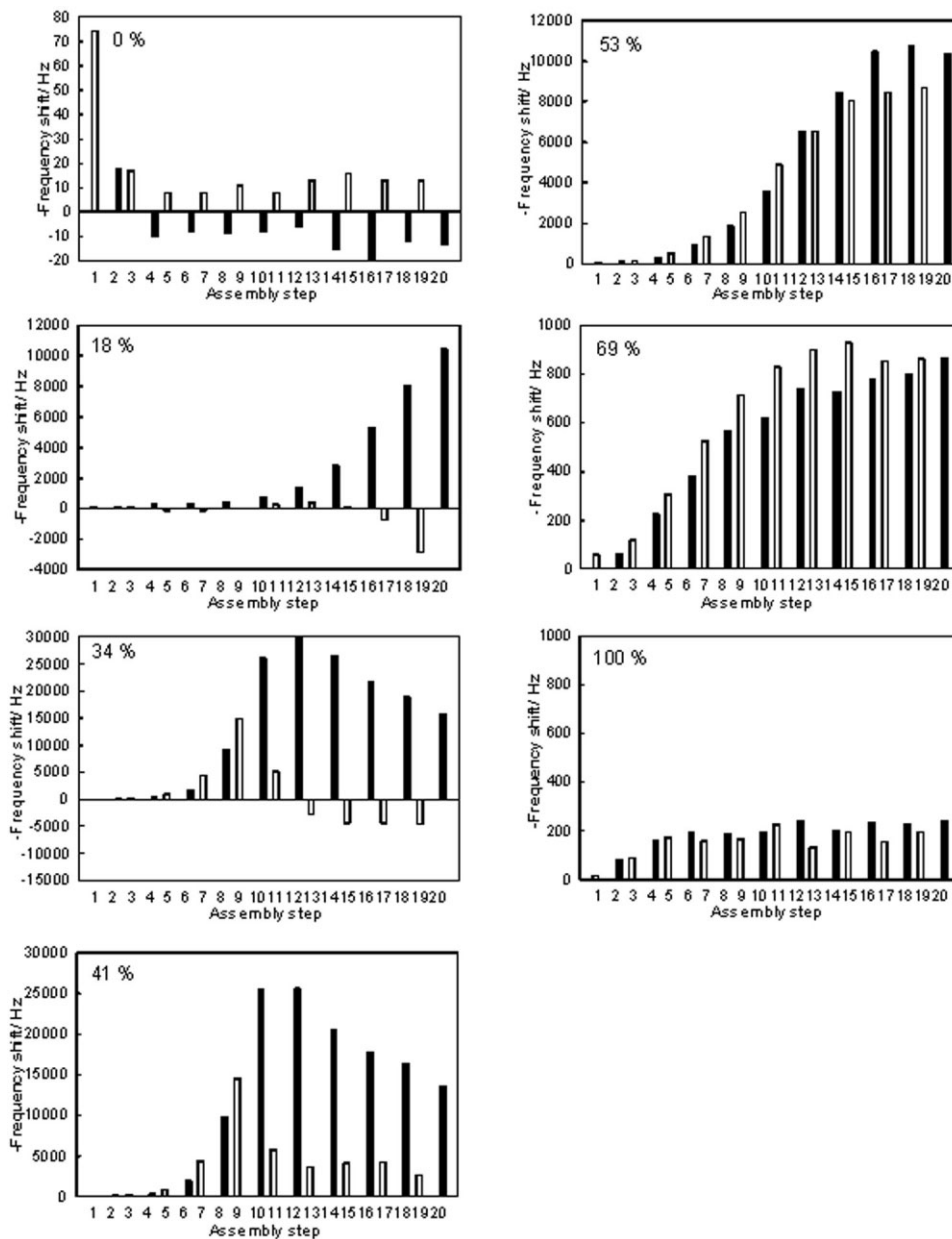


Figure 3 Frequency shifts at each step against assembly step. Open and closed bars indicate poly(VAm-co-NVF) and PAA steps, respectively.

bly between poly(VAm-co-NVF) and PSS.¹³ Since the increase in charge density of poly(VAm-co-NVF) resulted in a more extended conformation of poly(VAm-co-NVF) in the aqueous phase, then the mean thickness of poly(VAm-co-NVF) assembled at each assembly step was apparently decreased, although the amount of PAA assembled was also affected by the poly(VAm-co-NVF). Accordingly, poly(VAm-co-NVF)s with more than 69% VAm seemed to have a similar conformation on the film surface, and were characterized by a similar assembly amount. Furthermore, the increase in the assembly amount may be caused by the penetration of polymers into films with ion pair rear-

rangements,¹⁸ resulting in an exponential increase in the assembly amount (see Fig. 3).

On the other hand, the present threshold of 40% VAm was clearly greater than 30%, which was observed for assembly between poly(VAm-co-NVF) and PSS.¹³ Since the charge density of PAA was smaller than that of PSS (the degree protonation of polyAAc solubilized in water is more than 90%),¹⁸ thus the VAm value for LbL assembly seemed to be increased. This is the clear difference between weak and strong polyacids. Assembly between partially quaternized poly(vinylimidazole) and fully charged PAA at an ionic strength of 0.01M demonstrated that stable mul-

tilayers were formed above the quaternization of 18%, although the hydrodynamic layer thickness was maximal at $\sim 8\%$.⁹ Assembly between copolymers of diallyldimethylammonium chloride (DADMAC) and *N*-methyl-*N*-vinylacetamide (NMVA), and PSS in the presence of 0.1M NaCl revealed that film thickness after a 10-cycle assembly was steeply increased when the cationic unit fraction was increased from 50 to 75%, and was decreased slightly when the cationic fraction exceeded 75%.¹⁰ Assembly between copolymers of DADMAC and *N*-methyl-*N*-vinylformamide, and PSS at low ionic strength demonstrated three different assembly zones: below 9% DADMAC, the change in thickness was zero; between 10 and 30%, the increment strongly decreased with increasing DADMAC content; above 30%, the increment reached a constant value.¹¹ Assembly between copolymers of DADMAC and NMVA assembled with PSS at a higher ionic strength revealed preferential assembly at 75% DADMAC.¹² Accordingly, the threshold value of charge is strongly dependent on chemical structures of polymers and assembly conditions. In the present study, the difference between weak and strong polyacids was clearly demonstrated with regard to the shift in the threshold value.

The presence of both polymers in the assembly using a copolymer with 41% VAm content was analyzed by ATR spectra in air (original figures not shown). Amide I ($\nu_{\text{C=O}}$), amide II ($\delta_{\text{N-H}}$), and protonated primary amine bands assigned to NVF and VAm units were observed at 1661, 1538, and 3212 cm^{-1} , respectively, indicating the presence of poly(VAm-co-NVF). Carbonyl vibration bands ($\nu_{\text{C=O}}$) assigned to dimeric carboxyl groups and symmetric vibration bands assigned to carboxylates were observed at 1712 and 1384 cm^{-1} , respectively, indicating the presence of partially ionized PAA. This indicates that some carboxyl groups remained without electrostatic interactions with amines. These observations show that both polymers were assembled by alternate immersion of the substrate in both aqueous solutions.

The assembly profiles were analyzed in detail. The frequency shifts at each step are presented as a bar graph against the assembly step for all assembly combinations, as shown in Figure 3. The assembly between poly(VAm-co-NVF) and PAA can be divided into two different assembly patterns. One pattern was observed with a VAm content ranging from 0 to 34% and consisted of deposition at the PAA step and subsequent desorption of polymers at the poly(VAm-co-NVF) step (except for assembly from polyNVF), as previously shown. In other words, the copolymer partially desorbed PAA. The assembled poly(VAm-co-NVF) with a lower VAm content possibly formed water-soluble complexes with PAA, since the NVF units strongly interact with water molecules. On the other hand, the poly(VAm-co-NVF) with greater VAm

content forms water-insoluble complexes with PAA at the film surface.²⁰

Another pattern, which was observed at a VAm content above 53%, consisted of an initial exponential increase in the assembly amounts of both polymers with increasing number of assembly steps and subsequently the amounts became constant. The assemblies of 41 and 46% VAm were intermediate in characteristic between assemblies observed with 34 and 53% VAm content. Using the assembly between poly(L-lysine) and hyaluronic acid, Picart et al. suggested that the exponential increase in the apparent thickness was attributable to the diffusion of free poly(L-lysine) chains into the interior of the films with the expulsion of water after the formation of continuous films on surfaces.²¹⁻²³ Poly(VAm-co-NVF) might also diffuse into the films until a particular assembly step, which is dependent on the VAm content, as achieved. Since the copolymers with 53 and 69% VAm content should have a globular conformation because of relaxation of electrostatic repulsion, such globular polymers seemed to exponentially assemble. In fact, the present assemblies swelled with water molecules (see Fig. 5). It is noted that the step number of the exponential increase decreased with increasing VAm content.

AFM observation demonstrated not only surface topographic images of films but also cross-sectional views by scratching of the films with a cantilever. Figure 4 shows the AFM images of surfaces of 10- and 20-step assemblies between poly(VAm-co-NVF) with a VAm content of 53% or poly(VAm) (corresponding to a VAm content of 100%), and PAA in air. The mean roughness of the assemblies from the copolymer and polyVAm were estimated to be 11 and 21 nm, respectively. The surfaces of the former assembly were rougher than the latter. Rearrangement of the polymers might occur after assembly, resulting in a rougher surface. These roughness were larger than those of the assemblies from the copolymer and PSS, 4.4 and 5.1 nm, respectively. The difference in charge density clearly affected the surface topology of the films. Cross-sectional images were obtained for assemblies derived from the copolymer with 53% VAm and polyVAm in air and water, as shown in Figure 5. Films swelled with water molecules, as evidenced by an increase in film thickness in water. Since NVF units are hydrophilic and did not participate in electrostatic interactions, these units swell with water molecules. In addition, since free carboxyl groups that did not participate in ionic complexes remained, these similarly swelled. The percent increases in thickness were estimated to be 148 and 139% for 10- and 20-step assemblies, respectively, and the former was slightly greater than the latter. This indicates that the former film potentially interacted with water molecules, because of the presence of both NVF and free carboxyl units. It is noted that the film prepared from poly-

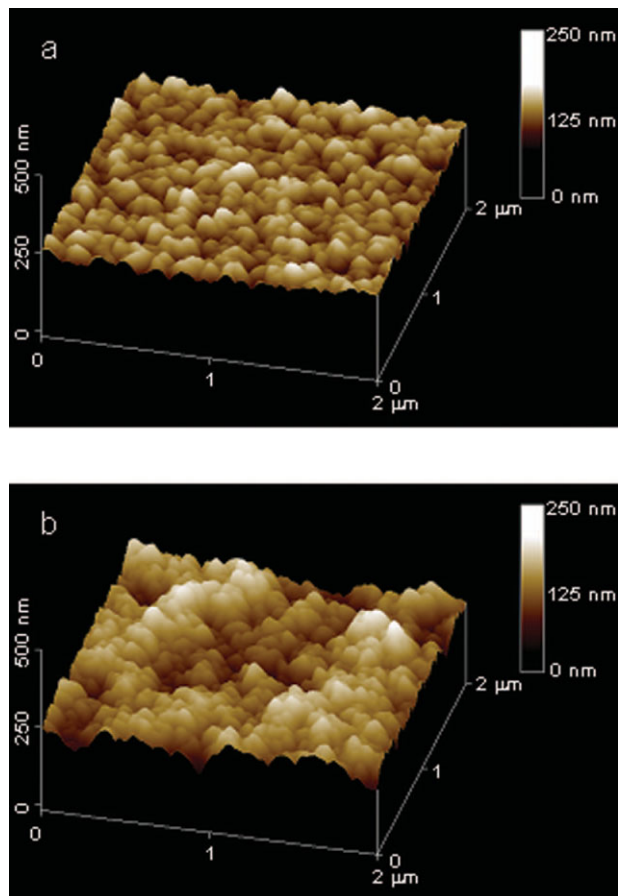


Figure 4 Surface AFM images of (a) 10- and (b) 20-step assemblies between poly(VAm-co-NVF) with a VAm content of 53% and PAA in air. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

VAm and PSS did not swell with water molecules.¹³ This is also a difference between weak and strong polyacids.

It was demonstrated that cytophilic and cytophobic properties of LbL assembled multilayer films can be controlled by the film structure.^{16,24} Especially, highly ionically stitched films attracted cells, whereas weakly ionically crosslinked films, which swell substantially in physiological conditions, resisted cell attachment. Hydrated poly(ethylene glycol) surfaces are also known to be cell-adhesion-resistant.^{25,26} Accordingly, the present films might have potential for controlling cell interactions. In addition, since thicker films are obtained at suitable VAm contents, homogeneous coating will be achieved by less assembly steps.

CONCLUSIONS

LbL assembly between poly(VAm-co-NVF) with variable VAm content and a weak polyacid PAA was demonstrated from their aqueous solutions. The assembly processes were quantitatively monitored by the QCM technique. The assembly was strongly affected by the VAm content. There was a threshold VAm content for zigzag and continuous assemblies. The assembly amount was maximal at the threshold VAm content, which was slightly greater than that obtained from a strong polyacid. The presence of both polymers in the assembly was confirmed by ATR spectra. Two different assembly patterns were characterized. The rougher surface was obtained from the assembly of poly(VAm-co-NVF) with lower VAm content. All of the assemblies analyzed swelled with water molecules, and the swelling was dependent on the VAm content. Since various types of polymers of *N*-vinylalkylamides can be synthesized, the charge effects on LbL assembly as well as potential applications of polyelectrolyte materials²⁷ will be demonstrated in detail in future studies.

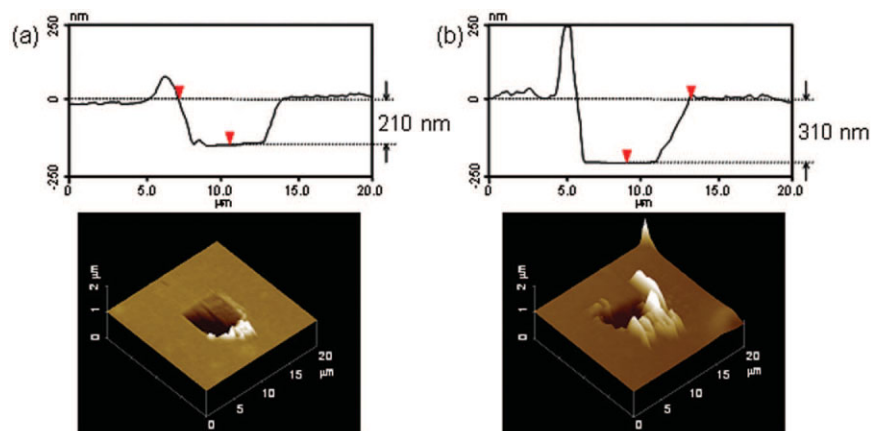


Figure 5 Scratching AFM images of a 10-step assembly between poly(VAm-co-NVF) with a VAm content of 53% and PAA (a) in air and (b) in water. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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