

NOTES

## Ultrathin Hydrogels Prepared from Poly(acrylic acid-co-acrylamide) and Poly(vinylamine) on a Solid Substrate

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Surface modification of material surfaces facilitates improvements in surface characteristics of the material without any change in mechanical properties. Nano-coating with polymeric thin films has potentially useful for biomedical and technological applications. Although conventional coating methods such as spin- or solution-casting can be used to fabricate the films, it is sometimes difficult to regulate film assembly structure at a macromolecular level. Recently developed layer-by-layer (LbL) assembly fabricates polyelectrolyte multilayers on solid material surfaces by simple alternate immersion of the material into aqueous solutions of oppositely charged polyelectrolytes, sequentially forming polyion complexes at the surface.<sup>1–4</sup> This method can be applied to various types of polyelectrolytes including biomacromolecules as well as synthetic polymers. Not only polymer complexes but also chemical reactions between polymers have been used for the fabrication of chemically stable films.

In our previous papers, sequential formation of amide linkages in LbL assemblies between primary amine containing copolymers of *N*-vinylalkylamides such as poly(vinylamine-co-*N*-vinylisobutyramide) (poly(VAm-co-NVIBA))<sup>5</sup> or poly(vinylamine-co-*N*-vinylformamide) (poly(VAm-co-NVF)),<sup>6</sup> plus poly(acrylic acid) (polyAAc) in the presence of a water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was used in the fabrication of ultrathin films on a solid substrate. Subsequent immersion into aqueous phase resulted in an increase in the film thickness as a result of swelling with water molecules due to the presence of hydrophilic *N*-vinylalkylamide units, thus fabricating ultrathin hydrogels. These findings indicated that ultrathin hydrogels could be prepared by sequential chemical reaction of certain copolymers having hydrophilic neutral units. Although copolymers of *N*-

vinylalkylamides are potentially available for the preparation of ultrathin hydrogels in conjunction with polyAAc, at least approximately 40 mol%<sup>5</sup> and 30 mol%<sup>6</sup> of VAm content in poly(VAm-co-NVIBA) and poly(VAm-co-NVF), respectively, were necessary for stepwise deposition with polyAAc. Otherwise, the films were not deposited in an LbL manner. In addition, since 5 mol% of EDC relative to AAc units of polyAAc was maximal for the activation of carboxyl groups due to the solubility of polyAAc, greater amounts of VAm were possibly necessary in the reaction.

In the present study, ultrathin hydrogels were prepared from the combination of commercially available poly(acrylic acid-co-acrylamide) (poly(AAc-co-AAm)) with an AAc content of 10 mol%, plus poly-VAm. Since amide linkages of acrylamide polymers are reversibly bound compared to polymers of *N*-vinylalkylamides, carboxyl groups are presented in the copolymer, poly(AAc-co-AAm). The assembly process was quantitatively analyzed using a quartz crystal microbalance (QCM) substrate. Not only amine-containing copolymers of *N*-vinylalkylamides but also carboxyl acid-containing copolymers of acrylamides were potentially available for the preparation of ultrathin hydrogels. Since greater amounts of EDC could be solubilized in an aqueous solution of poly(AAc-co-AAm), 10 mol% of AAc units was sufficient to prepare ultrathin hydrogels. These findings provide important information regarding the preparation of ultrathin hydrogels from various copolymers of acrylamides.

### EXPERIMENTAL

#### Materials

Poly(AAc-co-AAm) (*M*<sub>w</sub> 200000) with an AAc content of 10 mol% was purchased from Aldrich

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(USA), and used without further purification. PolyVAm ( $M_w$  289100) was prepared by hydrolysis of polyNVF, following methods described in previous papers.<sup>7</sup> EDC (>98.0%) was purchased from Wako (Japan), and used without further purification. Ultrapure distilled water ( $17.8 \text{ M}\Omega \text{ cm}^{-1}$ ) was provided by the MILLI-Q labo.

#### QCM Analysis

The assembly was analyzed quantitatively by a 9-MHz QCM, as reported in our previous studies.<sup>5,6</sup> The crystal (9 mm in diameter) was coated on both sides with gold electrodes 4.5 mm in diameter, the mean roughness of which was 1.7 nm. The frequency was monitored by an Iwatsu frequency counter (Model SC7201). The leads of the QCM were protected with a silicone-rubber gel in order to prevent degradation during immersion in the aqueous solutions. The frequency shift of the QCM corresponded quantitatively to the amount of polymer deposited on the electrode, as determined using Sauerbrey's equation.<sup>8</sup> Before the reaction, the QCM electrodes were treated 3 times with a piranha solution [conc.  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  (30 wt% in water) = 3/1, v/v] for 1 min, followed by rinsing with pure water and drying with  $\text{N}_2$  to blast clean the electrode surface.

#### Assembly

The cleaned QCM was immersed in an aqueous poly(AAc-co-AAm) solution (0.05 unitM) containing EDC (in adequate amounts relative to the total number of AAc units), in which the carboxyl groups had already been activated for 5 min before immersion. The QCM was immersed for 15 min, rinsed gently with pure water, then dried under  $\text{N}_2$  gas. The frequency shift was then measured. The QCM was immersed again into an aqueous polyVAm solution (0.05 unitM), and the same procedure was repeated. This sequential reaction cycle was repeated for the preparation of ultrathin hydrogels. The pHs of the aqueous solutions of poly(AAc-co-AAm) and polyVAm were 4.0 and 10.5, respectively. The  $\text{pK}_a$  of polyAAc and polyVAm have been estimated to be 4.3<sup>9</sup> and 10.0,<sup>10</sup> respectively. These values indicate that carboxyl and amino groups, which can be used for the reactions, were present in the polymers. The assembly was performed at 4 °C to maintain the activity of EDC. Although the assembly was started with poly(AAc-co-AAm), it was also possible to initiate assembly with polyVAm.

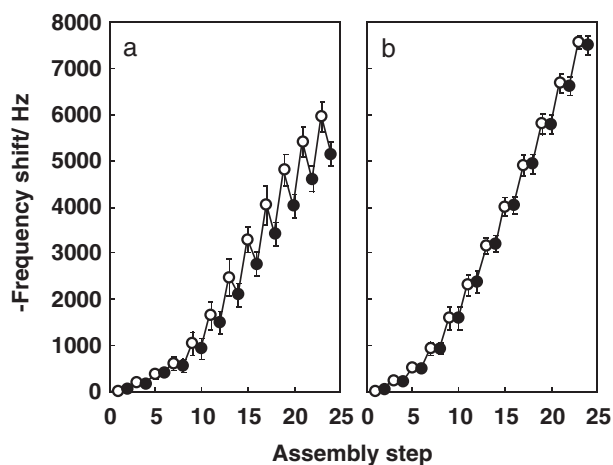
#### Characterization

Attenuated total reflection infrared (ATR-IR) spectra were obtained from the assembly coated QCM using a PerkinElmer Spectrum One (USA) in air at am-

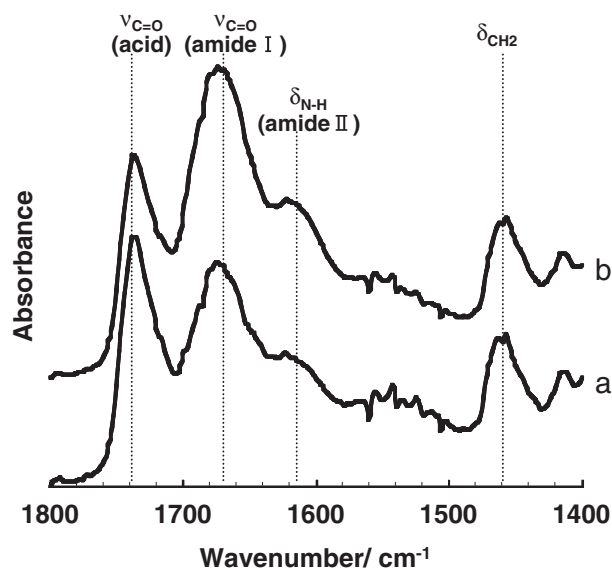
bient temperature. The interferograms were co-added 50 times, and Fourier transformed at a resolution of  $4 \text{ cm}^{-1}$ . Atomic force microscopic (AFM) images were obtained with a Digital Instruments NanoScope III that was operated in contact mode in both air and water at ambient temperature. We did not perform any image processing other than flat leveling. The static contact angle of the assembly was measured with a Kyowa Interface Science CA-X (Japan) at ambient temperature.

## RESULTS AND DISCUSSION

The assembly process was quantitatively analyzed by using a QCM substrate coated with gold electrodes, following methods described in our previous studies.<sup>5,6</sup> Figure 1 shows the frequency shifts of the QCM, corresponding to the amounts of assembled polymer, plotted against the assembly step, when poly(AAc-co-AAm) with a 10 mol% AAc content was sequentially assembled with polyVAm on the QCM substrate in the absence or presence of an amount of EDC equimolar to the AAc units. In both cases, the frequencies decreased with increased number of steps, indicating the stepwise deposition of the polymers. The amount assembled in the presence of EDC was greater than that in the absence. The assembly profiles were zigzag in shape, especially in the absence of EDC, followed by the desorption of some polymers at the polyVAm step. Since it is difficult to determine from the QCM analysis alone whether amide linkages were effectively formed between poly(AAc-co-AAm) and polyVAm in the presence of EDC, ATR-IR spectra of the 24-step assemblies were obtained, as shown in Figure 2. The carbonyl vibra-



**Figure 1.** QCM analysis of the assemblies of poly(AAc-co-AAm) with an AAc content of 10 mol% and polyVAm prepared in the (a) absence or (b) presence of 1.0 equimolar amount of EDC to AAc units. The open and closed symbols show the poly(AAc-co-AAm) and polyVAm steps, respectively.



**Figure 2.** ATR-IR spectra of the 24-step assemblies of poly(AAc-co-AAm) with an AAc content of 10 mol% and polyVAm prepared in the (a) absence or (b) presence of an equimolar amount of EDC to AAc units.

tion band, amide I and II bands were observed for both assemblies, indicating the presence of poly(AAc-co-AAm). The relative intensity of the amide I and II bands against the carbonyl vibration band of AAc units for the assembly prepared in the presence of EDC was clearly greater than that of assemblies prepared in the absence of EDC. This observation strongly indicates the formation of amide linkages between poly(AAc-co-AAm) and polyVAm during stepwise deposition in the presence of EDC. Both assemblies were stable in the aqueous phase, and slight amounts of the polymers were desorbed. Since the assembly was performed at 4 °C, the polymers were possibly deposited by noncovalent interactions such as hydrogen bonds as well as electrostatic interactions between the polymers in the absence of EDC, although these interactions could not be detected using the ATR-IR spectra. Note that the physicochemical properties of the polymers, such as water swelling, differed from each other (see below). When twice the molar amounts of EDC relative to the AAc units were added, the amount of polymer assembled was slightly smaller than that at equimolar concentrations, as shown in Table I. The addition of greater amounts

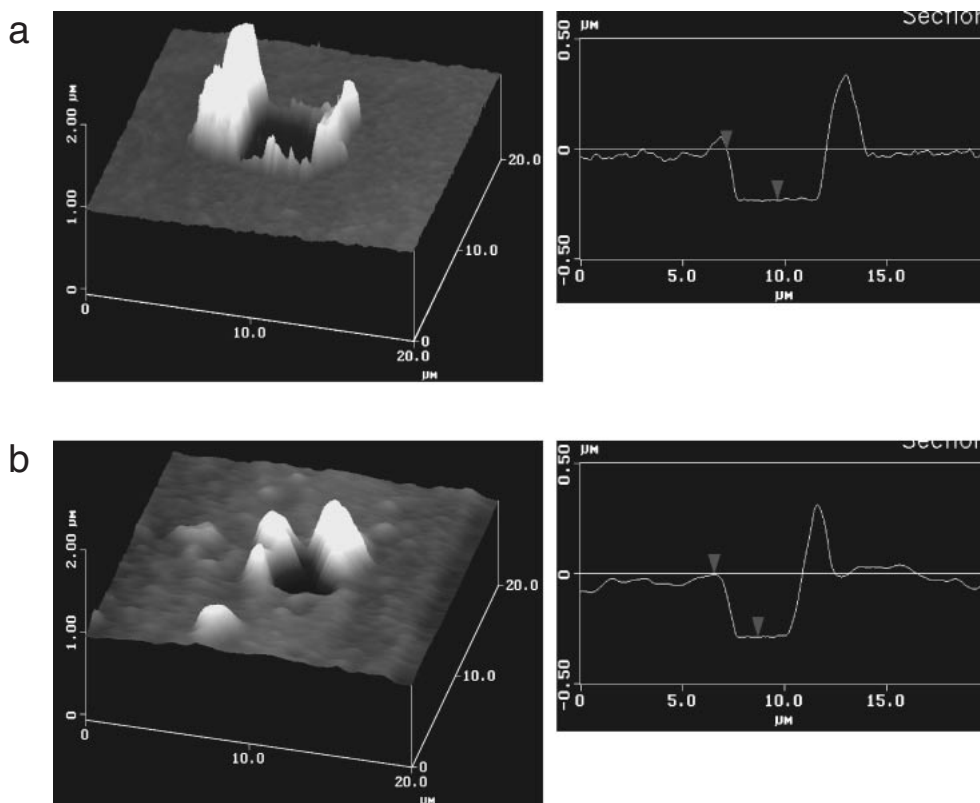
of EDC accelerated amide linkage formation between the polymers. This increase in the number of amide linkages resulted in a more extended conformation of the polymer at each assembly step, thus forming a thinner assembly. This hypothesized assembly mechanism is also supported by the assembly of poly(VAm-co-NVF) and polyAAc.<sup>6</sup> The amount of assembled polymer was also regulated by the concentration of poly(AAc-co-AAm). An increase in the polymer concentration resulted in an increase in the total assembly amount. As a consequence, poly(AAc-co-AAm), even with an AAc content as low as 10 mol%, could be chemically reacted with polyVAm in the presence of a water-soluble carbodiimide, with the formation of new amide linkages.

In order to understand the water swelling properties of the 24-step assemblies, the film thickness was estimated using the scratching mode of an AFM in air and water. Typical 3-dimensional and cross-sectional AFM images of the scratched films are shown in Figure 3, and the obtained data are summarized in Table I. The mean roughness of the assemblies prepared in the presence of EDC at 0, 1, and 2 equimolar amounts in air were 6.8, 7.3, and 5.9 nm, and those in water were 4.8, 9.4, and 7.6 nm, respectively, thus indicating smooth surfaces. The incremental increases in assembly thickness due to water were estimated as the apparent swelling ratio. The thickness was increased in water in all assemblies, and the percent incremental increases were ranged from 106–136%, indicating the fabrication of ultrathin hydrogels. These swelling ratios were similar to those reported for ultrathin hydrogels previously prepared using copolymers of *N*-vinylalkylamides.<sup>5,6</sup> Even in the absence of EDC, the film obtained swelled slightly with water molecules, possibly due to the presence of monomer units that did not related to the noncovalent interactions between the polymers. The percent incremental increase in assembly thickness was dependent on the amount of EDC, and increased with the amount of EDC. This observation is complicated, because an increase in the number of chemical bonds, corresponding to an increase in the number of cross-linkages, should decrease the swelling ratio. As previously discussed, the present polymers could be assembled even in the absence of EDC. Accordingly, the formation of

**Table I.** Characterization of ultrathin hydrogels prepared from poly(AAc-co-AAm) and polyVAm

WSC against AAc amounts (eq.)	$-\Delta F$ after 24-step assembly	Thickness in air (nm)	Thickness in water (nm)	Thickness <sup>a</sup> increment in water (%)
0	5167	119	126	106
1	7632	236	285	121
2	6477	141	192	136

<sup>a</sup> Estimated by (thickness in water/air)  $\times$  100.



**Figure 3.** 3-dimensional and cross-sectional AFM images of the scratched assembly of poly(AAc-co-AAm) with an AAc content of 10 mol% and polyVAm prepared in the presence of an equimolar amount of EDC to AAc units in (a) air and (b) water.

amide linkages between the polymers appears to cause the disappearance or suppression of noncovalent interactions such as hydrogel bonds and electrostatic interactions between the polymers in the assemblies. In other words, the number of newly formed amide linkages accelerated the swelling of assemblies with water. Previous observations have similarly supported this explanation.<sup>6</sup> Furthermore, the static contact angles of air bubbles in pure water for the 24-step assemblies prepared in the presence of EDC at 0, 1, and 2 equimolar amounts were  $60.7 \pm 0.4$ ,  $60.5 \pm 0.4$ , and  $60.6 \pm 1.0$  deg, respectively, which were the same within experimental error. On the other hand, assemblies immersed in aqueous HCl solution (pH 2.0) were characterized by contact angles of  $43.1 \pm 1.0$ ,  $44.3 \pm 0.7$ , and  $50.9 \pm 0.6$  deg, respectively. This suggests that the film surface became hydrophobic in an acidic solution, because the AAc units were protonated even in the assemblies with polyVAm on the outermost surfaces. It is notable that the percent decrease in contact angles decreased with increased amount of EDC. This also suggests that fewer AAc units remained in the assembly, supporting our observations regarding the percent incremental increase in the film thickness. It was confirmed that the relationship between chemical bond formation and noncovalent interactions between constituent polymers in assemblies governed the swelling properties

of ultrathin hydrogels prepared by stepwise LbL deposition.

Ultrathin hydrogels were prepared by the combination of poly(AAc-co-AAm) and polyVAm even at an AAc content as low as 10 mol%, which was much smaller than the functional group content necessary for the preparation of ultrathin hydrogels by copolymers of *N*-vinylalkylamides.<sup>5,6</sup> It was demonstrated that copolymers of acrylamides are also available as constituents in the formation of ultrathin hydrogels. Thickness analysis in air and water revealed the unique water swelling properties of the resulting ultrathin hydrogels. Although it is difficult to conclude that ultrathin hydrogels still have a multilayered and/or interdigitated structure, detailed analysis using various polymer combinations will demonstrate structural information. The present methodology will raise new possibilities in the functional surface modification of materials in both biomedical and technological fields.

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## REFERENCES

1. G. Decher and J.-D. Hong, *Makromol. Chem., Macromol. Symp.*, **46**, 321 (1991).
2. G. Decher and J.-D. Hong, *Ber. Bunsen-Ges. Phys. Chem.*, **95**, 1430 (1991).
3. G. Decher and J. B. Schlenoff, "Multilayer Thin Films", Wiley-VCH, Weinheim, 2003.
4. S. Tripathy, J. Kumar, and H. S. Nalwa, Ed., "Handbook of Polyelectrolytes and Their Applications", Vol. 1, American Scientific Publishers, Los Angeles, 2002.
5. T. Serizawa, K. Nanamaki, K. Yamamoto, and M. Akashi, *Macromolecules*, **35**, 2184 (2002).
6. T. Serizawa, Y. Nakashima, and M. Akashi, *Macromolecules*, **36**, 2072 (2003).
7. K. Yamamoto, T. Serizawa, Y. Muraoka, and M. Akashi, *Macromolecules*, **34**, 8014 (2001).
8. G. Sauerbrey, *Z. Phys.*, **155**, 206 (1959).
9. S. Chibowski, *J. Colloid Interface Sci.*, **140**, 444 (1990).
10. K. Sumaru, H. Matsuoka, and H. Yamaoka, *J. Phys. Chem.*, **100**, 9000 (1996).