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Stepwise Fabrication and Characterization of Ultrathin Hydrogels Prepared from Poly(vinylamine-*co*-*N*-vinylformamide) and Poly(acrylic acid) on a Solid Substrate

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ABSTRACT: Sequential amide formation between poly(vinylamine-*co*-*N*-vinylformamide)s [poly(VAm-*co*-NVF)s] with VAm contents of 20, 28, and 42 mol % plus polyAAc using a water-soluble carbodiimide was studied to prepare ultrathin hydrogels on a solid substrate. The analysis of assembly amounts at each assembly step using a quartz crystal microbalance revealed that at least 28 mol % of VAm was essential for stepwise reaction of both polymers on the substrate. The assembly amount was regulated not only by the VAm content but also by the concentration of the copolymer in the reaction solution. Reflection−absorption spectra of the assemblies showed typical peaks assigned to both polymers. The thickness of the assemblies, which was analyzed in the scratching mode using atomic force microscopy, increased in water to form ultrathin hydrogels. The increase in thickness was dependent on the VAm content, and the assembly prepared from poly(VAm-*co*-NVF) with a 42 mol % VAm content swelled with more water molecules compared to that with a 28 mol % VAm. Analysis using cyclic voltammetry revealed that $Fe(CN)_6^{3-}$ readily permeated the ultrathin hydrogels by the process of diffusion. The permeability was also dependent on the VAm content. Collectively, these results indicated that ion-permeable ultrathin hydrogels were successfully prepared on a solid substrate.

Introduction

The fabrication of ultrathin polymer films with nano-ordered thickness on material surfaces potentially improves the surface characteristics of the materials without any deformation of mechanical strength. Certain water-soluble substances can permeate these films when the films swell with water molecules, forming ultrathin hydrogels. Since bulk hydrogels have various potential applications, the coating of materials with ultrathin hydrogels is attractive for preparation of chemical sensors or surface modification of biomedical materials. It is therefore important to develop new techniques to prepare structurally regulated ultrathin hydrogels comprised of water-soluble polymers on material surfaces. Thin hydrogels have been prepared by surface graft polymerization in the presence of cross-linkers, by photopolymerization of cast monomers and cross-linkers, and by electropolymerization in solutions containing monomers and cross-linkers.[1] However, it is difficult to regulate the thickness of the resulting films to the nanometer level and the chemical structure at the macromolecular level.

Layer-by-layer (LbL) assembly, which can be performed by alternate immersion of materials into interactive polymer solutions, on material surfaces is an attractive method for the preparation of ultrathin polymer films.[2] Not only noncovalent interactions (electrostatic interactions,[2] charge-transfer interactions,[3] hydrogen bonding,[4] and van der Waals interactions[5]) but also chemical reactions[6] (e.g., amide, ester, or urethane bond formation) between adequate polymer combinations have been applied to LbL assembly. In a previous study, we demonstrated stepwise formation of amide linkages between poly(vinylamine-*co*-*N*-vinylisobutyramide) [poly(VAm-*co*-NVIBA)] with a VAm content of approximately 40 mol % plus poly(acrylic acid) (polyAAc) on a substrate and successfully fabricated ultrathin polymer films.[7] The film swelled with water molecules to form a hydrogel structure because the neutral and hydrophilic NVIBA units were not utilized in the chemical bonds. Furthermore, measurement of the static contact angle using an air bubble in water demonstrated the ultrathin hydrogel to be thermoresponsive, a property derived from the lower critical solution temperature of polyNVIBA. These observations suggest that sequential chemical reactions using certain copolymers with neutral and hydrophilic units create ultrathin hydrogels on substrates. Accordingly, we have successfully fabricated ultrathin hydrogels from a combination of thermoresponsive poly(acrylic acid-*co*-*N*-isopropylacrylamide) with a suitable AAc content plus poly(vinylamine) (polyVAm) in a similar manner.[8]

In our research group, polymers of *N*-vinylalkylamides with various alkyl side chains were synthesized by free radical polymerization of appropriate monomers.[9] Copolymers were also prepared by copolymerization of adequate combinations, in which the reactivity of the monomers was similar, resulting in highly random copolymers. Hydrolysis of the polymers under controlled conditions yielded VAm units, which can in turn be utilized for the sequential chemical reactions in the fabrication of ultrathin hydrogels. Furthermore, it is easy to alter the chemical structure of the alkyl side chain of the VAm-containing polymer to analyze the effect of the alkyl side chain on the reaction. Two methodologies have been developed to obtain the VAm-containing polymers: (1) controlled hydrolysis of homopolymers and (2) selective hydrolysis of copolymers (*N*-vinylalkylamide units with shorter alkyl side chains can be more readily hydrolyzed).

In the present study, sequential chemical reactions of poly(vinylamine-*co*-*N*-vinylformamide)s [poly(VAm-*co*-NVF)s] with variable VAm contents plus polyAAc were analyzed to fabricate ultrathin hydrogels on a

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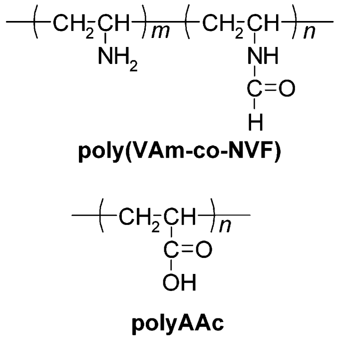
Scheme 1



$-(CH_2-CH(NH_2))_m-(CH_2-CH(NHCHO))_n-$

poly(VAm-co-NVF)

$-(CH_2-CH(COOH))_n-$

polyAAc

substrate. The effects of the alkyl side chains as well as the permeability of substances into the resulting ultrathin hydrogels were investigated. The copolymers were synthesized by controlled alkaline hydrolysis of polyNVF. The carboxyl groups of polyAAc were activated by 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) before the reaction. The alternate immersion of a substrate into an aqueous solution of the polymers demonstrated stepwise deposition of the polymers. The reaction was analyzed quantitatively by a quartz crystal microbalance (QCM) and was characterized by its attenuated total reflection (ATR) spectrum and atomic force microscopy (AFM). The results obtained were compared with those from the reaction between poly(VAm-*co*-NVIBA)s, which have larger alkyl side chains compared to poly(VAm-*co*-NVF)s and polyAAc.[7] Furthermore, ionic permeability into the ultrathin hydrogels was also analyzed by cyclic voltammetry (CV) using potassium ferricyanide. The chemical structures of the polymers used are shown in Scheme 1.

Experimental Section

Materials. Poly(VAm-*co*-NVF)s with a VAm content of 20, 28, and 42 mol % were prepared by hydrolysis of polyNVF (M_n 15 000, M_w/M_n 3.2) in aqueous 2 N NaOH solution at 60 °C for an adequate amount of time, as determined in our previous study.[9c,d] PolyAAc (M_w 2000) was purchased from Aldrich and was used without further purification. EDC (>98.0%) was purchased from Wako (Japan) and used without further purification. Ultrapure distilled water (17.8 ΩM cm^{-1}) was provided by the MILLI-Q labo system.

QCM. The stepwise assembly of the polymers was analyzed quantitatively by a 9 MHz QCM with polished gold electrodes 4.5 mm in diameter, of which the roughness was 1.8 nm, as reported in our previous studies.[5,7,8] The amount of polymer assembled, Δm, was estimated by measuring the frequency decrease of the QCM, ΔF, using Sauerbrey's equation as follows:[10]

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

where F_0 is the parent frequency of the QCM (9×10^6 Hz), A is the electrode area (0.159 cm^2), ρ_q is the density of the quartz (2.65 g cm^{-3}), and μ_q is the shear modulus (2.95×10^{11} dyn cm^{-2}). Before assembly, the QCM electrodes were treated three times with a piranha solution [concentrated H_2SO_4/H_2O_2 (30 wt % in water) = 3/1, v/v] for 1 min, followed by rinsing with pure water and drying with N_2 gas to clean the electrode surface.

Stepwise Assembly. The QCM was immersed in an aqueous polyAAc solution (50 unit mM, pH 3.5) containing EDC (5 mol % to the total units of polyAAc), in which the carboxyl group had already been activated for 5 min before the immersion, for 15 min at 4 °C, rinsed thoroughly with pure water, and then dried under N_2 gas. The frequency shift was then measured. The QCM was immersed again into an aqueous poly(VAm-*co*-NVF) solution (almost cases, 2.5 unit mM, pH 5.8) for 15 min at 4 °C, and the same procedure was repeated. This cycle was repeated for the appropriate number of steps in the assembly. The polymers were assembled at 4 °C in order to maintain the activity of the EDC. Since the EDC solution was saturated, it was difficult to alter the concentration for the analysis of cross-linking amounts. Although the assembly process was initiated with polyAAc, the starting polymer did not affect the stepwise assembly.

Characterization. ATR spectra were obtained with a Perkin-Elmer Spectrum One in air at ambient temperature. The QCM electrode with a refractive surface was used directly to observe the ATR spectra. The interferograms were co-added 50 times and Fourier transformed at a resolution of 4 cm^{-1}. AFM images were obtained with a Digital Instruments NanoScope III that was operated in contact mode in air or water at ambient temperature after scratching the assemblies with a cantilever. We did not perform any image processing other than flat leveling. The mean roughness (Ra) in a given observed area was estimated from the following equation:

$$Ra = \frac{1}{LxLy}\int_0^{Ly}\int_0^{Lx}|F(x,y)|\,\mathrm{d}x\,\mathrm{d}y$$

where $F(x,y)$ is the surface relative to the center plane that is a flat plane parallel to the mean plane and Lx and Ly are the dimensions of the surface. CVs were measured by ALS/CH-Instruments electrochemical analyzer model 660 at 25 °C using potassium ferricyanide under a nitrogen atmosphere. Potassium ferricyanide was dissolved into 0.1 M sodium perchlorate at a concentration of 10 mM. The double-sided QCM electrode was used as a working electrode. A polished Pt wire 1 mm in diameter and Ag/AgCl (saturated aqueous NaCl solution) were used as counter and reference electrodes, respectively.

Results and Discussion

Stepwise Assembly. Amide formation between the primary amino groups of VAm in poly(VAm-*co*-NVIBA)s and the carboxyl groups of polyAAc produced ultrathin films, which could swell with water molecules to form ultrathin hydrogels.[7] This observation suggests that derivative polymers of *N*-vinylalkylamides have potential applications in the preparation of various types of ultrathin hydrogels when prepared in conjunction with polymers having carboxyl groups. In the present study, we selected the derivatives poly(VAm-*co*-NVF)s with shorter alkyl side chain as amine polymers. Steric hindrance associated with the alkyl side chain seemed to affect the formation of amide bonds. As such, shortening of these chains may accelerate formation.

Figure 1 shows the frequency shift of the QCM against assembly steps, when the QCM was alternately immersed into aqueous solutions of poly(VAm-*co*-NVF)s with 20, 28, and 42 mol % VAm content plus polyAAc. Stepwise shifts were observed for the assembly of poly(VAm-*co*-NVF)s with 28 and 42 mol % VAm content, indicating the fabrication of ultrathin films. The assembly amount at each step gradually increased with increasing step number. Similar incremental increases were observed for the electrostatic LbL assemblies between chitosan and dextran sulfate under concentrated ionic solutions[11] and between poly(VAm-*co*-NVF) with a 30 mol % VAm content and poly(sodium styrenesulfonate) (PSS).[12] In all cases, the polymers should have a coiled conformation in solutions through relaxation of electrostatic repulsion. The deposition of coiled polymers seemed to increase the reaction site.

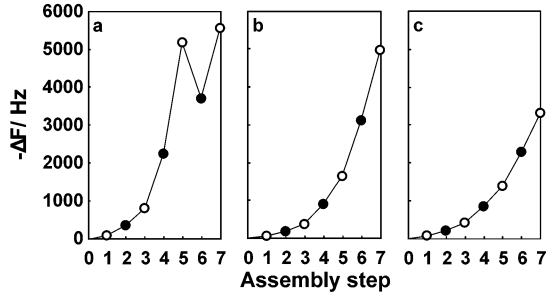


Figure 1. Dependence of the frequency shift on the number of assembly steps for assemblies of poly(VAm-*co*-NVF) with (a) 20, (b) 28, and (c) 42 mol % VAm content plus polyAAc. The concentrations of poly(VAm-*co*-NVF) and polyAAc were 2.5 and 50 unit mM, respectively. Even and odd steps indicate poly(VAm-*co*-NVF) and polyAAc assemblies, respectively.

The pHs of reaction solutions of poly(VAm-*co*-NVF)s and polyAAc were 5.8 and 3.5, respectively (see Experimental Section). Sequential chemical reactions between the polymers were potentially accelerated by electrostatic condensation of the polymers on the film surface. In fact, less assembly could be performed by alternate immersion of the substrate in the absence of EDC (approximately 70% to the total assembly amount prepared in the presence of EDC). Furthermore, electrostatic LbL assembly between polyAAc and primary amine-containing poly(allylamine hydrochloride) (PAH) prepared from aqueous solutions at pHs 3.5 and ≈6, respectively, demonstrated a greater assembly thickness.[13] Accordingly, the present assembly conditions seemed to be suitable for chemical reactions, which was accelerated by electrostatic deposition of the polymers.

The assemblies were stable in concentrated ionic solutions, and only approximately 10% of the polymers were desorbed from the assemblies in an aqueous 1 M NaCl solution. On the other hand, more than 90% of the assembly was desorbed in the NaCl solution, when the assembly was performed in the absence of EDC. This observation possibly indicates that amide linkages were effectively formed between the polymers in the presence of EDC. In the case of poly(VAm-*co*-NVF) with a 20 mol % VAm content, the frequency shifted continuously until the completion of five steps; however, the shift became zigzag in fashion after five steps had been completed. This suggests that the formation of covalent bonds between the poly(VAm-*co*-NVF) and polyAAc was insufficient, leading to desorption of the polymers.

The amount assembled from poly(VAm-*co*-NVF) with a 28 mol % VAm content was slightly larger than that from the copolymer with 42 mol %. This is possibly due to differences in the conformation of the poly(VAm-*co*-NVF)s. The lower VAm content may reduce ionic repulsion between VAm units, producing a copolymer with a more coiled conformation and increasing the thickness of each layer of poly(VAm-*co*-NVF)s in the assembly. In other words, the more extended poly(VAm-*co*-NVF) with greater VAm content initially adsorbed onto the surface of polyAAc by electrostatic interactions and then reacted to form amide linkages. In this case, the poly(VAm-*co*-NVF)s with greater number of VAm units seemed to react with carboxyl groups, resulting in a more linear conformation of the poly(VAm-*co*-NVF) and reduced thickness following adsorption. This assembly pattern resembles the incremental assembly of LbL with polyion complex formation in the presence of concentrated salt ions.[2]

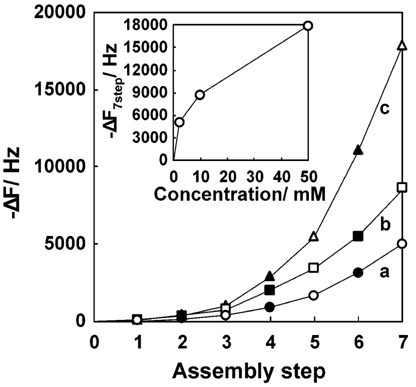


Figure 2. Dependence of frequency shifts on the number of assembly steps for an assembly between poly(VAm-*co*-NVF) with a 28 mol % VAm content and polyAAc (50 unit mM) at copolymer concentrations of (a) 2.5, (b) 10, and (c) 50 unit mM. Even and odd steps indicate poly(VAm-*co*-NVF) and polyAAc assemblies, respectively. The inset shows the dependence of the frequency shift at seven steps on polymer concentration.

Effects of charge densities on electrostatic LbL assembly have already been investigated using combinations between PAH and a weak polyacid, polyAAc under various pHs,[13] and combinations between copolymers with variable amine contents and fully charged poly(acrylic acid)[14] or PSS.[12,15] Delicate changes in charge densities of the polymers strongly affected the assembly thickness, although the threshold charge density for successfully performing LbL assembly was dependent on the assembly conditions and the chemical structure of the polymers. In fact, the assemblies between amine-containing copolymers such as poly(VAm-*co*-NVF)s[12] or poly(diallyldimethylammonium chloride-*co*-*N*-methyl-*N*-vinylforamide)[15b] and PSS at low ionic strength revealed that the assembly thickness was maximal at the amine content of approximately 30 mol %, of which the result is similar to the present chemical reactions. Electrostatic LbL assembly of poly(VAm-*co*-NVF)s with variable VAm contents and polyAAc at low ionic strength will also demonstrate that the assembly thickness is maximal at a 40 mol % VAm content.[16]

A VAm content of 40 mol % was necessary for the stepwise assembly of poly(VAm-*co*-NVIBA) and polyAAc. In the present case, there was a threshold VAm content between 20 and 28 mol %, which was smaller than that of the assembly from poly(VAm-*co*-NVIBA).[7] Since changing the side chain from butyl groups for NVIBA units to protons for NVF units reduced steric hindrance, cross-linking proceeded readily in the present combinations at a lower VAm content. In fact, twice the amount of polymer was assembled compared to assembly from poly(VAm-*co*-NVIBA), confirming the effective reaction between poly(VAm-*co*-NVF) and polyAAc.

Although the reaction parameters such as temperature and amounts of EDC could not be altered due to certain restrictions (see Experimental Section), a concentration of poly(VAm-*co*-NVF) with a 28 mol % VAm content was effectively changed from 2.5 to 50 unit mM by maintaining the concentration of polyAAc at 50 unit mM. It was found that the amount assembled increased with increased concentration and saturated above the largest concentration, as shown in Figure 2. Since a competitive reaction should occur between the poly(VAm-*co*-NVF) already reacted and the poly(VAm-*co*-NVF) in the solution, the amount assembled was accelerated by increasing the concentration. Although

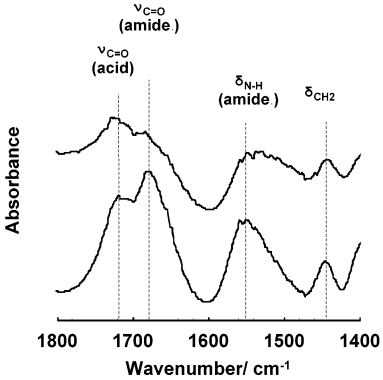


Figure 3. ATR spectra of 10-step assemblies of poly(VAm-*co*-NVF) with (a) 28 and (b) 42 mol % VAm content plus polyAAc. The concentrations of poly(VAm-*co*-NVF) and polyAAc were 2.5 and 50 unit mM, respectively.

the assemblies were stopped at seven steps, assemblies had been continued with more steps.

Spectral Analysis. To confirm the presence of both polymers on the substrate, the ATR spectra of the assemblies of poly(VAm-*co*-NVF)s with 28 and 42 mol % VAm content plus polyAAc were obtained by direct measurement of the QCM substrates after the 10-step assembly, as shown in Figure 3. Amide I ($\nu_{\mathrm{C=O}}$) and amide II ($\delta_{\mathrm{N-H}}$) bands assigned to NVF units were observed at approximately 1680 and 1550 cm^{-1}, respectively, indicating the presence of poly(VAm-*co*-NVF)s. In addition, the carbonyl vibration bands ($\nu_{\mathrm{C=O}}$) of carboxyl groups assigned to AAc units were observed at approximately 1720 cm^{-1}, also indicating the presence of polyAAc that did not participate in chemical cross-links. It is important to determine whether the observed amide bonds were newly formed between poly(VAm-*co*-NVF) and polyAAc. It is, however, difficult to quantitatively analyze the amount of cross-linking. Since the estimation of cross-linking in bulk hydrogels is also difficult, we did not proceed with any further analysis, similar to our previous study.[7,8] In fact, the intensity of the amide I band relative to the carbonyl vibration bands for an assembly of poly(VAm-*co*-NVF)s with a 42 mol % VAm content was greater than that of poly(VAm-*co*-NVF)s with a 28 mol % VAm content. This strongly suggests that more amide linkages were formed in the former assembly.

Thickness Analysis. The hydrogel structure of the assemblies was analyzed by increment in the thickness of the assemblies in water. AFM observation with a scratching mode was utilized to detect the thickness. The *Ra* value of a six-step assembly between poly(VAm-*co*-NVF) with a 28 mol % VAm content and polyAAc in air and water was 8 and 13 nm, respectively. Those of a six-step assembly between poly(VAm-*co*-NVF) with a 42 mol % VAm content and polyAAc were 3 and 4 nm, respectively (original images not shown). These observations showed that the present assemblies had a smooth surface. Figure 4 shows the thickness analysis of the assemblies between poly(VAm-*co*-NVF)s with 28 and 42 mol % VAm content plus polyAAc in air and water, as determined by AFM. The obtained data are summarized in Table 1. The apparent densities of the assemblies were estimated from the thickness, the QCM electrode area, and the assembly amount derived from the frequency shift. The thickness of the assemblies increased in water, while the density decreased, indicating that the assemblies swelled with water molecules. Since NVF units are neutral and hydrophilic and since the regulated amount of EDC (5 mol % to the total units of polyAAc) formed the suitable amount of cross-links between the polymers, water molecules readily penetrated into the assemblies. Furthermore, AAc units remained in the assemblies should be potentially protonated in water, since the degree protonation of polyAAc solubilized in water is more than 90%,[17] although the pK_{a} of polyAAc assembled might be decreased due to closely existed amino groups of poly(VAm-*co*-NVF)s. The protonated and ionized free carboxyl groups of polyAAc might also swell with water molecules. The increment in the thickness of the assembly obtained from poly(VAm-*co*-NVF) with a 42 mol % VAm content was larger than those of the assembly with a 28 mol % VAm content.

To quantitate the swelling behavior of the assemblies, the swelling ratio was estimated from the following equation: $(W_{\mathrm{water}}/W_{\mathrm{polymer}}) \times 100$, where W_{water} is the apparent weight of water molecules estimated from the hydrogel volume [(QCM electrode area) × (thickness in water)] in water assuming the density to be 1.0 g cm^{-3}, and W_{polymer} is the weight of the polymers estimated from the QCM analyses. Accordingly, the volume of the polymers in ultrathin hydrogels was ignored to estimate W_{water}. The swelling ratio of the assembly from poly(VAm-*co*-NVF) with a 42 mol % VAm content was approximately 3.5 times larger than that of the assembly with a 28 mol % VAm content. It is difficult to reasonably explain these results because a greater number of cross-links should be formed in the assembly from the poly(VAm-*co*-NVF) with the greater VAm content, leading to a lower swelling ratio. As already mentioned above, the present combinations of polymers could also be assembled by electrostatic interactions in the absence of EDC, although the resulting assemblies were unstable. In the case of poly(VAm-*co*-NVF) with a 42 mol % VAm content, amide linkages corresponding to cross-links should form readily. However, since poly(VAm-*co*-NVF) with a 28 mol % VAm content had a more coiled structure in the reaction medium, some VAm units may remain in the assembly even after the reaction. The remaining VAm units seemed to interact electrostatically with AAc units after possible rearrangement of the polymers in air to produce a hydrophobic environment that excludes water molecules. The rearrangement of constituent polymers has been known to occur during the drying processes in electrostatic LbL assembly.[18] In other words, the larger number of amide linkages in the assembly apparently accelerated water penetration into the assemblies. This interpretation is also supported by CV measurements (see below). The assemblies prepared from poly(VAm-*co*-NVF) with two different mol % VAm are schematically illustrated in Figure 5. Similar results have been obtained in the assemblies between poly(acrylic acid-*co*-*N*-isopropylacrylamide) with a suitable AAc content and polyVAm.[8] The greater AAc content and the larger EDC for the sequential reaction resulted in increased swelling with water molecules.

Ionic Permeability. CVs enable analysis of the permeability of electroactive ionic species across films deposited on an appropriate electrode on the basis of oxidation–reduction cycles. CVs with ferricyanide [Fe(CN)$_6^{3-}$] as a model ion using the QCM electrode uncoated or coated with the ultrathin hydrogels are shown in Figure 6. A suitable CV curve that contains

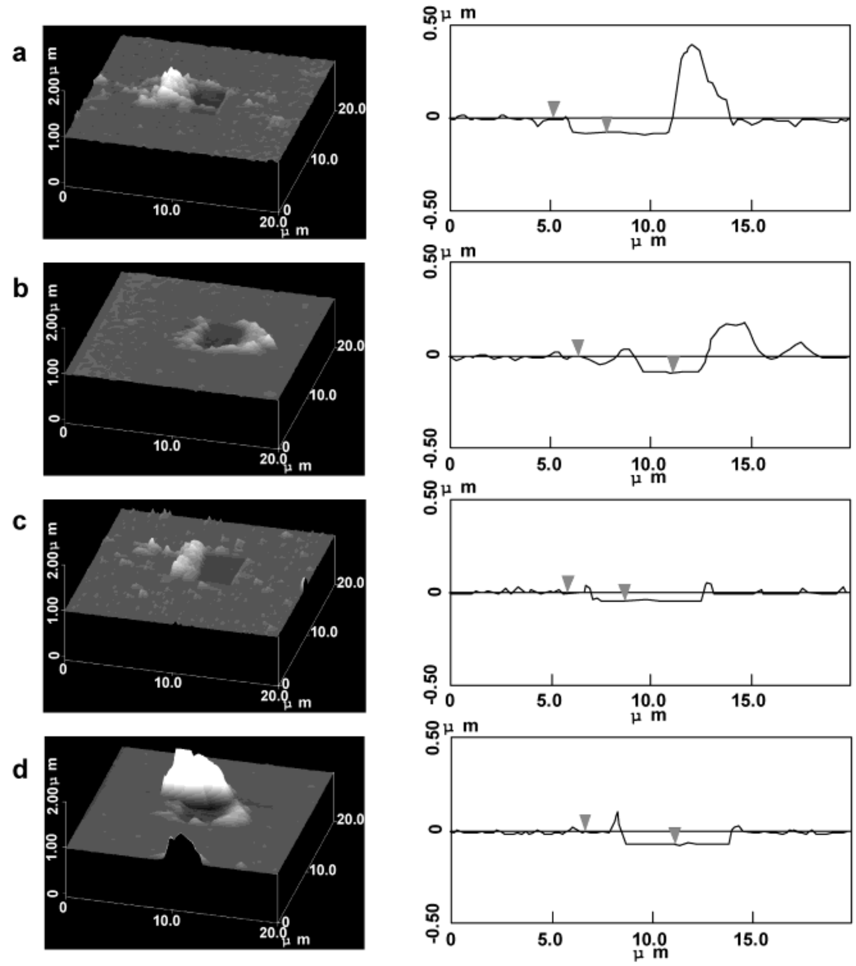


Figure 4. Scratching analysis of a six-step assembly between poly(VAm-*co*-NVF) with a 28 mol % VAm content and polyAAc in (a) air and (b) water and between poly(VAm-*co*-NVF) with a 42 mol % VAm content and polyAAc in (c) air and (d) water. The concentrations of poly(VAm-*co*-NVF) and polyAAc were 2.5 and 50 unit mM, respectively.

Table 1. Thickness Analysis of Ultrathin Hydrogels

	ultrathin film (in air: before swelling)				ultrathin hydrogel (in water: after swelling)		
VAm content (mol %)	freq shift (Hz)	polymer assembled (μg/cm^2)	thickness (nm)	density (g/cm^3)	thickness (nm)	density (g/cm^3)	swelling ratio ($W_{\text{water}}/W_{\text{polymer}}$, %)
28	3021	8.3	83	1.00	89	0.93	7.7
42	2107	5.8	49	1.18	73	0.79	27

oxidation and reduction processes was obtained for a bare QCM electrode. Similar curves were also observed for the electrodes coated with the ultrathin hydrogels. These observations indicate that $Fe(CN)_6^{3-}$ readily permeated the ultrathin hydrogels through the water in the films to react with the electrode. In fact, the peak currents to a bare QCM electrode, to the electrode coated with the ultrathin hydrogel prepared from poly(VAm-*co*-NVF) with a 42 mol % VAm content, and to the hydrogel prepared from poly(VAm-*co*-NVF) with a 28 mol % VAm content were 1.34, 1.56, and 1.74 mA cm^{-2}, respectively. The peak current increased when the electrode was coated with the ultrathin hydrogels. Since VAm units should remain in the ultrathin hydrogels, the $Fe(CN)_6^{3-}$ would seem to be condensed by electrostatic interactions. This means that it is possible to control the amounts of ions deposited in ultrathin hydrogels by regulating the chemical structure of the constituent polymers. Furthermore, the peak current increased with decreased VAm content in the poly(VAm-*co*-NVF) used, even though the apparent swelling ratio of the ultrathin hydrogels prepared from poly(VAm-*co*-NVF) with a lower VAm content was smaller, as shown in Figure 4. This may similarly be explained by the presence of VAm units remaining in the ultrathin hydrogels. As already shown in Figure 5, the ultrathin hydrogel prepared from poly(VAm-*co*-NVF) with a 28 mol % VAm content seemed to have more unreacted VAm units than that prepared from the copolymer with 42 mol %. Since $Fe(CN)_6^{3-}$ interacts with VAm units in the assembly, larger amounts of $Fe(CN)_6^{3-}$ accumulated in the former ultrathin hydrogel as determined by the larger peak currents. The permeability of substances across ultrathin films on functional materials is important for applications to sensors and for selective separation. Bruening et al. revealed that $Fe(CN)_6^{3-}$ could permeate an electrostatically assembled LbL film with a thickness as high as 44 nm when prepared under suitable conditions, although the peak current was smaller than that to a gold electrode coated with mercaptopropionoic acid.[19] The present results showed that ions could permeate ultrathin hydrogels with a greater thickness. It is notable that the frequency of the QCM coated with the ultrathin hydrogels did not change

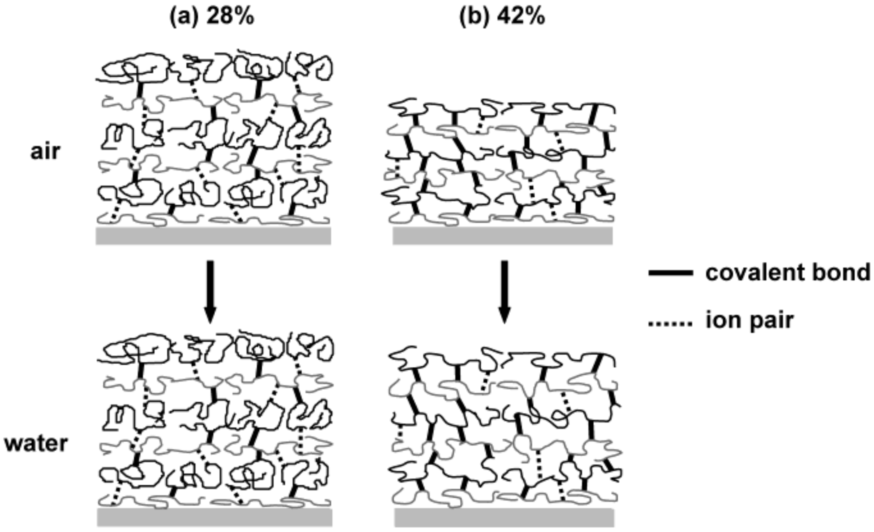


Figure 5. Schematic representation of assemblies of poly(VAm-*co*-NVF) with (a) 28 and (b) 42 mol % VAm content plus polyAAc.

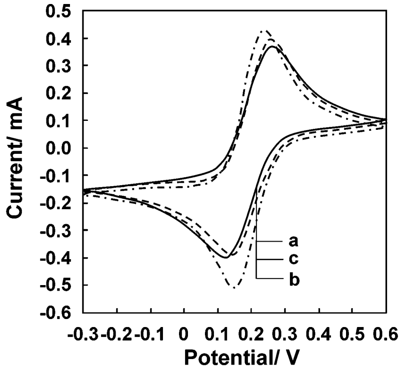


Figure 6. CVs of (a) a bare QCM and six-step assemblies of poly(VAm-*co*-NVF) with (b) 28 and (c) 42 mol % VAm content plus polyAAc at a sweep rate of 0.05 V s^{-1}. The concentrations of poly(VAm-*co*-NVF) and polyAAc for assemblies were 2.5 and 50 unit mM, respectively.

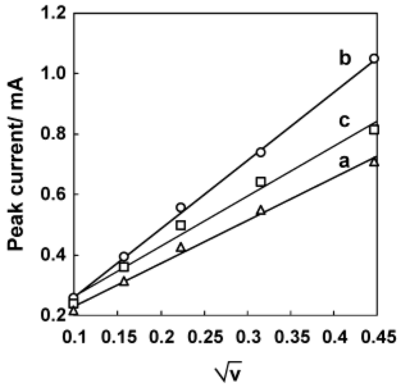


Figure 7. Dependence of peak current on the square root of sweep rate for (a) a bare QCM and six-step assemblies of poly(VAm-*co*-NVF) with (b) 28 and (c) 42 mol % VAm content plus polyAAc. The concentrations of poly(VAm-*co*-NVF) and polyAAc for assemblies were 2.5 and 50 unit mM, respectively.

after measuring the CVs, indicating that the ultrathin hydrogels were stable and did not peel off during CV measurement.

To obtain the CVs of electrodes coated with the ultrathin hydrogels (Figure 6b,c), there observed induction times that were dependent on the VAm content of poly(VAm-*co*-NVF) used. The peak currents were not observed at the initial stage, but current gradually increased with time. The saturation times were approximately 10 and 20 min for the ultrathin hydrogels prepared from poly(VAm-*co*-NVF)s with 28 and 42 mol % VAm content, respectively. This observation also suggests that $Fe(CN)_6^{3-}$ readily incorporated into the former hydrogel. Figure 7 shows the dependence of the saturated peak current against the square root of the sweep rate. In all cases, linear relationships were obtained. After $Fe(CN)_6^{3-}$ was incorporated into the ultrathin hydrogels, CVs were governed by diffusion of $Fe(CN)_6^{3-}$, indicating a water-swollen novel structure.

Conclusions

Poly(VAm-*co*-NVF)s with 20, 28, and 42 mol % VAm content plus polyAAc were sequentially reacted on a QCM substrate with gold electrodes through amide linkages using condensing reagents. The frequency analysis of the QCM revealed that at least 28 mol % of VAm was necessary for the sequential reaction. The total assembly amount increased slightly with decreasing VAm content. The assembly amount at each assembly step gradually increased with each assembly step possibly due to the conformational effect of poly(VAm-*co*-NVF)s in solutions. The assembly amount also increased with increasing the concentration of poly(VAm-*co*-NVF) in the reaction solution. The presence of both polymers in the assemblies was confirmed by ATR. The thickness of the assemblies, which was analyzed by the scratching mode of AFM, was increased in water compared to that in air, indicating the formation of an ultrathin hydrogel. The assemblies prepared from poly(VAm-*co*-NVF) with a greater VAm content absorbed more water molecules. $Fe(CN)_6^{3-}$ permeated the ultrathin hydrogels, as evidenced by CVs. We have demonstrated not only the preparation of ultrathin hydrogels from combinations of poly(VAm-*co*-NVF)s and polyAAc but also ionic permeability into the resulting ultrathin hydrogels. The present characteristics offer potential in the preparation of novel sensors and for loading of functional molecules into ultrathin hydrogels.

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