Studies on Stimuli-Responsive Nano-Structured Materials: Fabrication of Functional Surfaces with Ultrathin Hydrogel Films

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Chapter 1

General Introduction

Surface Modification

The macro- or nanometer-ordered modification of material surfaces has attracted much attention in the material sciences, because this method is very practical for fabricating functional material surfaces without sacrificing the physical properties of the materials. The surface properties of materials (such as wettability, adhesion ability, photosensitivity, themosensitivity, pH responsitivity) can be successfully controlled by terminally attaching various organic modifiers. To achieve this purpose, the physical or chemical methods, as well as their combination, have been extensively studied.

For example, the deposition of self-assembled monolayers (SAMs) offers one of the highest quality routes used to prepare chemically and structurally

Organic thin film

Self-assembled monolayers (SAMs)
Polymer grafts
Langmuir-Blodgett films (LB films)
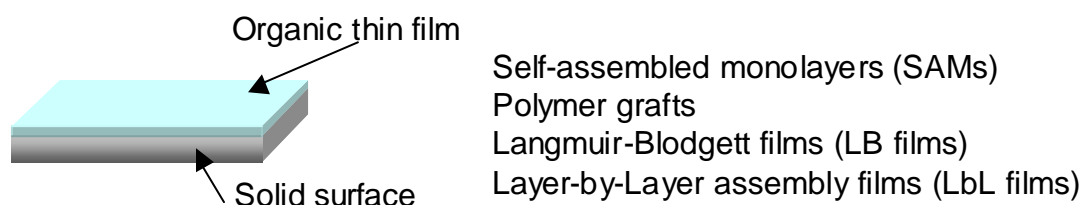Layer-by-Layer assembly films (LbL films)

Solid surface

Figure 1-1. Schematic illustration of surface modification of the solid materials by organic molecules.

well-defined surfaces.[1] SAMs are organic assemblies formed by the adsorption of molecular constituents from solution or the gas phase onto the material surfaces. The most intensively studied SAMs are derived from the adsorption of alkanethiols on gold, silver, copper, palladium and platinum. The high affinity of thiols for the surfaces of noble and coinage metals makes it possible to generate well-defined organic surfaces with useful and highly alterable chemical functionalities displayed at the exposed interface. SAMs can control the wettability and electrostatic nature of the interfaces of individual nanostructures and thus their ability to organize into large assemblies. SAMs add chemical functionality and thermodynamic stability to the surfaces of relatively simple inorganic nanostructures (quantum dots, superparamagnetic particles, nanowires) and make it possible to connect them to more complex systems (e.g. biological systems).

Fabrication of Stimuli-Responsive Switching Surfaces

Recently, there has been considerable interest in the development of materials whose surface properties can be dynamically modulated. These characteristic surfaces are so-called "switching surfaces" and have drawn increasing attention for its diverse applications in controlled assembly,[2,3] designed wettability alternation,[4] and programmed adsorption of proteins[5,6] or cells.[7] Various switchable surfaces initiated by external factors, including photon,[8,9] charge,[10,11] pH,[12] temperature[13,14] and chemical or electro-chemical energy,[15-18] have been extensively studied.
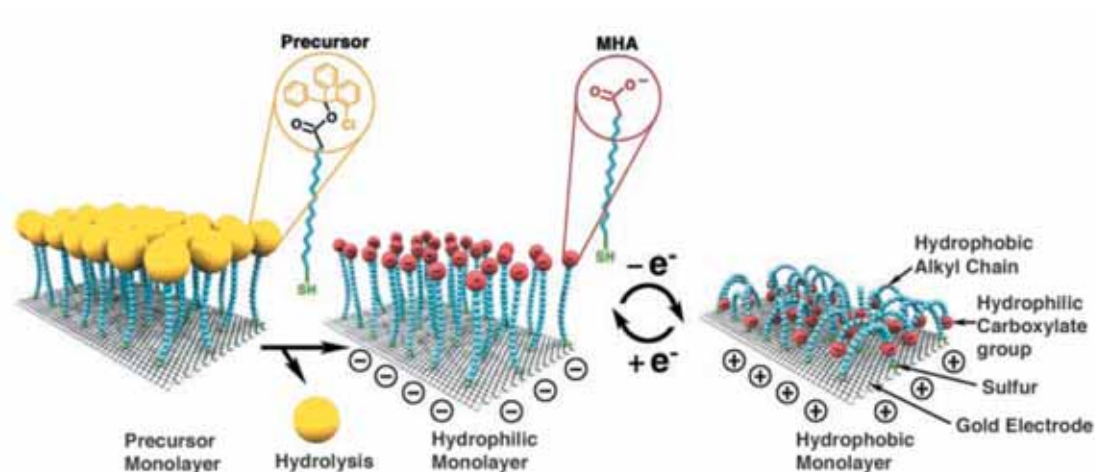
Figure 1-2. Idealized representation of the transition between straight (hydrophilic) and bent (hydrophobic) molecular conformations. [Lahann, J., *et. al.*, *Science,* **2003**, *299*, 371.]

Among this work, the low density SAM (LD-SAM) prepared on substrate is one of the most attractive discoveries.[17-19] To create the switching surface that could reversibly change from being hydrophilic to hydrophobic by applying an electric potential, Lahann *et al*. used the LD-SAM on a gold substrate.[17] To create the LD-SAM that has a negatively-charged terminus, they used the (16-mercapto)hexadecanoic acid capped alkanethiolates assemblied on Au following by dissociating the cap. The change in wetting behavior was caused by surface-confined, single layered molecules undergoing conformational transitions between a hydrophilic and hydrophobic state. The thus-prepared functional surface is predicted to have potential applications in dynamic regulation of macroscopic properties. Actually, this strategy has been used to induce the mechanical motions of molecules[18] and to control protein assembly.[19,20]

In addition, stimuli-responsive polymers are also one of the candidates

used for surface modification because of their interesting characteristics. Among them, one of the most intensively studied polymers is poly(*N*-isopropylacrylamide) (PNIPAAm), which exhibits a sharp phase transition in water at 32 °C.[21] PNIPAAm films were prepared by the polymerization of NIPAAm onto a functionalized surface via atom transfer radical polymerization,[22-24] plasma polymerization,[25,26] or photo-polymerization.[27,28] The surface wettability of these films can be reversibly changed around the lower critical solution temperature (LCST) of PNIPAAm in aqueous media. Dynamic control of the surface energy is of interest for controlling wettability,[29,30] biomolecular adsorption,[31] and cell adhesion.[32,33]

Ionov et al. have been reported a novel approach for the dynamic control of gliding microtubules motility by external stimuli.[34] This approach is based on the fabrication of a composite surface where functional kinesin
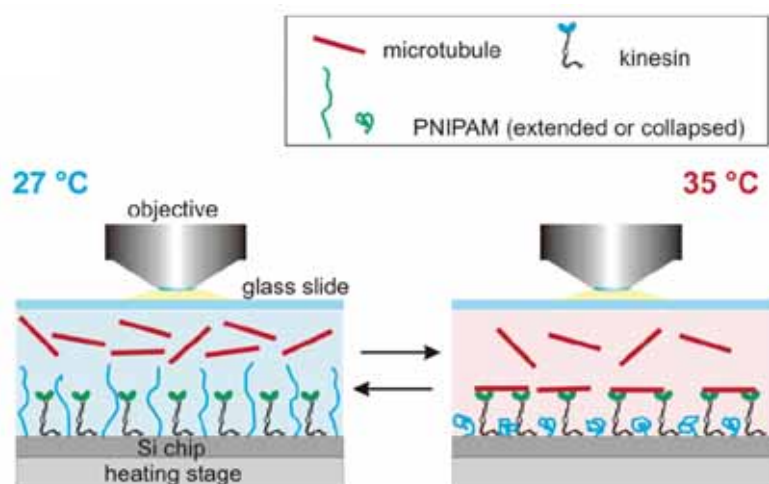

Figure 1-3. Schematic illustration of gliding motility of microtubules on a thermoresponsive PNIPAAm surface with adsorbed kinesin. [Ionov, L., *et. al.*, *Nano Lett.,* **2006**, *6*, 1982.]

motor-molecules are adsorbed onto a substrate between surface-grafted polymer chains of thermoresponsive poly(N-isopropylacrylamide). By external temperature control between 27 and 35 °C, they demonstrate the reversible landing, gliding, and releasing of motor-driven microtubules in response to conformational changes of the polymer chains. This method represents a versatile means to control the activity of biomolecular motors, and other surface-coupled enzyme systems, in bionanotechnological applications. Though these modifications are able to dramatically change the surface properties, however, the internal nano-space of organic modifications can hardly be effectively used.

Potential Value of Ultrathin Hydrogel Films

Hydrogels are soft materials that swell as they absorb large amounts of solvents. These unique materials have applications in both technological and biomedical fields. From a biomedical or biotechnological point of view, the surfaces of hydrogels proffer the potential to create bio-inert (e.g. non-activated/non-adhesive biomolecules) devices due to their water-swollen structures, which are similar to bio-interfaces such as proteins, cells, and tissues. In particular, the hydrogels, which undergo volume phase transitions in response to physical or chemical stimuli in the outer environment, are very interesting materials. PNIPAAm hydrogels show a reverse volume phase transition during the heating or cooling processes.[35] Furthermore, various hydrogels that are responsive to stimuli, such as pH,[36] ion strength,[37] light,[38] solvent composition,[39] and an electric
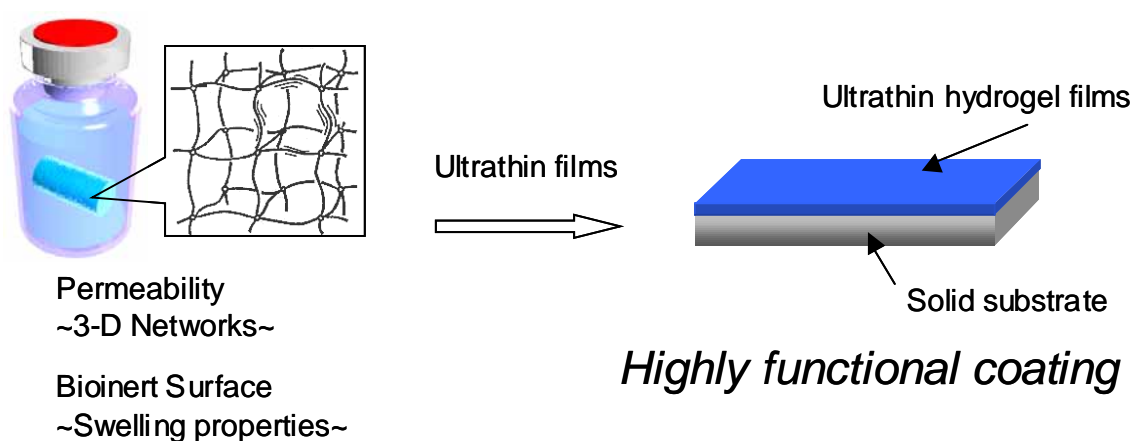
Figure 1-4. Schematic illustration of the fabrication of functional surfaces with ultrathin hydrogel films.

field[40] have been reported. These intelligent materials are quite suitable for designing devices such as separation, sensing, actuator, and controlled drug release. Accordingly, it is potentially valuable to fabricate material surfaces composed of stimuli-responsive ultrathin hydrogel films with a nanometer-ordered.

Recently, several investigations were performed to create stimuli-responsive hydrogel structures on various substrates. Matzelle *et al.* studied the mechanical properties of the PNIPAAm homopolymer and copolymer films prepared by simultaneous polymerization and cross-linking.[41] Another method of cross-linking PNIPAAm films was investigated by Kuckling *et al.*[42-45] Copolymers of PNIPAAm containing chromophres were photochemically cross-linked, and the swelling properties of the resulting films were investigated. Moreover, the use of plasma immobilization and electron beam irradiation were investigated for the preparation of stimuli-responsive hydrogel films.[46]

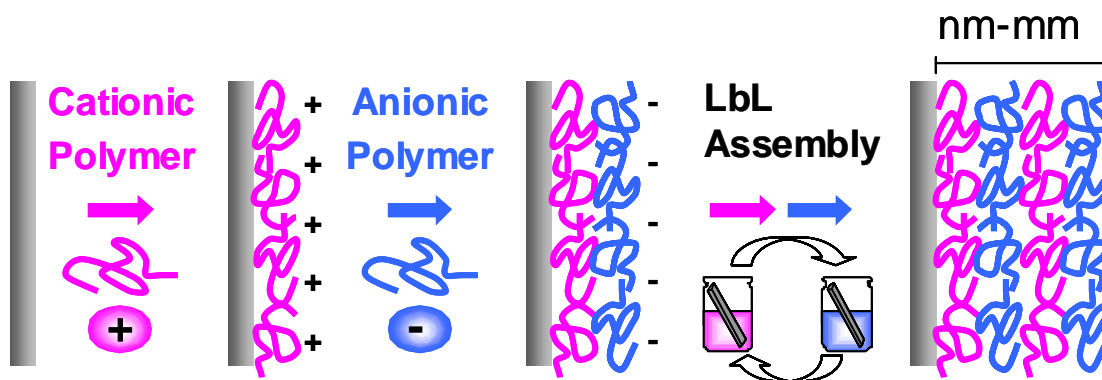Moreover, Serizawa et al. have reported the thermoresponsive ultrathin

Figure 1-5. Schematic of the Layer-by-Layer (LbL) technique. Simplified molecular concept of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. Counterions are omitted for clarity. The polyion conformation and layer interpenetration are an idealization of the surface charge reversal with each adsorption step.

hydrogel films using Layer-by-Layer (LbL) method.[47] This technique is based on sequential chemical reactions between activated polymers of themoresponsive copolymers containing amino or carboxylic groups.[48] Carbodiimide chemistry was used to produce chemical linkages between a thermoresponsive copolymer and counter polymer. Because of the lack of intermolecular adhesion between self-assembled polymers, crosslinked films of surface-bound ultrathin hydrogels were produced which preserved the temperature responsiveness of copolymers.

Objectives

The objective of this study is the preparation and characterization of stimuli-responsive ultrathin hydrogel films with two approaches (LbL method and photo-cross-link reaction). Ultrathin hydrogel films allow for novel surface modification for various technological applications.

This thesis includes the following:

Chapter 2 describes the preparation of ultrathin hydrogel films with regulated nanostructure by using LbL technique. Sequential surface chemical reactions of poly(acrylic acid-co-*N*-isopropylacrylamide) [poly(AAc-co-NIPAAm)] of which carboxyl groups activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), plus poly(vinylamine hydrochloride) through amide linkages produced ultrathin films on a solid substrate. Assembly processes were quantitatively monitored by a quartz crystal microbalance (QCM). Swelling properties of ultrathin hydrogel films were analyzed by atomic force microscopic (AFM) measurement. The effects of EDC amounts, ionic strength, solution pHs, and AAc contents on swelling properties were analyzed to reveal both internal structures and responses to external environments. Permeablities of ultrathin hydrogel films were observed by using cyclic voltammetries (CV) technique. In addition, thermoresponsive on-off ion permeabilities of ultrathin hydrogels, which derived from a LCST of polyNIPAAm, were also analyzed to reveal stimuli-responsive functions of ultrathin hydrogels.

Chapter 3 describes the tunable loading of charged dyes as model drugs into ultrathin hydrogel films, and the release of dyes depending on the aqueous ionic strength and the pH values. Loading and releasing of anionic charged dye were analyzed by ultraviolet-visible (UV-Vis) spectrometry. The amounts of loaded dyes were observed depending on the dye concentrations, film thickness, and AAc content of the copolymers. In addition, the release of loaded dyes from ultrathin hydrogel films was

observed in the different ionic strength, pH values of immersion solution.

In **Chapter 4**, there were investigated the preparation and characterization of PNIPAAm-based photo-cross-linkable copolymer as well as the corresponding hydrogel. Novel photo-cross-linkable polymers were synthesized for the preparation of the hydrogels with both pH- and temperature-responsive properties. The photo-cross-linkable copolymers [poly(NIPAAm-co-CIPAAm)-BP] were obtained by the condensation reaction of the carboxyl groups of poly(NIPAAm-co-CIPAAm) and the amino groups of 4-aminobenzophonone. To investigate the both pH- and temperature-responsive behavior, the volume phase transirion of the photo-cross-linked hydrogel was measured the equilibrium swelling ratios as the functions of pH and temperature.

In **Chapter 5**, based on Chapter 4, there were investigated the preparation and characterization of ultrathin hydrogel films by photo-cross-link reaction. After formation of the spin-coated polymer films on a solid substrate, UV light irradiation started the cross-linking reaction. The spin-coating processes and stability of the polymer films were quantitatively monitored by QCM and the thickness was estimated using AFM measurement. To investigate the both pH- and temperature-responsive behavior, the permeability changing were analyzed by using CV technique.

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Chapter 2

Stepwise Preparation and Characterization of Ultrathin Hydrogel Films by Layer-by-Layer Amide Formation

2.1 Summary

Sequential surface chemical reactions of poly(acrylic acid-co-*N*-isopropylacrylamide) [poly(AAc-co-NIPAAm)] with AAc contents of 5, 10, and 15 mol%, of which carboxyl groups were previously activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), plus poly(vinylamine hydrochloride) through amide linkages produced ultrathin films on a solid substrate. Assembly processes were quantitatively monitored by a quartz crystal microbalance as substrates. Assembled amounts increased with decreasing both AAc and EDC amounts. Subsequent immersion of ultrathin films into aqueous media resulted in the thickness increases, producing ultrathin hydrogels. Swelling ratios were estimated by percent increases in the thickness and increased with increasing AAc and EDC amounts. Swelling ratios were regularly changed by varying the ionic strength and pH of aqueous media. Swelling properties were interpreted based on structural information of ultrathin hydrogels.

Cyclic voltammetries using potassium ferricyanide revealed that ions permeated ultrathin hydrogels, and permeabilities were clearly suppressed above a lower critical solution temperature (LCST) of polyNIPAAm. Reversible on-off changes in permeabilities below and above a LCST were potentially observed. Not only structural control but also stimuli responsive functions of ultrathin hydrogels were realized within the present study.

2.2 Introduction

Hydrogels are soft materials that swell as they absorb large amounts of water molecules. These unique materials have applications in both technological and biomedical fields. Water-soluble functional molecules can be reserved and their release regulated via changes in the conditions of outer environments, concomitant with changes in physical properties of hydrogels. From a biomedical or biotechnological point of view,[1] the surfaces of hydrogels proffer the potential to create bio-inert (e.g. non-activated/non-adhesive biomolecules) devices due to their water-swollen structures, which are similar to bio-interfaces such as proteins, cells, and tissues. Accordingly, it is potentially valuable to fabricate material surfaces composed of ultrathin hydrogels with a regulated nanostructure. However, it is difficult to create functional coatings from hydrogels using conventional methodologies.

Assemblies composed of structurally regulated polymers on material surfaces enable not only functional modification of the materials but also

physicochemical analyses of polymeric characteristics in nanometer-ordered spaces. Layer-by-layer (LbL) assembly has recently been developed to create polyelectrolyte multilayers on surfaces by simple alternate immersion of materials into aqueous solutions of interactive polyelectrolytes including synthetic and biopolymers.[2] Not only ionic interactions but also hydrogen bonds,[3] charge transfer,[4] and van der Waals[5] interactions have been utilized to create functional ultrathin films. LbL assembly is normally applied using non-covalent interactions. However, several research groups have performed chemical reactions to create stable multilayers, in which the component polymers are covalently bound to each other at the interface between film layers.[6]

The swelling of polyelectrolyte multilayers in aqueous ionic solutions has already been investigated in detail.[7] Schlenoff *et al.*[7c] demonstrated that ionic pairs formed between constituent polyelectrolytes were partially dissolved in aqueous solutions of sodium chloride, resulting both in the increase in film thickness and change in surface topology of films. The swelling was strongly dependent on the salt concentration and the chemical structure of polymers. Rubner *et al.*[8] demonstrated that multilayers prepared either by electrostatic interactions or hydrogen bonds swelled in buffered physiological solutions depending both on the chemical structure of polymers and assembly conditions (pHs and ionic strength), and the amounts of adherent cells on multilayers were clearly modulated, resulting in expression of cytophilic and cytophobic surfaces. Accordingly, the swelling analysis of multilayers is significant not only for understanding

physical properties of multilayers but also for modifying biomedical material surfaces.

On the other hand, the formation of the small number of covalent bonds in multilayers composed of suitable water-soluble polymers permits fabrication of swelled multilayers on surfaces, due to strong hydration of hydrophilic polymer units, which did not join chemical bonds and inter-polymer interactions.[9] These multilayers are thought to be ultrathin hydrogels that spread two-dimensionally on surfaces. The creation of these multilayers requires the selection of suitable polymer combinations. The sequential amide formation between poly(vinylamine-co-*N*-vinylisobutyramide) [poly(VAm-co-NVIBA)] with suitable amounts of the VAm units and poly(acrylic acid) (polyAAc) was used to create ultrathin hydrogels.[9a] Furthermore, the obtained hydrogels showed reversible change in hydrophilicity, dependent on the temperature of the aqueous media. This thermoresponse was derived from the phase transition of the polyNVIBA units from coiled to globular states above a lower critical solution temperature (LCST). The sequential reaction was also applied to a combination of poly(vinylamine-co-*N*-vinylformamide) and polyAAc for the preparation of ultrathin hydrogels.[9b] Although 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was used for the activation of carboxyl groups in polyAAc, the maximum amount added to an aqueous polyAAc solution was 5 mol% relative to the total number of carboxyl groups due to polymer precipitation. Furthermore, approximately 40 and 30 mol% of VAm units in poly(VAm-co-NVIBA)

and poly(VAm-co-NVF), which were dependent on steric hindrance of lateral groups, were necessary for sequential chemical reactions. Accordingly, we could not analyze the detailed water-swelling structural properties of ultrathin hydrogels with different amounts of cross-links using the combinations described above. Although the combination of poly(acrylic acid-co-acrylamide) and poly(vinylamine) was recently utilized for the preparation of ultrathin hydrogels, some polymers were desorbed at each assembly step, resulting in the zigzag increase in assembly amounts.[9c]

In the present study, combinations of poly(acrylic acid-co-*N*-isopropylacrylamide)s [poly(AAc-co-NIPAAm)s] with different AAc contents and poly(vinylamine hydrochloride) (polyVAm) were selected for sequential chemical reactions on substrate surfaces to prepare ultrathin hydrogels with different structural properties. Then, effects of EDC amounts, ionic strength, solution pHs, and AAc contents on swelling properties were analyzed to reveal both internal structures and responses to external environments. In addition, thermoresponsive on-off ion permeabilities of ultrathin hydrogels, which derived from a LCST of polyNIPAAm,[11] were also analyzed to reveal stimuli-responsive functions of ultrathin hydrogels.

2.3 Experimental

2.3.1 Materials.

Poly(AAc-co-NIPAAm)s with AAc contents of 5, 10, and 15 mol% were synthesized according to methods described in a previous study.[11] Although it was difficult to estimate the molecular weight, AAc content was analyzed by ^{1}H-NMR spectra. PolyVAm (Mw 25000) was purchased from Polysciences. The pH of aqueous polyVAm solution was adjusted from 2 to 7.5 using aqueous 10 mM NaOH solution, dialyzed in water, and then lyophilized. Although vinylamine hydrochloride units are less reactive with activated carboxyl groups than free vinylamine, otherwise much greater amounts of polymers were deposited, resulting in difficulty of swelling analysis. EDC (>98.0 %) was purchased from Wako (Japan), and used without further purification. Methyl orange (Wako) and methylene blue (Aldrich) were used without further purification.

2.3.2 Quartz Crystal Microbalance (QCM).

The assembly was analyzed quantitatively by a 9-MHz QCM, as previously reported in our studies.[5,9] The crystal (9 mm in diameter) was coated on both sides with gold electrodes 4.5 mm in diameter, the 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 amount of polymer deposited, Δm

(ng), was estimated by measuring the frequency shift of the QCM, ΔF (Hz), using Sauerbrey's equation[12] as follows:

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

where F_o 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} dyne cm^{-2}). This equation was reliable when measurements were performed in air as described in this study. Before the reaction, the QCM electrodes were treated 3 times with a piranha solution [conc. 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 to blast clean the electrode surface.

2.3.3 Ultrathin Hydrogel Film Preparation by Sequential Chemical Reactions.

The cleaned QCM was immersed in an aqueous poly(AAc-co-NIPAAm) solution (0.05 unitM) containing EDC (in adequate amounts relative to the total AAc units), in which the carboxyl group had been already activated for 5 min before immersion. The QCM was immersed for 15 min, rinsed gently with pure water, and then dried under N_2 gas. The frequency shift was then measured in air. 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 aqueous solutions of poly(AAc-co-NIPAAm) with

AAc contents of 5, 10 and 15 mol%, and polyVAm were 4.3, 4.0, 4.0, and 7.5, respectively. Assembly was performed at 4 °C to maintain the activity of EDC. Although assembly was started with poly(AAc-co-NIPAAm), it was possible to initiate assembly with polyVAm. Assembly was stable even though polymers at the first step were physically adsorbed without any chemical reaction onto the QCM substrate.

2.3.4 Characterizations.

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. Swelling ratio was obtained by the following equation:

$$SW = \frac{d_{water}}{d_{air}} \times 100$$

where SW is swelling ratio (%), d_{water} is the film thickness in water, and d_{air} is the film thickness in air. Attenuated total reflection (ATR) spectra were obtained with a PerkinElmer Spectrum One (USA) in air at ambient temperature. One side of a poly(ethylene terephthalate) film was coated with gold to obtain a reflective surface. Polymers were then assembled using a method similar to that used in the QCM measurement. The interferograms were co-added 4 times, and Fourier transformed at a resolution of 4 cm^{-1}. Dye adsorption experiments were basically followed by a previous study.[13] Ultrathin hydrogels were similarly prepared on a transparent quartz substrate (45x9x1 mm). The coated substrate was immersed into dye solutions at 1 mM for 15 min at 20°C, rinsed with pure

water, and then dried with nitrogen gas. Dye amounts adsorbed were analyzed by an ultraviolet-visible (UV-Vis) spectrometry, Jasco Model V-550 at ambient temperature. Cyclic voltammetries (CVs) were measured by ALS/CH-Instruments Electrochemical Analyzer Model 660 at 25 °C using potassium ferricyanide under nitrogen atmosphere. Potassium ferricyanide was dissolved into 0.1 M sodium perchlorate at a concentration of 10 mM. Double-sided QCM electrodes were 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, as cited in our previous study.[9b]

2.4 Results and Discussion

Covalently constructed polyelectrolyte multilayers partially composed of polymers containing neutral and hydrophilic units potentially transformed into ultrathin hydrogels in aqueous media, due to the swelling of water molecules into multilayers.[9] For various applications of ultrathin hydrogels, it is important to control physicochemical properties (e.g. amounts of functional groups, swelling ratio, and permeation of molecules) on the basis of chemical strategies. A reasonable method is adequate selection of polymer components. We have already applied polymers of N-vinylalkylamides,[9a,b] of which the side groups could be partially hydrolyzed to introduce primary amines, and copolymers of acrylamide and AAc[9c] to a single component of multilayer formation. The preparation

of ultrathin hydrogels was potentially performed using those polymers from the point of a difference in chemical structures. Since preparative conditions were limited, and it was difficult to control physicochemical properties using those polymers, we selected the present combination of poly(AAc-co-NIPAAm)s and polyVAm, and characterized assembly processes, swelling properties, incorporation of charged dyes, and thermoresponsive ion permeabilities.

Figure 2-1 shows frequency shifts corresponding to assembly amounts plotted against assembly steps, when poly(AAc-co-NIPAAm)s with AAc contents of 5, 10, and 15 mol% were sequentially assembled with polyVAm on a QCM substrate in the presence of an equimolar EDC amount to the AAc units. The mean roughness analyzed by AFM were 2.0±0.5, 2.5±0.5, and 3.7±0.3 nm for 16-step assemblies of copolymers with AAc contents of 5, 10, and 15 mol%, respectively, resulting in the preparation of smooth ultrathin films. Assembly processes were reproducible in all cases, and frequencies decreased with increasing numbers of steps, indicating stepwise deposition of polymers. ATR spectra of assemblies demonstrated disappearance of a weak peak for carbonyl vibration bands at around 1710 cm^{-1}, corresponding to carboxyl groups in AAc units, which had been observed for a cast film of copolymers. This observation suggests the formation of amide linkages between poly(AAc-co-NIPAAm)s and polyVAm during assembly processes. Furthermore, the films obtained were stable and did not desorb in aqueous media, and the alternate immersion of the substrate in the absence of EDC did not demonstrate the stepwise
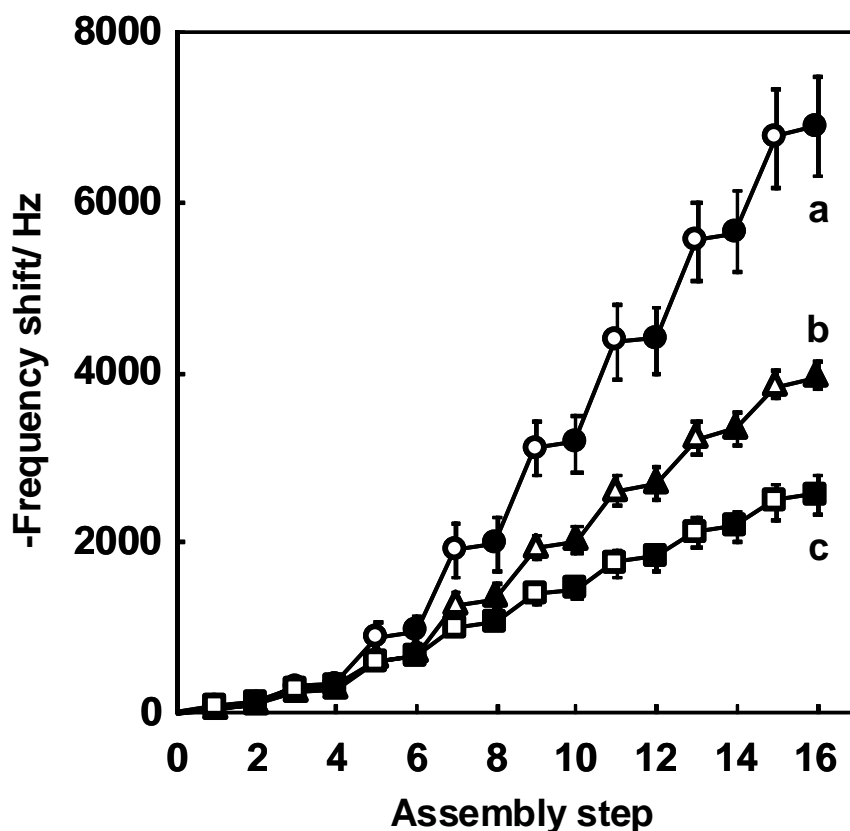
Figure 2-1. QCM analysis of the stepwise assembly of poly(AAc-co-NIPAAm) with AAc contents of (a) 5, (b) 10, and (c) 15 mol%, plus polyVAm in the presence of 1.0 equimolar amount of EDC to AAc units at 4 ℃. The open and closed symbols show the poly(AAc-co-NIPAAm) and polyVAm steps, respectively.

change in frequency (see Figure 2-3), also supporting the formation of chemical bonds between polymers. In the present system, the swelling of water molecules basically derives from the presence of neutral NIPAAm units of the copolymers. In other words, since electrostatically constructed conventional multilayers do not swell with water molecules in ordinary aqueous media, it is better to reduce ion complexes in assemblies for simple construction of ultrathin hydrogels. From this point of view, acrylamide copolymers containing small amounts of AAc units might be suitable for multilayer components, compared to copolymers of *N*-vinylalkylamides.[9a,b]

Total amounts assembled were dependent on AAc units and increased with decreasing AAc units. Although amounts of assembled polyVAm analyzed by the QCM method were almost the same in all cases, amounts of copolymers increased with decreasing AAc units. Accordingly, the difference was derived from increased assembly amounts of copolymers. This is possibly due to conformational differences of copolymers in reaction solution. A decrease in AAc content relaxes electrostatic repulsion of units, forming a more coiled conformation in aqueous media. When coiled polymers reacted with polyVAm on the assembly surface, the apparent monolayer thickness increases, assuming deposition with a similar two-dimensional density. In other words, extended polymers with a greater AAc content resulted in the construction of thinner monolayers on the assembly surface. These conformational effects have already been similarly demonstrated in cases of electrostatic LbL assembly.[13a,14] Furthermore, total

assembled numbers of AAc units, which were estimated from total assembly amounts of copolymers, percent contents of AAc units, and mean unit molecular weights of copolymers, were independent of AAc content, and were the same within experimental error for all copolymers. This estimation indicates that the increase in assembly amounts of copolymers is derived from an increase in coiled NIPAAm segments, which had not participated in film-based reactions, in the films. On the other hand, total assembled numbers of VAm units similarly estimated were approximately 1.5 times greater than those of AAc units in all cases, indicating an excess presence of amino groups within films. In fact, UV-vis spectral analyses demonstrated that an anionic dye, methyl orange was clearly incorporated into films, while a cationic dye, methylene blue was not incorporated at all under the same conditions. This observation also supports that cationic VAm units remain in films, and suggests that almost all AAc units react with VAm units during assembly. Note that the incorporation of methyl orange was independent of the outermost polymer component.

Apparent swelling ratios were estimated as percent increases in thickness in air and water at ambient temperature, as shown in Figure 2-2. The thickness in water increased compared to that in air in all cases, indicating swelling with water molecules. Dependence of swelling ratios against AAc content was characteristic for ultrathin hydrogels prepared by LbL assembly. The ratio increased with increasing AAc content. Since swelling of conventional bulk hydrogels is normally suppressed by increased cross-links, swelling of ultrathin hydrogels may decrease with increasing
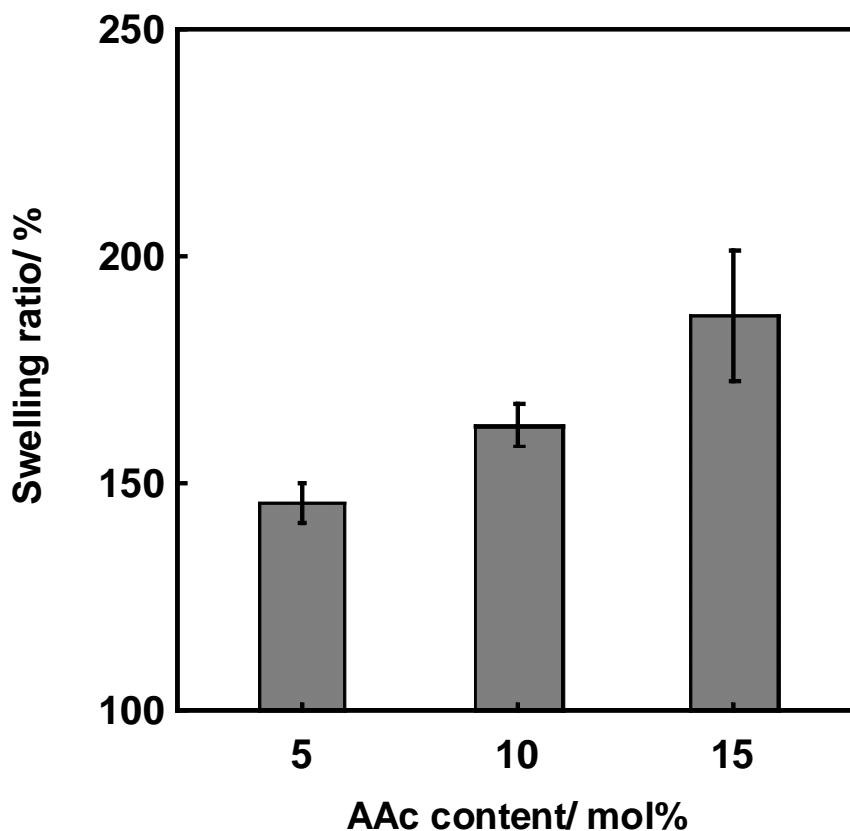
Figure 2-2. Swelling ratios of 16-step assembled ultrathin hydrogels prepared from poly(AAc-co-NIPAAm) with the different AAc contents and polyVAm in the presence of 1.0 equimolar amount of EDC in aqueous media, which were estimated by the following equation: [(thickness in water)/(thickness in air)x100].

AAc content. Accordingly, swelling properties cannot be simply interpreted by the number of cross-links. Conventional hydrogels are prepared from homogeneous solutions of monomers or polymers, while ultrathin hydrogels are prepared by stepwise two-dimensional deposition of polymer monolayers. In this case, it was not only cross-links that formed in films but also the conformation of deposited polymers possibly affected swelling properties. As already shown above, VAm, NIPAAm, and newly formed amide groups exist in ultrathin hydrogels. Considering that those units are hydrophilic, the conformational effect seemed to be a major factor for the difference in swelling ratios. Copolymers with greater AAc contents, which had been assembled under more extended conformations, had the ability to swell in water, because copolymers can be changed into coiled conformations after swelling. In other words, since the copolymers with smaller AAc contents were already comprised of coiled conformations in air, the thickness increase seemed to be suppressed. Based on these conformational effects, the difference in the apparent swelling ratios, depending on AAc contents, can be interpreted.

Changes in EDC amounts for stepwise reactions further represent assembly manners. Figure 2-3 shows frequency shifts corresponding to assembly amounts plotted against assembly steps, when poly(AAc-co-NIPAAm)s with 15 mol% AAc content, was sequentially assembled with polyVAm on the QCM substrate at various EDC amounts. Above an equimolar EDC to AAc units, assembly amounts were almost the same, indicating that similar assemblies had occurred. On the other hand,
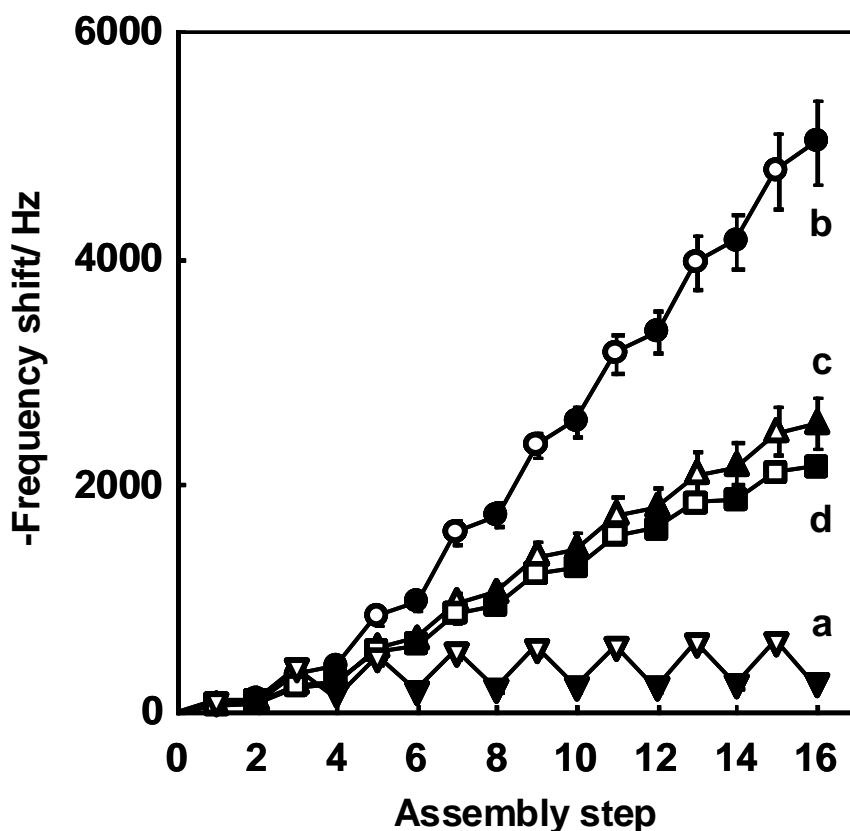
Figure 2-3. QCM analysis of the stepwise assembly between poly(AAc-co-NIPAAm) with an AAc contents of 15 mol% and polyVAm in the presence of (a) 0, (b) 0.5, (c) 1.0, and (d) 1.5 equimolar amounts of EDC. The open and closed symbols show the poly(AAc-co-NIPAAm) and polyVAm steps, respectively.

the amount was clearly increased at 0.5 equimolar EDC. Since the same copolymer was used here, the difference in polymer conformations in solutions cannot reasonably explain the increased amount. These data suggest conformational changes of copolymers on film surfaces depend on EDC amounts. The reaction of all AAc units with VAm units on the film should necessitate changes of the copolymer to more extended conformations. However, when EDC is insufficient, conformational changes are incomplete. Accordingly, greater amounts were assembled at 0.5 equimolar EDC. These analyses suggest another regulation of physicochemical properties of ultrathin hydrogels. In fact, the swelling ratio of the film prepared at 0.5 equimolar EDC was clearly smaller than others, as shown in Figure 2-4. This is possibly due to the presence of partially formed ionic complexes of remained AAc and VAm units (the film becomes hydrophobic due to complexes, similar to a manner of polyion complexes). The presence of AAc units within the film was also supported by slight incorporation of methylene blue into the film (data not shown). Furthermore, the swelling ratio was smaller than that of the film prepared from the copolymer with 5 mol% AAc content at an equimolar EDC, even though apparent cross-links for both films are similar. This also supports the presence of hydrophobic ionic complexes. It is noted that swelling ratios of films prepared at above an equimolar EDC are the same, also indicating fabrication of the same film. As a consequence, an equimolar EDC was sufficient to prepare ordinary ultrathin hydrogels, and decreasing amounts of EDC resulted in varied swelling properties.
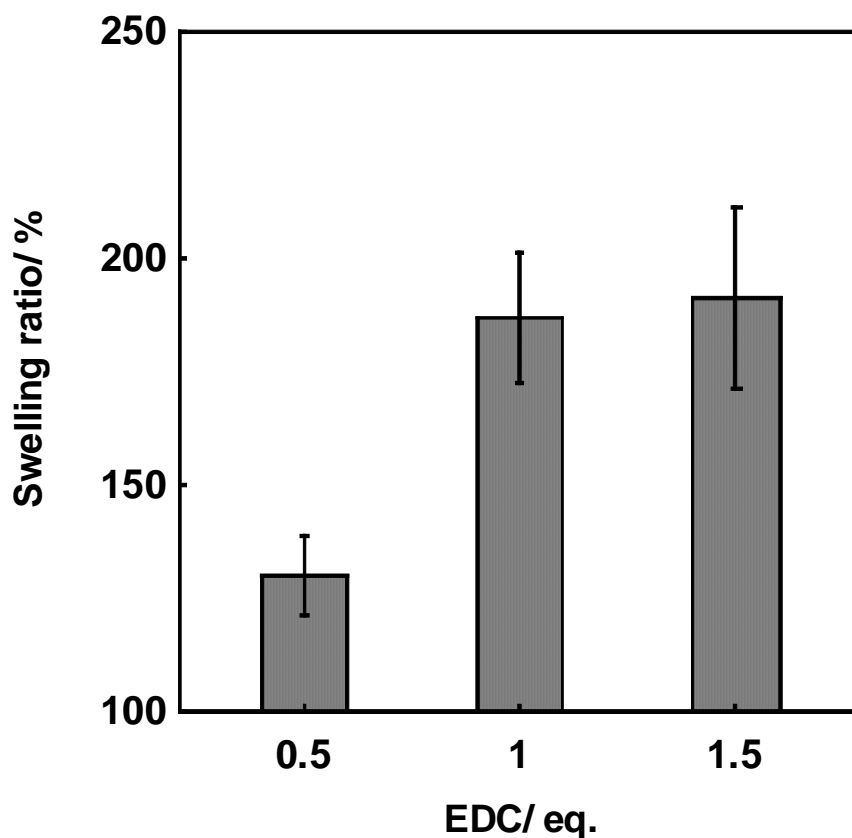
Figure 2-4. Swelling ratios of 16-step assembled ultrathin hydrogels prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of various equimolar amount of EDC in aqueous media, similarly estimated.

Swelling properties of polyelectrolyte multilayers determine their potential functions. Rubner *et al.* demonstrated that swelling of multilayers, of which the swelling ratios could be controlled by assembly conditions using a single polymer combination, can regulate cytophilic/cytophobic properties.[15] Accordingly, it is significant to analyze the swelling of ultrathin hydrogels under various atmospheres. Figure 2-5 shows swelling ratios of the ultrathin hydrogel prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of an equimolar EDC amount to AAc units, when it was immersed into various NaCl concentrations at ambient temperature. Swelling ratios gradually decreased with increasing NaCl concentration. This is possibly due to deswelling of water molecules from polymer components by coexistent inorganic ions. Furthermore, Figure 2-6 shows swelling ratios of the same film, when it was immersed into aqueous solutions at various pHs. The swelling was clearly suppressed at pH 10. As it has already discussed, VAm units remain in the film. In addition, the pK_a of VAm units is known to be 10.0.[16] Accordingly, deprotonation of VAm units occurr above this pH, and VAm units become more hydrophobic, thus resulting in a decrease in swelling. It is difficult to reasonably explain increased swelling at pH 12. In our previous study,[17] dehydrogenation reactions using polyVAm-protected platinum nanoparticles suggested further deprotonation of amino groups, resulting in anionically charged amines. Regeneration of charges seemed to be the reason for the increase. From these observations, the swelling of ultrathin hydrogels could be regulated by ionic strength and pH of aqueous

Figure 2-5. Swelling ratios of the 16-step assembled ultrathin hydrogel prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of 1.0 equimolar amount of EDC in aqueous media at various NaCl concentrations.
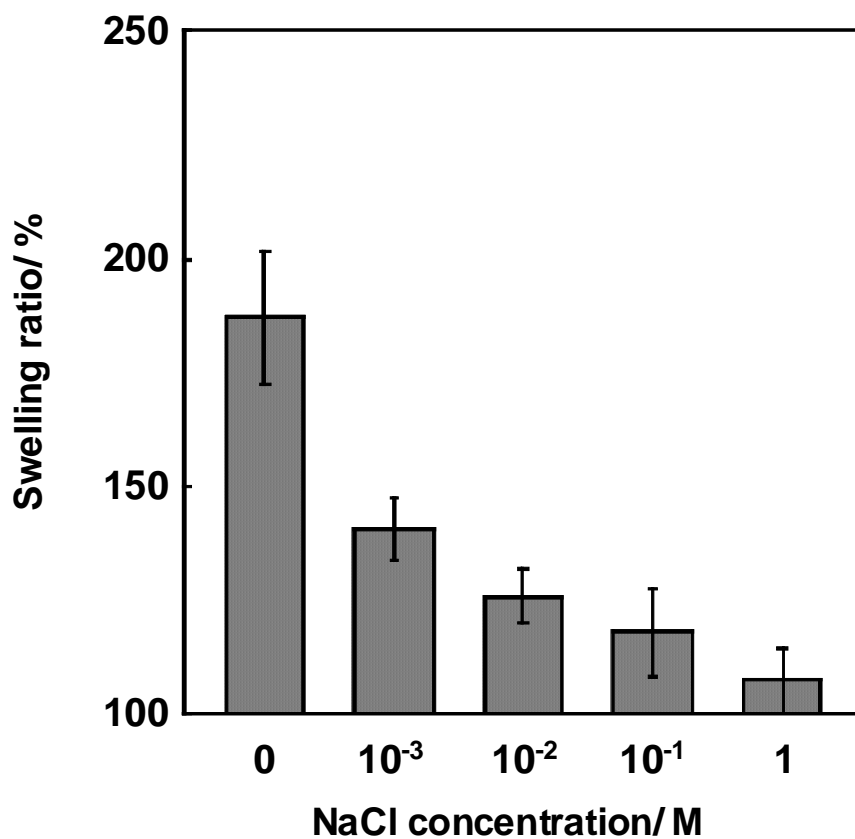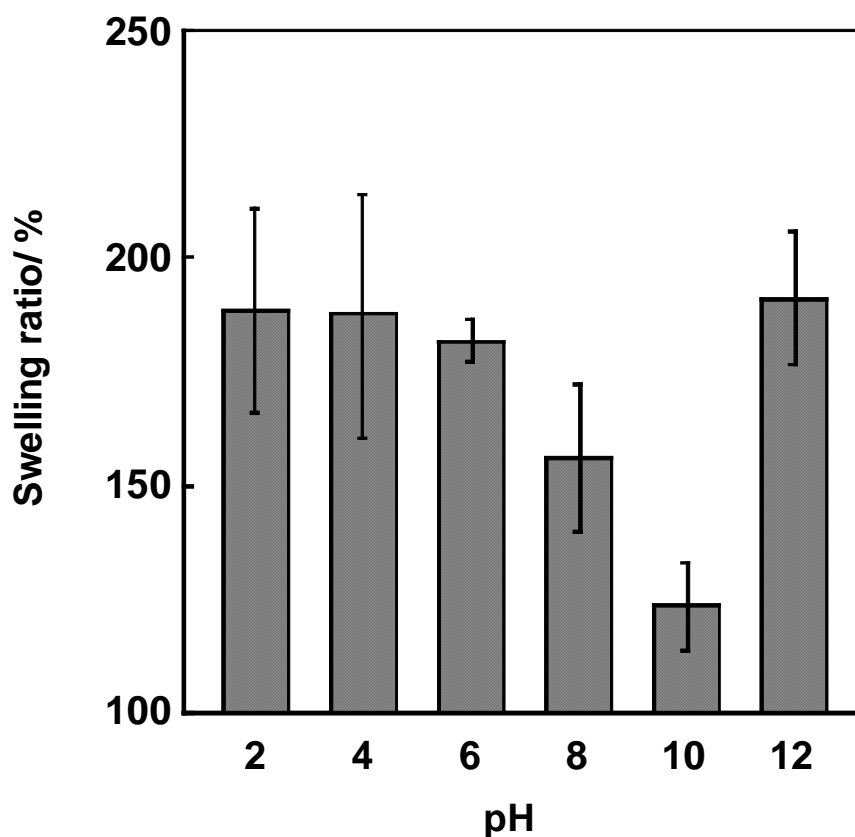
Figure 2-6. Swelling ratios of the 16-step assembled ultrathin hydrogel prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of 1.0 equimolar amount of EDC in aqueous media at various pHs. PH was adjusted by using aqueous 10 mM HCl and NaOH solutions, and then the ionic strength was maintained.

media, depending on the chemical structure of constituent polymers.

Permeabilities of small molecules are sensitive to the structure of ultrathin hydrogels. Since dyes could be incorporated into ultrathin hydrogels as shown above, these small molecules should be permeable. Furthermore, temperature-dependent permeabilities are expected due to thermoresponsible properties of polyNIPAAm segments. Here, we analyzed permeabilities of potassium ferricyanide using a CV technique, which analyzes permeabilities of electro-active ionic species across films deposited onto an appropriate electrode on the basis of oxidation-reduction cycles. QCM plates coated with ultrathin hydrogels were directly used for the electrode. Before analyses of on-off permeability, CVs of ultrathin hydrogels with different structures are obtained at 20 °C, of which the temperature is lower than a LCST of polyNIPAAm, approximately 32 °C.[18] Typical CVs are shown in Figure 2-7. A suitable CV curve that contains oxidation and reduction processes was obtained for a bare QCM electrode, and the peak current was 0.242 mA cm^{-2}. A similar curve containing the peak current of 0.132 mA cm^{-2} was observed for the electrode coated with ultrathin hydrogels. Latter peak currents were smaller than former ones, even though anionic ferricyanide ions may be electrostatically condensed to the present amine-rich ultrathin hydrogels. In fact, our previous study demonstrated the peak current for carboxyl group-rich ultrathin hydrogels was similar to a bare electrode.[9b] These observations suggest that initially adsorbed copolymers more strongly interact with the electrode surface, and then the electrode area seems to become apparently small. On the other
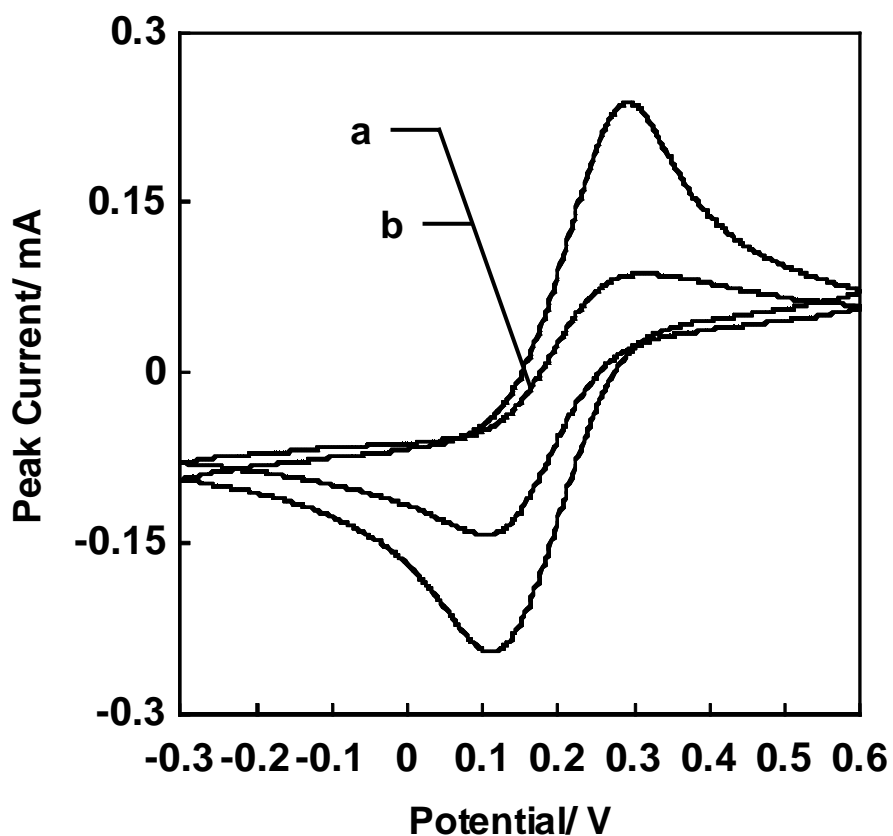
Figure 2-7. CV charts of (a) a bare QCM and the 8-step assembled ultrathin hydrogel prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of 1.0 equimolar amount of EDC at a sweep rate of 0.05 V s^{-1}.

hand, peak currents gradually decreased with increasing thickness of ultrathin hydrogels, indicating that ionic diffusion was suppressed with increasing thickness. It is also noted that the peak current of the ultrathin hydrogel prepared at 0.5 equimolar EDC was greater than those prepared at 1.0 and 1.5 equimolar EDC, of which the assembly amounts were adjusted to be almost the same by changing assembly steps. This observation indicates that ionic species can more readily permeate into ultrathin hydrogels with smaller amounts of covalent cross-links (see above). Furthermore, the dependence of peak currents against the square root of the sweep rate demonstrated linear relationships in all cases, thus indicating that CVs were governed by diffusion of electro-active ions into ultrathin hydrogels. As a consequence, potassium ferricyanide potentially permeated ultrathin hydrogels through water in films to react with the electrode.

For analyzing on-off ionic permeabilities, CVs were measured at various temperatures between 20-60 °C, at which temperature ultrathin hydrogels stably remained on the substrate. Peak currents steeply decreased between 30 and 35 °C, because of the deswelling of polyNIPAAm segments, and subsequent shrinkage of polymer chains on the substrate, resulting in the suppression of ionic permeabilities. Furthermore, reversible changes of peak currents at 20 and 40 °C were observed as shown in Figure 2-8. Permeabilities were significantly controlled by an on-off manner in all cases, and the films were stably remained after cycles. The on-off magnitude was greater for thinner hydrogels, because the peak current below a LCST was greater. Peak currents gradually decreased with
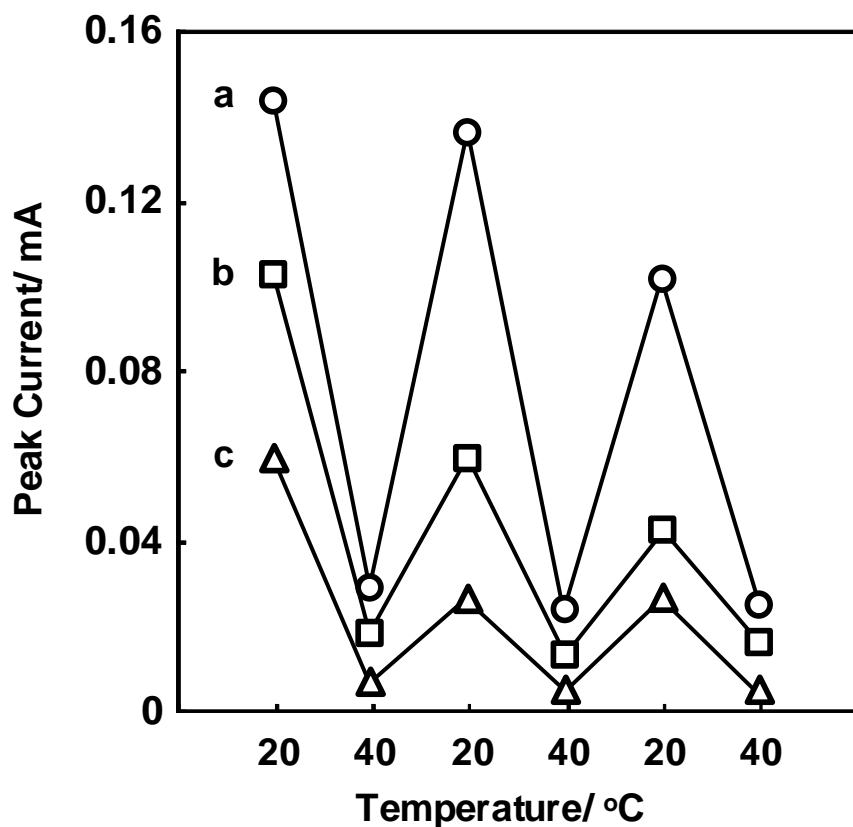
Figure 2-8. Reversible changes of CV peak currents at 20 and 40 ° C at a sweep rate of 0.05 V s^{-1} for the ultrathin hydrogel prepared from poly(AAc-co-NIPAAm) with an AAc content of 15 mol% and polyVAm in the presence of 1.0 equimolar amount of EDC: (a) 8, (b) 12, and (c) 16-step assembly.

increasing cycles. This is possibly due to gradual changes in internal nanostructures of ultrathin hydrogels, followed by the difficulty of complete regeneration of initial structures. Preliminary experiments of contact angles using air bubbles in water demonstrated that the surface became hydrophobic above the LCST, also indicating deswelling of water molecules. These observations suggest that ultrathin hydrogels can be used for various stimuli-responsive nanomaterials by modification with functional molecules in/on hydrogels.

2.5 Conclusions

Ultrathin hydrogels were prepared by stepwise monolayer deposition of poly(AAc-co-NIPAAm)s with different AAc contents and polyVAm on solid substrates through chemical reactions using EDC to activate AAc units, and were characterized by suitable methods. QCM substrates were used to quantitatively monitor assembly processes. Sequential reactions under various conditions demonstrated potential regulation of hydrogel structures. Smaller AAc contents led to greater assembly amounts; however swelling ratios increased with increasing contents. Less than an equimolar amount of EDC to AAc contents led to greater assembly amounts; then swelling ratios decreased. Swelling ratios were affected by ionic strength and pH of aqueous media. These physicochemical properties of ultrathin hydrogels were interpreted in terms of differences in chemical structures of constituent polymers, polymer conformations in aqueous media and on surfaces, polymer hydrophobicity, and reaction efficiencies between polymers. Electro-active ionic species permeated ultrathin hydrogels, and the permeation was suppressed above a LCST of polyNIPAAm. Furthermore, on-off permeation was reversibly observed. Preliminary experiments demonstrated facile incorporation of various organic small molecules and metal nanoparticles into ultrathin hydrogels, and these properties will be reported in the near future. Ultrathin hydrogels allow for novel surface modification for various technological applications.

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Chapter 3

Control of Loading/Releasing of Organic Charged Dyes in/from Ultrathin Hydrogels

3.1 Summary

The anionic dyes methyl orange (MO) and allura red (AR) were used as model drugs to assess the loading and release by layer-by-layer assembled ultrathin hydrogels prepared via the amide formation of poly(acrylic acid-co-N-isopropylacrylamide) with AAc contents of 5, 10, and 15 mol% plus poly(vinylamine hydrochloride). The amount of MO loaded was potentially controlled by changing the dye concentrations, film thickness, and AAc content of the copolymers. The release of AR was controlled by the NaCl concentration and pH. We conclude that the polymeric matrices of ultrathin hydrogels have great potential for the loading and release of charged drugs.

3.2 Introduction

Sustainable and controllable drug release enhances the effective treatment of disease, due to lower toxicity, tunable targeting, and improved patient convenience.[1,2] It is essential to chemically design polymeric matrices, which can readily embed various types and amounts of drugs, and then to release them in response to changes in external environment. It is therefore important to regulate not only the chemical structures of the matrices, but also the assembled structures of the component polymers at the macromolecular level.

Layer-by-layer (LbL) assembled polyelectrolyte multilayer films,[3] prepared by the alternate immersion of solid substrates into aqueous solutions containing oppositely charged polyelectrolytes, are potential matrices for drug loading and release. Researchers have previously reported the loading and release of drugs (sometimes model dyes), from assembled structures of multilayer films[4,5] and the regulation of these components by LbL assembly.[6-9] The LbL assembly of weak polyacids under suitable acidic conditions can generate acidic functional groups, and the subsequent immersion of these films into aqueous dye solutions at neutral pH values resulted in the loading of cationic dyes against the newly generated charges in the films.[4,5] Next, the re-immersion into an acidic solution or a suitable polymer solution released the dyes. The use of sulfonated cyclodextrins in the LbL assembly process produced films that incorporated dyes through host-guest chemistry.[6] The use of charged

microgels as film components produced insulin release from the films.[7] Hydrolytically degradable LbL films also demonstrated tunable drug release.[8] Furthermore, drug-conjugated polyelectrolytes as prodrugs were used for the components of the films, and the reduction of cell viabilities was revealed.[9] Although the loading and release of drugs has been achieved using LbL assembled films, further analysis using other films with different chemical properties remains to be performed.

LbL assembled films prepared by the chemical cross-linking of copolymers with neutral and hydrophilic monomer units swell with water molecules in their aqueous phase, thus forming ultrathin hydrogels.[10-13] The swelling properties as well as the chemical compositions can be delicately modified by changing the component polymers used, as well as by changing the assembly conditions. Since the component polymers are chemically cross-linked, ultrathin hydrogels are stable even in concentrated ionic solutions,[10] although LbL assembled polyelectrolyte multilayer films conventionally prepared by weak polyacids were decomposed by ions.[14] In addition, small molecules such as ions and dyes are readily incorporated into swollen ultrathin hydrogels[13] through suitable interactions between functional groups remaining in the ultrathin hydrogels and the incorporating species. Accordingly, ultrathin hydrogels have great potential as drug matrices. Here, we report the tunable loading of charged dyes as model drugs into ultrathin hydrogels, and the release of dyes depending on the aqueous ionic strength and the pH values. LbL amide formation between poly(acrylic acid-co-*N*-isopropylacrylamide)

[poly(AAc-co-NIPAAm)] with AAc contents of 5, 10, and 15 mol%, plus poly(vinylamine hydrochloride) (polyVAm) containing suitably cationic VAm units in ultrathin hydrogels resulted in the electrostatic loading of anionic dyes. Accordingly, methyl orange (MO) with a single sulfonyl charge unit and allura red (AR) with double sulfonyl charge units were used for comparison. The schematic representation of this study is shown in Figure 3-1.
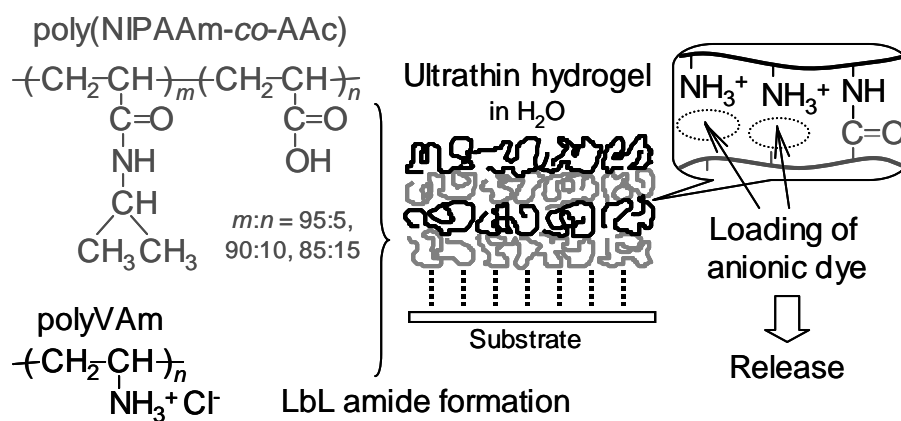

Figure 3-1. Schematic representation of this study.

3.3 Experimental

3.3.1 Materials.

Poly(AAc-co-NIPAAm)s were synthesized according to methods described in a previous study.[15] 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC) (>98.0 %) was purchased from Wako (Japan), and was used without further purification. MO (Wako) and AR (Wako) were used without further purification.

3.3.2 Preparation of Ultrathin Hydrogel on Solid Substrate.

The preparation of the ultrathin hydrogels was essentially the same as described in our previous study.[13] Briefly, a quartz substrate (35x9x1 mm) was immersed in an aqueous poly(AAc-co-NIPAAm) solution (0.05 unitM) containing EDC (equimolar amounts to the total AAc units), in which the carboxyl group had been already activated for 5 min before immersion. The substrate was immersed for 15 min, rinsed gently with pure water, and then dried under N_2 gas. The substrate was immersed again into an aqueous polyVAm solution (0.05 unitM), and the same procedure was repeated. This sequential reaction cycle was repeated for a specific number of steps. Assembly was started with poly(AAc-co-NIPAAm)s. Although the substrate was not modified before assembly, the ultrathin hydrogels remained stable on the substrate. For the quantitative analysis of the amount assembled, a quartz crystal microbalance (QCM) with gold electrodes was used as the substrate.[10-13] The amount of polymer deposited,

Δm (ng), was estimated by measuring the frequency shift of the QCM, ΔF (Hz), using Sauerbrey's equation[16] as follows:

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

where F_o 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} dyne cm^{-2}). The frequency was detected in air after the assembled polymers were sufficiently dried under nitrogen gas, so as not to detect swollen water molecules, following the methods described in previous studies.[10-13]

3.3.3 Characterizations.

For the loading of the dyes, the coated substrate was immersed into dye solutions at the specified concentrations for 15 min at 20 °C, rinsed with pure water, and then dried under nitrogen gas. Dye absorption was analyzed by ultraviolet-visible (UV-Vis) spectrometry using a Jasco Model V-550 at ambient temperature. Attenuated total reflection (ATR) spectra (Specrum One, Perkin Elmer) were obtained using a refractive surface of the QCM gold electrode in air. The interferograms were co-added 50 times, and Fourier transformed at a resolution of 4 cm^{-1}. For the release of dyes from the ultrathin hydrogels, the substrate coated with dye loaded ultrathin hydrogels was immersed into aqueous solutions with various NaCl concentrations and various pH values at 20 °C, and then the absorption of the aqueous phase was analyzed by UV-Vis spectroscopy.

3.4 Results and Discussion

In a previous study,[13] anionic MO (instead of cationic dyes such as methylene blue) was preferentially incorporated into ultrathin hydrogels prepared from poly(AAc-co-NIPAAm)s and polyVAm, indicating that the hydrogels were suitable for the loading of anionic dyes. In other words, since almost all AAc units from the copolymers reacted with the VAm units of the polyVAm for LbL chemical reactions, only cationic VAm units remained in the ultrathin hydrogels. In the present study, we analyzed the controlled loading of MO into ultrathin hydrogels under various conditions.

The change in the concentration of MO regulates the amount of MO loaded. We observed two absorption peaks at 275 nm and 375 nm for MO after loading into 16-step assembled ultrathin hydrogels prepared from poly(AAc-co-NIPAAm) with a 15 mol% AAc content and polyVAm, as shown in Figure 3-2a. The maximum absorbance at 375 nm (λ_{max}) was largely blue-shifted as compared to the λ_{max} in aqueous solution (467 nm), suggesting that MO electrostatically interacted with the VAm units in the ultrathin hydrogels. ATR spectra of the MO-loaded film revealed the peak of symmetric vibration bands for sulfonyl groups of MO at 1119 cm^{-1}, while the peak of a MO powder was observed at 1112 cm^{-1}. This shift also suggests the electrostatic interaction of MO with the VAm unit. From the release analysis (see below), 62 nmol of MO per quartz substrate were loaded into the ultrathin hydrogel in 1 mM MO.

The increase in the MO concentration resulted in an increase in the absorbance, with the same peak positions. The λ_{max} plotted against the MO concentration showed a saturation curve, and the amount of MO loaded was saturated at approximately 10 mM, as shown in Figure 3-2b. These observations indicated that the loading amount can be controlled by the MO concentration. From the saturation curve, the binding constant of MO to the ultrathin hydrogel was estimated by assuming the presence of a

$$\frac{[MO]}{Abs} = \frac{1}{Abs_{max}}[MO] + \frac{1}{Abs_{max}K}$$

Langmuirian type adsorption as follows:

where Abs_{max} is the maximum absorbance and K is the binding constant. The data could be fitted to the equation with the coefficient of variation of 0.99, and the binding constant was estimated to be 8.0×10^2 M^{-1}.

The film thickness for assembly (8, 12, and 16 steps) can also change the loading amounts, as shown in Figure 3-2c. An increase in film thickness, which was estimated in air,[13] resulted in an almost linear increase in the amount loaded. These observations indicated that the amount of MO loaded can be regulated by the film thickness, and that the internal structure of ultrathin hydrogels are homogeneous even after the increased thickness. Furthermore, a change in the AAc content of poly(AAc-co-NIPAAm)s for assembly resulted in characteristic changes in the amounts loaded (note that the assembly step numbers were changed to obtain similar assembly amounts, 14.6 μg cm^{-2}). The amount loaded decreased with an increasing AAc content, as shown in Figure 3-2d. Since the assembly of
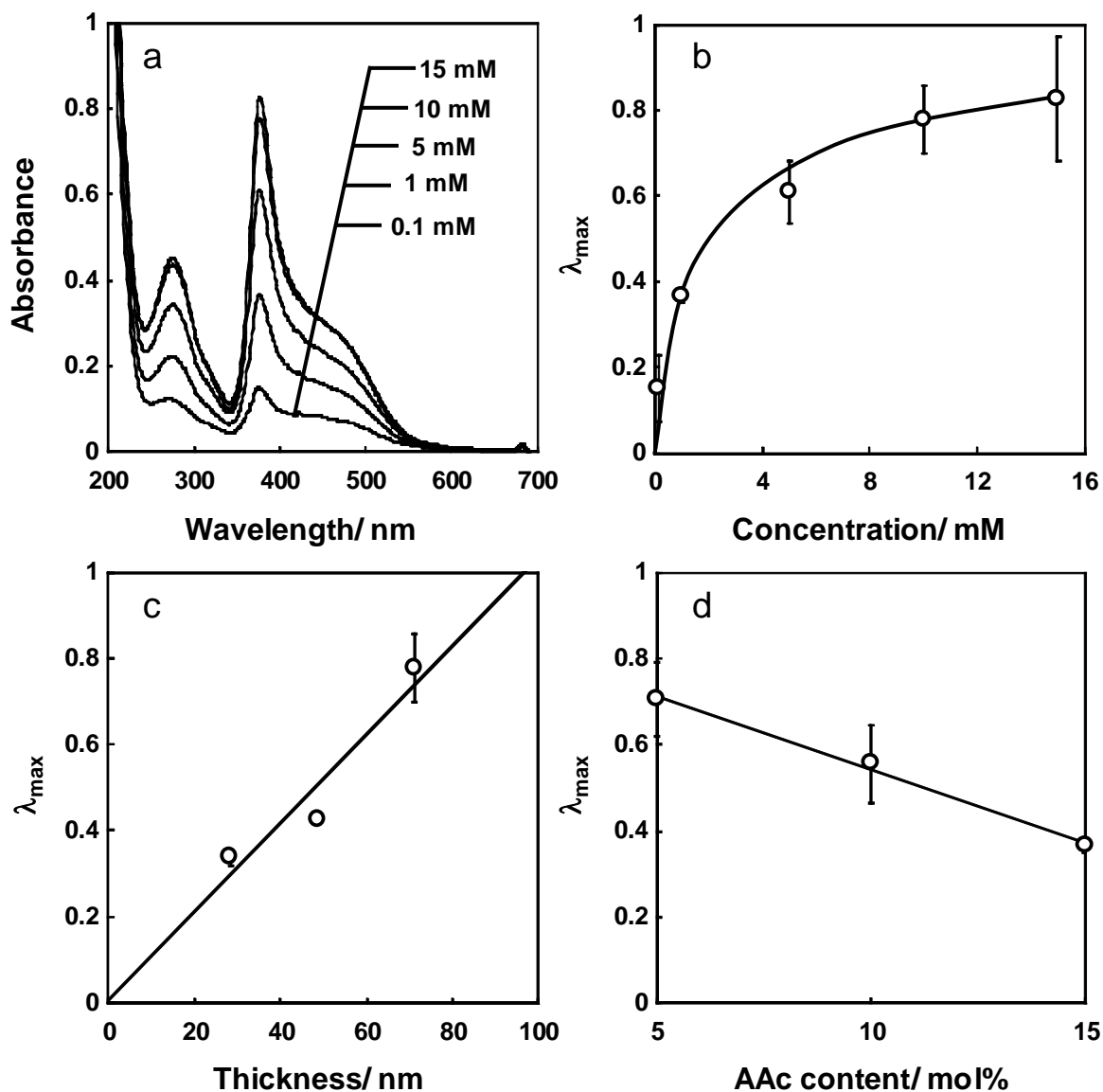
Figure 3-2. (a) UV-Vis spectra of MO loaded into ultrathin hydrogels prepared by a 16-step assembly from poly(AAc-co-NIPAAm) with a 15 mol% AAc content and polyVAm at various MO concentrations, (b) the λ_{max} of the MO loaded into 16-step ultrathin hydrogels against the MO concentration, (c) the λ_{max} of MO loaded into 16-step ultrathin hydrogels at 10 mM MO against the film thickness, and (d) the λ_{max} of MO loaded into ultrathin hydrogels at 1 mM MO against the AAc content of poly(AAc-co-NIPAAm)s, in which 8-, 12-, and 16 steps were used to assemble copolymers with 5, 10, and 15 mol% AAc contents.

poly(AAc-co-NIPAAm)s with a greater AAc content utilized greater amounts of VAm, the total amount of VAm units remaining in the ultrathin hydrogels should be decreased with increasing AAc content. This decrease in the VAm amount resulted in a decrease in the amounts of MO loaded. As a consequence, the MO concentrations, the film thickness of the assembly, and the AAc contents of the copolymers potentially regulated the amounts of MO loaded. We found that changes in these parameters readily changed the loading amounts.

Electrostatically loaded dyes can be released into ionic solutions (hindrance of electrostatic interactions) and at suitable pH (disappearance of the VAm charges). Although the release of MO was analyzed in ionic solutions, the release rates as well as the amount of MO released could not be controlled (all of the MO could be released even in the presence of 0.1 mM NaCl), possibly due to the fact that the interactions of MO with VAm were weakly responsive to the external environment. Although the release of dyes with a single charge unit were demonstrated using conventionally assembled polyelectrolyte multilayer films,[4,5] the interactions of MO with VAm in the water-swelling environment of the ultrathin hydrogels seemed to be weak. Therefore, we selected AR, an anionic dye with double charge units, to strengthen the interactions with the VAm units. In fact, AR was similarly loaded into the ultrathin hydrogels. A total of 130 nmol of AR per quartz substrate was loaded into ultrathin hydrogels prepared by poly(AAc-co-NIPAAm) with a 15 mol% AAc content and polyVAm in 1 mM AR. The loading amount of AR was greater than that of MO. Since AR

has double charges, AR was more readily loaded to ultrathin hydrogels through electrostatic interactions. Since AR has double charges, the amounts of the VAm units interacting with AR were approximately 4 times greater than those interacting with MO (62 nmol) at the same dye concentration. Considering the data in Figure 3-2b, AR could interact with the VAm units that were not used for the interaction with MO. In other words, the free VAm units seemed to be remained in the case of MO loading even at higher MO concentrations.

Figure 3-3a shows the time dependence of the amounts of AR released (loaded at 1 mM) from the ultrathin hydrogel when the NaCl concentration was changed from 1 mM to 1 M (pH 5.6). For the initial 20 min, AR was rapidly released, and then the AR was gradually released. The release rates increased with increasing NaCl concentrations, and thus the concentration could control the release rates. In addition, sustainable release was also realized. Even after 60 min in 1 M NaCl, approximately 50 % of the AR still remained in the ultrathin hydrogels. These observations suggest that drugs loaded into ultrathin hydrogels can be more slowly and continuously released under physiological ionic conditions. Release at suitable pH values is more dramatic. Although AR was not released under acidic pH conditions, almost all of the AR was rapidly released after 30 min at pH values 10, as shown in Figure 3-3b. Since polyVAm has a pK_a of 10,[17] these observations strongly suggest that the disappearance of the cationic VAm charges triggered the release of AR. In fact, the release profiles could be fitted to the terms of Fickian diffusion, suggesting that the release was
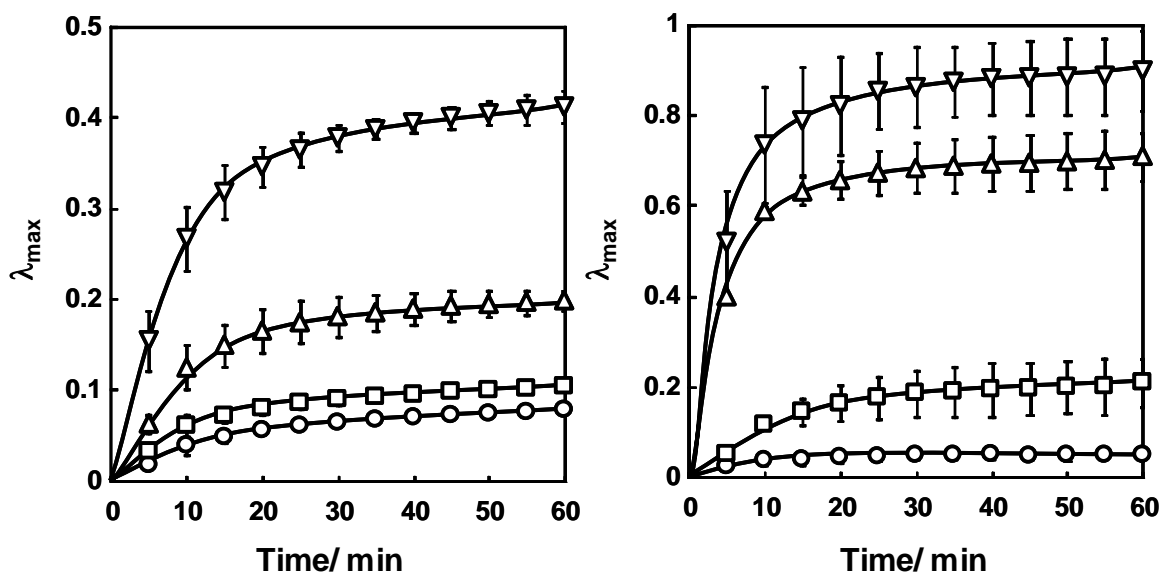
Figure 3-3. Release of AR loaded at 1 mM AR into ultrathin hydrogels prepared by 16-step assembly from poly(AAc-co-NIPAAm) with a 15 mol% AAc content and polyVAm, (a) against the NaCl concentration (circle, square, triangle, and reversed triangle indicate the release in water containing 1 mM, 10 mM, 100 mM, and 1 M NaCl, respectively) and (b) against the solution pH (circle, square, triangle, and reversed triangle indicate the release in water with pHs of 6, 8, 10, and 12, respectively).

governed by classical diffusion of AR (see below and Figure 3-4, 3-5). As a consequence, we found that the release of AR can be performed by changing the ionic strength and/or pH.

The ultrathin hydrogels prepared from poly(AAc-co-NIPAAm)s and polyVAm showed reversible change in permeabilities of ions below and above a lower critical solution temperature (LCST) of the polyNIPAAm units.[13] Although we preliminarily analyzed the release of dyes below and above a LCST, the LCST properties did not affect the release. Further delicate analysis is necessary to reveal the thermoresponsive release of small molecules from the ultrathin hydrogels.

Fick's second law is expressed as follows:

$$\partial C/\partial t = D (\partial^2 C/\partial x^2)$$

D : diffusion coefficient
x : diffusive dimension
C : concentration

From the solution of Fick's second law,

$$M_t/M_\infty = 4 (Dt/\pi l^2)^{1/2} \qquad [0 \leq M_t/M_\infty \leq 0.6]$$

$$M_t/M_\infty = 1 - 8/\pi^2 \exp(-\pi^2 Dt/l^2) \qquad [0.4 \leq M_t/M_\infty \leq 1.0]\text{-------eq. (1)}$$

Eq. (1) was transformed as follows, and then the data were fitted.

$$ln (1-M_t/M_\infty) = -\pi^2 Dt/l^2 + ln 8/\pi^2$$

M_t : released amount at time t $\qquad$ M_∞ : released amount at equilibrium

D : diffusion coefficient $\qquad$ l : film thickness

t : time
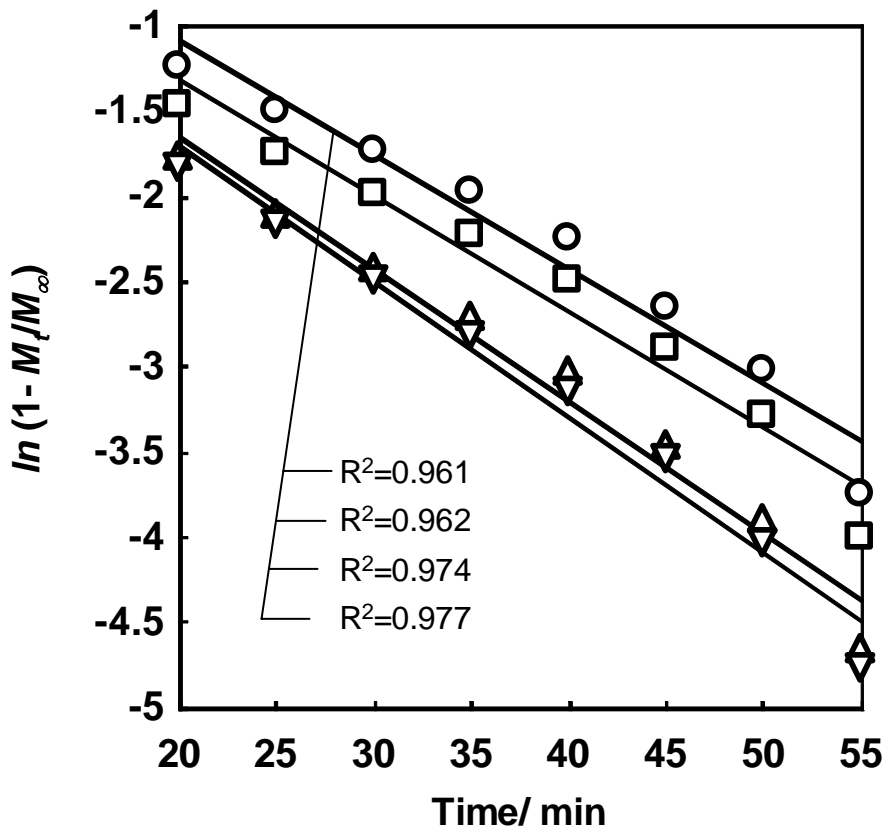
Figure 3-4. Fitting to the Fickian diffusion equation [eq. (1)] of the AR release in water containing various NaCl concentrations. Circle, square, triangle, reverse triangle indicated the release in water containing 1 mM, 10 mM, 100 mM, and 1 M NaCl, respectively.

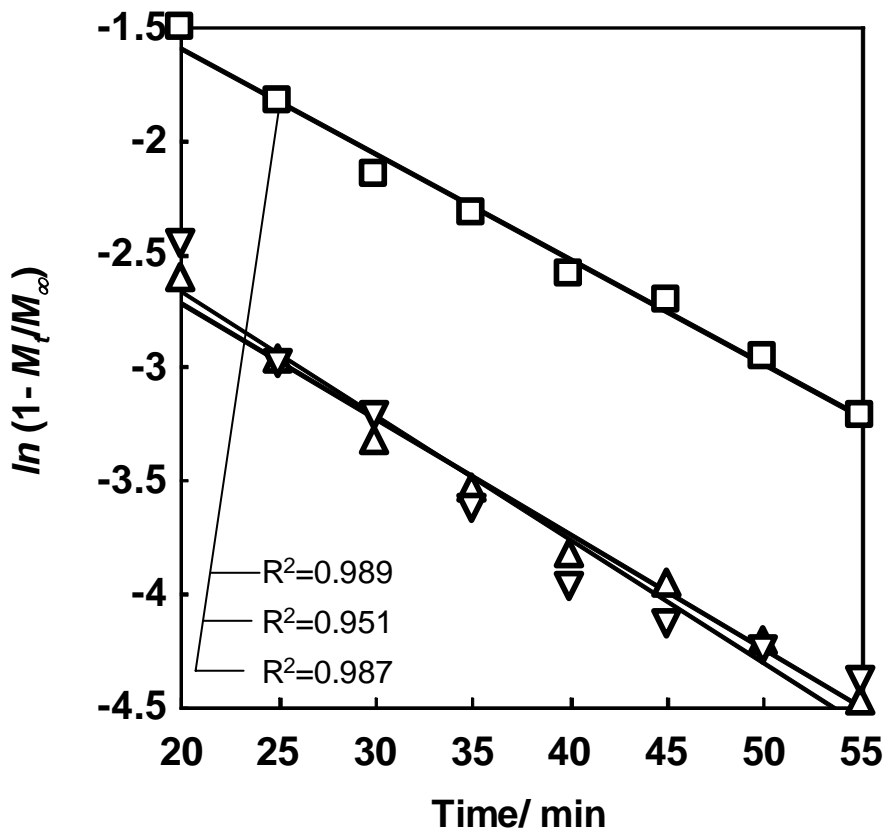**Figure 3-5.** Fitting to the Fickian diffusion equation [eq. (1)] of the AR release in water with various pHs. Square, triangle, and reverse triangle indicate the release in water with pHs 8, 10, 12, respectively.

3.5 Conclusions

We demonstrated that anionic dyes as model drugs were electrostatically loaded into ultrathin hydrogels prepared by LbL chemical reactions between poly(AAc-co-NIPAAm)s and polyVAm. The amounts loaded could be altered by changing the dye concentrations, film thickness, and AAc content of the copolymers. The sustainable release of loaded dyes was demonstrated for dyes with plural charges. The release was governed by the ionic strength and pH of the immersing solution. Ultrathin hydrogels have potential applications as controlled releasing matrices for charged drugs. Although we demonstrated a system for anionic dyes, the suitable selection of component polymers should result in anionically charged ultrathin hydrogels.[9,10] The tuning of delicate structures within these ultrathin hydrogels will further regulate loading and release of small molecules.

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Chapter 4

Novel Photo-Reactive Acrylamide-Based Copolymers Instantly Gave the Sensitive Stimuli-Responsive Hydrogels

4.1 Summary

Novel photo-cross-linkable polymers were synthesized for the preparation of the hydrogels with both pH- and temperature-responsive properties. Photo-reactive 4-aminobenzophenone (BP) was introduced into the side groups of poly(*N*-isopropylacrylamide-co-2-carboxyisopropyl-acrylamide). This copolymer was designed for the highly random sequences of comonomers. The introduction degree of BP was controlled by the changing ratio between the BP and the carboxyl groups. The cylinder-shaped hydrogel was easily prepared by UV irradiation for a few minutes. The Photo-cross-linked hydrogels exhibited clear volume phase transitions in response to both the pH and temperature changes and expected to be used for drug delivery systems.

4.2 Introduction

Recently, stimuli-responsive polymers can provide a variety of applications to the scientific and technological fields and the interest in these polymers has exponentially increased due to their promising potential.[1] Among them, temperature and pH responsive properties have been considerably investigated because they are very useful for many applications such as drug delivery system and so on. One of the most intensively studied polymers is poly(*N*-isopropylacrylamide), that is abbreviated as PNIPAAm. It exhibits a sharp phase transition in water at 32 °C that is called a lower critical solution temperature (LCST).[2] The polymer conformation changes completely reversibly from an extended coil to a globular structure, around the LCST. On the basis of this behavior, the hydrogels that is derived from the cross-linked PNIPAAm show a reverse volume phase transition during the heating or cooling processes.[3] Not only the PNIPAAm hydrogel but also various hydrogels that are responsive to the stimuli, such as the pH,[4] ion strength,[5] light,[6] solvent composition,[7] and electric field[8] have been widely reported. These hydrogels are so-called "intelligent materials" and quite suitable for the designing devices such as separation, sensing, actuator and controlled drug release.[9]

We have already reported that the poly(*N*-isopropylacrylamide-co-2-carboxyisopropylacrylamide) [poly(NIPAAm-co-CIPAAm)] is the ideal random copolymer,[10] because the polymerization reactivity ratios are

approximately equivalent. Such polymers are undoubtedly favorable for obtaining random sequence of the carboxyl groups and both clear and sensitive response.

In this study, we investigated the preparation and characterization of PNIPAAm-based photo-cross-linkable polymer as well as the corresponding hydrogel. The photo-cross-linkable polymers [poly(NIPAAm-co-CIPAAm)-BP] were obtained by the condensation reaction of the carboxyl groups of poly(NIPAAm-co-CIPAAm) and the amino groups of 4-aminobenzophonone. In terms of the cross-linking reactions, photo-cross-linking by the UV light is one of very convenient technique for preparation of three-dimensional polymer network. So far, this method has been utilized in various applications actually and recent interest has expanded to the fields of surface modifications and biotechnology.

To investigate the both pH- and temperature-responsive behavior, of the hydrogel prepared by the photo-cross-linking, we measured the equilibrium swelling ratios as the functions of pH and temperature.

4.3 Experimental

4.3.1 Materials.

Poly(NIPAAm-co-CIPAAm) with 30 mol% of CIPAAm content was synthesized according to the methods described in a previous study.[10] N,N'-dicyclohexyl carbodiimide (DCC), 4-amino- benzophonone (BP)

were purchased from Wako (Tokyo, Japan), and used without further purification. The suitable addition of hydrochloric acid (HCl) and sodium hydroxide (NaOH) aqueous solutions adjusted the pH of the media containing the hydrogels.

4.3.2 Introduction of Benzophenone moieties.

Poly(NIPAAm-co-CIPAAm) (379.1 mg, 3.0 mmol) was dissolved in 20 mL of N,N'- dimethylformamide (DMF) at 4 ^{o}C for 12 h. Then, 222.8 mg of DCC (1.08 mol; 1.2 equivalent molar against the total CIPAAm units) was added and the reaction mixture was stirred at room temperature. After 10 min, the adequate amount (0.5, 1.0 and 2.0 equivalent molar against the total CIPAAm units) of BP was added and then the solution was stirred at room temperature for 24 h. The resulting solution was dialyzed against DMF and water to remove the unreacted BP and the impurities, and then lyophilized. The introduction degree of the BP was determined by ^{1}H-NMR spectra (JEOL FX 400, Tokyo, Japan) in DMSO-d_6. Furthermore, the amount of BP introduction was quantified using chloroform solution containing 20 μg/ml of poly(NIPAAm-co-CIPAAm)-BP by means of UV-vis spectrometer (Jasco Model V-550, Tokyo, Japan).

4.3.4 Hydrogel synthesis.

The cylinder-shaped hydrogels were prepared by UV irradiation using an ultrahigh pressure Hg lamp (a 250 W, USHIO SX-UI251HQ, Tokyo, Japan). The DMF solution containing 5 w/v% of the photo-reactive polymer was

poured into a capillary glass tube with a diameter of 1 mm, and then UV light was irradiated for 20 min at room temperature. The gel was washed by immersion in DMF for 3 days, and then it was soaked in the pH-adjusted aqueous solution. The cylinder-shaped hydrogels were cut into piece with 10 mm length for the analysis of stimuli-responsive properties.

4.3.5 Equilibrium swelling ratio.

The hydrogel size was measured for estimation of the equilibrium swelling ratios $(D/D_0)^3$. D was the tubular gel diameter in equilibrium swollen state at the corresponding conditions (various pHs and temperature) and D_0 was the hydrogel diameter in equilibrium swollen at pH 12 and 15 °C. Three-dimensional equilibrium swelling ratios can be evaluated by cubing D/D_0 because the gel swelling and deswelling has isotropy.[11] We preliminarily have confirmed that the equilibrium swelling ratios as a function of pH and temperature reached evenly within 12 h. The diameter of cylinder-shaped gels was measured by a Stemi DV4 microscope (Carl Zeiss, Germany) connected to a DCR-TRV33K digital video recorder (SONY, Japan).

4.4 Results and Discussion

Poly(NIPAAm-co-CIPAAm)-BP was prepared by the condensation reaction between the DCC-activated carboxyl groups and amino groups of BP (Figure 4-1). A typical ^{1}H-NMR spectrum of after condensation reaction
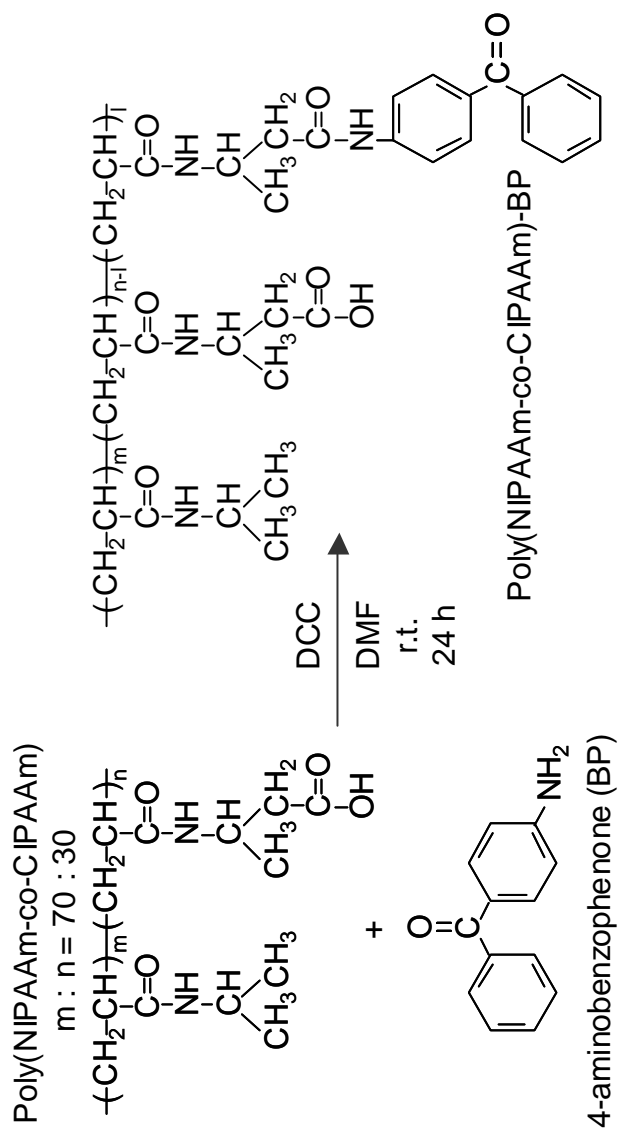
Figure 4-1. Synthetic Scheme for Benzophenone-Substituted Poly(NIPAAm-co-CIPAAm).

is shown Figure 4-2. Based on the ^{1}H-NMR measurement, the introduction ratio was determined using the signals of 7.5-8.5 ppm assigned to BP. These observations suggest the success in the BP introduction into poly(NIPAAm-co-CIPAAm).

Additionally, the introduction degree of BP could be controlled by the amount of BP adding into reaction mixture. Table 4-1 summarized preparation results of poly(NIPAAm-co-CIPAAm)-BP. According to ^{1}H-NMR spectra measurement, the signals of BP increased as the added BP amount were increased. Figure 4-3 shows the UV-vis adsorption spectra of poly(NIPAAm-co-CIPAAm)-BP chloroform solution. The absorbance at 300 nm is attributed to the π-π* transition of the BP groups.[12] In contrast, when only poly(NIPAAm-co-CIPAAm) was used, the peak was not observed. This result shows that BP was undoubtedly introduced into poly(NIPAAm-co-CIPAAm). And then the peak max at 300 nm has increased as the amount of the introduction of BP increases into polymers. These observations suggest that the introduction of BP into the polymers could be controlled easily.

Next, we studied the photochemical reaction of poly(NIPAAm-co-CIPAAm)-BP by UV irradiation. Figure 4-4 shows the UV-vis spectra of the polymer with an 11.1 mol% BP content as a function of UV irradiation time. During UV irradiation, the absorbance at 300 nm decreased continuously and almost disappeared after only 5 min. Benzo- phenone is indirectly excited to the lowest triplet state (π-π*) by the direct absorption into the singlet state (π-π*) upon UV irradiation. The hydrogen
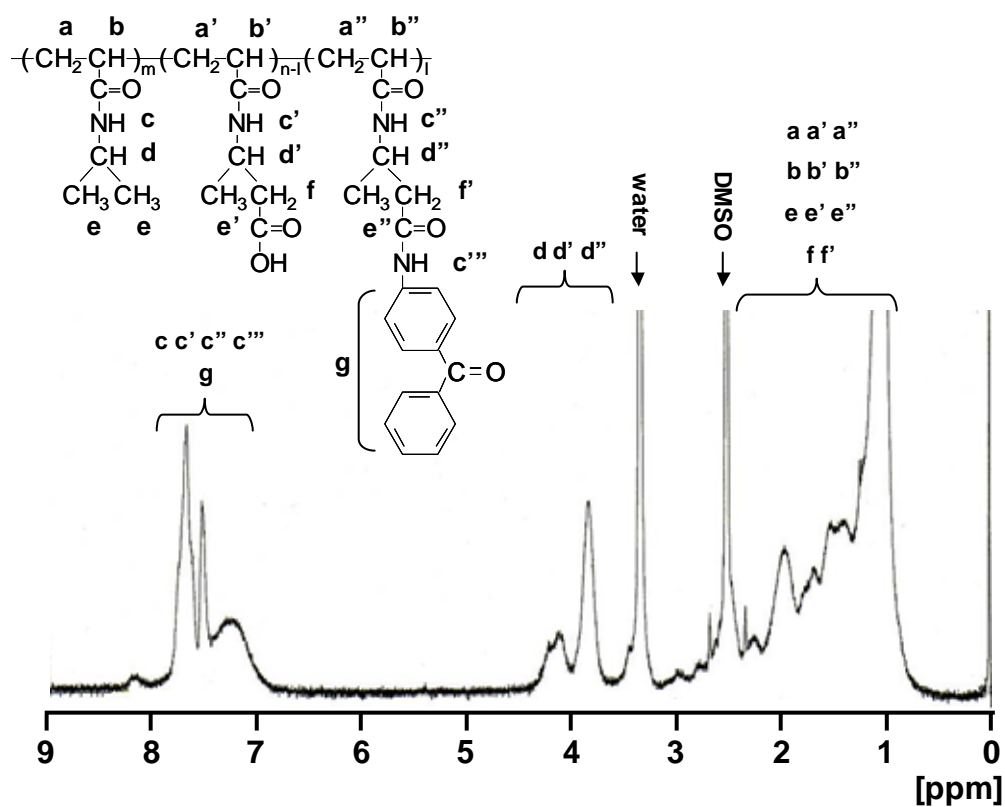
Figure 4-2. ¹H-NMR spectrum of poly(NIPAAm-co-CIPAAm)-BP in DMSO-d$_6$ at room temperature.

Table 4-1. Preparation of poly(NIPAAm-co-ClPAAm)-BP.

Run	ClPAAm (mol%)	Polymer		DCC		4-aminobenzophenone		In copolymer (mol%)		
	(mol%)	(mmol)	(mg)	(mmol)	(mg)	(mmol)	(mg)	ClPAAm	BP
1						0.45	88.8	26.3	3.7
2	30	3	379.1	1.08	222.8	0.9	177.5	24.4	5.6
3						1.8	355.0	18.9	11.1

Reaction conditions: solvent = DMF, 18 ml; T = r.t.

Figure 4-3. UV-vis spectra of the poly(NIPAAm-co-CIPAAm)-BP chloroform solution: Circle, square, triangle and reverse triangle indicate 0, 3.7, 5.6, and 11.1 mol% BP introduced, respectively.
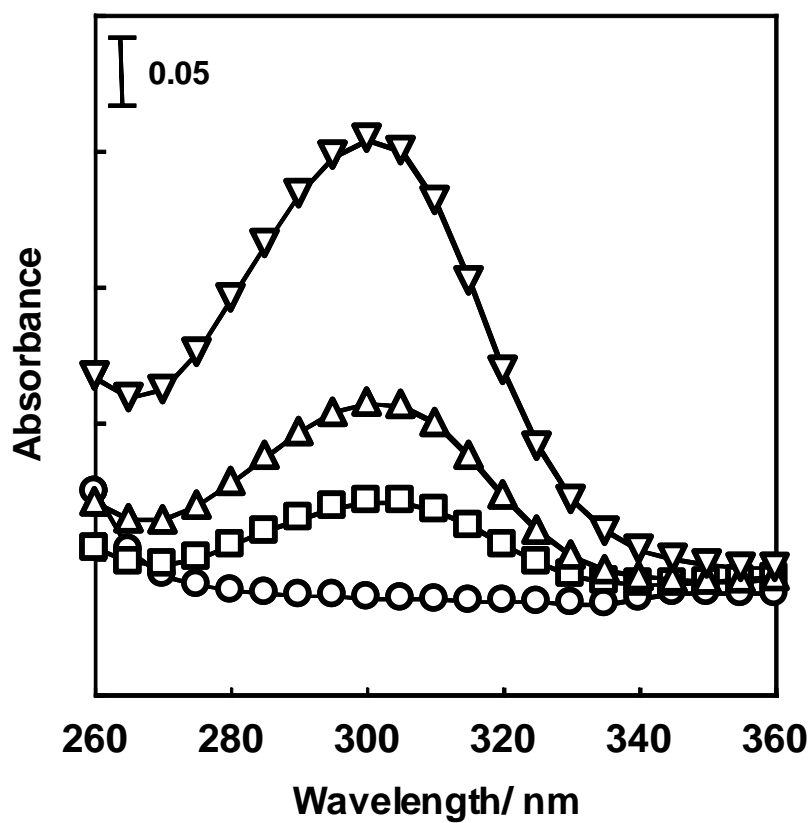
atom present in polymer solution is easily abstracted by benzophenone triplet (3BP*). The benzophenone ketyl radical and a polymer on-chain radical readily recombine to generate a new C-C bond[13] followed by the cross-linking. In the separate experiment, we also confirmed the no progress of the reaction without the photo-reactive BP.

As described above, BP moieties can play the role of cross-linking agent by UV irradiation. Then, we prepared the cylinder-shaped hydrogels by UV irradiation using 5 w/v% of DMF solution of photo-reactive polymer with an 11.1 mol% of BP. The hydrogel prepared in this condition could be provided to the estimation of the pH- and temperature-responsive properties. In separate experiments, we checked the suitable condition to prepare the hydrogels possible to provide the following estimation. As a result, the use of low content of BP in the polymer or the preparation of low concentration of polymer solution is not appropriate to them. This result means the BP moieties dominated the hydrogel formation.

The stimuli-responsive properties of the poly(NIPAAm-co-CIPAAm)-BP hydrogel were investigated by the measurement of equilibrium swelling ratios as a function of pH change. Figure 4-5 shows equilibrium swelling ratios of the hydrogels at each pH condition at fixed 15 °C. The equilibrium ratio of the hydrogels increased with increasing pH value, as seen in the figure. Poly(NIPAAm-co-CIPAAm)-BP is terpolymer as shown in Figure 4-1, that is, contains three kinds of side chain structure in the polymer chains; unmodified isopropyl groups, carboxyisopropyl groups and BP-introduced isopropyl groups. At the higher pH, carboxyl groups are
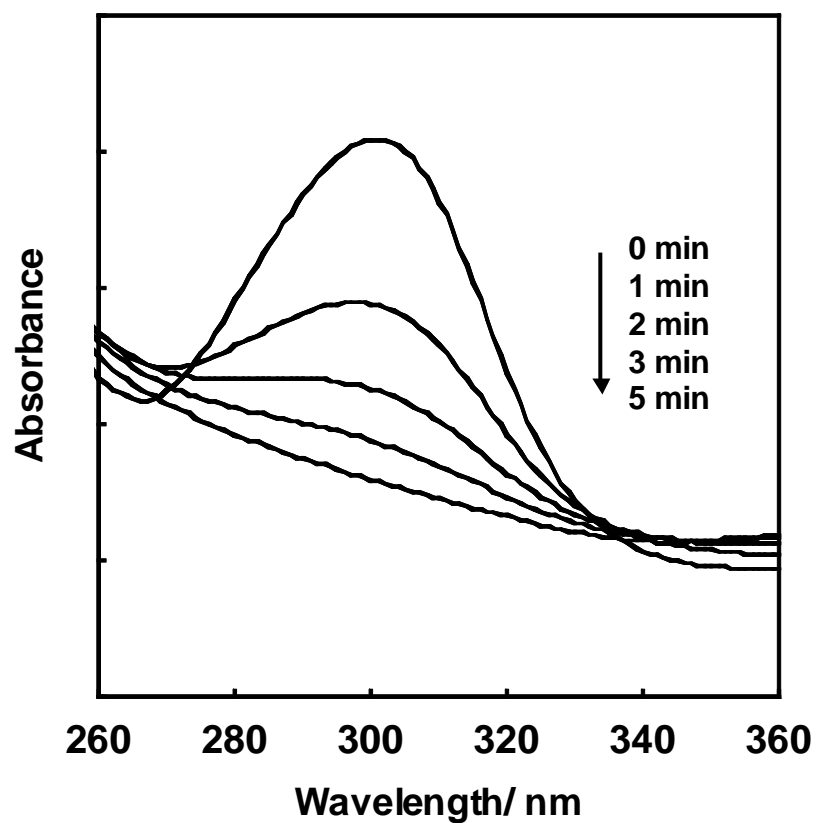
Figure 4-4. UV-vis spectra of the poly(NIPAAm-co-CIPAAm)-BP (BP content 11.1 mol%) chloroform solution as a function of UV irradiation time.
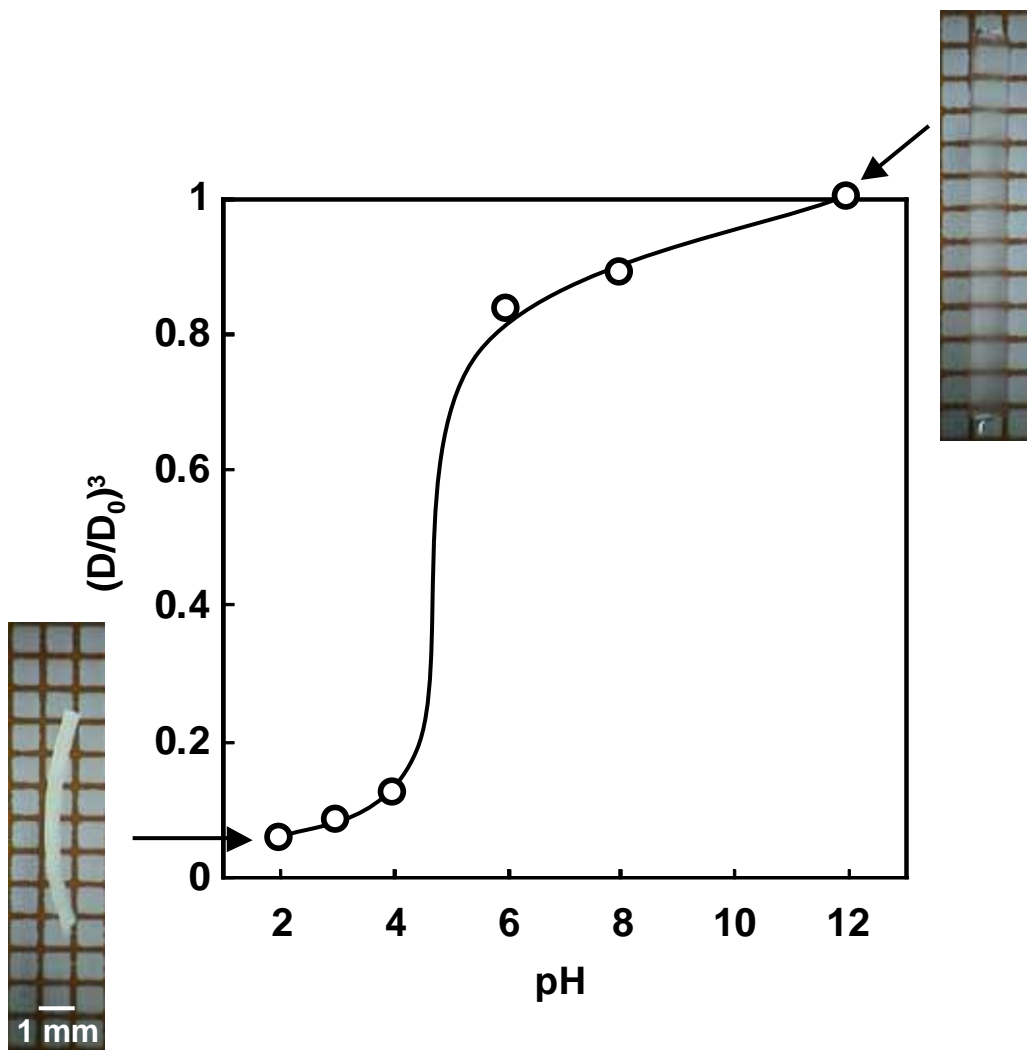
Figure 4-5. Equilibrium swelling ratio, $(D/D_0)^3$, for poly(NIPAAm-co-CIPAAm)-BP (BP content 11.1 mol%) cylinder-shaped gel as a function of various pH. The inset show photograph of the gels at pH 2 and 12.

approximately ionized and the hydrogel can swell by electrostatic repulsion of the groups. It is due to Donnan ion exclusion primarily, following by swelling changes by electrostatic repulsion.[14] In contrast, at a lower pH, the repulsion would be canceled and furthermore the formation of the hydrogen bond between the carboxyl groups would additionally occur. By our previous paper,[15] it was reported that the LCST of the linear poly(NIPAAm-co-CIPAAm) shifts to a lower temperature in lower pH than its pKa value. Moreover, the LCST shifting depended on the carboxyl group content closely. These results corresponds to the data that was previously reported by Nakayama et al.[16] The clear response to the pH change of the hydrogel suggests that the properties of linear poly(NIPAAm-co-CIPAAm) reflects in the resulting hydrogel.

Next, we observed equilibrium swelling ratios of the poly(NIPAAm-co-CIPAAm)-BP hydrogel as the function of temperature at pH 2, 3, 6, 8, 10 and 12 and the volume phase transition profiles are shown in Figure 4-6. As overall tendency, the equilibrium swelling ratio of the hydrogel has decreased with increasing temperature. This result is due to the thermo-sensitive deswelling of the poly(NIPAAm-co-CIPAAm) segments above the LCST. As seen in the figure, we can understand the clear pH-dependency on the swelling ratios. The largest change was observed at pH 6. The much carboxylate ion would inhibit the shrinking of the polymer network in the gel by electrostatic repulsion. While, at lower pH, carboxyl groups cannot dissociate and can form the hydrogen bonding between the polymer chains. As a result, physical cross-linking points
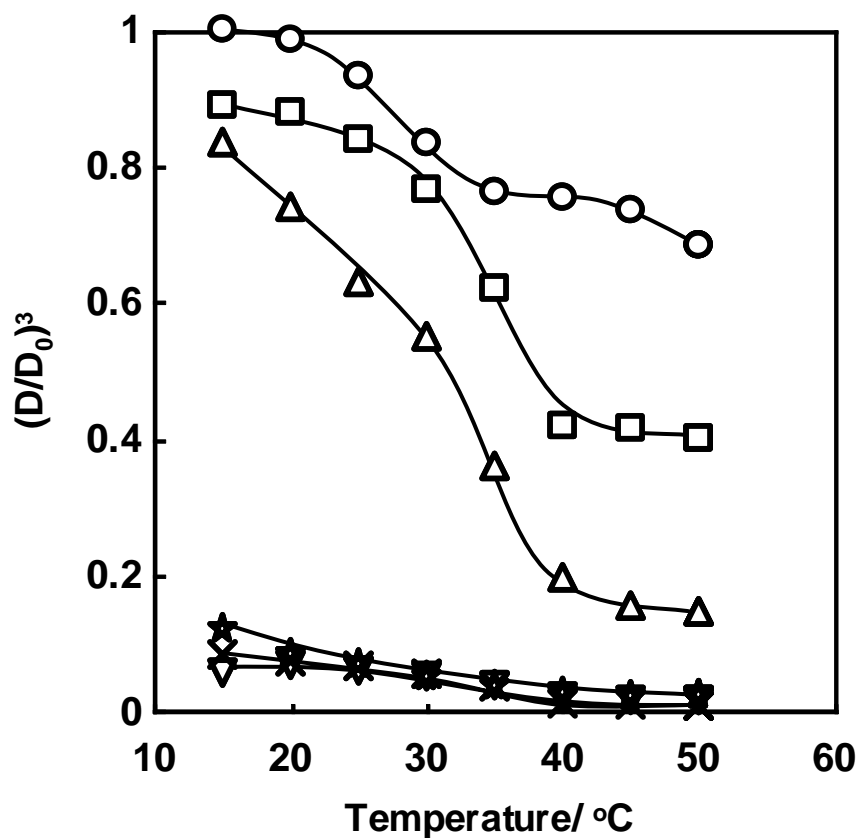
Figure 4-6. Equilibrium swelling ratio, $(D/D_0)^3$, for poly(NIPAAm-co-CIPAAm)-BP (BP content 11.1 mol%) cylinder-shaped gel as a function of temperature at various pH; pH 2 ( ), pH 3 (✗), pH 4 (☆), pH 6 ( ), pH 8 (□), pH 12 (○).

would increase and prevent the hydrogel from swelling. That's why the hydrogel showed lower swelling ratios at low pH condition.

The photo-cross-linkable polymer would be useful to fabrication of thermo-responsive drug delivery device for biological medicine such as proteins. The loading of the macromolecular compounds into the hydrogel is usually not easy. In general, the immersing of the dried gels into the protein solution is performed but the loading does not reach the high level. The use of the photo-cross-linkable hydrogel would solve this problem. UV irradiation to the mixed solution of the photo-reactive polymer and the protein could achieve the protein-loaded hydrogel without loss of the valuable drugs.

4.5 Conclusions

In this study, we newly developed the very useful photo-cross-linkable polymers. Those were prepared by the condensation reaction between the carboxylated NIPAAm copolymers and 4-aminobenzophenone (BP). The introduction degree of BP was controlled by changing the ratio of BP against the carboxyl groups in the polymer. The cylinder-shaped hydrogel was successfully prepared by photo-cross-linking using UV irradiation. Photo-cross-linked hydrogels exhibited volume phase transitions in response to both pH and temperature changes. These result suggest that the preparation of novel stimuli-responsive polymers and their materials. These materials are expected for application in a controlled delivery device.

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Chapter 5

Stimuli-Responsive Ultrathin Hydrogel Films Prepared by Photo-Reactive Acrylamide-Based Copolymers

5.1 Summary

To develop stimuli-responsive ultrathin polymer films on a solid substrate, a novel photo-cross-linkable polymer with both temperature- and pH-responsive properties was prepared. The photo-reactive 4-aminobenzophenone (BP) was introduced onto the side groups of poly(*N*-isopropylaclylamide-co-2-carboxyisopropylaclylamide) [poly(NIPAAm-co-CIPAAm)]. This copolymer was designed for the highly random sequences of comonomers. After formation of the spin-coated polymer films on a solid substrate, UV light irradiation started the cross-linking reaction. The spin-coating processes and stability of the polymer films were quantitatively monitored by a quartz crystal microbalance (QCM) and the thickness was estimated using an atomic force microscope (AFM). These measurements confirmed the formation of a very plain polymer film and the film thickness was precisely controlled by the concentration of the polymer solution used for the spin-coating.

Moreover, the obtained films showed a high stability due to the cross-liking reaction due to the UV irradiation. Cyclic voltammetries using potassium ferricyanide revealed that the ions could permeate into the photo-cross-linked ultrathin polymer films. The permeability of the ultrathin hydrogel films was dramatically changed by varying the pH and temperature of the aqueous media. These observations suggest that the preparation of isopropylacrylamide-based stimuli-responsive ultrathin hydrogel films was possible.

5.2 Introduction

The macro- or nanometer-ordered modification of material surfaces has attracted much attention in the material sciences, because this method is very practical for fabricating functional material surfaces without sacrificing the physical properties of the materials. To achieve this purpose, the physical or chemical methods, as well as their combination, have been extensively studied. For example, the deposition of self-assembled monolayers (SAMs) offers one of the highest quality routes used to prepare chemically and structurally well-defined surfaces.[1] Langer *et al*. used SAMs on a gold substrate to create surfaces that could reversibly switch from being hydrophilic to hydrophobic by applying an electric potential.[2] The design feature of the system is a low density SAM that has a negatively-charged terminus. This strategy has been used to induce the mechanical motions of molecules[3] and to control protein assembly.[4, 5]

Recently, there has been considerable interest in the development of materials whose surface properties can be dynamically modulated. Especially, stimuli-responsive polymers are one of the candidates used for surface modification because of their interesting characteristics. One of the most intensively studied polymers is poly(*N*-isopropylacrylamide) (PNIPAAm), which exhibits a sharp phase transition in water at 32 °C.[6] PNIPAAm films were prepared by the polymerization of NIPAAm onto a functionalized surface via atom transfer radical polymerization,[7-9] plasma polymerization,[10, 11] or photopolymerization.[12, 13] The surface wettability of these films can be reversibly changed around the lower critical solution temperature (LCST) of PNIPAAm in aqueous media. Dynamic control of the surface energy is of interest for controlling wettability,[14, 15] biomolecular adsorption,[16] and cell adhesion.[17, 18]

In particular, the hydrogels, which undergo volume phase transitions in response to physical or chemical stimuli in the outer environment, are very interesting materials. PNIPAAm hydrogels show a reverse volume phase transition during the heating or cooling processes.[19] Furthermore, various hydrogels that are responsive to stimuli, such as pH,[20] ion strength,[21] light,[22] solvent composition,[23] and an electric field[24] have been reported. These intelligent materials are quite suitable for designing devices such as separation, sensing, actuator, and controlled drug release.

Recently, several investigations were performed to create stimuli-responsive hydrogel structures on various substrates. Matzelle *et al.* studied the mechanical properties of the PNIAAm homopolymer and

copolymer films prepared by simultaneous polymerization and cross-linking.[25] Another method of cross-linking PNIPAAm films was investigated by Kuckling *et al.*[26-29] Copolymers of PNIPAAm containing chromophres were photochemically cross-linked, and the swelling properties of the resulting films were investigated. Moreover, the use of plasma immobilization and electron beam irradiation were investigated for the preparation of stimuli-responsive hydrogel films.[30] Furthermore, temperature-responsive hydorogel films can also be prepared by the layer-by-layer method. Serizawa *et al.* reported that LbL assembled films prepared by the sequential chemical cross-linking between the amino and carboxyl groups on respective surfaces swell and deswell in their aqueous media, thus forming ultrathin hydrogels.[31-33] This result depends on the neutral, hydrophilic and temperature-responsive units that exist in the copolymer. The responsive properties as well as the chemical compositions can be delicately modified by changing the component polymers. Since the component polymers are chemically cross-linked, ultrathin hydrogels are stable even in concentrated ionic solutions. In addition, ultrathin hydrogels have a controllable loading amount of charged dyes, and the release of these dyes depend on the aqueous ionic strength and the pH values.[34]

In the present study, we investigated the molecular design, preparation and characterization of the stimuli-responsive ultrathin hydrogel film by photo-cross-linking. Photo-cross-linkable polymers [poly(NIPAAm-co-CIPAAm)-BP] were obtained by the condensation reaction of the carboxyl groups of poly(NIPAAm-co-CIPAAm) and the

amino groups of 4-aminobenzophonone. First, the spin-coated films using poly(NIPAAm-co-CIPAAm)-BP were prepared and then the photo-cross-linking reaction was preformed by UV light irradiation. In addition, the film stability and stimuli-responsive ion permeability change were studied using the QCM method. In terms of the kinds of cross-linking reactions, the use of UV light is one of very convenient technique for preparing of three-dimensional network films. So far, this method has been utilized in various applications and recent interest has expanded to the fields of surface modifications and biotechnology. In this study, a stimuli-responsive polymer is modified with photoreactive benzophenone groups in order to obtain a stable cross-linkage in the polymer thin films.

5.3 Experimental

5.3.1 Materials.

Poly(NIPAAAm-co-CIPAAm) with a 20 mol% CIPAAm content, was synthesized according to the methods described in a previous study.[35, 36] *N,N'*-dicyclohexyl carbodiimide (DCC) (95.0 %), 4-aminobenzophonone (BP) (98.0 %), potassium ferricyanide and sodium perchlorate were purchased from Wako (Japan), and used without further purification. The quartz crystal microbalance (QCM) electrode (frequency = 9 MHz, AT-cut) was purchased from USI (Japan). Before the film preparation, the QCM electrodes were cleaned by treatment with a piranha solution [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 to clean the electrode surface by blasting. The suitable addition of hydrochloric acid (HCl) and sodium hydroxide (NaOH) aqueous solutions adjusted the pH of the solutions for the cyclic voltammetry measurement.

5.3.2 Synthesis of benzophenone-introduced poly(NIPAAm-co-CIPAAm).

Poly(NIPAAm-co-CIPAAm) (607.65 mg, 5.0 mmol) was dissolved in 20 mL of *N,N'*-dimethylformamide (DMF) at 4 °C for 12 h. Then, 247.58 mg of DCC (6.0 mmol) and 236.69 mg of 4-aminobenzophenone (BP) were added and the reaction mixture was stirred at room temperature for 24 h. The solution was dialyzed against DMF and water to remove the unreacted BP and the impurities, then lyophilized. The introduction degree of the BP was determined by ^{1}H-NMR spectrum (JEOL FX 400, Tokyo, Japan).

5.3.3 Film preparation by spin-coating.

The polymer films were prepared by spin-coating (MIKASA 1H-DXII, Tokyo, Japan). Ten µL DMF solutions containing 1, 3 and 5 w/v% polymer were dropped on a QCM substrate, followed by the spin-coating at 1500 rpm for 60 sec. After air-dried, the frequency shift was then measured by an Iwatsu frequency counter (Model SC7201). The polymer film formation and stability were estimated using a QCM method as described below.

5.3.4 Photo-cross-linking.

The cross-linking reaction by UV irradiation was performed using an ultrahigh pressure Hg lamp (a 250 W, USHIO SX-UI251HQ, Tokyo, Japan). The polymer films on a substrate were irradiated in air without a filter at a distance of 40 cm from the source. Using the polymer films on a quartz substrate (35x9x1 mm), the photo-cross-link reaction process was monitored by a UV-vis spectrometer (Jasco Model V-550, Tokyo, Japan).

5.3.5 QCM Substrate.

In this experiment, the QCM substrate of the quartz crystal with a 9 mm diameter was used. The 4.5 mm gold electrodes were coated in the center of both sides. The roughness was estimated to be 1.7 nm. The frequency was monitored by the same frequency counter. The leads of the QCM were protected with a silicone-rubber gel in order to prevent degradation during immersion in the various solutions. The amount of the deposited polymer, Δm (ng), was estimated by measuring the frequency shift of the QCM, ΔF (Hz), using Sauerbrey's equation[37] as follows:

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

where F_o 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} dyne cm^{-2}). This equation was reliable when the measurements were performed in air.
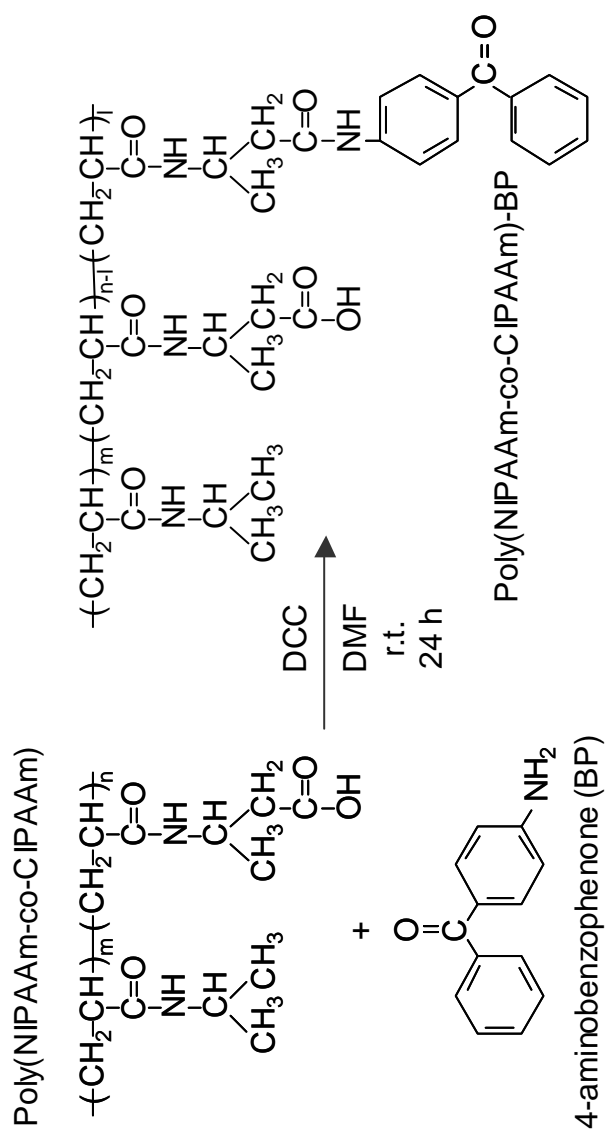
5.3.6 Atomic force microscopy.

The morphology of the air-dried photo-cross-linked polymer films on the QCM substrate was estimated by Atomic Force Microscopy (Digital Instruments NanoScope III). The images were obtained in the contact mode at ambient temperature.

5.3.7 Cyclic voltammetry.

Cyclic voltammetry (CV) was carried out using a HSV-100 (HOKUTO-DENKO, Tokyo, Japan) with a three-electrode system consisting of an Ag/AgCl (saturated aqueous NaCl solution) reference electrode, a platinum wire as the counter-electrode, and a photo-cross-linked polymer films supported on a QCM substrate as the working electrode. The scan rate was 50 mVs^{-1}. Potassium ferricyanide was dissolved into 0.1 M sodium perchlorate at a concentration of 10 mM using the pH adjusted solution. Before the CV measurement, potassium ferricyanide solution was purged with flowing nitrogen to remove the dissolved oxygen.

5.4 Results and Discussion

Poly(NIPAAm-co-CIPAAm)-BP was prepared by the condensation reaction between the DCC-activated carboxyl groups and amino groups of BP (Scheme 5-1).[38, 39] The CIPAAm content was 20 mol% and we intended to obtain the thin film with both temperature and pH-sensitive properties.

Poly(NIPAAm-co-ClPAAm)

$\text{(CH}_2\text{CH)}_m\text{(CH}_2\text{CH)}_n$

C=O   C=O
| |
NH   NH
| |
CH   CH
/\   /\
CH₃ CH₃ CH₃ CH₂
           |
           C=O
           |
           OH

+

4-aminobenzophenone (BP)

O=C, with two phenyl rings, NH₂

DCC
DMF
r.t.
24 h

$\text{(CH}_2\text{CH)}_m\text{(CH}_2\text{CH)}_{n-l}\text{(CH}_2\text{CH)}_l$

C=O   C=O   C=O
| | |
NH   NH   NH
| | |
CH   CH   CH
/\   /\   /\
CH₃ CH₃ CH₃ CH₂   CH₃ CH₂
            | |
            C=O   C=O
            | |
            OH   NH — benzophenone

Poly(NIPAAm-co-ClPAAm)-BP

Scheme 5-1. Synthetic Scheme for Benzophenone-Substituted Poly(NIPAAm-co-ClPAAm).

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Too much or too low content would be possible to decline the sensitiveness. Moreover, we used the relatively low concentration of polymer solution (5 mmol/20 mL), because the viscosity of the solution increases with increasing the polymer concentration and it could obstruct the smooth reaction for the introduction of BP into the polymer. Based on the ^{1}H-NMR measurement, the introduction ratio was determined using the signals of 7.5-8.5 ppm assigned to BP and the degree of substitution of the BP group was estimated to be 3.2 mol%. In addition, we initially checked the introduction of the BP moieties into the polymer chains. By UV irradiation, the DMF solution of the resulting polymer became very viscous compared to the starting poly(NIPAAm-co-CIPAAm) and eventually gelation occurred. These results confirmed the usefulness of the BP moieties for the cross-linking reaction. BP as a cross-linking agent has been extensively studied. Hydrogen abstraction via an excited BP unit is a classic reaction in organic photochemistry.[40-42] UV irradiation causes an n-π* transition in the carbonyl group. The resulting biradical reacts with the C-H groups of the near polymer chain and then caused cross-linking.

For the cross-linkable polymer preparation, IPAAm-CIPAAm was intentionally used because we considered that the random introduction of the BP moieties could produce a uniform cross-linking and the resulting polymer films would be smooth. We have already reported that the poly(*N*-isopropylaclylamide-co-2-carboxyisopropylaclylamide) [poly(NIPAAm-co-CIPAAm)] that would be used in this study is the ideal random copolymer,[35, 36] because the reaction activity ratios are

approximately equivalent. Such polymers are undoubtedly favorable for obtaining a clear and sensitive response. Moreover, the spin-coated polymer films showed a high stability in DMF by a 15 min UV irradiation (region B in Figure 5-1a). On the other hand, the films that were not UV irradiated quickly flaked off in DMF. This result suggests the successful photo-cross-linking and the very stable polymer film formation by UV irradiation.

We investigated the polymer layer formation onto the solid surfaces by the QCM method. The QCM frequency shifts were accompanied by the poly(NIPAAm-co-CIPAAm)-BP adsorption. The shifting profiles are shown in Figure 5-1a. In this experiment, the spin-coating was repeated five times, just in case, to confirm the adsorption profiles. Interestingly, the frequency did not change after once or twice coatings and the adsorption of poly(NIPAAm-co-CIPAAm)-BP reached equilibrium since then. The frequency shift did not change and it indicates no further polymer layers build-up in the repeating coatings. This result denotes the formation of a uniform polymer film. By the spin-coating method, we could form a very smooth polymer surface with reduced roughness, which was confirmed by atomic force microscopy (AFM) as described below. The thickness of the spin-coated polymer films changed depending on the polymer concentration (region A of Figure 5-1b). In all cases, the adsorption of poly(NIPAAm-co-CIPAAm)-BP reached equilibrium by repeated 5 spin-coating. The amount of polymer layers could be controlled by changing the polymer concentration. On the polymer solution with 5 w/v%,
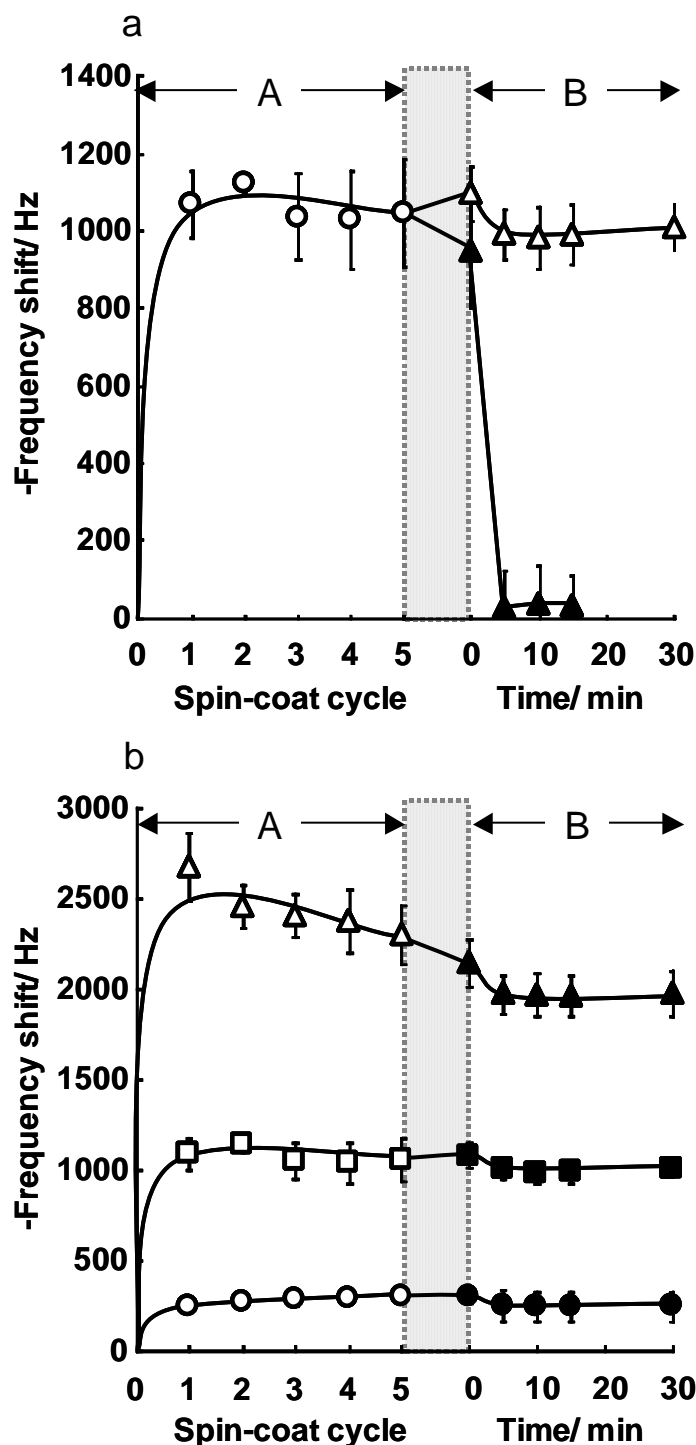
Figure 5-1. QCM analysis of the poly(NIPAAm-co-CIPAAm)-BP spin-coated films: (a) polymer concentration is 3 w/v% (region A). The right side of the figure (region B) shows the stability of the spin-coated films in DMF: open triangle and closed triangle are the 15 min UV irradiation and non-irradiation, respectively. (b) Circle, square and triangle indicate the polymer concentration of 1, 3 and 5 v/w%, respectively (region A). Closed symbols indicate the stability of the spin-coated films in DMF after the 15 min UV irradiation (region B).

the frequency shift slightly dropped down at higher number of spin-coat cycles. This result suggests that additional contact of the polymer solution might peel out the unstably-adsorbed polymer layers by use of highly viscous polymer solution.

Table 5-1 summarizes the frequency shifts by the QCM and surface characterization by the AFM analysis. As the polymer concentration increases, the QCM frequency shifts proportionally increased and the thickness and the roughness of the polymer films that were estimated by AFM apparently improved. Nevertheless, the ratio of roughness/thickness surely decreased with increase of the concentration of polymer solution and the surface becomes smoother overall. Moreover, roughness of the hydrogel film has not been changed before and after UV irradiation.

As seen in region B of Figure 5-1b, the UV-irradiated film did not show any frequency change even after soaking in DMF which indicates that the layered polymers turned into the highly stable hydrogels, while the frequency in the non-irradiated film significantly decreased and the layered polymers flaked off. The photo-cross-linking process was monitored by UV-vis spectroscopy. Figure 5-2 shows the UV-vis spectra of the spin-coated films having the polymer concentration of 3 w/v% on the quartz substrate as a function of the UV irradiation time. During UV irradiation, the absorbance at 300 nm decreased and almost disappeared after 3 min. Benzophenone is indirectly excited to the lowest triplet state ($\pi\pi^*$) by direct absorption into the singlet state ($\pi\pi^*$) upon UV irradiation, and the benzophenone ketyl radical readily recombines to generate a new

Table 5-1. Characterization of spin-coated polymer thin films in air after UV irradiation.

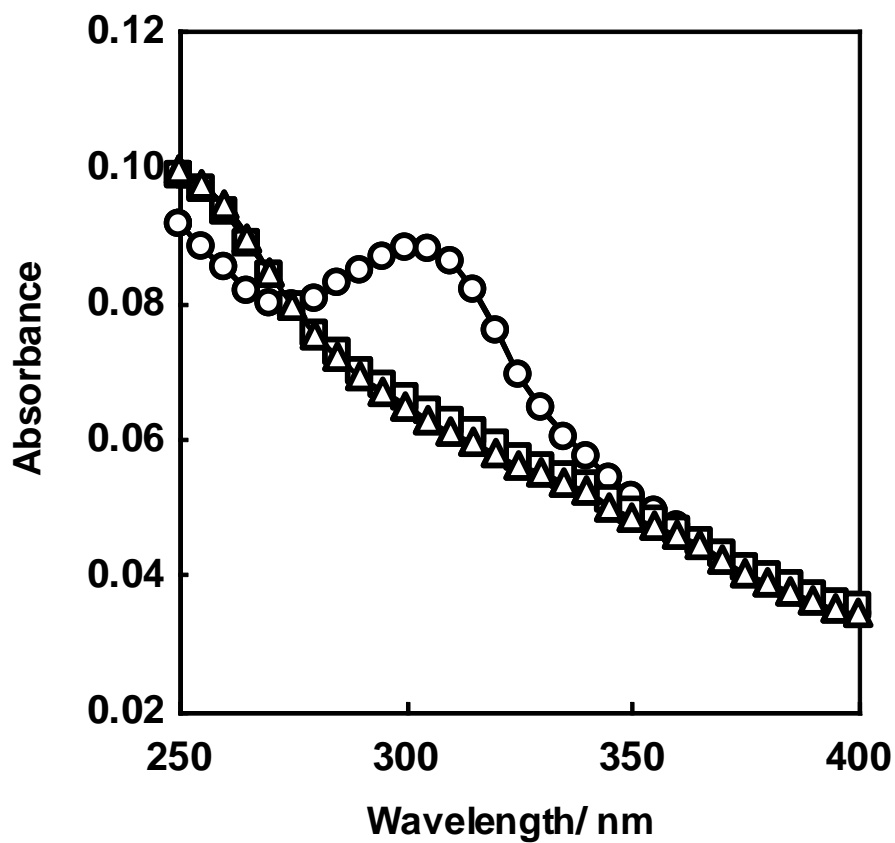| polymer conc. (w/v%)	Frequency shift (Hz)	Roughness (nm)	Thickness (nm)	Roughness/Thickness (-)
1	295 ± 28	3.8 ± 0.2	13.7 ± 1.7	0.28
3	1051 ± 122	8.4 ± 1.4	46.8 ± 7.2	0.18
5	2295 ± 166	10.8 ± 7.1	74.7 ± 10.0	0.14

Figure 5-2. UV-vis spectra of the 3 w/v% spin-coated film on quartz slide as a function of UV irradiation: Circle, square and triangle indicate 0, 3, and 5 min after UV irradiation.

C-C bond.[43] Consequently, the polymer films obtained a high stability. Furthermore, the spin-coated films of the DMF solution of only poly(NIPAAm-co-CIPAAm) and the mixture of the copolymer and BP were not stabilized by the UV irradiation.

Next, we studied the stimuli-responsive properties of the ultrathin hydrogel films using a CV technique in aqueous media. The electroactive ionic species, potassium ferricyanide, was deposited across the films onto the appropriate electrode on the basis of the oxidation-reduction cycles. The QCM coated with the photo-cross-linked polymer films were directly used for the electrode. CV chart at 20 °C (pH = 8) with the polymer films coated on both sides of the QCM substrate is shown in Figure 5-3. A suitable CV curve that contains the oxidation and reduction processes was obtained for a bare QCM electrode, and the peak current was 0.312 mA cm^{-2}. A similar curve containing the peak current of 0.211 mA cm^{-2} was observed for the electrode coated with the photo-cross-linked polymer films. This result suggests that potassium ferricyanide ions can permeate the photo-cross-linked polymer films. In other words, the photo-cross-linked polymer films made in this study can swell in aqueous media, and produce ultrathin hydrogel films.

As the poly(NIPAAm-co-CIPAAm) contained the homopolymer-like backbone and carboxylic groups in the side chains, it shows not only a sensitive temperature-response, but also a pH-response. The lower critical solution temperature (LCST) of this copolymer changes by varying the pH. Therefore, we investigated the response by such stimuli using the
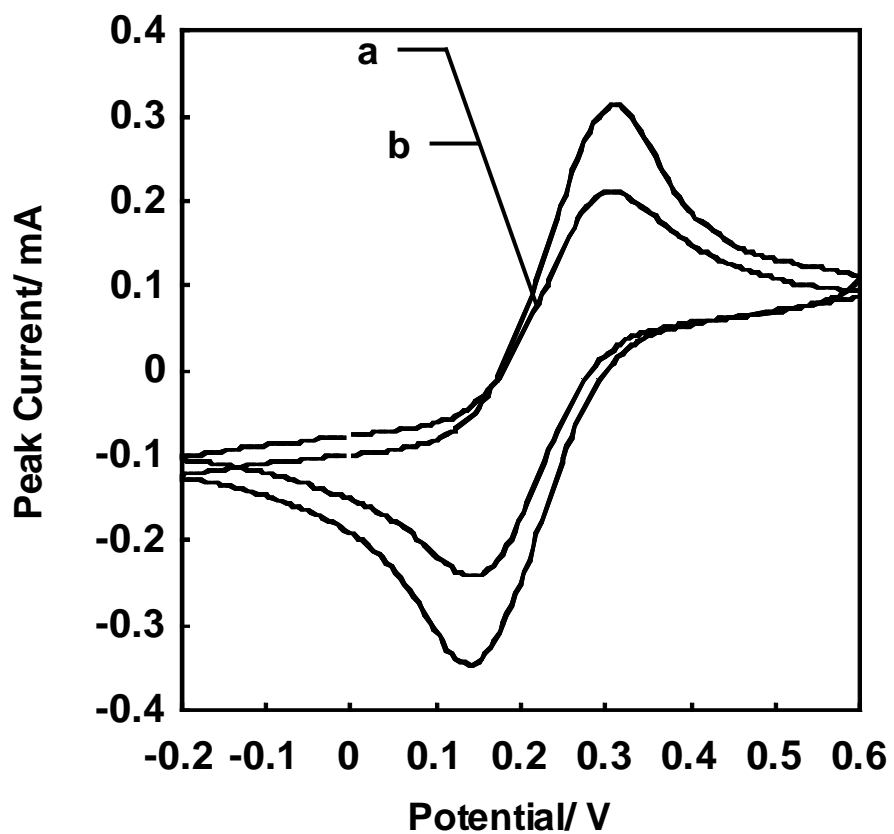
Figure 5-3. Cyclic voltammograms of (a) a bare QCM, and (b) the 3 w/v% spin-coated film in an aqueous 0.1 M sodium perchlorate solution at pH 12 containing 10 mM potassium ferricyanide.

permeability estimation of the hydrogel films with the same method described above. Figure 5-4 shows the CV charts using thin hydrogel films coated on the QCM electrode under various pH conditions and 20 °C. As seen in the figure, ion permeation was observed at a high pH (pH > 8), while the ion could not penetrate at a low pH (pH < 6). This result suggests that the association and dissociation of carboxyl groups of this polymer would take place around 6. According to our previous report, the apparent pKa of the poly(NIPAAm-co-CIPAAm) is estimated to be 6.2.[44] The ultrathin hydrogel film can clearly response to the pH change and shows a swelling-deswelling change. At the higher pH, carboxyl groups are approximately ionized and the thin hydrogel film can swell by electrostatic repulsion of the ionized carboxyl groups. In contrast, at a lower pH, the repulsion would be canceled and also the formation of the hydrogen bond between the carboxyl groups would additionally occur. In our separate experiment, the LCST of the linear poly(NIPAAm-co-CIPAAm) shifts to a lower temperature in the aqueous solution the lower pH. Moreover, the LCST shift depended on the carboxyl group content. This result corresponds to the previous description of Nakayama *et al.*[45] The ultrathin hydrogel film can sensitively control the ion permeation in response to the pH change.

Next, we checked the temperature-responsive properties of the thin hydrogel film. The cyclic voltammograms at various temperatures from 10 °C to 50 °C at pH 8 are shown in Figure 5-5. The redox peak currents increased between 10 and 20 °C and it is due to the simple improvement of
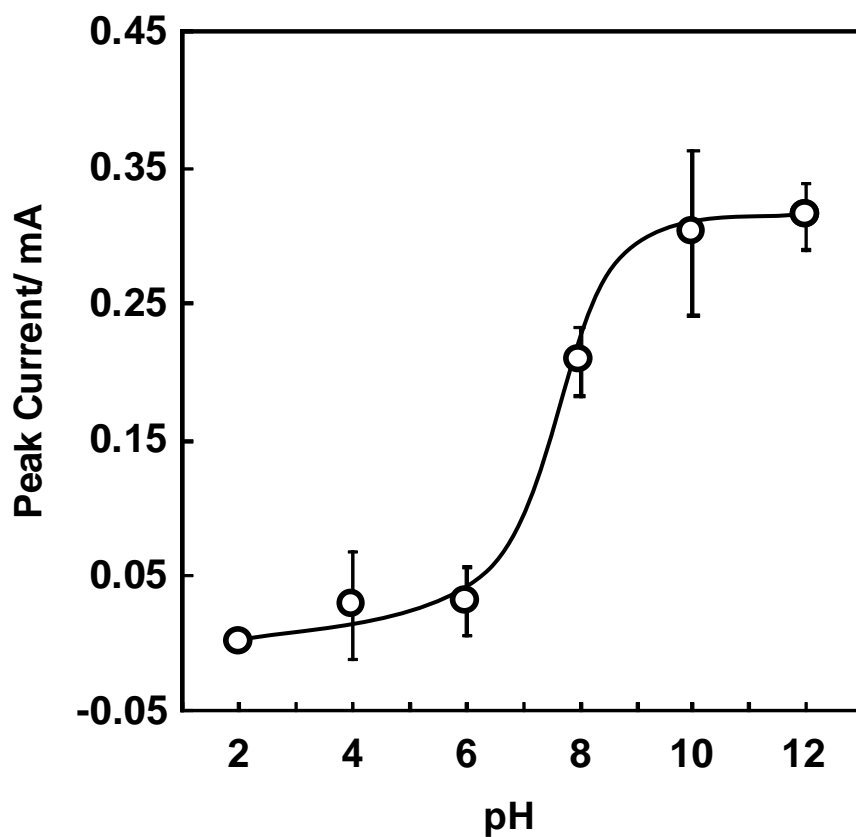
Figure 5-4. Cyclic voltammograms of the ultrathin hydrogel film (polymer concentration: 3 w/v%) coated electrode in an aqueous 0.1 M sodium perchlorate solution containing 10 mM potassium ferricyanide at various pHs (temp: 20 °C).
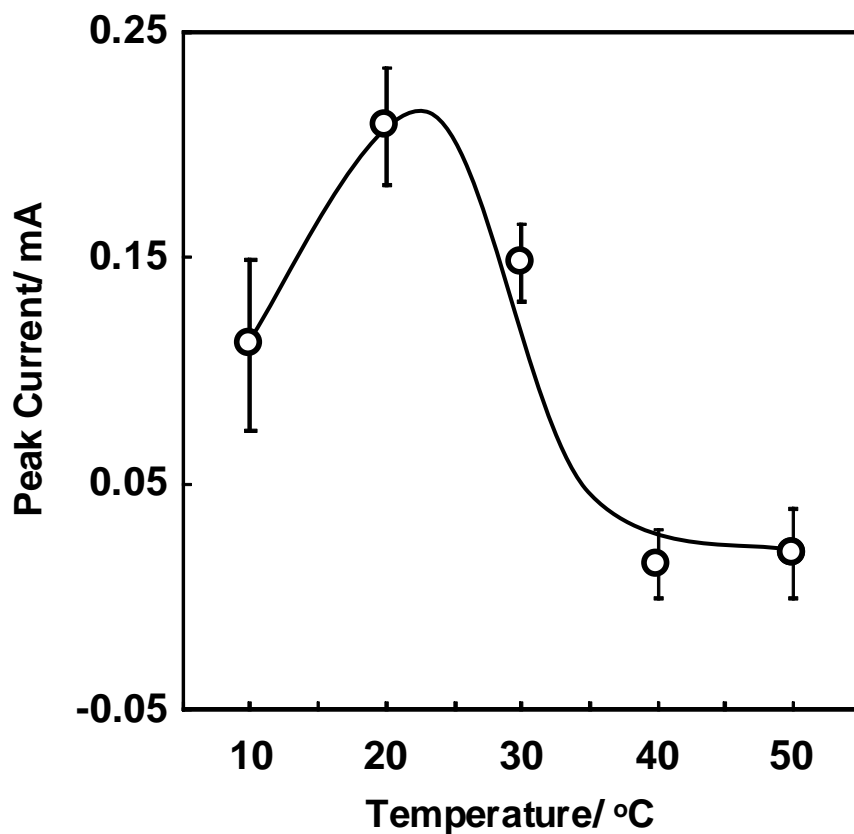
Figure 5-5. Cyclic voltammograms of the ultrathin hydrogel film (polymer concentration: 3 w/v%) coated electrode in an aqueous 0.1 M sodium perchlorate solution containing 10 mM potassium ferricyanide at various temperatures (pH = 8).

the ions movement by higher temperature. The peak currents significantly decreased between 20 °C and 40 °C. This result indicated that by the deswelling of the poly(NIPAAm-co-CIPAAm) segments above the LCST, the polymer network would shrink and free diffusion of the ions became difficult. However, the permeability of the film at high pH (pH > 10) did not change at the various temperatures. As seen in Scheme 5-1, the poly(NIPAAm-co-CIPAAm)-BP contains the free carboxyl groups which did not participate in the cross-liking reaction, and it would dissociate and form the carboxylate at pH 10. The higher carboxylate ion content would inhibit the shrinking of the polymer network in the hydrogel films by electrostatic repulsion.[46, 47]

Based on these results, we can conclude that the photo-cross-linkable poly(NIPAAm-*co*-CIPAAm)-BP is very useful for the functionalization of the solid material surfaces and could be utilized for a controlled delivery device.

5.5 Conclusions

In summary, a photo-cross-linkable polymer [poly(NIPAAm-co-CIPAAm)-BP] was prepared by the condensation reaction between the DCC-activated carboxyl groups in the side chains and the amino groups of BP. The spin-coating of the DMF solution of poly(NIPAAm-*co*-CIPAAm)-BP successfully formed ultrathin and smooth polymer layers, and the thickness depended on the polymer concentration. The photo-cross-linking reaction occurred very quickly, and the obtained film demonstrated a high stability. The cyclic voltammetries using potassium ferricyanide revealed that ions could permeate through the photo-cross-linked polymer film, producing ultrathin hydrogel films. Furthermore, the permeability of the ultrathin hydrogel films changed as the pH and temperature varied. These films are expected for application in a controlled delivery device and the control led attachment for biomaterials.

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Concluding Remarks

The objective of this thesis is the preparation of stimuli-responsive ultrathin hydrogel films using Layer-by-Layer (LbL) assembly and photo-cross-linking, and the characterization of application for controlled devise of these film and the observation of the characteristic change of these according to change in environmental. The results obtained through this work are summarized as follows.

In **Chapter 2**, the ultrathin hydrogel films were prepared by stepwise monolayer deposition of poly(AAc-co-NIPAAm)s with different AAc contents and polyVAm on solid substrates through sequential chemical reactions using EDC to activate AAc units, and were characterized by suitable methods. QCM substrates were used to quantitatively monitor assembly processes. Sequential reactions under various conditions demonstrated potential regulation of hydrogel structures. Swelling ratios were estimated by percent increases in the thickness and increased with increasing AAc and EDC amounts. Swelling ratios were regularly changed by varying the ionic strength and pH of aqueous media. Swelling properties were interpreted based on structural information of ultrathin hydrogel films. Additionally, the stimuli-responsive properties of the ultrathin hydrogel films were cleared by the observation of ions permeability of these using by cyclic voltanmetry method.

Chapter 3 describes the tunable loading of charged dyes as model drugs into ultrathin hydrogel films, and the release of dyes depending on the

aqueous ionic strength and the pH values. The amount of methyl orange (MO) loaded was potentially controlled by changing the dye concentrations, film thickness, and AAc content of the copolymers. The release of allura red (AR) was controlled by the NaCl concentration and pH. These results suggest that the polymeric matrices of ultrathin hydrogels have great potential for the loading and release of charged drugs.

In **Chapter 4**, novel photo-cross-linkable polymers [poly(NIPAAm-co-CIPAAm)-BP] were synthesized for the preparation of the photo-cross-linked bulk hydrogels with both pH- and temperature-responsive properties. Photo-reactive 4-aminobenzophenone (BP) was introduced into the side groups of poly(*N*-isopropylacrylamide-co-2-carboxyisopropylacrylamide). The cylinder-shaped bulk hydrogel was easily prepared by UV irradiation for a few minutes. The Photo-cross-linked hydrogels exhibited clear volume phase transitions in response to both the pH and temperature changes.

Chapter 5 describes the preparation and characterization of the stimuli-responsive ultrathin hydrogel film consist of poly(NIPAAm-co-CIPAAm)-BP by photo-cross-linking reaction. After formation of the spin-coated polymer films on a solid substrate, UV light irradiation started the cross-linking reaction. The obtained films showed a high stability due to the cross-liking reaction due to the UV irradiation. Cyclic voltammetries using potassium ferricyanide revealed that the ions could permeate into the photo-cross-linked ultrathin polymer films. The permeability of the ultrathin hydrogel films was dramatically changed by

varying the pH and temperature of the aqueous media. These observations suggest that the preparation of acrylamide-based stimuli-responsive ultrathin hydrogel films was possible.

List of Publications

Chapter 2:

Takeshi Serizawa, **Daisuke Matsukuma**, Kazuhisa Nanameki, Masami Uemura, Fumiyo Kurusu, and Mitsuru Akashi, "Stepwise Preparation and Characterization of Ultrathin Hydrogels Composed of Thermoresponsive Polymers", *Macromolecules*, **2004**, *37*, 6531.

Chapter 3:

Takeshi Serizawa, **Daisuke Matsukuma** and Mitsuru Akashi, "Loading and Releasing of Charged Dyes Using Ultrathin Hydrogels", *Langmuir*, **2005**, *21*, 7739.

Chapter 4:

Daisuke Matsukuma, Kazuya Yamamoto and Takao Aoyagi, "Novel Photo-Reactive Acrylamide-Based Copolymers Instantly Gave the Sensitive Stimuli-Responsive Hydrogels", *J. Photopolym. Sci. Technol.*, **2006**, *19*, 445.

Chapter 5:

Daisuke Matsukuma, Kazuya Yamamoto and Takao Aoyagi, "Stimuli-Responsive Properties of N-Isopropylacrylamide-Based Ultrathin Hydrogel Films Prepared by Photo-Cross-Linking", *Langmuir*, **2006**, *22*, 5911.

List of Presentations

1. <u>Daisuke Matsukuma</u>, Kazuhisa Nanameki, Takeshi Serizawa, Mitsuru Akashi, "*Loading of Organic Low Molecules into Ultrathin Hydrogels Prepared by Sequential Chemical Reactions*", **52th SPSJ Annual Meeting**, May 28-30, 2003, Nagoya, Japan.

2. <u>Daisuke Matsukuma</u>, Takeshi Serizawa, Mitsuru Akashi, "*Control of Loading/ Releasing of Organic Small Molecules in/ from Ultrathin Hydrogels*", **52th SPSJ Symposium on Macromolecules**, September 24-26, 2003, Yamaguchi, Japan.

3. Hiroo Yamashita, <u>Daisuke Matsukuma</u>, Takeshi Serizawa and Mitsuru Akashi, "*Stepwise Preparation of Ultrathin Polymer Hydrogerls and Their Biocompatibility*", **Pusan-Kyengnam/Kyushu-Seibu Joint Symposium on High Polymers (11 th) and Fibers (9 th)**, November 3-5, 2003, Pusan, Korea.

4. <u>Daisuke Matsukuma</u>, Takeshi Serizawa, Mitsuru Akashi, "*Stepwise Preparation and Swelling/ Penetrating Properties of Ultrathin Hydrogels*", **53th SPSJ Annual Meeting**, May 25-27, 2004, Kobe, Japan.

5. <u>Daisuke Matsukuma</u>, Takeshi Serizawa, Mitsuru Akashi, "*Stepwise

Preparation and Characterization of Ultrathin Hydrogels", **53th SPSJ Symposium on Macromolecules**, September 15-17, 2004, Hokkaido, Japan.

6. <u>Daisuke Matsukuma</u>, Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi, *"Stimuli-Responsive Properties of Multilayer Thin Films Dependent on Intramolecular Structure"*, **54th SPSJ Symposium on Macromolecules**, September 20-22, 2005, Yamagata, Japan.

7. <u>Daisuke Matsukuma</u>, Kazuya Yamamoto and Takao Aoyagi, *"Preparation and Characterization of Stimuli-Responsive Thin Films Stabilized by Chemical Bond"*, **54th SPSJ Symposium on Macromolecules**, September 20-22, 2005, Yamagata, Japan.

8. Kazuya Yamamoto, <u>Daisuke Matsukuma</u>, Yumi Kuboshima and Takao Aoyagi, *"Stimuli-Responsive Properties of Multilayer Thin Films Dependent on Intramolecular Structure"*, **14th SPSJ Polymer Material Forum**, November 15-16, 2005, Tokyo, Japan.

9. <u>Daisuke Matsukuma</u>, Koji Yamashita, Kazuya Yamamoto and Takao Aoyagi, *"Stimuli-Responsive Properties of N-Isopropylacrylamide-Based Thin Hydrogel Films Prepared by Photo-Cross-Linking"*, **55th SPSJ Annual Meeting**, May 24-26, 2006, Nagoya, Japan.

10. Daisuke Matsukuma, Kazuya Yamamoto and Takao Aoyagi, "*Stimuli-Responsive Properties of Photo-Reactive Acrylamide-Based Polymers*", **The 23rd Conference of Photopolymer Science and Technology**, June 27-30, 2006, Chiba, Japan.

11. Daisuke Matsukuma, Kazuya Yamamoto and Takao Aoyagi, "*Preparation and Characterization of Ultrathin Hydrogel Films of Photo-Cross-Linkable Stimuli-Responsive Copolymer*", **55th SPSJ Symposium on Macromolecules**, September 20-22, 2006, Toyama, Japan.

12. Daisuke Matsukuma, Kazuya Yamamoto and Takao Aoyagi, "*Characterization of Stimuli-Responsive Hydrogel Films Prepared by Photo-Cross-Linking*", **56th SPSJ Annual Meeting**, May 29-31, 2007, Kyoto, Japan.

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