

Studies on Well-Defined Nano-Complexes Derived from Stimuli-Responsive Polymers

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Chapter 1

General Introduction

The nano-sized aggregates with well-defined structures and functionalities have attracted much attention in the many fields as medical science and industries, because these materials are expected as stabilizers, reservoirs, and drug carriers. In particular, it is well known that those aggregates consisting of polymers are concise to be controlled conformations, structures, and functionalities.

Amphiphilic block copolymers consisting of a hydrophobic and hydrophilic segment have attracted considerable attention because these copolymers can self-assemble into micelles, vesicles, or other aggregates with a hydrophobic core and hydrophilic shell in aqueous solutions.^{1,2} The conformation and size of the aggregates depend on many factors such as the chemical structure, molecular weight, and solvent composition.³⁻⁵ In

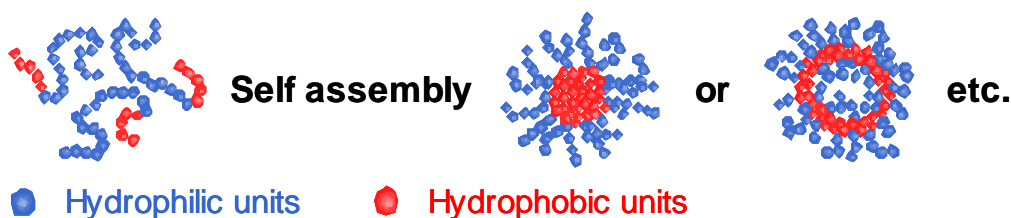


Figure 1-1. Schematic representation of the aggregate formations for amphiphilic block copolymers.

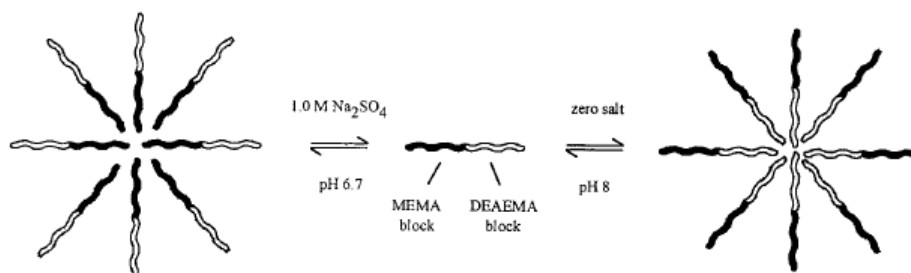


Figure 1-2. Schematic representation of the formation of micelles and reverse micelles for an AB diblock copolymer. [Bütün, V., et al., *J. Am. Chem. Soc.* **1998**, *120*, 11818]

addition, the hydrophobic core enables an incorporation of components insoluble in aqueous solutions and the hydrophilic shell stabilizes the aggregation structure.⁶⁻⁸ As such interesting behavior are possessed, the amphiphilic block copolymers are widely expected applications in various fields.

Recently, Armes et al. reported that a hydrophilic AB diblock copolymer can self-assemble in aqueous solutions to form a reversible micelle, and is described as “schizophrenic” diblock copolymer.⁹⁻¹³ This diblock copolymer consisted of 2-(*N*-morpholino)ethyl methacrylate (MEMA) and 2-(diethylamino)ethyl methacrylate (DEA), and reversibly exhibited three conformation as a molecularly dissolved state, a micelle with a MEMA core, and a micelle with a DEA core depending on pH or ionic strength in aqueous solutions.⁹ Laschewsky et al. reported that a hydrophilic AB diblock copolymer exhibited a double thermo-responsive property with schizophrenic character in aqueous solutions.^{14,15} It was comprised of poly(*N*-isopropylacrylamide) (PNIPAAm) with the lower critical solution temperature (LCST) and zwitterionic poly(3-[*N*-(3-methacrylamidopropyl)-

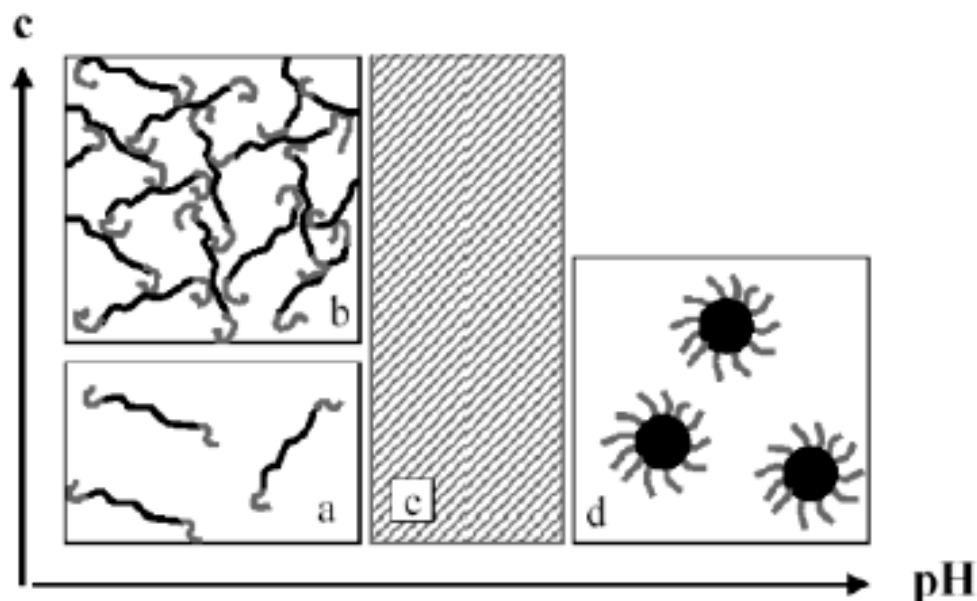


Figure 1-3. Schematic representation of phase behavior of the $\text{PAA}_{134}\text{-}b\text{-P2VP}_{628}\text{-}b\text{-PAA}_{134}$ aqueous solutions as a function of pH: (a) molecular dissolution; (b) transient network; (c) precipitation region; (d) compact micelles.

[Sfika, V., et al., *Macromolecules* **2003**, 36, 4983]

N,N-dimethyl]ammonio propane sulfonate) (PSPP) with upper critical solution temperature (UCST), and that molecularly dissolve in aqueous solution between the UCST of PSPP and the LCST of PNIPAAm. At low temperature below the UCST of PSPP, it formed the aggregate with a PSPP core attributed to electrostatic interaction of zwitterionic groups. On the other hand, it formed the aggregate with a hydrophobic PNIPAAm core due to dehydration at high temperature above the LCST of PNIPAAm. These aggregates were able to reversibly switch a core and shell of aggregates in response to temperature. Furthermore, Sfika et al. reported the aggregation behavior of a hydrophilic ABA triblock copolymer poly(acrylic acid)-*b*-poly(2-vinylpyridine)-*b*-poly(acrylic acid) in aqueous solutions.

¹⁶⁻¹⁸ It possessed the interesting properties which exhibited not only aggregates formations but also gelation with three-dimensional network architecture in aqueous solution depending on pH and concentration. These self-assembly behavior are reversibly caused, and corresponding diblock copolymers were not observed those aggregation properties.¹⁹⁻²²

The block copolymers that the each segment shows different characters can form very interesting associates or micelles by self-assembly in the optimal conditions.¹⁻³ The characteristics of them are tunable by the kinds of components, chemical structures, and chain lengths of each blocks.^{4,5} Moreover, complex formations of polyions are also practical as the driving force for another unique polymeric micelles or associates. The polyion complex micelles are formed by the electrostatic interactions between oppositely charged block copolymers in aqueous solutions.²³ The ionic block copolymers with a polyethylene oxide (PEO) segment exhibit

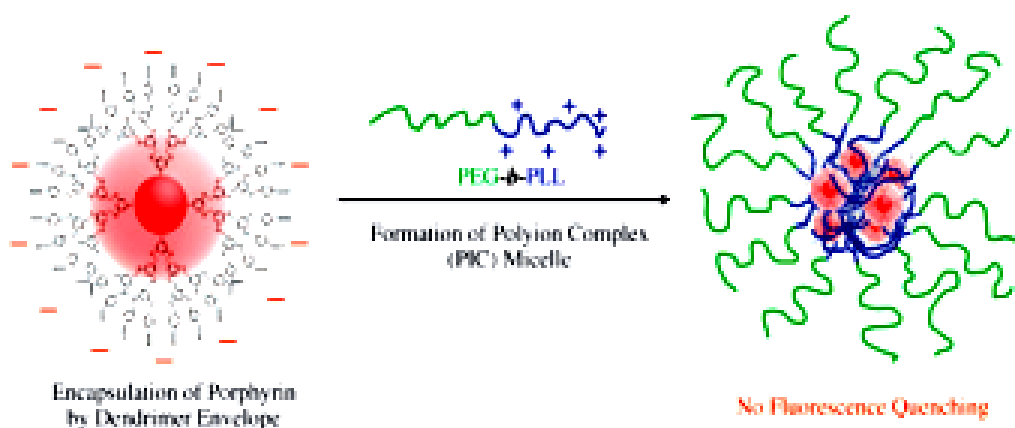
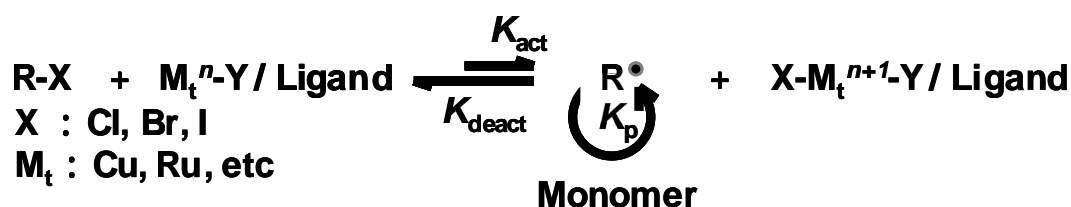


Figure 1-4. Schematic representation formation of polyion complex micelles through electrostatic assembly of anionic dendrimer porphyrins and PEG-*b*-PLL copolymers.

[Jang, W., et al., *Angew. Chem. Int. Ed.* **2005**, *44*, 419]

exclusively self-assemble behaviors. Moreover, their micelles are very stable nanometer-sizes sphere with mono-disperse. In other cases, the associates by charged block copolymers could be formed with the multivalent metal ions or supermolecules.²⁴⁻²⁹

The block copolymers have been conventionally prepared by anionic, cationic, and group transfer polymerization. However, these methods require stringent conditions and limit a small number of monomers which are available. Recently, controlled radical polymerization techniques referred to living radical polymerizations have been studied such as nitroxide-mediated polymerization, atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer polymerization.³⁰⁻³³ These techniques are more concise than ionic polymerizations, it is possible to prepare polymers controlled architecture with predetermined molecular weights (M_n) and low molecular weight distributions (M_w/M_n). In particular, ATRP is versatile and able to polymerize a wide range of monomer. Crescenzi et al. reported the controlled polymerization of NIPAAm via ATRP in a water/DMF mixture at 20 °C and successful preparation of the block copolymer with NIPAAm, regarded as difficult to polymerize them in the past.³⁴ It was demonstrated



Scheme 1-1. Atom transfer radical polymerization.

that this process enabled the preparations of block copolymers consisting of NIPAAm and other monomers.

The objective of this thesis is the preparations and characterizations of aggregates and nano-sized complexes derived from stimuli-responsive ABA triblock copolymers. The stimuli-responsive ABA triblock copolymers as a component exhibit the aggregation behaviors in response to external stimuli. Those comprised of the anionic electrolytic segments, form complexes with cationic compounds. The results obtained through this study are described in outline as follows.

In chapter 2, the stimuli-responsive ABA triblock copolymers were prepared by ATRP and controlled M_n with low M_w/M_n . Those were able to be prepared with predetermined molecular weight depending on concentration of an initiator. The stimuli-responsive ABA triblock copolymers exhibited behaviors depended on the characters of each segments in aqueous solutions. Those possessed thermo-responsive property of PNIPAAm in a middle block, in spite of the introduction of hydrophobic *Pt*BMA segments or hydrophilic PMAA segments in end blocks.

Chapter 3 describes the aggregation behaviors of stimuli-responsive triblock copolymers in response to external stimuli. A stimuli-responsive ABA triblock copolymer consisting of *Pt*BMA of thermo-responsive segment and PMAA of hydrophobic segments in end blocks, *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA changed aggregation conformation in response to temperature. PMAA-*b*-PNIPAAm-*b*-PMAA were composed of

pH-responsive PMAA and PNIPAAm, and controlled to invert a core and a shell of aggregates depending on both pH and temperature. Those are able to be controlled the aggregation conformations in response to external stimuli.

In Chapter 4, preparation and characterization of complex for PMAA-*b*-PNIPAAm-*b*-PMAA and silica particles with amino groups conducted by surface modification. It was prepared by stepwise monolayer deposition of them with QCM substrate. We observed the formation of complex layer by electrostatic interaction between PMAA-*b*-PNIPAAm-*b*-PMAA and silica particles with amino groups. Those depended on molecular weight of PMAA-*b*-PNIPAAm-*b*-PMAA as a component. The obtained complex layer exhibited thermo-responsive properties of PNIPAAm segments on surface.

Chapter 5 describes the preparation and aggregation behavior of nano-sized complex by electrostatic interaction between PMAA-*b*-PNIPAAm-*b*-PMAA and cationic compound as multivalent metal ions or PAMAM dendrimer. It was difficult to form complex of PMAA-*b*-PNIPAAm-*b*-PMAA and multivalent metal ions since PMAA segments were too short to stabilize complex. However, nano-complex with PAMAM dendrimer was formed with many binding sites for electrostatic interactions. It was used PNIPAAm-*b*-PMAA as AB diblock copolymer to examine the effects of polymer structures for nano-complex formations. Nano-complex derived from PNIPAAm-*b*-PMAA exhibited association like aggregation behavior, which is a diameter change of aggregate depending on PAMAM

concentration. On the other hands, that from PMAA-*b*-PNIPAAm-*b*-PMAA which a micelle like aggregation behavior was independent on it. We were able to prepare and control a complex with various conformations and stimuli-responsive properties by those methods.

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Chapter 2

Preparations and Characterizations of Well-Defined Stimuli-Responsive ABA Triblock Copolymers by Atom Transfer Radical Polymerization

2.1 Summary

Stimuli-responsive ABA triblock copolymers consisting of thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) in a middle block and hydrophobic poly(*t*-buthylmethacrylate) (PtBMA) or pH-responsive poly(methacrylic acid) (PMAA) in end blocks, reversibly exhibit the hydrophilic or hydrophobic properties depending on temperature and pH. An amphiphilic and thermo-responsive ABA triblock copolymer, PtBMA-*b*-PNIPAAm-*b*-PtBMA was polymerized by atom transfer radical polymerization (ATRP) using diethyl *meso*-2,5-dibromoadipate as a bifunctional initiator, copper (I) bromide as a catalyst, and tris(2-dimethylaminoethyl)amine as a ligand in a water/DMF mixture at 20 °C. It was determined that the molecular weights and molecular weight distributions of them were controlled by gel permeation chromatography. We calculated the PtBMA contents in PtBMA-*b*-PNIPAAm-*b*-PtBMA by

^1H NMR spectra. pH- and thermo-responsive PMAA-*b*-PNIPAAm-*b*-PMAA was also prepared by hydrolysis of PtBMA segments in end blocks of PtBMA-*b*-PNIPAAm-*b*-PtBMA. It was confirmed that PMAA segments were derived from PtBMA segments by ^1H NMR spectra. Both PtBMA-*b*-PNIPAAm-*b*-PtBMA and PMAA-*b*-PNIPAAm-*b*-PMAA exhibited the thermo-responsive properties caused by the phase transition of a PNIPAAm segment in a middle block.

2.2 Introduction

The block copolymers that the each segment shows different characters can form very interesting associates or micelles by self-assembly in the optimal conditions.¹⁻³ The characteristics of them are tunable by the kinds of components, chemical structures, and chain lengths of each blocks.^{4,5}

Recently, stimuli-responsive polymers have attracted much attention as the component for micelle- or associates-forming block copolymers. Because the resulting stimuli-responsive polymeric micelles become candidates for fabrication of the novel drug delivery systems with higher potentials. Actually, temperature-responsive polymeric micelles can be easily derived from poly(*N*-isopropylacrylamide) (PNIPAAm) -based block copolymers with hydrophobic segment.⁶ They can form micellar structure below Lower Critical Solution Temperature (LCST). Under heated condition, the thermo-responsive shell layers surrounding the hydrophobic core effectively dehydrate and the micelles would turn into hydrophobic

aggregates. Using this unique system, we could selectively accumulate the drug carrier by local heating. Not only the surface character and association behavior but also the drug loading or release would be controlled.^{7,8}

The block copolymers have been conventionally prepared by anionic, cationic, and group transfer polymerization. However, these methods require stringent conditions and limit a small number of monomers which are available. Recently, controlled radical polymerization techniques referred to living radical polymerizations have been studied such as nitroxide-mediated polymerization, atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer polymerization.⁹⁻¹² These techniques are more concise than ionic polymerizations, it is possible to prepare polymers controlled architecture with predetermined molecular weights and low molecular weight distributions. In particular, ATRP is versatile and able to polymerize a wide range of monomer. Crescenzi et al. reported the controlled polymerization of NIPAAm via ATRP in a water/DMF mixture at 20 °C and successful preparation of the block copolymer with NIPAAm, regarded as difficult to polymerize them in the past.¹³ It was demonstrated that this process enabled the preparations of block copolymers consisting of NIPAAm and other monomers.

In this study, we report the preparations and characterization of well-defined stimuli-responsive ABA triblock copolymer comprising PNIPAAm segment in a middle block and poly(*t*-butylmethacrylate) (PtBMA) or poly(methacrylic acid) (PMAA) segment in end blocks by

ATRP. *PtBMA-b-PNIPAAm-b-PtBMA* is an amphiphilic triblock copolymer and examined the aggregation behaviors affected of hydrophobic *PtBMA* segment and thermo-responsive *PNIPAAm* segment. *PMAA-b-PNIPAAm-b-PMAA* is a double hydrophilic triblock copolymer comprised of pH-responsive *PMAA* segments and thermo-responsive *PNIPAAm* segments.

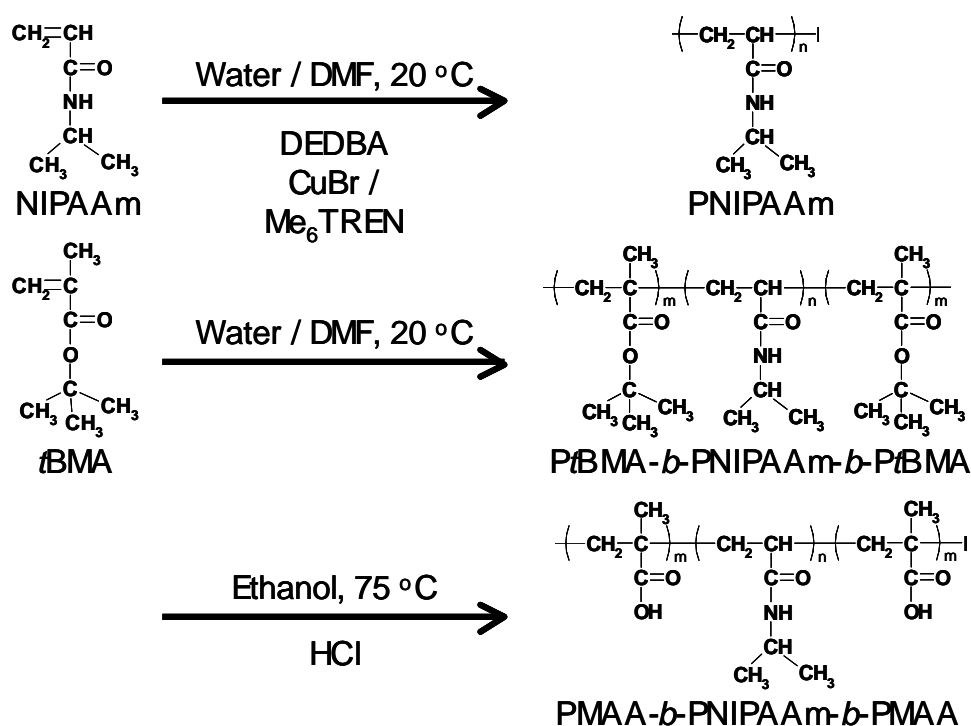
2.3 Experimental Section

2.3.1 Materials.

Diethyl *meso*-2,5-dibromoadipate (DEDBA, Sigma-Aldrich, St. Louis, USA), copper (I) bromide (CuBr, Sigma-Aldrich, St. Louis, USA), 36 % hydrochloric acid (HCl, Wako Pure Chemicals, Osaka, Japan) aqueous solution, *N,N'*-dimethyl formamide (DMF, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and ethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as received. Tris(2-dimethylaminoethyl)amine (Me₆TREN) was prepared as described in the literature.^{14,15} *NIPAAm* (kindly supplied by KOHJIN, Tokyo, Japan) was recrystallized from a benzene/hexane mixture and dried in vacuo. *tBMA* (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was purified by vacuum distillation.

2.3.2 Polymerization of *NIPAAm*.

A typical ATRP was carried out as follows.¹³ DEDBA as a bifunctional initiator and *NIPAAm* were dissolved in a water/DMF mixture. CuBr as



Scheme 2-1. Preparations of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

catalyst and Me₆TREN as ligand dissolved in water were put in a Schlenk tube and formed the CuBr/Me₆TREN complex. After the NIPAAm and CuBr/Me₆TREN complex solution were respectively degassed, the NIPAAm solution was added to the CuBr/Me₆TREN complex solution stirred and started the polymerization. The polymerization was carried out at 20 °C. After 1 h, the monomer conversion of NIPAAm was estimated by analyzing ¹H NMR spectrum of the polymerization mixture. We obtained PNIPAAm purified via dialysis and lyophilization.

2.3.3 Synthesis of PtBMA-*b*-PNIPAAm-*b*-PtBMA.

The first PNIPAAm segment in a middle block was polymerized as described in the previous section. After 1 h, *t*BMA dissolved in DMF was degassed and added to the polymerization mixture stirred.¹³ After 24 h, the monomer conversions of NIPAAm and *t*BMA were estimated by analyzing ¹H NMR spectrum of the polymerization mixture. We obtained PtBMA-*b*-PNIPAAm-*b*-PtBMA purified via dialysis and lyophilization.

2.3.4 Hydrolysis of PtBMA-*b*-PNIPAAm-*b*-PtBMA.

PtBMA-*b*-PNIPAAm-*b*-PtBMA was subjected to acid hydrolysis with 36 % HCl aqueous solution and ethanol. The hydrolysis was carried out at 75 °C for 12 h. After the hydrolysis, we obtained PMAA-*b*-PNIPAAm-*b*-PMAA purified via dialysis and lyophilization.

2.3.5 Characterization.

¹H NMR spectra. ¹H NMR spectra were recorded using a 400 MHz JEOL JNM-GSX400 spectrometer (JEOL Ltd., Tokyo, Japan). The monomer conversions and *t*BMA or MAA contents were determined in dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) or pyridine-*d*₅ at 50 °C.

Gel Permeation Chromatography. The molecular weights (*M*_n) and molecular weight distributions (*M*_w/*M*_n) of PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA were determined by gel permeation chromatography (GPC) using TOSOH TSK-GEL α-2500 and α-4000 columns (TOSOH CORPORATION, Tokyo, Japan) connected to a JASCO RI-2031 refractive index detector (JASCO Corporation, Tokyo, Japan) at 40 °C. The eluent was DMF containing of 10 mM lithium bromide and poly(ethylene oxide) standards were used for calibration.

Differential scanning calorimetry. Differential scanning calorimetry (DSC, EXSTAR 6000, Seiko Instruments, Tokyo, Japan) was used to estimate the thermo-responsive profiles of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA. DSC measurements were carried out between 0 °C and 60 °C at 1.0 °C/min in the heating process. 10 w/v% polymer solutions were placed in silver pans and carried out DSC measurements.

Investigation of thermo-responsive behavior by transmittance measurement. The thermo-responsive profiles of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA were investigated by the transmittance measurements using a JASCO V-550

UV-vis spectrometer (JASCO Corporation, Tokyo, Japan) with a temperature-controlled. 0.5 w/v% aqueous solutions of them were heated at 1.0 °C/min and measured at the wavelength 500 nm. The transmittance measurement of PMAA-*b*-PNIPAAm-*b*-PMAA was carried out in aqueous solutions at pH 12.

2.4 Results and Discussion

2.4.1 Preparations of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

PNIPAAm with different M_n were synthesized by ATRP using DEDBA as a bifunctional initiator, CuBr as catalyst, and Me₆TREN as ligand with feed ratio [DEDBA]/[CuBr]/[Me₆TREN] = 1:2:2 mol ratio (Scheme 2-1). DEDBA and NIPAAm were dissolved in a water/DMF mixture. CuBr and Me₆TREN dissolved in water were put in a Schlenk tube and turned into green with a bluish tone as they formed the CuBr/Me₆TREN complex. After the NIPAAm and CuBr/Me₆TREN complex solution were respectively degassed, the NIPAAm solution was added to the CuBr/Me₆TREN complex solution stirred and started the polymerization at 20 °C. After 1 h, the monomer conversion of NIPAAm was estimated by analyzing ¹H NMR spectrum of the polymerization mixture in DMSO-*d*₆ at 50 °C. Table 2-1 shows the monomer conversion of NIPAAm polymerized with different mol ratio relative to initiator. They reached more than 90 % in all cases. It indicates that the number of propagating radicals is constant

Table 2-1. Polymerization Kinetics of PNIPAAm Prepared by ATRP at 20 °C.

Polymer	Monomer	Time (h)	Conv. ^a (%)	M_n^a (theory)
PNIPAAm	NIPAAm ₂₀₀	1	98	22200
PNIPAAm	NIPAAm ₅₀₀	1	90	50900
PNIPAAm	NIPAAm ₁₀₀₀	1	92	104000

^a Estimated by ¹H-NMR.

Table 2-2. Polymerization Kinetics of *Pt* BMA-*b* -PNIPAAm-*b* -*Pt* BMA Prepared by ATRP at 20 °C.

Polymer	Monomer	Time (h)	Conv. ^a (%)	M_n^a (theory)
<i>Pt</i> BMA- <i>b</i> -PNIPAAm- <i>b</i> - <i>Pt</i> BMA	NIPAAm ₂₀₀	25	98	23800
	<i>t</i> BMA ₅₀		23	
<i>Pt</i> BMA- <i>b</i> -PNIPAAm- <i>b</i> - <i>Pt</i> BMA	NIPAAm ₅₀₀	25	90	55100
	<i>t</i> BMA ₁₂₅		24	
<i>Pt</i> BMA- <i>b</i> -PNIPAAm- <i>b</i> - <i>Pt</i> BMA	NIPAAm ₁₀₀₀	25	92	115300
	<i>t</i> BMA ₂₅₀		32	

^a Estimated by ¹H-NMR.

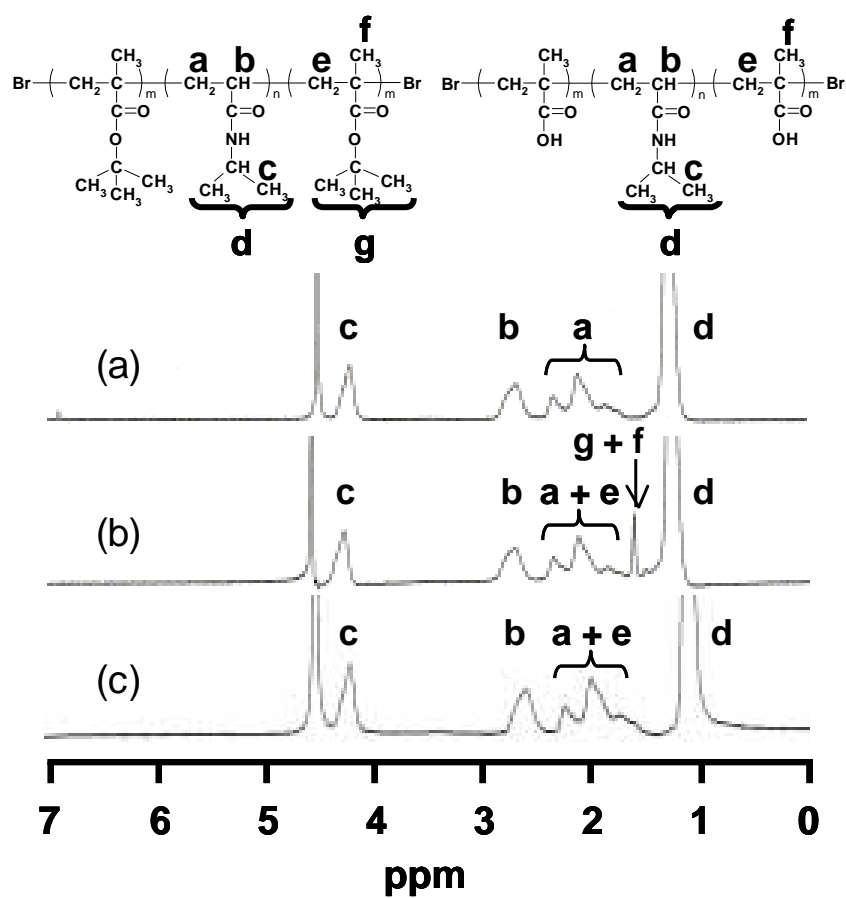


Figure 2-1. ^1H NMR spectra of PNIPAAm (a), PtBMA-*b*-PNIPAAm-*b*-PtBMA (b), and PMAA-*b*-PNIPAAm-*b*-PMAA (c) in pyridine- d_5 at 50 °C.

and termination does not occur.

PtBMA-*b*-PNIPAAm-*b*-PtBMA was prepared by sequential monomer addition of ATRP in a water/DMF mixture at 20 °C (Scheme 2-1).^b The PNIPAAm of a middle block was polymerized with a bifunctional initiator, subsequently, *t*BMA that dissolved in DMF and degassed was added to the polymerization mixture after 1 h. The monomer conversions of NIPAAm and *t*BMA were respectively more than 90 % and 20 % in 25 h (Table 2-1). The monomer conversions of NIPAAm after 25 h were almost same compared to them after 1 h. These results suggest that the polymerization of *t*BMA preferentially proceeded after *t*BMA had been added to the polymerization mixture. The monomer conversions of *t*BMA remained approximately less than about 30 % after 25 h. Furthermore, all PtBMA-*b*-PNIPAAm-*b*-PtBMA were translucent in water during dialysis. We consider that the termination would be occurred because PtBMA is hydrophobic and insoluble in a water/DMF mixture. ¹H NMR spectra of PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA obtained after purification are shown in Figure 2-1. PtBMA-*b*-PNIPAAm-*b*-PtBMA compositions were estimated by comparing the peak area of methyl protons (g, f) of *t*BMA with methine protons (c) of NIPAAm. We confirmed that all PtBMA-*b*-PNIPAAm-*b*-PtBMA with different [initiator]/[NIPAAm]/[*t*BMA] mol ratio were introduced PtBMA segments (Table 2-3, 2-4, and 2-5). However, *t*BMA contents estimated by ¹H NMR spectra were very low compared to [initiator]/[NIPAAm]/[*t*BMA] mol ratio. M_n and M_w/M_n of PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA are shown in Table 2-3,

**Table 2-3. Molecular Characteristics of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA,
and PMAA-*b*-PNIPAAm-*b*-PMAA.**

Code	Polymer	Content ^a (mol%)	M_n^b (GPC)	M_w/M_n^b (GPC)
N ₂₀₀	PNIPAAm ₂₀₀		17800	1.09
B ₇ N ₂₀₀ B ₇	PtBMA ₇ - <i>b</i> -PNIPAAm ₂₀₀ - <i>b</i> -PtBMA ₇	PtBMA 6.8	16900	1.10
M ₇ N ₂₀₀ M ₇	PMAA ₇ - <i>b</i> -PNIPAAm ₂₀₀ - <i>b</i> -PMAA ₇	PMAA 6.8		

^a Estimated by ¹H NMR. ^b Measured by GPC.

Table 2-4. Molecular Characteristics of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

Code	Polymer	Content ^a		M_n^b		M_w/M_n^b
		(mol%)		(GPC)		
N ₄₅₀	PNIPAAm ₄₅₀			44500		1.18
B ₁₄ N ₄₅₀ B ₁₄	PtBMA ₁₄ -b-PNIPAAm ₄₅₀ -b-PtBMA ₁₄	PtBMA	5.9	46800		1.37
M ₁₄ N ₄₅₀ M ₁₄	PMAA ₁₄ -b-PNIPAAm ₄₅₀ -b-PMAA ₁₄	PMAA	5.9			

^a Estimated by ¹H NMR. ^b Measured by GPC.

Table 2-5. Molecular Characteristics of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

Code	Polymer	Content ^a (mol%)	M_n^b (GPC)	M_w/M_n^b (GPC)
N ₉₂₀	PNIPAAm ₉₂₀		73800	1.52
B ₂₀ N ₉₂₀ B ₂₀	PtBMA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PtBMA ₂₀	PtBMA 4.1	60900	1.33
M ₂₀ N ₉₂₀ M ₂₀	PMAA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PMAA ₂₀	PMAA 4.1		

^a Estimated by ¹H NMR. ^b Measured by GPC.

2-4, and 2-5, respectively. M_n of both PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA were increased with NIPAAm and *t*BMA mol ratio relative to initiator increased. Moreover, M_w/M_n of them were also increased with their mol ratio relative to initiator increased. It is that because PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA with higher M_n increase with increasing viscosity of polymerization mixture under reaction.

Preparation of PMAA-*b*-PNIPAAm-*b*-PMAA by hydrolysis of PtBMA-*b*-PNIPAAm-*b*-PtBMA was similarly identified by disappearance of methyl proton (g, f) (Figure 2-1). The triblock copolymers are able to be synthesized with one step by this concise procedure and easily introduced the second blocks with addition of monomer.

2.4.1 Investigation of Thermo-Responsive Properties of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

The thermo-responsive properties of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA with different M_n in aqueous solutions were investigated by DSC and transmittance measurements. Figure 2-2 and 2-3 show the DSC curves of PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA in aqueous solutions, respectively. In all PNIPAAm with different M_n , the endothermic peaks due to phase transitions were observed at the same temperature, about 34 °C. It indicates that the LCST of them dose not depend on M_n . Moreover, the endothermic

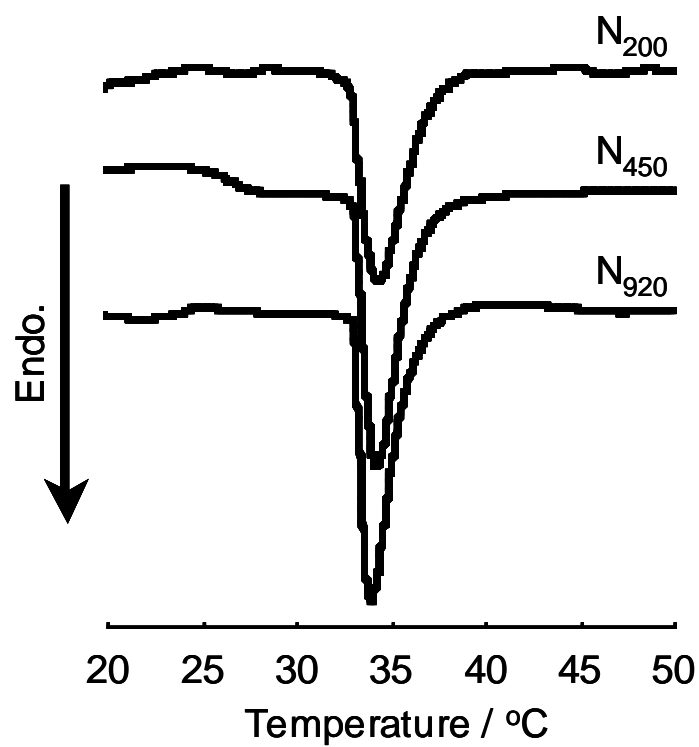


Figure 2-2. DSC curves for 10 w/v% aqueous solutions of $B_7N_{200}B_7$, $B_{14}N_{450}B_{14}$, and $B_{20}N_{920}B_{20}$; programming rate = 1 °C/min.

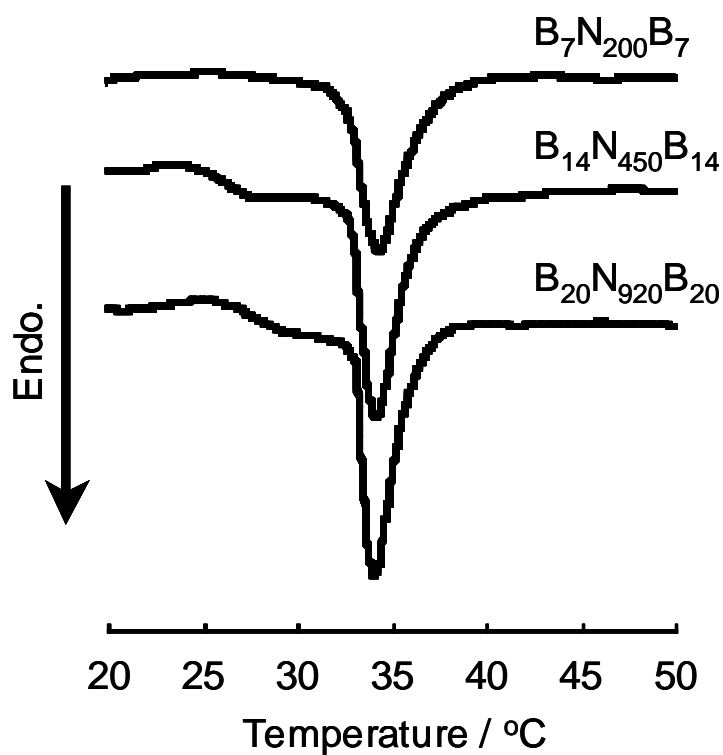


Figure 2-3. DSC curves for 10 w/v% aqueous solutions of $B_7N_{200}B_7$, $B_{14}N_{450}B_{14}$, and $B_{20}N_{920}B_{20}$; programming rate = 1 °C/min.

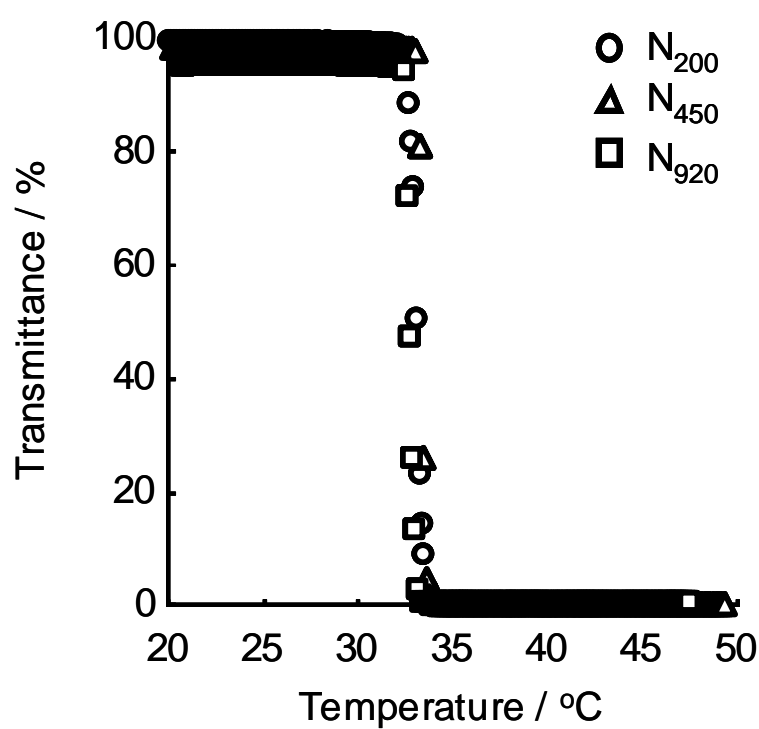


Figure 2-4. Transmittance measurements of 0.5 w/v% aqueous solutions of N_{200} (circles) , N_{450} (triangles) , and N_{920} (squares) ; programming rate = 1 °C/min.

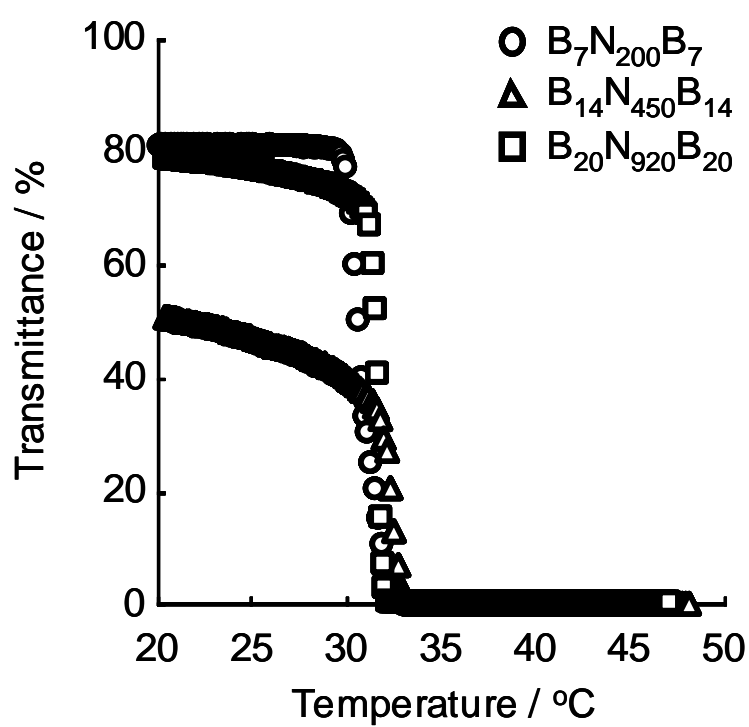


Figure 2-5. Transmittance measurements of 0.5 w/v% aqueous solutions of $B_7N_{200}B_7$ (circles) , $B_{14}N_{450}B_{14}$ (triangles) , and $B_{20}N_{920}B_{20}$ (squares) ; programming rate = 1 °C/min.

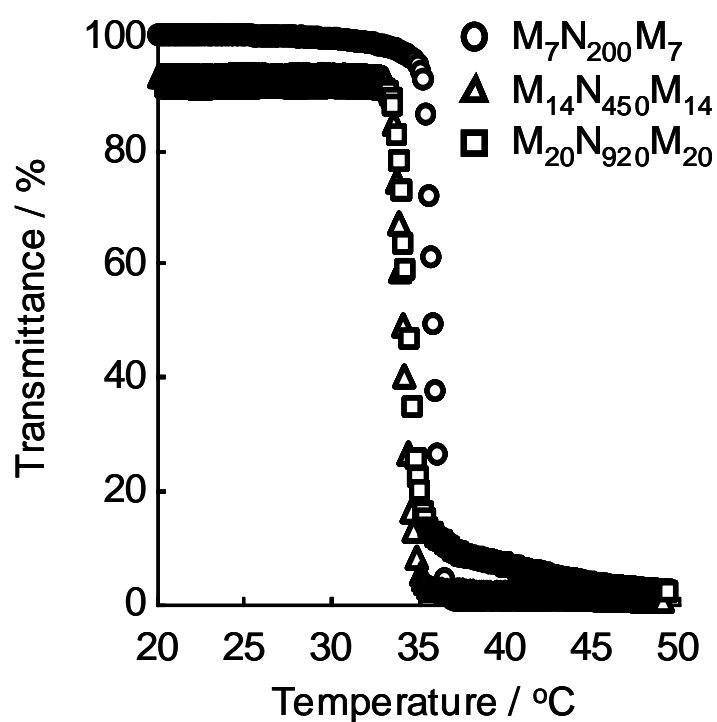


Figure 2-6. Transmittance measurements of 0.5 w/v% aqueous solutions of $M_7N_{200}M_7$ (circles) , $M_{14}N_{450}M_{14}$ (triangles) , and $M_{20}N_{920}M_{20}$ (squares) at pH 12; programming rate = 1 °C/min.

peaks were also observed for all *PtBMA-b-PNIPAAm-b-PtBMA* in aqueous solutions at the same temperature to PNIPAAm. In case of the random copolymers composed NIPAAm with hydrophobic comonomer, the LCST shift to a lower temperature because the hydrophobicity of them increases.¹⁶ That behavior was not observed as the *PtBMA* segments were incorporated in end blocks. This result suggests that the thermo-responsive properties of a PNIPAAm segment in a middle block is not influenced by hydrophobic *PtBMA* segments incorporated in end blocks of *PtBMA-b-PNIPAAm-b-PtBMA*.

The plots of the transmittance as a function of temperature are shown in Figure 2-4, 2-5 and 2-6. It was seen that the transmittance of *PtBMA-b-PNIPAAm-b-PtBMA* sharply decreased at 33 °C, which was consistent with the LCST of PNIPAAm (Figure 2-4). Furthermore, the aqueous solutions of *PtBMA-b-PNIPAAm-b-PtBMA* were slightly translucent below the LCST. These results suggest that *PtBMA-b-PNIPAAm-b-PtBMA* individually possesses characteristics of a thermo-responsive PNIPAAm segment in a middle block and hydrophobic *PtBMA* segments in end blocks, which corresponds to results of DSC measurements. The transmittance measurements of *PMAA-b-PNIPAAm-b-PMAA* were investigated in aqueous solutions at pH = 12 (Figure 2-6). The transmittance changes in response to temperature were observed in all *PMAA-b-PNIPAAm-b-PMAA* aqueous solutions at pH = 12. Carboxyl groups of PMAA segments in end blocks are dissociate and occur to electrostatic repulsion at higher pH. However, the phase transition of

PMAA-*b*-PNIPAAm-*b*-PMAA was exhibited in aqueous solutions at pH = 12 as the block length of PNIPAAm in a middle block was extremely long.

2.5 Conclusions

We reported the preparations and thermo-responsive properties of the amphiphilic and double hydrophilic ABA triblock copolymers. The amphiphilic ABA triblock copolymer, PtBMA-*b*-PNIPAAm-*b*-PtBMA consisting of thermo-responsive PNIPAAm in a middle block and hydrophobic PtBMA in end blocks was polymerized by ATRP in a water/DMF mixture at 20 °C. Furthermore, pH- and thermo-responsive PMAA-*b*-PNIPAAm-*b*-PMAA was also prepared by hydrolysis of PtBMA segments in end blocks of PtBMA-*b*-PNIPAAm-*b*-PtBMA. Both PtBMA-*b*-PNIPAAm-*b*-PtBMA and PMAA-*b*-PNIPAAm-*b*-PMAA exhibited the thermo-responsive properties caused by the phase transition of a PNIPAAm segment in a middle block.

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Chapter 3

Investigations of Aggregation Behavior of Stimuli-Responsive ABA Triblock Copolymers in Response to External Stimuli

3.1 Summary

An amphiphilic ABA triblock copolymer consisting of thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) in a middle block and hydrophobic poly(*t*-buthylmethacrylate) (*Pt*BMA) in end blocks was synthesized by atom transfer radical polymerization using a bifunctional initiator in a water/DMF mixture at 20 °C. A double hydrophilic block copolymer consisting of thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) in a middle block and pH-responsive poly(methacrylic acid) (PMAA) in end blocks was prepared by hydrolysis of *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA. *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA exhibited a very sensitive phase transition in response to temperature in an aqueous solution similar to PNIPAAm. PMAA-*b*-PNIPAAm-*b*-PMAA showed the thermo-responsive behavior depending on pH. Both *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA and PMAA-*b*-PNIPAAm-*b*-PMAA at low pH were observed aggregate formations below lower critical solution temperature in aqueous solutions by dynamic light scattering measurements and atomic

force microscopy. *PtBMA-*b*-PNIPAAm-*b*-PtBMA* formed a micelle with hydrophobic core of *PtBMA* segments and hydrophilic shell of *PNIPAAm* segments. Furthermore, *PMAA-*b*-PNIPAAm-*b*-PMAA* was confirmed the aggregation behavior in response to both temperature and pH.

3.2 Introduction

Amphiphilic block copolymers consisting of a hydrophobic and hydrophilic segment have attracted considerable attention because these copolymers can self-assemble into micelles, vesicles, or other aggregates with a hydrophobic core and hydrophilic shell in aqueous solutions.^{1,2} The conformation and size of the aggregates depend on many factors such as the chemical structure, molecular weight, and solvent composition.³⁻⁵ In addition, the hydrophobic core enables an incorporation of components insoluble in aqueous solutions and the hydrophilic shell stabilizes the aggregation structure.⁶⁻⁸ As such interesting behavior are possessed, the amphiphilic block copolymers are widely expected applications in various fields.

Recently, Armes et al. reported that a hydrophilic AB diblock copolymer can self-assemble in aqueous solutions to form a reversible micelle, and is described as “schizophrenic” diblock copolymer.⁹⁻¹³ This diblock copolymer consisted of 2-(*N*-morpholino)ethyl methacrylate (MEMA) and 2-(diethylamino)ethyl methacrylate (DEA), and reversibly exhibited three conformation as a molecularly dissolved state, a micelle with a MEMA

core, and a micelle with a DEA depending on pH or ionic strength in aqueous solutions.⁹ Laschewsky et al. reported that a hydrophilic AB diblock copolymer exhibited a double thermo-responsive property with schizophrenic character in aqueous solutions.^{14,15} It was comprised of poly(*N*-isopropylacrylamide) (PNIPAAm) with the lower critical solution temperature (LCST) and zwitterionic poly(3-[*N*-(3-methacrylamidopropyl)-*N,N*-dimethyl]ammoniopropane sulfonate) (PSPP) with upper critical solution temperature (UCST), and that molecularly dissolve in aqueous solution between the UCST of PSPP and the LCST of PNIPAAm. At low temperature below the UCST of PSPP, it formed the aggregate with a PSPP core attributed to electrostatic interaction of zwitterionic groups. On the other hand, it formed the aggregate with a hydrophobic PNIPAAm core due to dehydration at high temperature above the LCST of PNIPAAm. These aggregates were able to reversibly switch a core and shell of aggregates in response to temperature. Furthermore, Sfika et al. reported the aggregation behavior of a hydrophilic ABA triblock copolymer poly(acrylic acid)-*b*-poly(2-vinylpyridine)-*b*-poly(acrylic acid) in aqueous solutions.¹⁶⁻¹⁸ It possessed the properties exhibited not only aggregates formations but also gelation with three-dimensional network architecture in aqueous solution depending on pH and concentration. These self-assembly behavior are reversibly caused, and corresponding diblock copolymers were not observed those aggregation properties.¹⁹⁻²²

The block copolymers have been conventionally prepared by anionic, cationic, and group transfer polymerization. However, these methods

require stringent conditions and limit a small number of monomers which are available. Recently, controlled radical polymerization techniques referred to living radical polymerizations have been studied such as nitroxide-mediated polymerization, atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer polymerization.²³⁻²⁶ These techniques are more concise than ionic polymerizations, it is possible to prepare polymers controlled architecture with predetermined molecular weights (M_n) and low molecular weight distributions (M_w/M_n). In particular, ATRP is versatile and able to polymerize a wide range of monomer. Crescenzi et al. reported the controlled polymerization of NIPAAm via ATRP in a water/DMF mixture at 20 °C and successful preparation of the block copolymer with NIPAAm, regarded as difficult to polymerize them in the past.²⁷ It was demonstrated that this process enabled the preparations of block copolymers consisting of NIPAAm and other monomers.

In this study, we report the aggregation behavior of well-defined stimuli-responsive ABA triblock copolymer comprising PNIPAAm segment in a middle block and poly(*t*-butylmethacrylate) (PtBMA) or poly(methacrylic acid) (PMAA) segment in end blocks prepared by ATRP. PtBMA-*b*-PNIPAAm-*b*-PtBMA is an amphiphilic triblock copolymer and examined the aggregation behaviors affected of hydrophobic PtBMA segments and a thermo-responsive PNIPAAm segment. PMAA-*b*-PNIPAAm-*b*-PMAA is a double hydrophilic triblock copolymer comprised of pH-responsive PMAA segments and a thermo-responsive PNIPAAm

segment, and exhibited the aggregation behavior and conformations such as schizophrenic block copolymers in response to pH and temperature. We investigated the aggregate formations of these triblock copolymers in aqueous solutions depending on external stimuli by the use of transmittance measurements, dynamic light scattering (DLS) measurements, ^1H NMR spectroscopy, and atomic force microscopy (AFM) observations.

3.3 Experimental Section

3.3.1 Materials.

NIPAAm was kindly supplied from Kojin (Tokyo, Japan) and purified by recrystallization from *n*-hexane. *t*-Butylmethacrylate (*t*BMA) was purchased from Wako Pure Chemicals (Osaka, Japan) and purified by distillation under reduced pressure. Other chemicals for polymer synthesis and characterization were of analytical grade and used without further purification.

PNIPAAm and *Pt*BMA-*b*-PIPAAm-*b*-*Pt*BMA were synthesized by atom transfer radical polymerization (ATRP) according to the literature.²⁷ PMAA-*b*-PNIPAAm-*b*-PMAA was prepared by hydrolysis of corresponding precursors, *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA (Table 5-1). *Pt*BMA-*b*-PIPAAm-*b*-*Pt*BMA and PMAA-*b*-PNIPAAm-*b*-PMAA are 920 units for a PNIPAAm segment in a middle block and 20 units for each *Pt*BMA or PMAA segments in end blocks, referred to as $\text{M}_{14}\text{N}_{450}\text{M}_{14}$. The molecular weight and its distribution are $M_n = 60900$ and $M_w/M_n = 1.33$,

Table 3-1. Molecular Characteristics of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

Code	Polymer	Content ^a		M_n^b		M_w/M_n^b	
		(mol%)		(GPC)		(GPC)	
N ₉₂₀	PNIPAAm ₉₂₀				73800		1.52
B ₂₀ N ₉₂₀ B ₂₀	PtBMA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PtBMA ₂₀	PtBMA	4.1		60900		1.33
M ₂₀ N ₉₂₀ M ₂₀	PMAA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PMAA ₂₀	PMAA	4.1				

^a Estimated by ¹H NMR. ^b Measured by GPC.

respectively. The corresponding PNIPAAm is 920 units, and M_n and M_w/M_n of it is 73800 and 1.52.

3.3.2 Characterization.

¹H NMR spectroscopy. To analyze the thermo-responsive properties, ¹H NMR spectroscopy was used. ¹H NMR spectra were recorded using a 400 MHz JEOLJNM-GSX400 spectrometer (JEOL Ltd., Tokyo, Japan). The stimuli-responsive properties and micelle formation were studied in D₂O at pD = 12 and room temperature or 50 °C.

Gel Permeation Chromatography. The M_n and M_w/M_n of PNIPAAm and PtBMA-*b*-PNIPAAm-*b*-PtBMA were determined by gel permeation chromatography (GPC) using TOSOH TSK-GEL α -2500 and α -4000 columns (TOSOH CORPORATION, Tokyo, Japan) connected to a JASCO RI-2031 refractive index detector (JASCO Corporation, Tokyo, Japan) at 40 °C. The eluent was DMF containing of 10 mM lithium bromide and poly(ethylene oxide) standards were used for calibration.

Investigation of thermo-responsive behavior by transmittance measurement. The thermo-responsive of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA profiles were investigated by the transmittance measurements using a JASCO V-550 UV-vis spectrometer (JASCO Corporation, Tokyo, Japan) with a temperature-controlled. 0.5 w/v% aqueous solutions of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA were heated at 1.0 °C/min and measured at the wavelength 500 nm. The

transmittance of PMAA-*b*-PNIPAAm-*b*-PMAA was measured in aqueous solutions at various pH.

Dynamic Light Scattering. The dynamic light scattering (DLS) measurements were conducted with Otsuka Electronics Photol FPAR-1000HL spectrometer (Otsuka Electronics Co., Ltd., Osaka, Japan). A light source was a 10 mW He-Ne laser and the wavelength of the laser was 632.8 nm. The PtBMA-*b*-PNIPAAm-*b*-PtBMA in an aqueous solution was determined at different concentrations, and PMAA-*b*-PNIPAAm-*b*-PMAA in an aqueous solution was determined at various pH.

Atomic Force Microscopy. The atomic force microscopy (AFM) images were obtained with a Digital Instruments NanoScope III (Nihon Veeco KK, Tokyo, Japan) that was operated in tapping mode. The samples were prepared by depositing and spin-coating 0.5 w/v% aqueous solutions of PtBMA-*b*-PNIPAAm-*b*-PtBMA or PMAA-*b*-PNIPAAm-*b*-PMAA at pH 2 on the quartz crystal microbalance.

3.4 Results and Discussion

3.4.1 Investigation of Thermo-Responsive Properties of PNIPAAm, PtBMA-*b*-PNIPAAm-*b*-PtBMA, and PMAA-*b*-PNIPAAm-*b*-PMAA.

The thermo-responsive properties of N₉₂₀, B₂₀N₉₂₀B₂₀, and M₂₀N₉₂₀M₂₀ in aqueous solutions were investigated by transmittance measurements. The plots of the transmittance as a function of temperature are shown in Figure

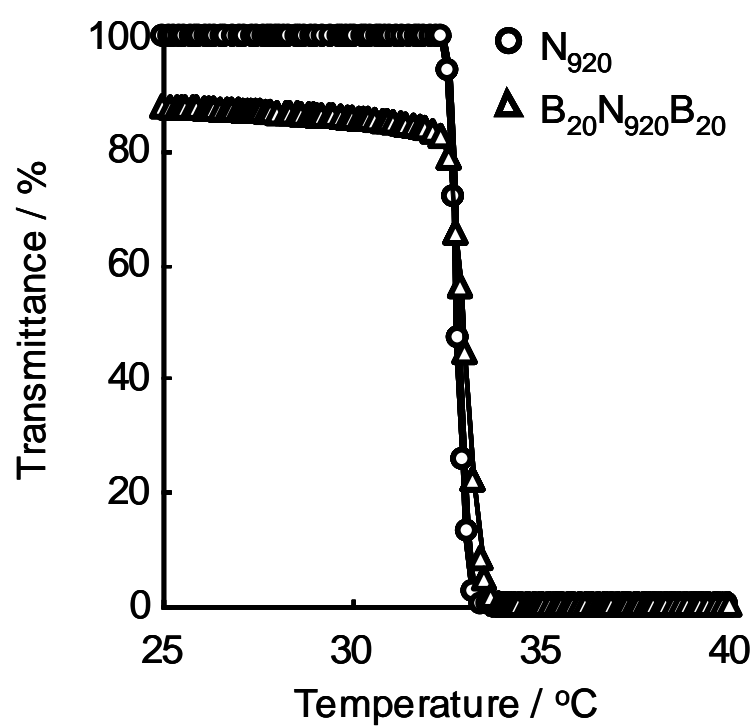


Figure 3-1. Transmittance measurements of 0.5 w/v% aqueous solutions of N₉₂₀ and B₂₀N₉₂₀B₂₀ ; programming rate = 1 °C/min.

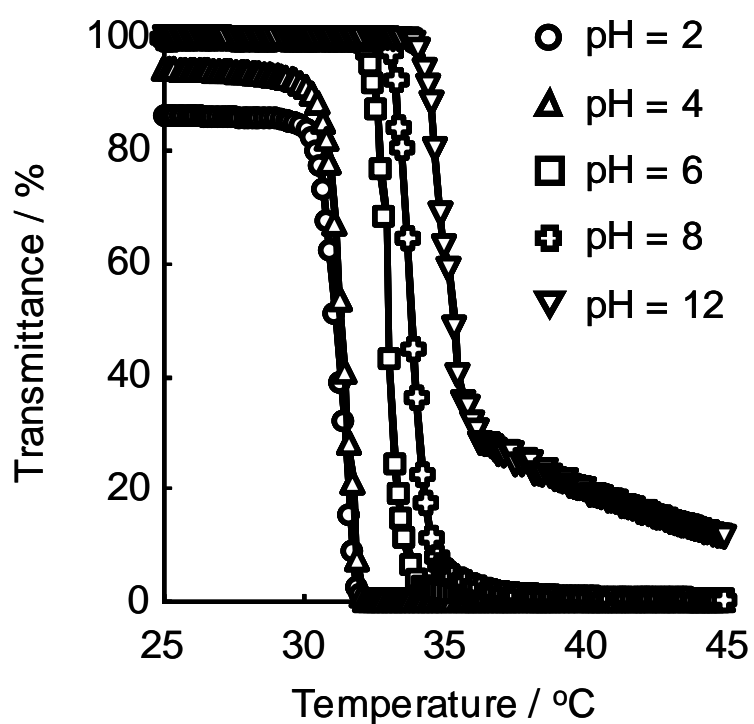


Figure 3-2. Transmittance measurements of 0.5 w/v% aqueous solutions of $M_{20}N_{920}M_{20}$ at different pH ; programming rate = 1 $^{\circ}C/min$.

3-1 and 3-2. It was seen that the transmittance of $B_{20}N_{920}B_{20}$ sharply decreased at 33 °C, which was consistent with the LCST of PNIPAAm (Figure 3-1). In case of the random copolymers composed NIPAAm with hydrophobic comonomer, the LCST shift to a lower temperature because the hydrophobicity of them increases.²⁸ Furthermore, the aqueous solution of $B_{20}N_{920}B_{20}$ was slightly translucent below the LCST. These results suggest that $B_{20}N_{920}B_{20}$ individually possesses characteristics of thermo-responsive PNIPAAm and hydrophobic PtBMA, and forms flower-like micelles with hydrophilic PNIPAAm shells and hydrophobic PtBMA cores below the LCST. The transmittance measurements of $M_{20}N_{920}M_{20}$ were investigated in aqueous solutions at various pH (Figure 3-2). The transmittance changes in response to temperature were observed at all pH, the LCST of them increased with increasing pH. The aqueous solutions of $M_{20}N_{920}M_{20}$ were slightly translucent at pH = 2 and 4, transparent at above pH = 6 below the LCST. PMAA in end blocks are weak polyelectrolyte and carboxyl groups of them can dissociate or protonate in dependence on pH. Therefore, it is suggested that $M_{20}N_{920}M_{20}$ in aqueous solutions formed hydrogen bonds between carboxyl groups or between a carboxyl group and an amide group because carboxyl groups protonated at lower pH.²⁸ Intermolecular and intramolecular hydrogen bonds in $M_{20}N_{920}M_{20}$ lead to the formation of aggregates, so transmittance was decreased in aqueous solutions at lower pH below the LCST. These results agree with them reported in the literature. However, the phase transition of $M_{20}N_{920}M_{20}$ was exhibited in aqueous solutions at all pH as the

block length of PNIPAAm in a middle block was extremely long. In addition, the transmittance above the LCST did not reached 0 % at pH = 12. We consider that $M_{20}N_{920}M_{20}$ forms micelles composed of a PNIPAAm core and a PMAA shell above the LCST due to the hydrophobic interaction of dehydrated PNIPAAm segments and the electrostatic repulsion of dissociate PMAA segments. These results indicate that $B_{20}N_{920}B_{20}$ and $M_{20}N_{920}M_{20}$ exhibit aggregation behaviors in response to pH and temperature, since each segment of them possess respective characters in aqueous solutions.

3.4.2 Observations of Aggregates Derived from *PtBMA-b-PNIPAAm-b-PtBMA* and *PMAA-b-PNIPAAm-b-PMAA*.

We conducted DLS measurements and AFM observations for translucent aqueous solutions of $B_{20}N_{920}B_{20}$ and $M_{20}N_{920}M_{20}$ below the LCST. Figure 3-3 and 3-4 show the results of DLS measurements. The diameter of $B_{20}N_{920}B_{20}$ was about 130 nm (Figure 3-3). The diameter of $M_{20}N_{920}M_{20}$ was about 90 nm at pH = 2 (Figure 3-4). $B_{20}N_{920}B_{20}$ formed flower-like micelles with a core of the hydrophobic *PtBMA* segments. As aggregates of $M_{20}N_{920}M_{20}$ were composed of hydrogen bonds between carboxyl groups or between a carboxyl group and an amide group, the hydrodynamic radius of $M_{20}N_{920}M_{20}$ was smaller than that of $B_{20}N_{920}B_{20}$. AFM images of $B_{20}N_{920}B_{20}$ and $M_{20}N_{920}M_{20}$ correlated with the results of DLS measurements (Figure 3-5). The average diameters of $B_{20}N_{920}B_{20}$ were about 90 nm and those of $M_{20}N_{920}M_{20}$ were about 70 nm. The shapes of

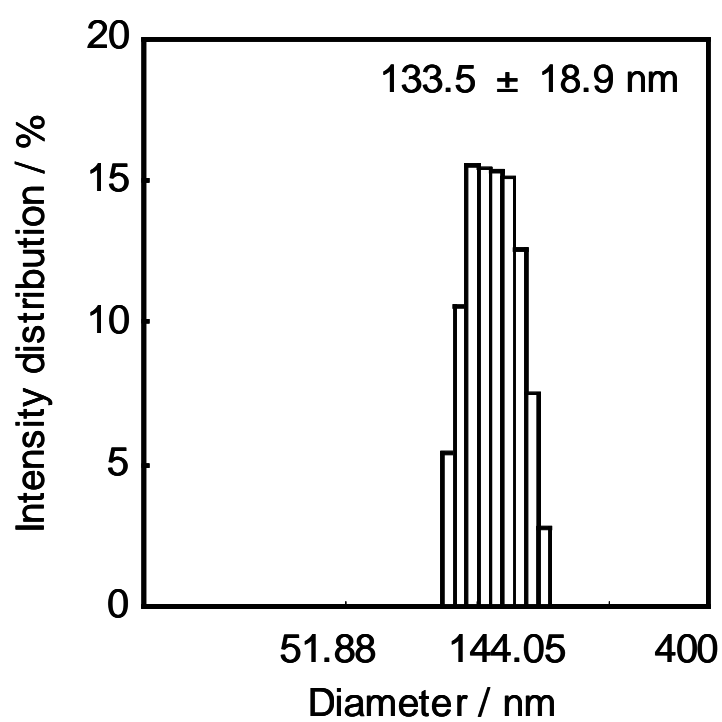


Figure 3-3. Diameter histogram of 0.5 w/v% aqueous solutions of $B_{20}N_{920}B_{20}$; $T = 21\text{ }^{\circ}\text{C}$.

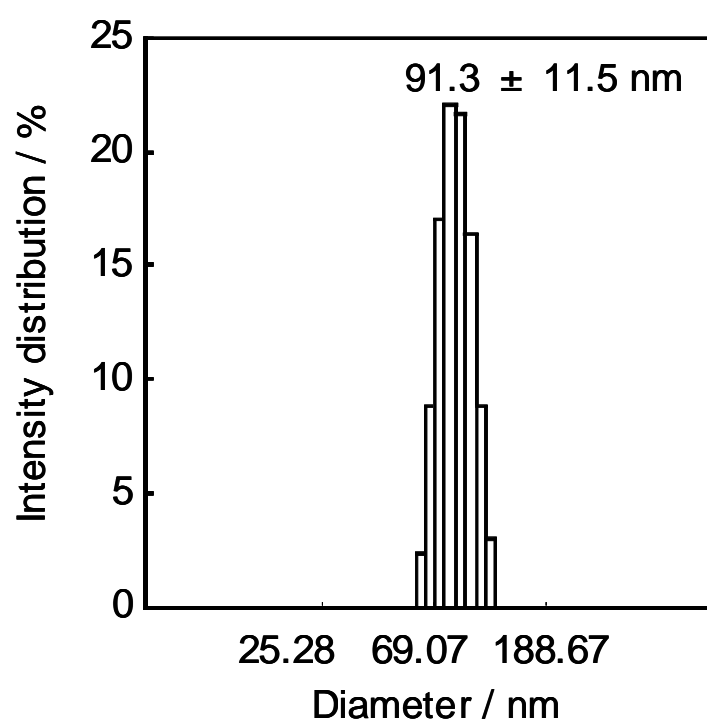


Figure 3-4. Diameter histogram of 0.5 w/v% aqueous solutions of $M_{20}N_{920}M_{20}$ at pH 2; $T = 21^\circ\text{C}$.

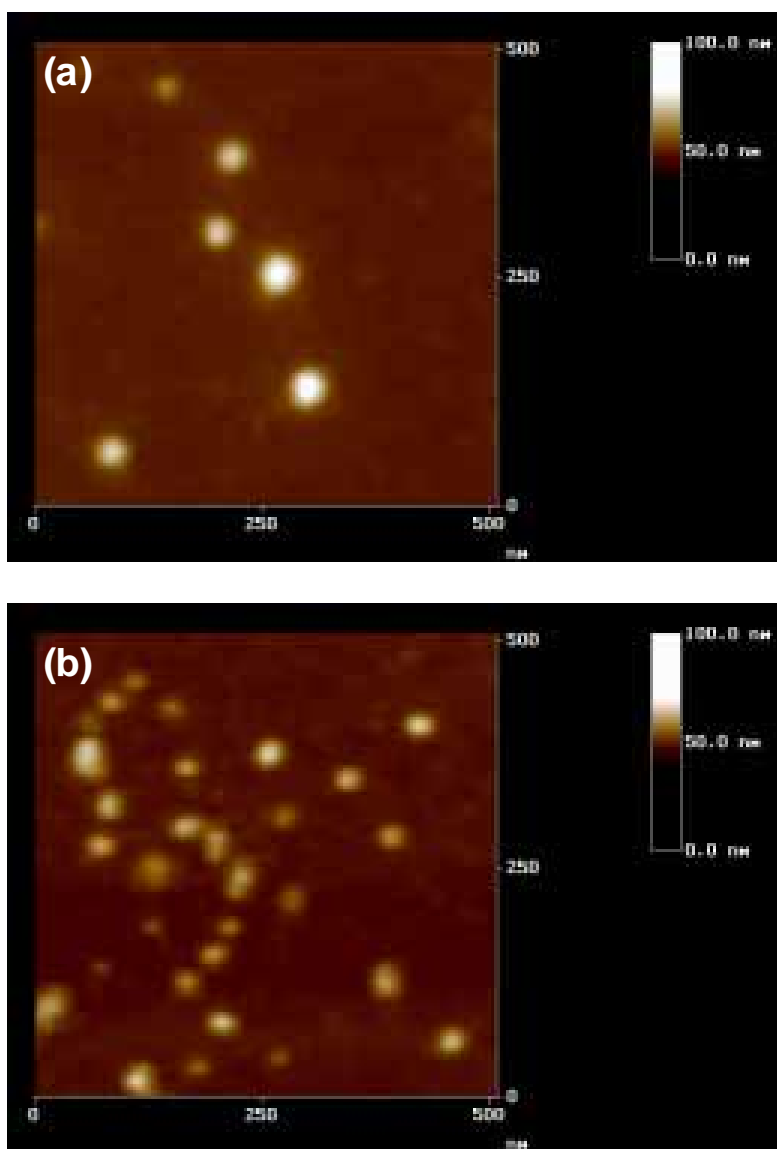


Figure 3-5. AFM images for the micelles of (a) $B_{20}N_{920}B_{20}$ and (b) $M_{20}N_{920}M_{20}$ at pH 2; $T = 21\text{ }^{\circ}\text{C}$.

them were spherical both ways. These aggregates size was smaller than the results determined by DLS measurements. It was assumed that the aggregates were contractile by dehydration to conduct AFM observations.

3.4.3 Thermo-Responsive Properties of Aggregates Derived from *PtBMA-b-PNIPAAm-b-PtBMA* and *PMAA-b-PNIPAAm-b-PMAA*.

Aggregation behaviors depending on temperature of $B_{20}N_{920}B_{20}$ or $M_{20}N_{920}M_{20}$ in aqueous solutions were observed by DLS measurements (Figure 3-6 and 3-7). We studied concentration dependency of the hydrodynamic diameters of $B_{20}N_{920}B_{20}$ in aqueous solutions (Figure 3-6). Those of $B_{20}N_{920}B_{20}$ were constant about 130 nm and monodisperse size distribution below the LCST at all concentration but increased with increasing concentration above the LCST in aqueous solutions. It was observed that $B_{20}N_{920}B_{20}$ formed micelles which were independent at concentration above 0.1 w/v% below the LCST. This shows that flower-like micelles formed according to the hydrophobic interaction of *PtBMA* segments possess of a hydrophilic *PNIPAAm* shell. *PNIPAAm* segments of a shell are dehydrated and hydrophobic above the LCST, and induce further aggregation due to hydrophobic interactions of inter-aggregates. Therefore, the hydrodynamic diameters of $B_{20}N_{920}B_{20}$ increased above the LCST. These micelle structures can reversibly change in dependence on temperature. Temperature dependence of hydrodynamic diameters of $M_{20}N_{920}M_{20}$ was observed in aqueous solutions at various pH

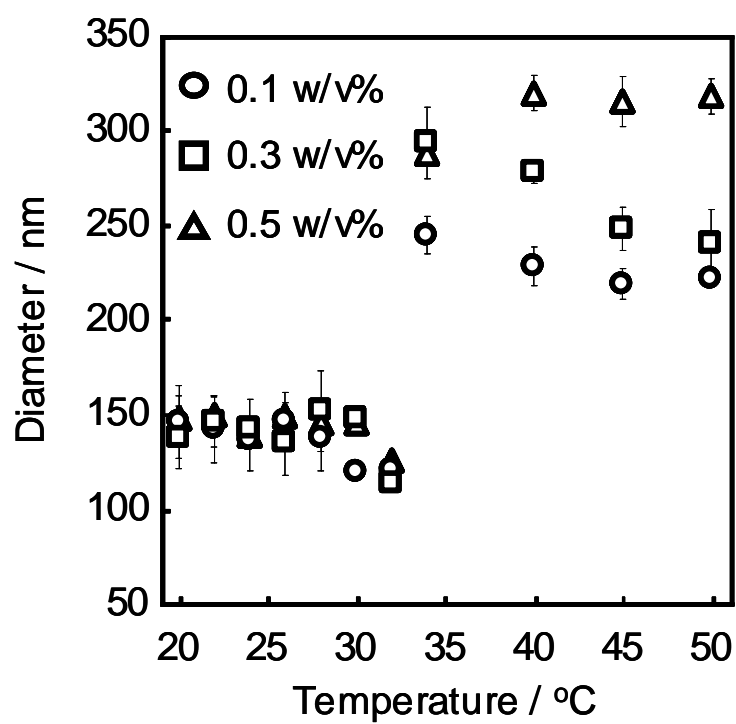


Figure 3-6. Temperature dependence of diameters for aqueous solutions of $B_{20}N_{920}B_{20}$ at different concentrations.

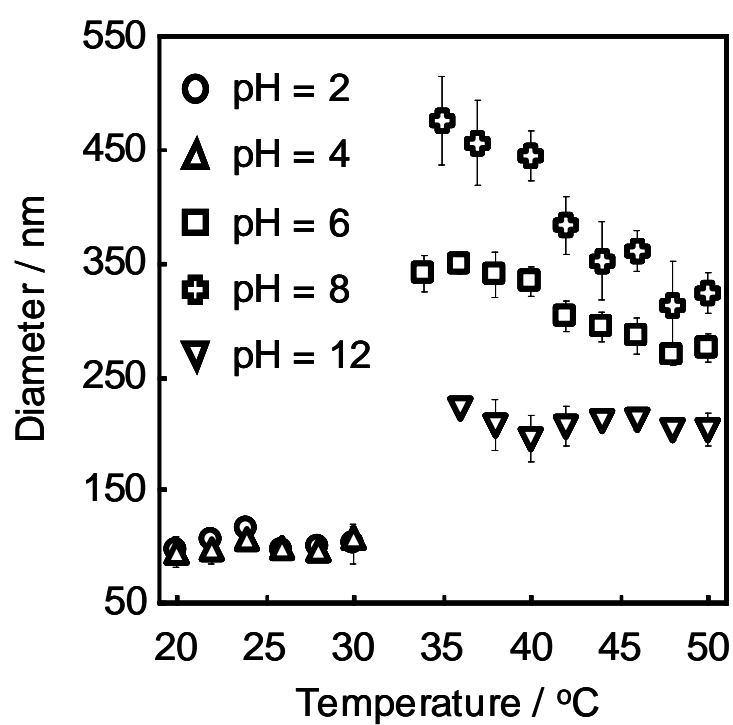


Figure 3-7. Temperature dependence of diameters for aqueous solutions of $M_{20}N_{920}M_{20}$ at different pH.

(Figure 3-7). The hydrodynamic diameters were estimated about 90 nm at pH = 2 and 4 below the LCST and not determined at higher pH as scattering light intensity was not enough. $M_{20}N_{920}M_{20}$ in aqueous solutions forms hydrogen bonds due to the protonation of carboxyl groups of PMAA segments at lower pH.²⁹ Therefore, intermolecular and intramolecular hydrogen bonds between $M_{20}N_{920}M_{20}$ lead to the aggregate formations. However, $M_{20}N_{920}M_{20}$ did not form aggregates at higher pH because it dissolved in aqueous solution due to the dissociations of carboxyl groups. Above the LCST, hydrodynamic diameters were also observed at higher pH as PNIPAAm segments collapse and exhibited phase transition with increasing temperature. The aggregates of $M_{20}N_{920}M_{20}$ formed at lower pH were precipitated above the LCST. We consider that the precipitate are easily formed as PNIPAAm segments become hydrophobic with increasing temperature and protonated PMAA segments form hydrogen bond between aggregates. On the other hand, the hydrodynamic diameters of $M_{20}N_{920}M_{20}$ at pH = 6 and 8 were almost similar to those of PNIPAAm in aqueous solution and hydrodynamic diameters of them were 200 - 300 nm and heterogeneous above the LCST. It is estimated that the aggregates are difficult to be stabilized as carboxyl groups of PMAA segments are composed of both the protonation and dissociation in aqueous solutions at pH = 6 and 8. Above the LCST at pH 12, the hydrodynamic diameter was about 150 nm and homogeneous in contrast to pH = 6 and 8. It indicates that $M_{20}N_{920}M_{20}$ forms micelles composed of PMAA shell and PNIPAAm core as carboxyl groups of PMAA segments completely dissociate at pH =

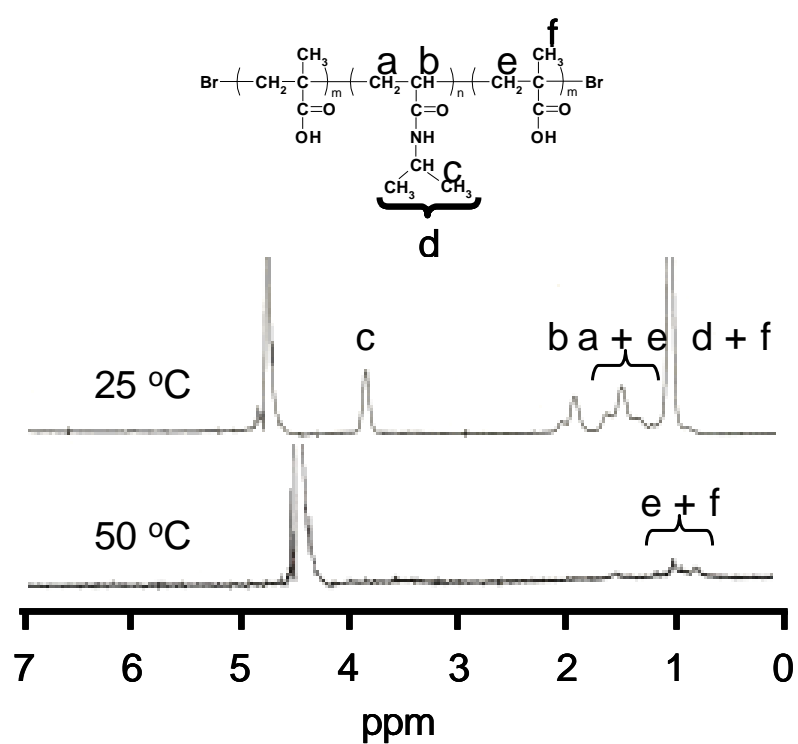


Figure 3-8. ^1H NMR spectra of $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ in D_2O of $\text{pD} = 12$ at 25 and 50 °C.

12 and PNIPAAm segments are dehydrated and hydrophobic above the LCST.

^1H NMR spectra of $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ in aqueous solution at pH 12 were recorded to reveal the details of the aggregation conformation (Figure 3-8). All proton signals of $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ were observed both PMAA and PNIPAAm segments soluble in D_2O of pD = 12 at 25 °C. However, the proton signals attributed to PNIPAAm segments almost completely attenuated at 50 °C. These results agree with DLS measurements of $\text{M}_{20}\text{N}_{920}\text{M}_{20}$, that is, the micelles were formed with the cores of dehydrated PNIPAAm segments and the hydrophilic shells of PMAA at pH 12 above the LCST. These results indicate that PMAA-*b*-PNIPAAm-*b*-PMAA reversibly inverts a core and a shell of aggregation conformations in response to pH and temperature.

These results are schematically represented in Figure 3-9 and 3-10. $\text{B}_{20}\text{N}_{920}\text{B}_{20}$ forms flower-like micelles in aqueous solutions below the LCST. The flower-like micelles exhibit a thermo-responsive properties due to PNIPAAm segment in a middle block and independent of concentration above 0.1 w/v% below the LCST (Figure 3-9). $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ exhibits various aggregation conformations in response to pH and temperature in aqueous solutions (Figure 3-10). At lower pH, $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ formed aggregates because $\text{M}_{20}\text{N}_{920}\text{M}_{20}$ was induced intramolecular or intermolecular hydrogen bonds due to the protonation of carboxyl groups of PMAA segments in aqueous solutions. Above the LCST, the precipitates were occurred as protonated PMAA segments formed hydrogen bond between

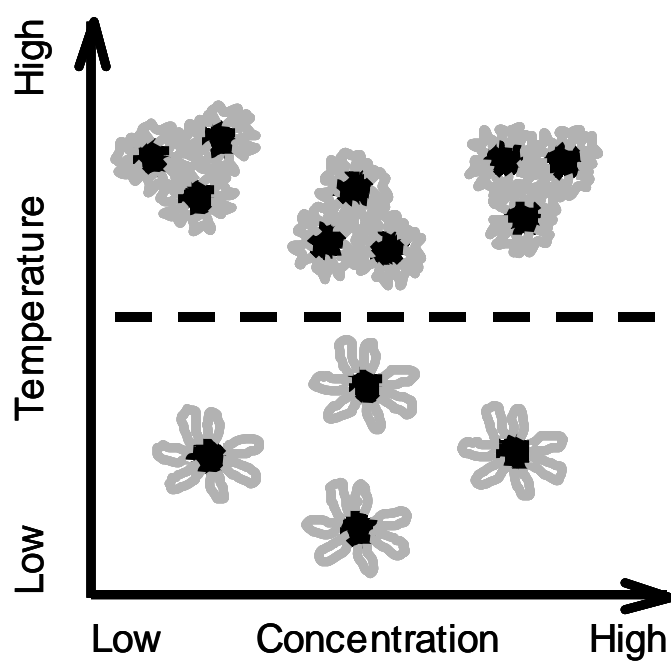


Figure 3-9. Schematic representation of stimuli-responsive micelle formations for $B_{20}N_{920}B_{20}$ in aqueous solutions.

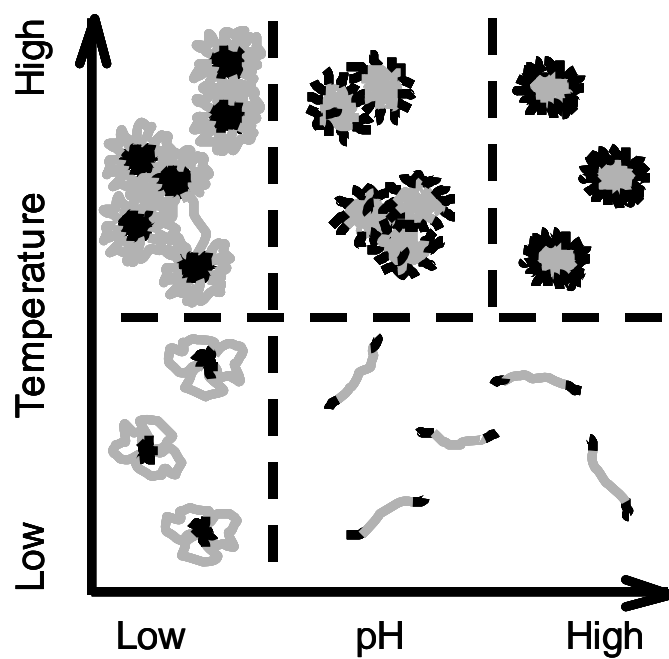


Figure 3-10. Schematic representation of stimuli-responsive micelle formations for $M_{20}N_{920}M_{20}$ in aqueous solutions.

aggregates depending on dehydration of PNIPAAm. However, $M_{20}N_{920}M_{20}$ molecularly dissolved due to the dissociations of carboxyl groups in aqueous solutions at above pH = 6 below the LCST.^{??} Above the LCST, micelles or aggregates were observed at higher pH. It was suggested that the aggregation sizes of $M_{20}N_{920}M_{20}$ were almost similar to that of PNIPAAm in aqueous solutions at pH = 6 and 8. Above the LCST at pH = 12, $M_{20}N_{920}M_{20}$ forms homogeneous micelles that are composed of dissociated PMAA shell and dehydrated PNIPAAm core. These micelles at pH = 12 above the LCST are formed with inverting a core and a shell for aggregation conformation of $M_{20}N_{920}M_{20}$ formed at pH = 2 below the LCST.

3.5 Conclusions

We reported the stimuli-responsive properties and aggregation behaviors of the amphiphilic and double hydrophilic triblock copolymers. The amphiphilic triblock copolymers, $B_{20}N_{920}B_{20}$ formed flower-like micelles independent of concentrations in aqueous solutions below the LCST. Those flower-like micelles exhibit thermo-responsive property due to shell of PNIPAAm segments in a middle block. The double hydrophilic triblock copolymers, $M_{20}N_{920}M_{20}$ had particularly interesting properties such as both pH- and thermo-responsive properties. At pH 2 below the LCST, $M_{20}N_{920}M_{20}$ formed aggregation conformation with a core of protonated PMAA including PNIPAAm and a shell of hydrophilic PNIPAAm. At pH

12 above the LCST, $M_{20}N_{920}M_{20}$ formed with a core of hydrophobic PNIPAAm by dehydration and a shell of dissociated PMAA. PMAA-*b*-PNIPAAm-*b*-PMAA exhibited the switch character of aggregation conformations in dependence on pH and temperature. These stimuli-responsive triblock copolymers are prospective as stabilizer and carrier to be able to control loading and release of agent in response to external stimuli.

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Chapter 4

Preparations and Characterizations of Organic-Inorganic Multilayer Films Consisting of Stimuli-Responsive ABA Triblock Copolymers and Silica Particles with Amino Groups

4.1 Summary

We studied the preparations and characterizations of organic-inorganic multilayer films consisting of pH- and thermo-responsive ABA triblock copolymers, PMAA-*b*-PNIPAAm-*b*-PMAA with carboxyl groups and silica particles with amino groups by the electrostatic interaction. It was prepared by stepwise monolayer deposition of them, and the assembly processes were quantitatively monitored by a quartz crystal microbalance as substrates. Assembled amounts increased with increasing the molecular weights of PMAA-*b*-PNIPAAm-*b*-PMAA as a component. The obtained organic-inorganic multilayer films were stabilized through induction of amide linkages with 1-Ethyl-3-(3-dimethylamino propyl)carbodiimide hydrochloride. The thermo-responsive properties of them were investigated by water contact angle measurements.

4.2 Introduction

The complexes formed by assembly of various compounds have received considerable attention in many fields. In particular, the complex with polymer is practical as the facility of a modification and controlled structure. The polyion complex micelles are formed by the electrostatic interactions between oppositely charged block copolymers in aqueous solutions.¹ The ionic block copolymers with a polyethylene oxide (PEO) segment exhibit exclusively self-assemble behaviors. Moreover, their micelles are very stable nanometer-sizes sphere with mono-disperse. In other cases, the associates by charged block copolymers could be formed with the multivalent metal ions or supermolecules.²⁻⁷ It is expected that the functionalities of those complexes.

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.⁸⁻¹¹ Not only ionic interactions but also hydrogen bonds,¹²⁻¹⁵ charge transfer,¹⁶⁻¹⁸ and van der Waals¹⁹⁻²² interactions have been utilized to create functional ultrathin films.

In this study, we prepared the organic-inorganic multilayer films consisting of pH- and thermo-responsive ABA triblock copolymers, PMAA-*b*-PNIPAAm-*b*-PMAA with carboxyl groups and silica particles with amino groups by the electrostatic interaction. It was prepared by stepwise monolayer deposition of them, and the assembly processes were

quantitatively monitored by a quartz crystal microbalance as substrates. We used the LbL to investigate intermolecular interactions of between PMAA-*b*-PNIPAAm-*b*-PMAA and aminated silica particles. The obtained organic-inorganic multilayer films were stabilized through induction of amide linkages with 1-Ethyl-3-(3-dimethylamino propyl)carbodiimide hydrochloride. The thermo-responsive properties of them were investigated by water contact angle measurements.

4.3 Experimental

4.3.1 Materials.

NIPAAm was kindly supplied from Kojin (Tokyo, Japan) and purified by recrystallization from *n*-hexane. *t*-Butylmethacrylate (*t*BMA) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and purified by distillation under reduced pressure. 3-aminopropyl trimethoxysilane (Sigma-Aldrich, St. Louis, USA) and acetic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as received. Silica particles dispersion solution was kindly supplied from NISSAN CHEMICAL INDUSTRIES, LTD. (Tokyo, Japan) and used as received. 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC) was obtained from Tokyo Chemical Ind. (Tokyo, Japan) and used without purification. Other chemicals for polymer synthesis and characterization were of analytical grade and used without further purification.

PNIPAAm and *Pt*BMA-*b*-PIPAAm-*b*-*Pt*BMA were synthesized by atom

**Table 4-1. Molecular Characteristics of Pt BMA-*b* -PNIPAAm-*b* -Pt BMA
and PMAA-*b* -PNIPAAm-*b* -PMAA.**

Code	Polymer	Cont. ^a (mol%)	M_n^b (GPC)	M_w/M_n^b (GPC)
B ₇ N ₂₀₀ B ₇	PtBMA ₇ - <i>b</i> -PNIPAAm ₂₀₀ - <i>b</i> -PtBMA ₇	PtBMA	6.8	16900
M ₇ N ₂₀₀ M ₇	PMAA ₇ - <i>b</i> -PNIPAAm ₂₀₀ - <i>b</i> -PMAA ₇	PMAA	6.8	
B ₁₄ N ₄₅₀ B ₁₄	PtBMA ₁₄ - <i>b</i> -PNIPAAm ₄₅₀ - <i>b</i> -PtBMA ₁₄	PtBMA	5.9	46800
M ₁₄ N ₄₅₀ M ₁₄	PMAA ₁₄ - <i>b</i> -PNIPAAm ₄₅₀ - <i>b</i> -PMAA ₁₄	PMAA	5.9	
B ₂₀ N ₉₂₀ B ₂₀	PtBMA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PtBMA ₂₀	PtBMA	4.1	60900
M ₂₀ N ₉₂₀ M ₂₀	PMAA ₂₀ - <i>b</i> -PNIPAAm ₉₂₀ - <i>b</i> -PMAA ₂₀	PMAA	4.1	

^a Estimated by ¹H NMR. ^b Measured by GPC.

transfer radical polymerization (ATRP) according to the literature.²³ PMAA-*b*-PNIPAAm-*b*-PMAA was prepared by hydrolysis of corresponding precursors, PtBMA-*b*-PNIPAAm-*b*-PtBMA. Molecular characteristics of PtBMA-*b*-PIPAAm-*b*-PtBMA and PMAA-*b*-PNIPAAm-*b*-PMAA with different molecular weights (M_n) show in Table 5-1.

Aminated silica particles were prepared by a silane coupling reaction with 3-aminopropyl trimethoxysilane in acetic acid at pH = 2.5. After reaction, we obtained Aminated silica particles dispersion solution purified via dialysis in acetic acid at pH = 2.5.

4.3.2 Quartz Crystal Microbalance.

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, IWATSU ELECTRIC CO., LTD., Tokyo, Japan). The leads of the quartz crystal microbalance (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 as follows:

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

where F_0 is the parent frequency of the QCM (9×10^6 Hz), A is the electrode

area (0.159 cm^2), ρ_q is the density of the quartz (2.65 g cm^{-3}), and μ_q is the shear modulus ($2.95 \times 10^{11} \text{ dyne cm}^{-2}$). 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. $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (30 wt% in water)=3/1, v/v] for 1 min, followed by rinsing with pure water and drying with N_2 to blast clean the electrode surface.

4.3.3 Preparations of Organic-Inorganic Multilayer Films Consisting of PMAA-*b*-PNIPAAm-*b*-PMAA and Aminated Silica Particles.

The cleaned QCM was immersed in an aminated silica particles dispersion solution (1.0 w/v%) at pH = 2.5. The QCM was immersed for 5 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 solution of PMAA-*b*-PNIPAAm-*b*-PMAA (0.02 unitM), and the same procedure was repeated. This sequential cycle was repeated for the preparation of organic-inorganic multilayer films. Assembly was performed at room temperature. Assembly was stable even though polymers at the first step were physically adsorbed without any chemical reaction onto the QCM substrate.

4.3.4 Stabilization of Organic-Inorganic Multilayer Films Consisting of PMAA-*b*-PNIPAAm-*b*-PMAA and Aminated Silica

Particles.

The organic-inorganic multilayer film was prepared as described in the previous section. Then, the QCM was immersed in an aqueous solution of EDC (in adequate amounts relative to the total MAA units). The QCM was immersed for 24 h, rinsed gently with pure water, and then dried under N₂ gas. The frequency shift was then measured in air. The condensation reaction was performed at 4 °C to maintain the activity of EDC.

4.4 Results and Discussion

4.4.1 Preparations of Organic-Inorganic Multilayer Films Consisting of PMAA-*b*-PNIPAAm-*b*-PMAA and Aminated Silica Particles.

Table 4-1 shows compositions of stimuli-responsive ABA triblock copolymers, PMAA-*b*-PNIPAAm-*b*-PMAA as components of organic-inorganic multilayer films. We prepared them consisting of PMAA-*b*-PNIPAAm-*b*-PMAA with different M_n and aminated silica particles by the electrostatic interaction and examined their M_n dependency of the assembly processes. Silica particles with amino groups were prepared by a silane coupling reaction.

To investigate the intermolecular interaction of between PMAA-*b*-PNIPAAm-*b*-PMAA with carboxyl groups and aminated silica particles, we prepared organic-inorganic multilayer films by stepwise monolayer deposition of them, and the assembly processes were quantitatively

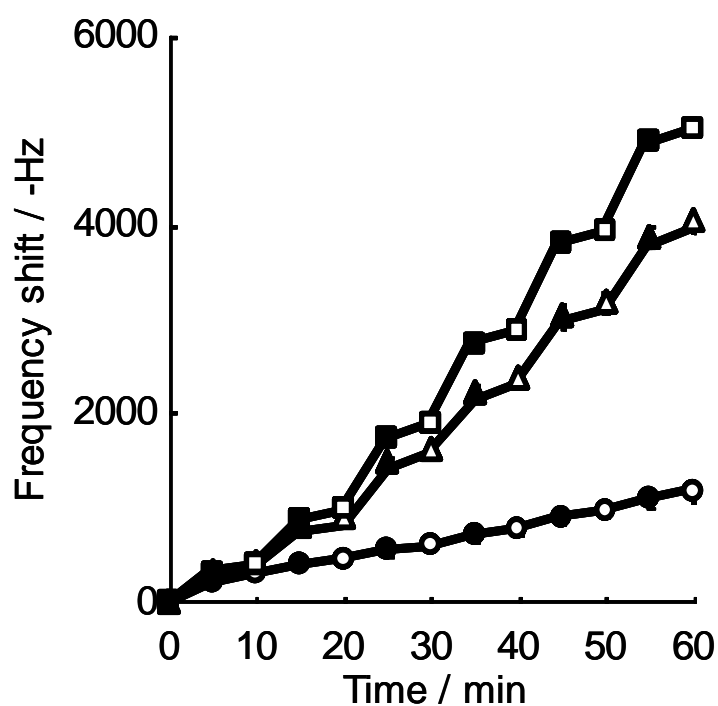


Figure 4-1. QCM analysis of the stepwise assembly of $M_7N_{200}M_7$ (open circles) , $M_{14}N_{450}M_{14}$ (open triangles) , or $M_{20}N_{920}M_{20}$ (open squares) and silica particles with amino groups (closed symbols) .

monitored by a QCM. Figure 4-1 shows frequency shifts corresponding to assembly amounts plotted against assembly steps, when PMAA-*b*-PNIPAAm-*b*-PMAA with different M_n were sequentially assembled with aminated silica particles on a QCM substrate. Assembly processes were reproducible in all cases, and frequencies decreased with increasing numbers of steps, indicating stepwise deposition of PMAA-*b*-PNIPAAm-*b*-PMAA and aminated silica particles. It shows that those multilayer films were prepared by electrostatic interaction between carboxyl groups of PMAA-*b*-PNIPAAm-*b*-PMAA and amino groups of aminated silica particles. Moreover, frequencies decreased with increasing M_n of PMAA-*b*-PNIPAAm-*b*-PMAA as components. It indicates that the assembly amounts of both PMAA-*b*-PNIPAAm-*b*-PMAA and aminated silica particles increased. The assembly amounts of not only PMAA-*b*-PNIPAAm-*b*-PMAA but also aminated silica particles increased, as the chain length of PMAA segments increased with increasing M_n of PMAA-*b*-PNIPAAm-*b*-PMAA.

4.4.2 Stabilization of Organic-Inorganic Multilayer Films Consisting of PMAA-*b*-PNIPAAm-*b*-PMAA and Aminated Silica Particles.

The organic-inorganic multilayer films were introduced amido linkages by a condensation reaction, and we examined the stability of them compared to ones prepared by the electrostatic interaction. Figure 5-2 shows the stabilities of organic-inorganic multilayer films before and after

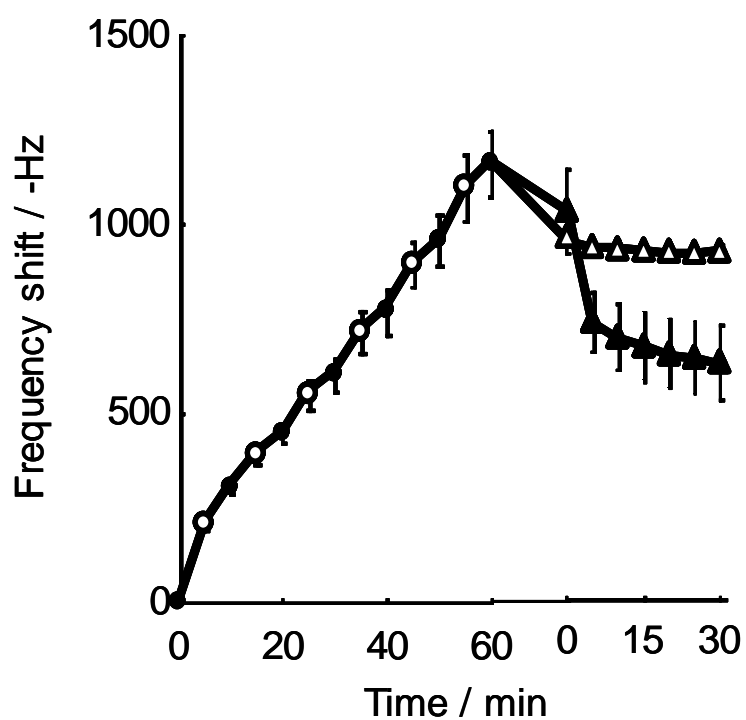


Figure 4-2. QCM analysis of the stepwise assembly of $M_7N_{200}M_7$ (open circles) and silica particles with amino groups (closed circles) with (open triangles) or without a condensation reaction (closed triangles) .

condensation reaction. After preparations of multilayer films, the QCM was immersed in an aqueous solution of EDC as those multilayer films were incorporated amido linkages interiorly. We investigated frequency shifts of those multilayer films in the alkaline solutions. These organic-inorganic multilayer films immersed in water or an aqueous solution of EDC, and frequency shifts of them were measured after 24 h. In both of them, it was investigated that frequencies decreased. This result indicates that $M_7N_{200}M_7$ or aminated silica particles physically adsorbed flaked off. However, only the multilayer films immersed in water but an aqueous solution of EDC flaked off in an alkaline solution. It shows that the organic-inorganic multilayer film was stabilized by incorporation of amido linkages by a condensation reaction.

4.4.2 Stabilization of Organic-Inorganic Multilayer films Consisting of PMAA-*b*-PNIPAAm-*b*-PMAA and Aminated Silica Particles.

To investigate of thermo-responsive properties of the organic-inorganic multilayer films, we measured water contact angles of them (Figure 5-3). Water contact angles of them decreased at between 30 and 35 °C with increasing temperature. At lower temperature below the lower critical solution temperature (LCST), a PNIPAAm segment in a middle block hydrate and is hydrophilic. It is considered that the multilayer film become hydrophobic as a PNIPAAm segment dehydrates at higher temperature above the LCST. These results suggest that the obtained organic-inorganic

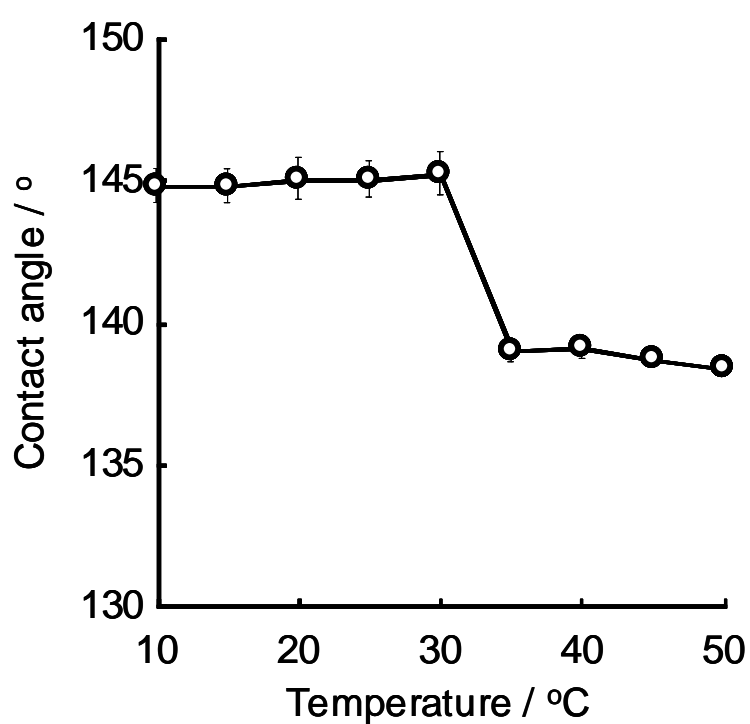


Figure 4-3. Contact angles of the organic-inorganic multilayer film consisting of $M_7N_{200}M_7$ and silica particles with amino groups.

multilayer films exhibit thermo-responsive properties.

4.5 Conclusions

We prepared the organic-inorganic multilayer films consisting of pH- and thermo-responsive ABA triblock copolymers, PMAA-*b*-PNIPAAm-*b*-PMAA with carboxyl groups and silica particles with amino groups by the electrostatic interaction. These organic-inorganic multilayer films were prepared by stepwise monolayer deposition of them, and the assembly processes were quantitatively monitored by a quartz crystal microbalance as substrates. Assembled amounts increased with increasing the molecular weights of PMAA-*b*-PNIPAAm-*b*-PMAA as a component, . The obtained organic-inorganic composite membranes were stabilized through induction of amide linkages with 1-Ethyl-3-(3-dimethylamino propyl)carbodiimide hydrochloride. The thermo-responsive properties of them were investigated by water contact angle measurements.

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Chapter 5

Preparations and Characterizations of Nano-Sized Complexes Derived from Stimuli-Responsive ABA Triblock Copolymers and Cationic Compounds

5.1 Summary

We have studied the preparations and characterizations of nano-sized complexes derived from stimuli-responsive AB or ABA block copolymers and G3 poly(amidoamine) dendrimers. The block copolymers consisted of thermo-responsive poly(*N*-isopropylacrylamide) and poly(methacrylic acid), respectively. The nano-sized complexes were consequent on the electrostatic interactions between carboxyl groups of the block copolymers and amino groups of dendrimers in aqueous solutions. We confirmed the effective of nano-sized complex formation by dynamic light scattering measurement and transmission electron microscope. Interestingly, the nano-sized complex derived from ABA triblock copolymer had smaller size than that from AB diblock copolymer. The condensation reactions between the block copolymers and the dendrimers effectively improved the stability of the nano-sized complexes in aqueous solutions. Furthermore, we also confirmed the sensitive response in temperature change.

5.2 Introduction

The block copolymers that the each segment shows different characters can form very interesting associates or micelles by self-assembly in the optimal conditions.¹⁻³ The characteristics of them are tunable by the kinds of components, chemical structures, and chain lengths of each blocks.^{4,5}

Recently, stimuli-responsive polymers have attracted much attention as the component for micelle- or associates-forming block copolymers. Because the resulting stimuli-responsive polymeric micelles become candidates for fabrication of the novel drug delivery systems with higher potentials. Actually, temperature- responsive polymeric micelles can be easily derived from poly(*N*-isopropylacrylamide) (PNIPAAm) -based block copolymers with hydrophobic segment.⁶ They can form micellar structure below Lower Critical Solution Temperature (LCST). Under heated condition, the thermo-responsive shell layers surrounding the hydrophobic core effectively dehydrate and the micelles would turn into hydrophobic aggregates. Using this unique system, we could selectively accumulate the drug carrier by local heating. Not only the surface character and association behavior but also the drug loading or release would be controlled.^{7,8}

Moreover, complex formation of polyions is also practical as the driving force for another unique polymeric micelles or associates. The polyion complex micelles are formed by the electrostatic interactions between oppositely charged block copolymers in aqueous solutions.⁹ The ionic block copolymers with a polyethylene oxide (PEO) segment exhibit

exclusively self-assemble behaviors. Moreover, their micelles are very stable nanometer-sizes sphere with mono-disperse. In other cases, the associates by charged block copolymers could be formed with the multivalent metal ions or supermolecules.¹⁰⁻¹⁵

In this study, we prepared the nano-sized complexes derived from ionic stimuli-responsive block copolymers with carboxyl groups and poly(amidoamine) (PAMAM) dendrimers with amino groups by the electrostatic interactions and characterized them in the aqueous media. Figure 5-1 shows schematic representation of aggregation behaviors of stimuli-responsive block copolymers and dendrimer in aqueous solution.

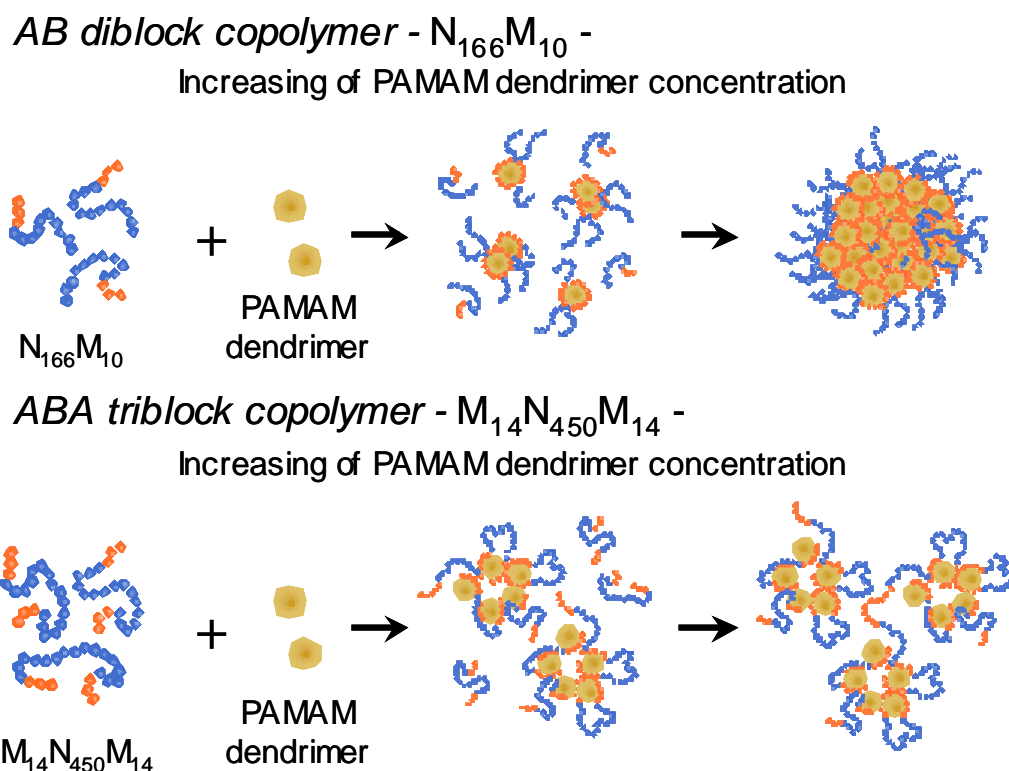


Figure 5-1. Schematic representation of the aggregation behavior of the stimuli-responsive block copolymers and PAMAM dendrimers.

The stimuli-responsive block copolymers consisted of two segments, PNIPAAm and poly(methacrylic acid) (PMAA) formed the AB or ABA block copolymers. Furthermore, the effective nano-sized complex stabilization was carried out by the condensation reactions between the block copolymers and the dendrimers.

Dendrimers are known to act as host for guest molecules such as metal ion, organic and inorganic compounds.^{16,17} Recently, it was reported that the complex derived from PAMAM dendrimers and block copolymers with a PEO segment showed high stability in aqueous solutions by surface modification of PAMAM dendrimer.¹³⁻¹⁵ It is expected that aggregation behavior of dendrimer would be controlled by using the stimuli-responsive copolymers as components.

5.3 Experimental Section

5.3.1 Materials.

NIPAAm was kindly supplied from Kojin (Tokyo, Japan) and purified by recrystallization from *n*-hexane. *t*-Butylmethacrylate (*t*BMA) was purchased from Wako Pure Chemicals (Osaka, Japan) and purified by distillation under reduced pressure. 1-Ethyl-3- (3-dimethylamino propyl) carbodiimide hydrochloride (EDC) was obtained from Tokyo Chemical Ind. (Tokyo, Japan) and used without purification. Other chemicals for polymer synthesis and characterization were of analytical grade and used without further purification.

Table 5-1. Molecular Characteristics of PNIPAAm-*b*-PMAA, PMAA-*b*-PNIPAAm-*b*-PMAA, and their precursors.

Code	Polymer	Content ^a		M_n^b	
		(mol%)		(GPC)	
N ₁₆₆ B ₁₀	PNIPAAm ₁₆₆ - <i>b</i> -PtBMA ₁₀	PtBMA	5.4	18900	1.32
N ₁₆₆ M ₁₀	PNIPAAm ₁₆₆ - <i>b</i> -PMAA ₁₀	PMAA	5.4		
B ₁₄ N ₄₅₀ B ₁₄	PtBMA ₁₄ - <i>b</i> -PNIPAAm ₄₅₀ - <i>b</i> -PtBMA ₁₄	PtBMA	5.9	46800	1.37
M ₁₄ N ₄₅₀ M ₁₄	PMAA ₁₄ - <i>b</i> -PNIPAAm ₄₅₀ - <i>b</i> -PMAA ₁₄	PMAA	5.9		

^a Estimated by ¹H NMR. ^b Measured by GPC.

PNIPAAm-*b*-PMAA as AB diblock copolymer and PMAA-*b*-PNIPAAm-*b*-PMAA as ABA triblock copolymer were prepared by hydrolysis of corresponding precursors, PNIPAAm-*b*-PtBMA and PtBMA-*b*-PNIPAAm-*b*-PtBMA (Table 5-1). They were synthesized by atom transfer radical polymerization (ATRP) according to the literature.¹⁸ PNIPAAm-*b*-PMAA contains 166 units for a PNIPAAm segment and 10 units for a PMAA one, abbreviated to as N₁₆₆M₁₀. The molecular weight and its distribution are $M_n = 18900$ and $M_w/M_n = 1.32$, respectively. PMAA-*b*-PNIPAAm-*b*-PMAA is 450 units for a PNIPAAm segment in a middle block and 14 units for each PMAA segments in end blocks, referred to as M₁₄N₄₅₀M₁₄. The molecular weight and its distribution are $M_n = 18900$ and $M_w/M_n = 1.32$, respectively. G3 PAMAM dendrimer was purchased from Sigma-Aldrich (St. Louis, USA). It was evaporated under reduced pressure before use and diluted with 50 mM tris HCl buffer solutions at a concentration of 10 mg/mL with adjusted pH. The theoretical molecular weight of G3 PAMAM dendrimer is $M_w = 6906$, and it has 32 -NH₂ and 30 -N=.

5.3.2 Preparations of Nano-Sized Complexes.

Nano-sized complexes were prepared in the following way. First, the block copolymers were dissolved in 50 mM tris HCl buffer solutions at a concentration of 1.0 mg/mL with adjusted pH. Then, the PAMAM dendrimer solution described above was added to the block copolymer solutions with stirring. As described below, we defined the molar mixing

ratio as $[-\text{NH}_2]/[-\text{COOH}]$.

5.3.3 Stabilization of Nano-Sized Complexes.

For stabilization of nano-sized complex, adequate amount of the condensation agent, EDC was added to the nano-sized complexes solution and it was stirred for 12 h at 4 °C. After the reaction, nano-sized complexes were purified by repeated washing with purified water.

5.3.4 Characterizations.

Dynamic light scattering spectroscopy. The dynamic light scattering (DLS) measurements were conducted with FPAR-1000HL spectrometer (Otsuka Electronics Co., Ltd., Osaka, Japan) to estimate the complex sizes and associates formation.

Electrophoretic light scattering spectroscopy. The z-potentials of the nano-sized complexes were estimated using ELS-6100 (Otsuka Electronics Co., Ltd., Osaka, Japan).

Transmission electron microscopic studies. The transmission electron microscopy (TEM) was also performed for evaluation of association size on a JEOL JEM-3010 (JEOL Ltd., Tokyo, Japan) at acceleration voltage of 300 kV. The sample was prepared by air-drying of the diluted solution on the collodion films.

Investigation of thermo-responsive behavior by transmittance measurement. The thermo-responsive profiles of the association forming behavior of nanoparticles were investigated by the transmittance

measurements using a JASCO V-55 UV-Vis spectrometer (JASCO Corporation, Tokyo, Japan) with a temperature-controlled.

5.4 Results and Discussion

5.4.1 Nano-Sized Complex Formations Derived from AB Block Copolymers and PAMAM Dendrimers.

First, DLS measurements were carried out to investigate the nano-sized complex formation by simple mixing of the block copolymer and the dendrimer solutions. Figure 5-2 and 5-3 show the scattering intensity and diameters of the nano-sized complexes derived from $N_{166}M_{10}$ and dendrimer. As seen in Figure 5-3, the diameters steeply increased with the $[-NH_2]/[-COOH]$ ratios increasing, and then were equilibrated in every sample at the specific $[-NH_2]/[-COOH]$ ratios. The equilibrated values of the diameters closely depended on pH values of aqueous solutions used for the complex formation.¹⁹ The profiles of scattering intensities were same to results of diameters in Figure 5-3 (Figure 5-2).

$-NH_2$ and $-N=$ protonate at $pH = 6$ and interact with all $-COO^-$ of the block copolymers (Figure 5-4). As described above, the number of $-NH_2$ and $-N=$ are 32 and 30 in a dendrimer molecule, respectively. As seen in Figure 5-3, the diameter reached the equilibrated value when the ratio of $[-NH_2]/[-COOH]$ was around 0.5. This result means the not only $-NH_3^+$ but also almost same number of $-NH^+=$ would participate in the complex formation. That is why the threshold was observed to be near 0.5 in the

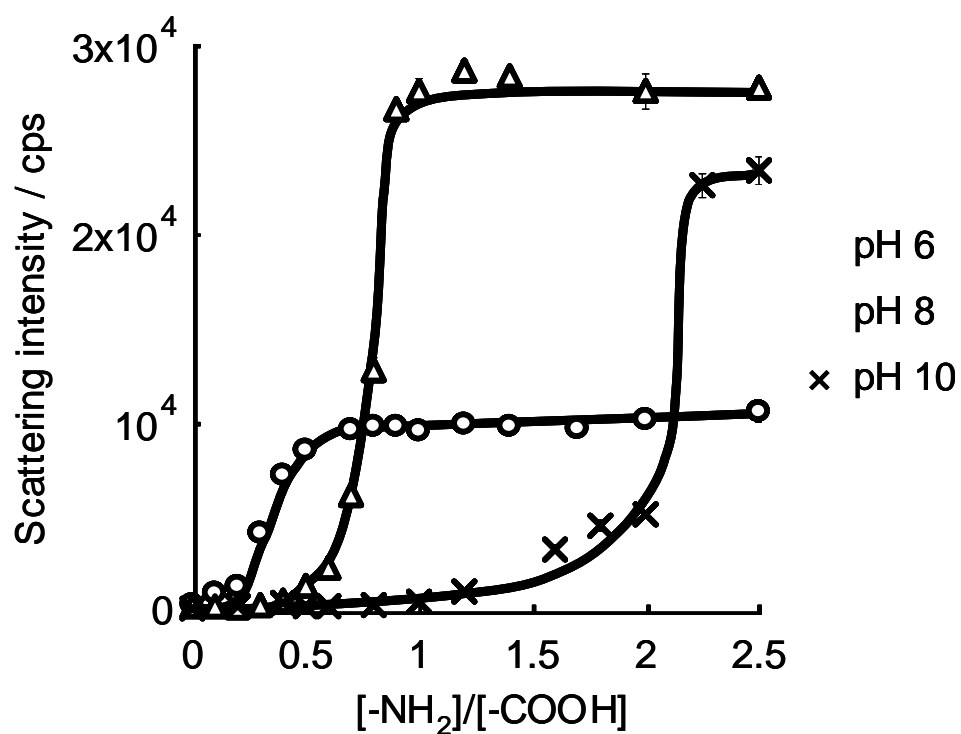


Figure 5-2. Scattering intensities of the nano-sized complexes for $N_{166}M_{10}$ solutions as a function of the PAMAM dendrimer concentration at pH = 6 (circles), 8 (triangles), 10 (crosses) ; polymer concentration = 0.1 w/v%, $T = 21$ °C.

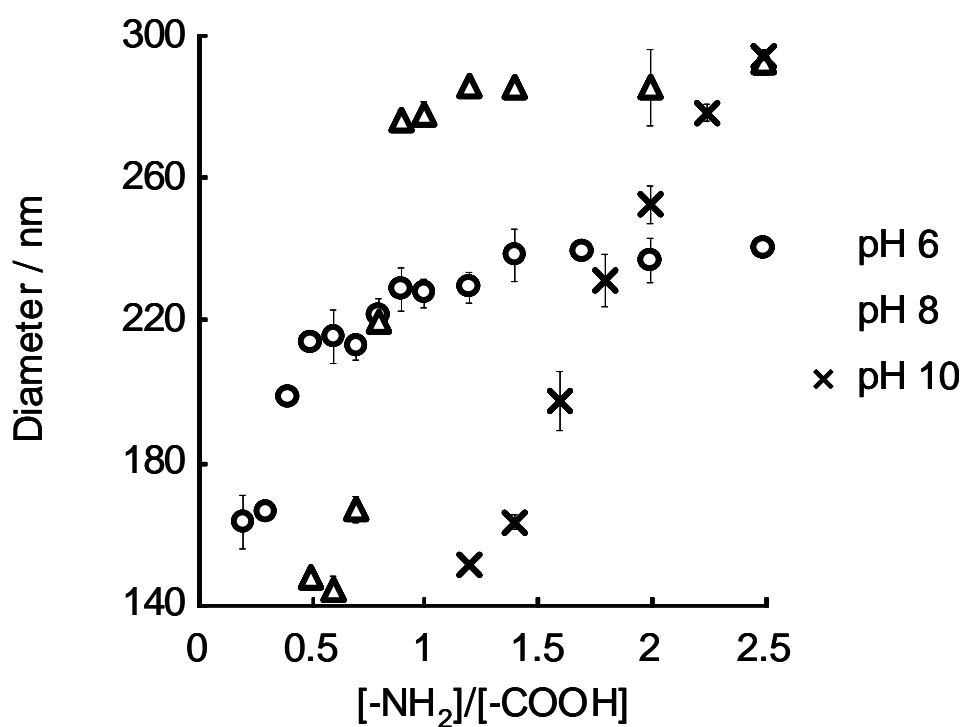


Figure 5-3. Diameters of the nano-sized complexes for N₁₆₆M₁₀ solutions as a function of the PAMAM dendrimer concentration at pH 6 = (circles), 8 (triangles), 10 (crosses) ; polymer concentration = 0.1 w/v%, $T = 21\text{ }^{\circ}\text{C}$.

solution adjusted pH = 6.

Taking the stoichiometry of the ionic interaction into the consideration, $[-NH_2]/[-COOH]$ threshold of around 1 on pH = 8 and 2 on pH = 10 could be easily understood. The pKa values of $-NH_2$ and $-N=$ in dendrimer are 6.5 and 9.9 respectively (Figure 5-4). At pH = 8, $-NH_3^+$ could only interact with $-COO^-$. At pH = 10, a half $-NH_3^+$ could react because the pH value is very near pKa of $-NH_2$. The results clearly mean that nano-sized complexes formed through electrostatic interaction.²⁰

$-COO^-$ of $N_{166}M_{10}$ are 10 in each chain above pH = 6. There are 32 $-NH_2$ and 30 $-N=$ in dendrimer that protonate or deprotonate dependent on pH. $-COO^-$ of $N_{166}M_{10}$ interact with protonated amino groups of dendrimer by electrostatic interactions. And then, the remaining deprotonated amino groups would induce the association between the complexes gradually by hydrogen bonds and reach thermodynamically equilibrated complexes resultantly. Considering the complex formation process, in initial stage, the dendrimer could immediately react with the polymer chains by electrostatic interactions and would form the modified complexes with the smaller size. These small complexes would gradually grow until they formed the stable complexes with larger size. As a result, the diameter would become larger

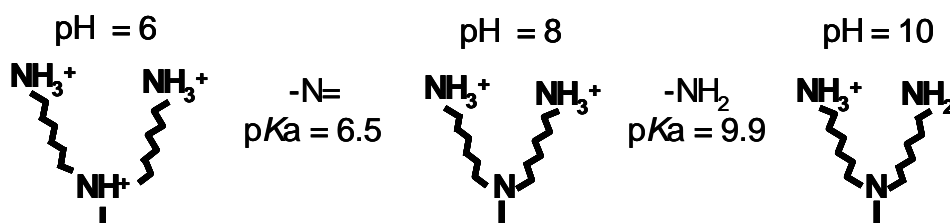


Figure 5-4. Schematic representation of protonations of PAMAM dendrimer dependent on pH.

by hydrogen bond between remaining deprotonated amino groups. The reason why smallest size was observed in pH = 6 might be that remaining deprotonated amino groups was smaller amount than that in pH = 8 and 10.

5.4.2 Nano-Sized Complex Formations Derived from ABA Block Copolymers and PAMAM Dendrimers.

Figure 5-5 and 5-6 shows the scattering intensity and diameters of the nano-sized complexes derived from ABA block copolymer $M_{14}N_{450}M_{14}$ in dependence on $[-NH_2]/[-COOH]$. The profiles were totally different from those from $N_{166}M_{10}$. In terms of the stoichiometry between amino and $-COO^-$, the thresholds $[-NH_2]/[-COOH]$'s that reached plateau levels were larger than those from $N_{166}M_{10}$. As indicated in Figure 5-5 and 5-6, the intensities as well as diameters were considerably smaller than those in the former cases. These results suggest that the constituting number of block copolymers and dendrimers would be fewer in resulting complexes. Interestingly, the complex diameters were almost same as around 100 nm independent of $[-NH_2]/[-COOH]$ ratio (Figure 5-6).

While, in case of the $M_{14}N_{450}M_{14}$, the reactive $-COO^-$ on the both side in the chain are capable of interacting with the dendrimer. Nevertheless, the scattering intensity was considerably low and the complex sizes were constant and smaller compared with those from $N_{166}M_{10}$. Moreover, the z-potentials at the $[-NH_2]/[-COOH] = 1.0$ and 1.5 were -3.0 mV and -1.9 mV (pH = 8). These results suggest the presence of free $-COO^-$. The one side segment of the block copolymer would only react with the dendrimer

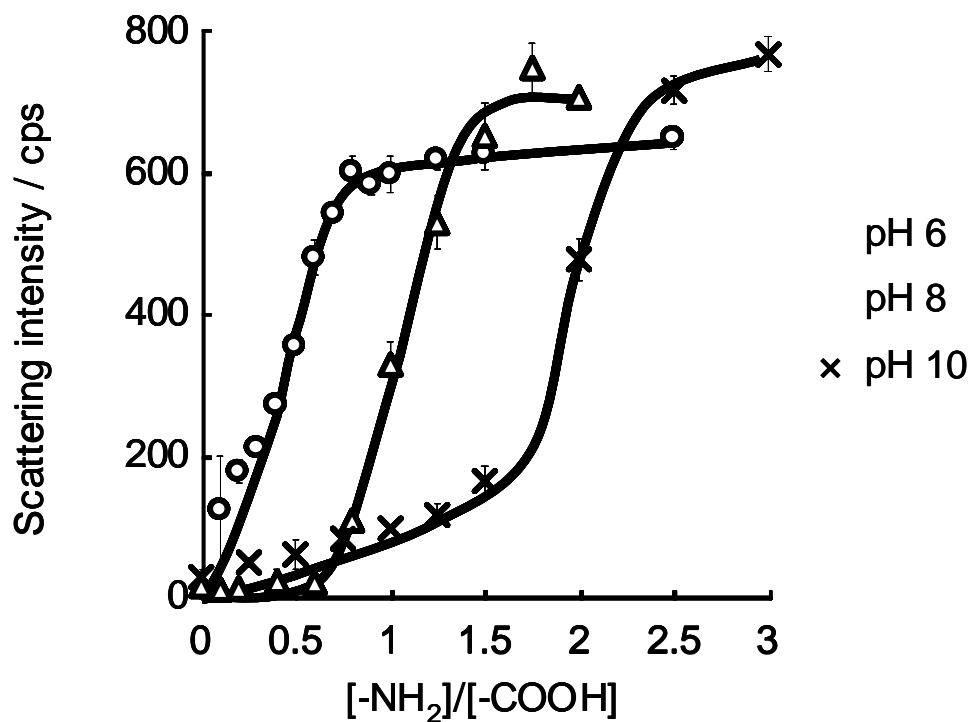


Figure 5-5. Scattering intensities of the nano-sized complexes for $M_{14}N_{450}M_{14}$ solutions as a function of the PAMAM dendrimer concentration at pH = 6 (circles), 8 (triangles), 10 (crosses); polymer concentration = 0.1 w/v%, $T = 21\text{ }^{\circ}\text{C}$.

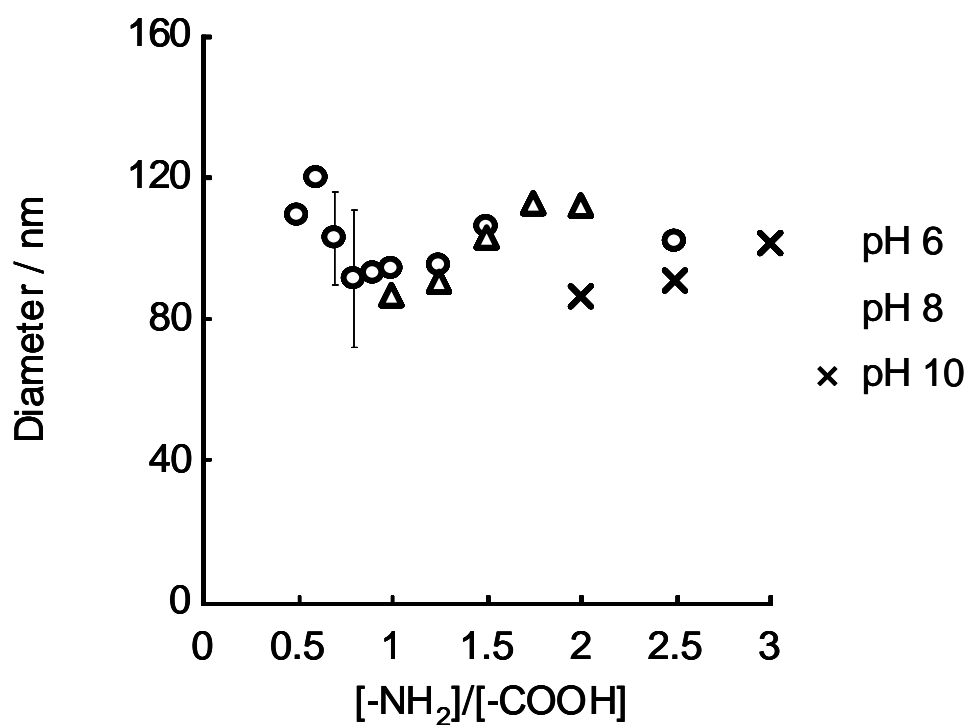


Figure 5-6. Diameters of the nano-sized complexes for $M_{14}N_{450}M_{14}$ solutions as a function of the PAMAM dendrimer concentration at pH = 6 (circles), 8 (triangles), 10 (crosses) ; polymer concentration = 0.1 w/v%, $T = 21$ °C.

and some free mobile chains with -COO^- would remain. Therefore, the modified dendrimer could increase with difficulty by even the charge canceling. This charge change induce the complex growth, however, the formation would be effectively interfered by the sterically hindered and negative charge. That is why the scattering intensity and diameters were considerably low and small compared with those from AB copolymer.

5.4.3 Stabilizations of Nano-Sized Complexes.

Prior to the stabilization, we have checked the stability by the turbidity observation. In the condition with $\text{pH} = 8$, the sample solution from $\text{N}_{166}\text{M}_{10}$ was slightly opaque due to the larger complex size (Figure 5-7, (a)). In the same condition, the solution prepared with $\text{M}_{14}\text{N}_{450}\text{M}_{14}$ looks very slightly blue based on the light diffraction. At $\text{pH} = 13$, both samples solution prepared with $\text{N}_{166}\text{M}_{10}$ and $\text{M}_{14}\text{N}_{450}\text{M}_{14}$ are completely transparent (Figure 5-7, (b)). Since this condition is over pK_a value of -NH_2 groups in the dendrimer, the complexes dissociate into the block copolymer with -COO^- and the non-charged dendrimers. Then, to improve the stability in wide pH condition, we carried out the chemical reaction.

After the reaction, as expected, the both solutions have maintained similar properties and the nano-sized complexes could not dissociate even though it was exposed in $\text{pH} = 13$ (Figure 5-8). Obviously, the amide bonding formation contributed to improve the stability in severe condition.

We also studied the TEM and DLS measurements of the nano-sized complexes and the obtained images were shown in Figure 5-9, 5-10, and

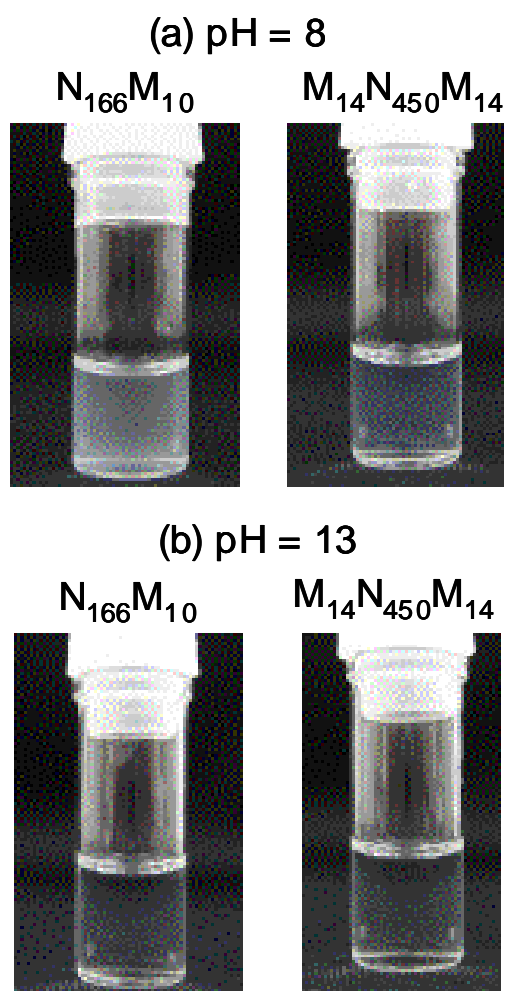


Figure 5-7. Photographs of the nano-sized complexes for $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ solutions at pH = 8 (a) and 10 (b) ; polymer concentration = 0.1 w/v%, $T = 21\text{ }^{\circ}\text{C}$.

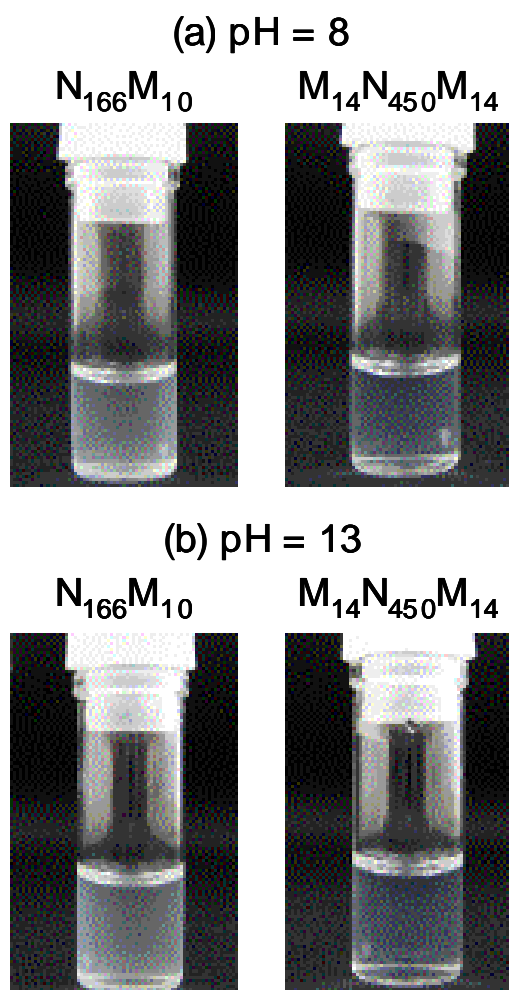


Figure 5-8. Photographs of the nano-sized complexes for $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ solutions at pH = 8 (a) and 10 (b) after stabilization; polymer concentration = 0.1 w/v%, $T = 21\text{ }^{\circ}\text{C}$.

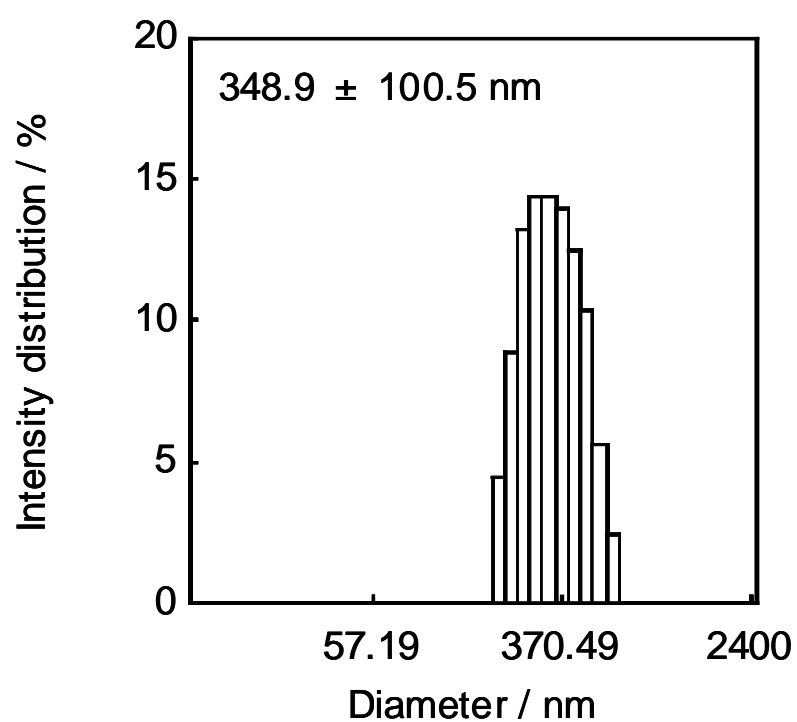


Figure 5-9. Diameter histogram of $N_{166}M_{10}$ nanoparticles with PAMAM dendrimer in aqueous solutions at pH = 8 after purification; $T = 21\text{ }^{\circ}\text{C}$.

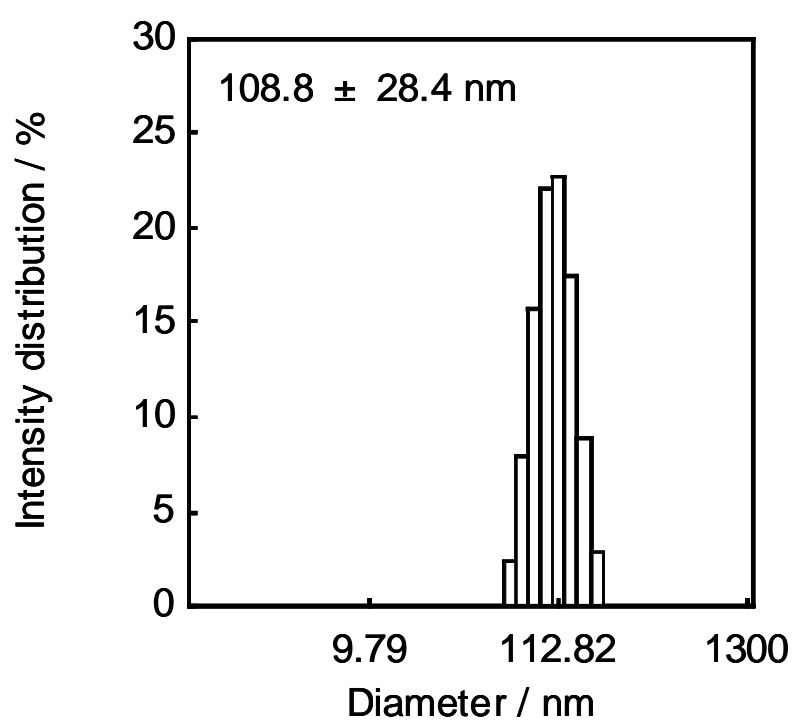


Figure 5-10. Diameter histogram of $M_{14}N_{450}M_{14}$ nanoparticles with PAMAM dendrimer in aqueous solutions at pH = 8 after purification; $T = 21^\circ\text{C}$.

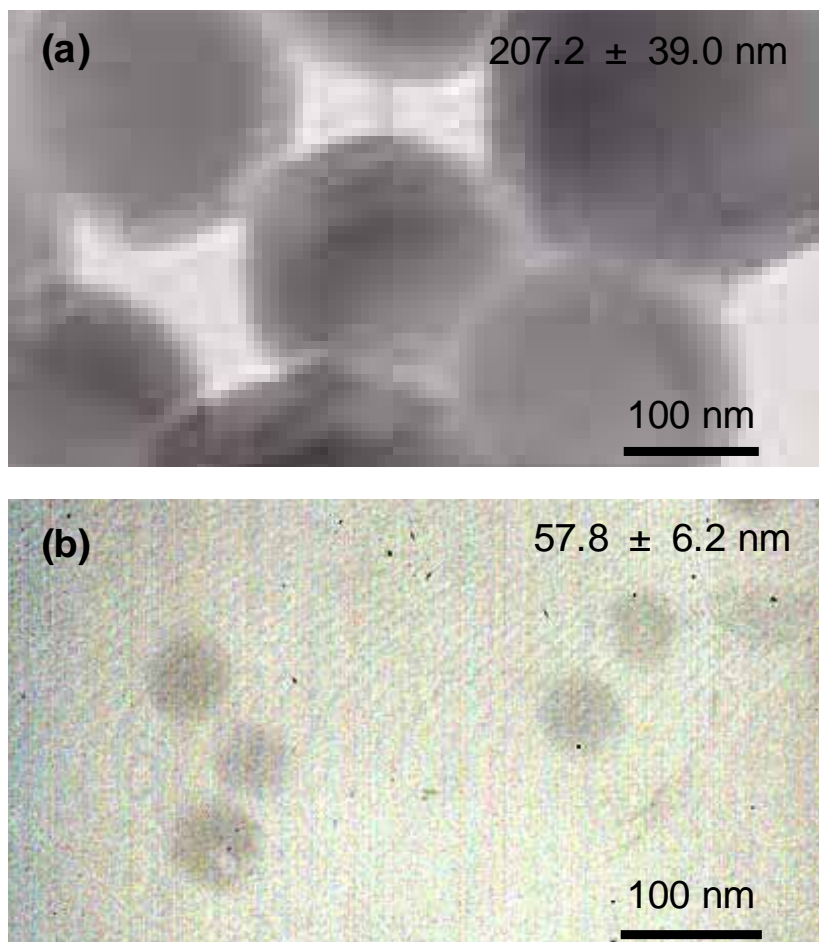


Figure 5-11. TEM images of the nanoparticles derived from $N_{166}M_{10}$ (a) and $M_{14}N_{450}M_{14}$ (b) with PAMAM dendrimer in aqueous solutions at pH = 8 after purification; $T = 21\text{ }^{\circ}\text{C}$.

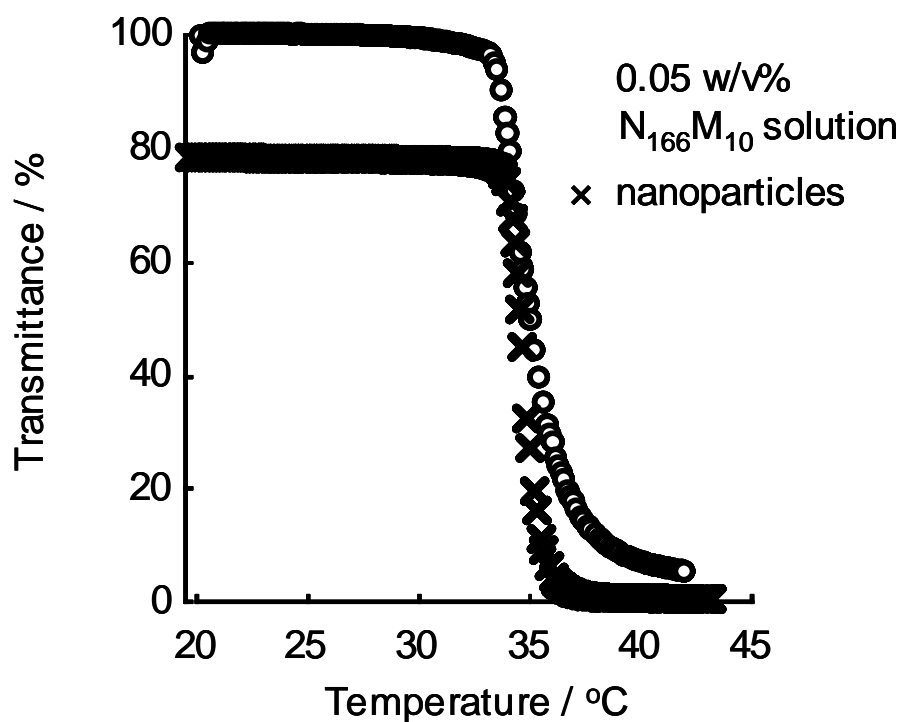


Figure 5-12. Transmittance measurements of N₁₆₆M₁₀ solution (circles) and N₁₆₆M₁₀ nanoparticles with PAMAM dendrimer (crosses) at pH = 8 ; polymer concentration = 0.05 w/v%, programming rate = 1 °C/min.

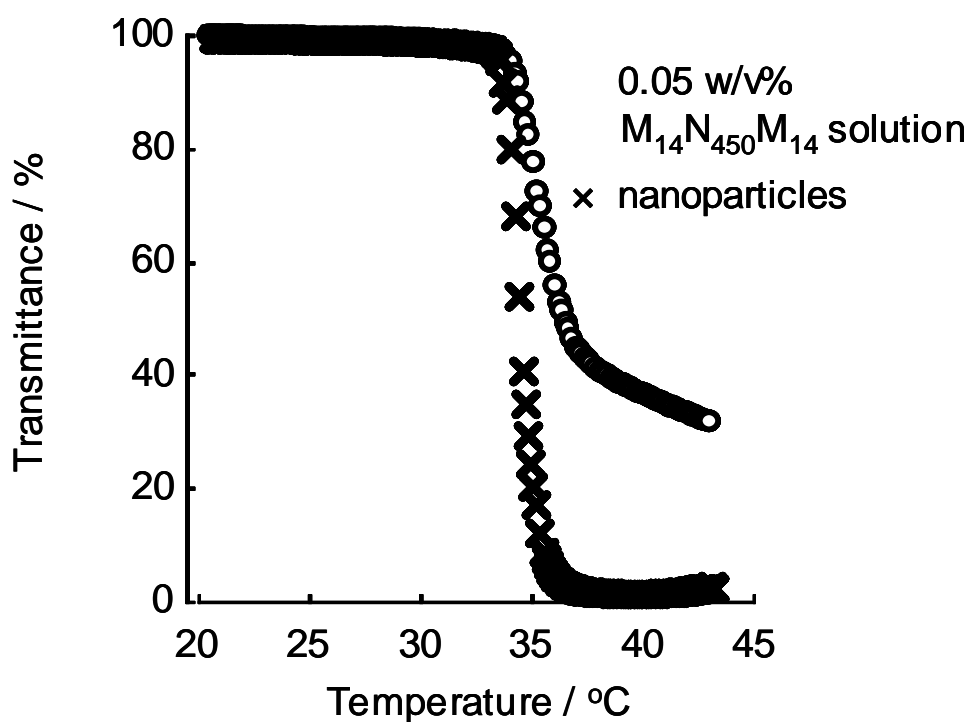


Figure 5-13. Transmittance measurements of M₁₄N₄₅₀M₁₄ solution (circles) and nanoparticles derived from M₁₄N₄₅₀M₁₄ with PAMAM dendrimer (crosses) at pH = 8 ; polymer concentration = 0.05 w/v%, programming rate = 1 °C/min.

5-11. They supported the round-shaped complexes and the reasonable sizes of the complexes. The sizes obtained from TEM were smaller than those from DLS because of drying of samples.

5.4.4 Thermo-Responsive Profiles of the Nano-Sized Complexes.

We examined the thermo-responsive profiles of prepared nano-sized complexes in aqueous solutions by turbidity measurements and the results are shown in Figure 5-12 and 5-13. The nano-sized complexes with and without stabilization shows the sensitive responsive behavior. The cloud points are the almost same as that of the starting $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ themselves. In some case, cloud points of the thermo-responsive polymer were changeable by the introduction of hydrophilic or hydrophobic moieties.²¹ For example, the introduction of hydrophobic moieties tends to shift to lower temperature. In this study, we observed no change in the cloud points and it might be due to the relatively hydrophilic nature of the amide bonding and dendrimer surface.

5.5 Conclusions

We succeeded in preparation of the nano-sized complexes comprised of the stimuli-responsive $N_{166}M_{10}$ and $M_{14}N_{450}M_{14}$ and dendrimers by the electrostatic interaction. Interestingly, the sizes of the obtained complexes were different depending on the types of starting block copolymers. The nano-sized complexes derived from $M_{14}N_{450}M_{14}$ had smaller size than that

from diblock copolymers. It might be due to the difference in the complex formation process. Furthermore, we could improve the stability by simple reaction. The stabilized nano-sized complexes were not disrupted in higher pH. Moreover, these obtained nano-sized complexes exhibited sensitive thermo-responsive properties.

The polymer modification with stimuli-response in this study can surely contribute to the bio-conjugate chemistry and drug delivery system because of not only the easy preparation but also the size and charge control of the complexes. In near future, we will report the complex formation process in detail.

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Concluding Remarks

The objective of this thesis is the preparations and characterizations of aggregates and nano-complexes derived from stimuli-responsive ABA triblock copolymers. The stimuli-responsive ABA triblock copolymers as a component exhibit the aggregation behaviors in response to external stimuli. Those comprised of the anionic electrolytic segments, form complexes with cationic compounds. The results obtained through this study are described in outline as follows.

In chapter 2, the stimuli-responsive ABA triblock copolymers were prepared by atom transfer radical polymerization and controlled molecular weight with low molecular weight distribution. Those were able to be prepared with predetermined molecular weight depending on concentration of an initiator. The stimuli-responsive ABA triblock copolymers exhibited behaviors depended on the characters of each segments in aqueous solutions. Those possessed thermo-responsive property of PNIPAAm in a middle block, in spite of the introduction of hydrophobic *Pt*BMA segments or hydrophilic PMAA segments in end blocks.

Chapter 3 describes the aggregation behaviors of stimuli-responsive triblock copolymers in response to external stimuli. A stimuli-responsive ABA triblock copolymer consisting of *Pt*BMA of thermo-responsive segment and PMAA of hydrophobic segments in end blocks, *Pt*BMA-*b*-PNIPAAm-*b*-*Pt*BMA changed aggregation conformation in response to temperature. PMAA-*b*-PNIPAAm-*b*-PMAA were composed of

pH-responsive PMAA and PNIPAAm, and controlled to invert a core and a shell of aggregates depending on both pH and temperature. Those are able to be controlled the aggregation conformations in response to external stimuli.

In Chapter 4, preparation and characterization of complex for PMAA-*b*-PNIPAAm-*b*-PMAA and silica particles with amino groups conducted by surface modification. It was prepared by stepwise monolayer deposition of them with QCM substrate. We observed the formation of complex layer by electrostatic interaction between PMAA-*b*-PNIPAAm-*b*-PMAA and silica particles with amino groups. Those depended on molecular weight of PMAA-*b*-PNIPAAm-*b*-PMAA as a component. The obtained complex layer exhibited thermo-responsive properties of PNIPAAm segments on surface.

Chapter 5 describes the preparation and aggregation behavior of nano-complex by electrostatic interaction between PMAA-*b*-PNIPAAm-*b*-PMAA and cationic compound as multivalent metal ions or PAMAM dendrimer. It was difficult to form complex of PMAA-*b*-PNIPAAm-*b*-PMAA and multivalent metal ions since PMAA segments were too short to stabilize complex. However, nano-complex with PAMAM dendrimer was formed with many binding sites for electrostatic interactions. It was used PNIPAAm-*b*-PMAA as AB diblock copolymer to examine the effects of polymer structures for nano-complex formations. Nano-complex derived from PNIPAAm-*b*-PMAA exhibited association like aggregation behavior, which is a diameter change of aggregate depending on PAMAM

concentration. On the other hands, that from PMAA-*b*-PNIPAAm-*b*-PMAA which a micelle like aggregation behavior was independent on it. We were able to prepare and control a complex with various conformations and stimuli-responsive properties by those methods.

List of Publications

Chapter 2:

Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “Preparations and Characterizations of Well-Defined Stimuli-Responsive ABA Triblock Copolymers by Atom Transfer Radical Polymerization”, *in preparation*.

Chapter 3:

Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “Investigations of Aggregation Behavior of Stimuli-Responsive ABA Triblock Copolymers in Response to External Stimuli”, *in preparation*.

Chapter 4:

Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “Preparations and Characterizations of Organic-Inorganic Multilayer Films Consisting of Stimuli-Responsive ABA Triblock Copolymers and Silica Particles with Amino Groups”, *in preparation*.

Chapter 5:

Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “Preparations and Characterizations of Nano-Sized Complexes Consisting of Stimuli-Responsive Block Copolymers and PAMAM dendrimers”, *Transactions of the Materials Research Society of Japan, in press*.

Other Publications

Tatuo Kaneko, Kazuhiro Hmada, Yumi Kuboshima and Mitsuru Akashi
“Reversible Thermoresponsive Aggregation/Deaggregation of
Water-Dispersed Polymeric Nanoparticles Exhibiting Structural
Transformation ” *Langmuir* **2005**, *21*, 9698-9703

Youhei Kotsuchibashi, Yumi Kuboshima, Kazuya Yamamoto and Takao
Aoyagi “Synthesis and Characterization of Double Thermo-Responsive
Block Copolymer Consisting N-isopropylacrylamide by Atom Transfer
radical Polymerization” *Journal of Polymer Science Part A: Polymer
Chemistry*, *submitted*.

List of Presentations

1. Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi
“Three-Dimensional Structures Composed of ABA Triblock Copolymers Prepared by ATRP”, **54th SPSJ Annual Meeting**, May 2005, Kanagawa, Japan.
2. Kazuya Yamamoto, Keiko Ide, Yumi Kuboshima, and Takao Aoyagi
“Stimuli-Responsive Properties of Polyacrylamide Block copolymers Synthesized by Atom Transfer Radical Polymerization”, **The 8th SPSJ International Polymer Conference**, July 2005, Fukuoka, Japan
3. Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi *“Dimensional Structures Prepared by Stimuli-Responsive ABA Triblock Copolymer”*, **54th SPSJ Symposium on Macromolecules**, September 2005, Yamagata, Japan.
4. Daisuke Matsukuma, Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi *”Stimuli-Responsive Properties of Multilayer Thin Films Dependent on Intramolecular Structure”*, **54th SPSJ Symposium on Macromolecules**, September 2005, Yamagata, Japan.
5. Kazuya Yamamoto, Daisuke Matsukuma, Yumi Kuboshima and Takao Aoyagi *”Stimuli-Responsive Properties of Multilayer Thin Films*

Dependent on Intramolecular Structure”, **14th SPSJ Polymer Material Forum**, November 2005, Tokyo, Japan.

6. Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “*Preparation and Characterizations of Stimuli-Responsive ABA Triblock Copolymers*”, **55th SPSJ Symposium on Macromolecules**, September 2006, Toyama, Japan.
7. Kazuya Yamamoto, Youhei Kotsuchibashi, Yumi Kuboshima and Takao Aoyagi “*Preparation and Characterizations of Stimuli-Responsive ABA Triblock Copolymers*”, **55th SPSJ Symposium on Macromolecules**, September 2006, Toyama, Japan.
8. Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “*Preparation and Aggregation Behavior of Stimuli-Responsive Block Copolymers*”, **56th SPSJ Annual Meeting**, May 2007, Kyoto, Japan.
9. Youhei Kotsuchibashi, Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “*Preparation of Double Thermo-Responsive Block Copolymer by ATRP*”, **56th SPSJ Annual Meeting**, May 2007, Kyoto, Japan.
10. Yumi Kuboshima, Youhei Kotsuchibashi, Kazuya Yamamoto and Takao Aoyagi “*Preparation and Characterizations of Architectures Constructed of Stimuli-Responsive block Copolymers*”, **56th SPSJ**

Symposium on Macromolecules, September 2007, Nagoya, Japan.

11. Youhei Kotsuchibashi, Yumi Kuboshima, Kazuya Yamamoto and Takao Aoyagi “*Estimation of Solution Behavior of AB Type Block Copolymers Consisting Different Thermo-Responsive Segments*” **56th SPSJ Symposium on Macromolecules**, September 2007, Nagoya, Japan.

12. Yumi Kuboshima, Kazuya Yamamoto, and Takao Aoyagi “Preparations and Characterizations of Nano-Complexes Consisting of Stimuli-Responsive Block Copolymers and PAMAM dendrimers”, **18th MRS-J Symposium**, December 2007, Tokyo, Japan

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