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Dual-Temperature and pH Responsive (Ethylene Glycol)-Based Nanogels via Structural Design

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Abstract

We report here the preparation of dual-temperature and pH responsive (ethylene glycol)-based nanogels via the reversible addition-fragmentation chain transfer (RAFT) polymerization under aqueous conditions. The core-shell structures of the nanogels were composed of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA), oligo(ethylene glycol) methacrylate (OEGMA), and di(ethylene glycol) dimethacrylate as cross-linker. Both the core and shell structures of the nanogels showed lower critical solution temperature (LCST). Interestingly, the LCST of the shell was strongly affected by the solution pH and salt concentration due to the presence of carboxyl groups at the nanogel surface. These dual temperature and pH responsive nanogels are expected to be non-toxic and therefore can be used as novel biomaterials for drug delivery applications.

Introduction

It is well known that the architectures (linear, branched, cyclic, network) of polymeric materials dictate the physicochemical properties even though they have the same chemical compositions.[1] Over the last decade, polymeric materials with excellent control over molecular weight, composition, structure, functionality, and conjugation have been made simpler with the development of unique synthetic such as controlled/living radical polymerization (CLRP) and click chemistry.[2,3] Smart polymers are polymers with stimuli-responsive properties (*e.g.* temperature, pH, salt concentration, light, and molecules) and have been actively produced using a combination of these synthetic techniques and applied in a wide range of fields.[4] The biomedical applications of these 'smart' polymers have been the most popular area. Living systems have very complex metabolism, and therefore designing 'smart' polymeric materials that can mimic biological entities in the living system is of great interest.[4(a),5]

Poly(ethylene glycol) (PEG) is a well-known polymer, FDA-approved and extensively used in the fields of biotechnology and biomedicine.[6] These PEG polymers are generally cheap, uncharged, water-soluble, non-toxic and non-inflammatory. In addition, the circulation time of biological macromolecules such as proteins and nucleic acids is prolonged by PEGylation.[4b] Nanoparticles

formulation, such as micelles and gels having PEG chains, are used as drug carriers because of their high stability in blood and these nanoparticles are found to eventually accumulate at cancer site by enhanced permeability and retention (EPR) effect and the reticuloendothelial system (RES) due to their long circulation time and desirable sizes.[7]

Some (ethylene glycol)-based polymers have been found to show reversible hydration/dehydration behavior in aqueous solution (*i.e.* temperature responsive property) at lower critical solution temperature (LCST) which depends on the number of side chain of ethylene glycol in the monomer unit.[8] The LCSTs are increased via increasing chain lengths of the hydrophilic ethylene glycol. Copolymerization with monomers having different numbers of ethylene glycol can also control the LCSTs.[9]

Recently, block copolymers having two LCSTs ($LCST_1$<$LCST_2$) in the structures have been prepared.[1d,1e,10] These dual-temperature responsive block copolymers dissolve in aqueous solution at temperatures below $LCST_1$ because of their hydrophilic-hydrophilic nature ($T<LCST_1$). On the other hand, the property of the block copolymers turns amphiphilic when the temperature is between the two LCSTs ($LCST_1<T<LCST_2$) and they revealed to form core-shell type micelles. Finally, the shell of the micelles also turns hydrophobic when the temperature is greater than $LCST_2$ ($LCST_2<T$). Amphiphilic block copolymers generally require organic

solvents for their micelle formation and incorporation of hydrophobic drug into the core. By controlling the temperature, these dual-temperature responsive block copolymers are expected to execute micelle-formation, micelle-collapse, drug loading, and drug release without using additional chemicals. However, there is still the possibility in improving the design to have structural stability below the critical micelle concentration (cmc) and hence better drug loading ability.[11]

Nanogels (NGs) are cross-linked structures formed by covalent bonds, hydrophobic interaction, electrostatic interaction, and coordinate bond, and they have been used as carriers due to their high stability, high drug loading capacity, and for their ability to incorporate various materials such as peptides, proteins, nucleic acids, and inorganic particles.[12] Cai *et al.* prepared micro/nanogels consisting of P(MEO_2MA-*co*-OEGMA) by free radical emulsion polymerization with surfactants as stabilizer.[13] Surfactant is generally considered toxic, which has to be removed from polymeric materials via dialysis and/or centrifugation before use as biomaterials. Reversible addition-fragmentation chain transfer (RAFT) polymerization has been employed extensively in the preparation of biomaterials since they do not require the use of toxic metal ions.[2d,2e,14] Using hydrophilic macro-chain transfer agent (macro-CTA) initially polymerized via the RAFT process, the preparation of NGs in one step is now possible in aqueous solution without the use of surfactants.

Examples of macro-CTAs used in NGs synthesis are poly(dimethylacrylamide) (PDMA),[15a] poly(ethylene oxide) (PEO),[15b] poly(glycerol monomethacrylate) (PGMA),[15c] and poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC).[16] Therefore, NG has high prospects to be applied as biomaterials due to their easy structural/functional design, bioinert polymerization conditions, and versatile encapsulations.

Very recently, Shen *et al.* prepared (ethylene glycol)-based NGs using macro-CTA of PEG and POEGMA in aqueous solution at 70 °C and their stabilities in salt and proteins solution from few days to several months have been evaluated.[17] The NGs can display two LCSTs are by P(MEO_2MA-*co*-OEGMA) core and POEGMA shell (LCST 90 °C) [9b], respectively. The LCST of the shell is however too high for any potential use in drug loading/release in biological conditions. Reaction temperature of normal RAFT polymerization is usually within 60~70 °C with thermal initiators such as 2,2'-azobisisobutyronitrile (AIBN) and 4,4'-azobis-4-cyanovaleric acid (ACVA) (10 hrs. half-life temperature; AIBN = 65 °C, ACVA = 68 °C). In aqueous solution, macro-CTA having LCST below the reaction temperature will precipitate due to the hydrophobic interaction before they form clear NG structures. It is therefore a challenge to prepare dual-temperature responsive NGs with polymers having LCSTs (<60~70 °C) in aqueous solution. RAFT polymerization has been

reported at room temperature with redox-initiator,[18a,b] photo-initiator,[18c-e] and radical initiator with low half-life temperature[18f-i]. Water soluble radical initiators which can decompose at low temperature, such as 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70)[18g] and 4,4'-Azobis[2-(imidazolin-2-yl)propane] dihydrochloride (VA-044),[18h,i] are particularly convenient for room temperature RAFT polymerization. VA-044 (half-life at 25 °C: ~130 h)[19] is previously used to prepare bioconjugates of bovine serum albumin (BSA)-polymer (polymers: poly(*N*-isopropylacrylamide)(PNIPAAm)[19] and PNIPAAm-*b*-poly(*N*,*N*-dimethylacrylamide)(PDMA)[18i]) by grafting-from RAFT polymerization in aqueous solution at 25 °C.

In this study, dual-temperature and pH responsive (ethylene glycol)-based NGs are synthesized by RAFT polymerization in aqueous solution at 37 °C having different compositions without the use of surfactant (Figure 1). Macro-CTAs (shell) are prepared at different polymer chain lengths but their LCSTs are set around 55 °C. Structures of the NGs were strongly affected by their synthetic conditions such as macro-CTAs chain length, composition of the core structure, and cross-linker contents. Also, NGs showed good re-dispersion in aqueous solution after freeze-drying. Interestingly, NGs were found to show pH responsive properties due to the presence

of carboxylic acid groups on the surface of the NGs. To the best of our knowledge, this is the first report for (ethylene glycol)-based NGs with double LCSTs and pH responsive behavior. The NGs are analyzed by a range of techniques including ^{1}H nuclear magnetic resonance (^{1}H NMR), gel permeation chromatography (GPC), transmittance measurement, dynamic light scattering (DLS), and transmission electron microscopy (TEM).

(Figure 1)

Experimental

Materials

2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA), oligo(ethylene glycol) methacrylate (OEGMA, M_n = 475 g/mol), and di(ethylene glycol) dimethacrylate (cross-linker: CL) were purchased from Sigma-Aldrich and purified by passing through basic alumina column. Initiator, 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) were purchased from Wako and purified by recrystallization from methanol. 4-Cyanopentanoic acid dithiobenzoate (CTP) was synthesized and purified according to the protocol given in previous reports.[20] All other chemicals and solvents were used as received. Distilled water used in this study was purified with a Millipore Milli-Q system.

Preparation of P(MEO_2MA-*co*-OEGMA)s as macro-chain transfer agents (macro-CTAs) with different chain lengths

P(MEO_2MA-*co*-OEGMA)s are macro-CTAs were prepared with different chain lengths by reversible addition-fragmentation chain transfer (RAFT) polymerization. A typical polymerization method of P(MEO_2MA-*co*-OEGMA) was shown below (Scheme 1(A)). MEO_2MA (1.78 g, 9.48 mmol), OEGMA (1.20 g, 2.52 mmol), CTP (44.6 mg, 0.16 mmol), and 4,4'-azobis-4-cyanovaleric acid (ACVA) (17.6 mg, 6.40×10^{-2} mmol) ($[MEO_2MA]_0/[OEGMA]_0/[CTP]_0/[ACVA]_0$ = 59.3/15.8/1/0.4) were dissolved in 4 mL methanol. After degassing with nitrogen gas for 30 min, the mixture was allowed to polymerize for 6 h at 60 °C. The polymerization container was soaked into liquid nitrogen to stop the reactions. The resulting P(MEO_2MA-*co*-OEGMA) was purified by reprecipitation using diethyl ether and was dried under reduced pressure. The collected high viscous P(MEO_2MA-*co*-OEGMA) is used as macro-CTA to polymerize nanogels. Chain lengths of P(MEO_2MA-*co*-OEGMA)s were controlled by changing ratios of $[monomers]_0/[CTA]_0/[ACVA]_0$.

Preparation of (ethylene glycol)-based nanogels (NGs)

Using the polymerized P(MEO_2MA-*co*-OEGMA)s as macro-CTAs, nanogels (NGs)

were prepared at 37 °C with various conditions such as solvent, differential macro-CTAs, core amounts, and cross-linker contents to obtain a well-defined structure (**NG1**~**11**). A typical polymerization method of NG (*i.e.* **NG11**) was shown below (Scheme 1(B)). Macro-CTA of P(MEO_2MA-*co*-OEGMA) (114 mg, 3.96×10^{-3} mmol), MEO_2MA (119.3 mg, 0.63 mmol), cross-linker (CL) of di(ethylene glycol) dimethacrylate (38.4 mg, 0.16 mmol), and VA-044 (4.27×10^{-1} mg, 1.32×10^{-3} mmol) ($[MEO_2MA]_0/[CL]_0/[\text{Macro-CTA}]_0/[\text{VA-044}]_0$ = 160/40/1/0.33) were dissolved in a 5 mL water/2-propanol (1/4 (mL)) solution at 0 °C. After degassing with nitrogen gas for 30 min at 0 °C, the mixture was allowed to polymerize for 20 h at 37 °C. The polymerization container was soaked into liquid nitrogen to stop the reactions. NG was purified by dialysis (MWCO: 12~14 kDa) in distilled water for 3 days at 4 °C and was collected by freeze-drying (Yield: 54%).[21] Preparation conditions of other NGs (**NG1**~**10**) were shown in supporting information.

Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra of polymers were recorded with a 500 MHz Varian spectrometer to confirm the chemical composition of the synthesized polymers.

The molecular weight and polydispersity of polymers were determined by gel

permeation chromatography (GPC) with I-MBLMW-3078 and I-MBHMW-3078 columns and Viscotek model 250 dual detector. As eluent, 10 mM LiBr DMF was used through a flow rate of 1.0 mL/min. Calibration curve was calculated by five monodispersed PEO standards.

Transmission electron microscopy (TEM) images were obtained on a transmission electron microscope Morgaghi 268 (Fei) with an accelerating voltage of 80 kV. The samples were prepared by adding a drop of NG suspension on carbon coated copper grids and were allowed to dry before analysis.

Transmittance change of polymers and NGs were measured by irradiation of light (wavelength 500 nm) using UV-Vis spectrometer (V-630, JASCO). Lower critical solution temperature (LCST) was defined at the temperature with 50 % of transmittance in this study.

Dynamic light scattering (DLS) was performed with a ZetaPlus-Zeta Potential Analyzer (Brookhaven Instruments Corporation) at a scattering angle θ = 90°. All samples were kept in a given temperatures to reach the equilibrium prior to the measurements.

Results and discussion

Preparation of poly(2-(2-methoxyethoxy)ethyl methacrylate-*co*-oligo(ethylene

glycol) methacrylate) (P(MEO_2MA-*co*-OEGMA)) as macro-chain transfer agents (macro-CTAs) with different chain lengths

Poly(2-(2-methoxyethoxy)ethyl methacrylate-*co*-oligo(ethylene glycol) methacrylate) (P(MEO_2MA-*co*-OEGMA)) were synthesized by reversible addition-fragmentation chain transfer polymerization (RAFT) and used as macro-chain transfer agents (macro-CTAs) (Scheme 1(A)). CTAs (or RAFT agents) are composed of Z-C(=S)S-R structures, and the Z and R substituents strongly affect not only polymerization but also properties of polymers.[22] The -C(=S)S-R located at the polymer chain ends, for instance, has been applied to thiol-ene click chemistry for its easy modification.[3b,23] Polymer chain ends having the Z groups located on the nanogel (NG) surface were of interest. 4-Cyanopentanoic acid dithiobenzoate (CTP) having a carboxylic acid group is used as chain transfer agent. Lower critical solution temperature (LCST) of P(MEO_2MA-*co*-OEGMA)s is easily controlled by the polymer compositions.[9b] Content of OEGMA in copolymers was adjusted to 21 mol% to have the LCSTs around 55 ℃. Table 1 shows characterization of the three polymers, P(MEO_2MA-*co*-OEGMA)s, with different chain lengths. OEGMA contents in the copolymers were 20.8, 20.6, and 20.3 mol% respectively, which were close to that of the feed compositions. The chain length is found to be an important factor when the copolymers work as the shell in NGs. Molecular weights (M_n) of

P(MEO_2MA-*co*-OEGMA)s were determined by GPC, and they were found to be 12,200, 20,900, and 28,900 g/mol, respectively, with relatively narrow molecular weight distributions (M_w/M_n: 1.27~1.34). Using the OEGMA content and molecular weight, the composition of the copolymers are P(MEO_2MA_{38}-*co*-$OEGMA_{10}$), P(MEO_2MA_{66}-*co*-$OEGMA_{17}$), and P(MEO_2MA_{92}-*co*-$OEGMA_{24}$), respectively. These copolymers have many ethylene glycol units in their structures which could be useful in biological systems.[17] The calculated weight-percent of ethylene glycol units in the P(MEO_2MA-*co*-OEGMA)s were 64~65 wt% and these high ethylene glycol contents are expected to lead high biocompatibilities to NGs. All copolymers were dissolved in milliQ water at 0.1 wt% and their transmittances were found to decrease rapidly with increasing solution temperature (Figure S1). The LCSTs of P(MEO_2MA_{38}-*co*-$OEGMA_{10}$), P(MEO_2MA_{66}-*co*-$OEGMA_{17}$), and P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) were 56.7, 56.9 and 55.2 ℃, respectively. LCST is defined at the temperature with 50% of transmittance in this study.

(Scheme 1, and Table 1)

Preparation of NGs with different compositions

Emulsion polymerization has been employed for the preparation of polymers and polymeric particles, which usually require the addition of surfactants as stabilizers.

These surfactants are often toxic and can be hard to remove after the polymerization by dialysis and/or centrifugation. As surfactant-free emulsion polymerization, other stabilizers such as ionic initiators and hydrophilic monomers have been used.[24] Using an amphoteric initiator, Fang *et al.* successfully prepared copolymers consisting of styrene and acrylamide by emulsion polymerization in absence of surfactants.[25] Recently, hydrophilic macro-CTAs have been used in the preparation of NGs by RAFT polymerization without the addition of surfactants.[15,16,21] For the polymerization, a mixture solution of water and alcohol is selected in the preparation of NGs. As the LCSTs of synthesized P(MEO_2MA-*co*-OEGMA)s (*i.e.* macro-CTAs) were 55.2~56.9 °C, it is difficult to polymerize clear NGs in aqueous solutions at temperature over the LCSTs due to their dehydration/aggregation behavior. Typical radical initiators such as AIBN and ACVA, however, are allowed to polymerize at 60~70 °C. Convertine *et al.* successfully achieved the preparation of di- and tri-block copolymers by RAFT polymerization at 25 °C using a water-soluble radical initiator VA-044 (half-life at 25 °C: ~130 h).[18h] The VA-044 is also used to modify proteins with copolymers by grafting-to method for the bio-inert synthesis conditions.[18i,19] In this study, (ethylene glycol)-based NGs was polymerized in water/2-propanol solution using VA-044, macro-CTAs, MEO_2MA, and cross-linker (CL) (Scheme 1(B)). As the CL, di(ethylene glycol) dimethacrylate was selected to

maintain ethylene glycol unit in the gel structures. P(MEO_2MA) homopolymer is known to show its LCST around 28 °C.[9b] The (ethylene glycol)-based NGs, therefore, were expected to have two LCSTs ($LCST_1$ and $LCST_2$) in both core and shell. First, **NG1**~**NG11** were prepared under different polymerization conditions (solvents, chain length of macro-CTAs, amount of core units, and CL contents) to investigate the relationship between these conditions and NG formation.

Characterization of PEG-based NGs

Table 2 shows diameters of NGs measured by DLS at 15, 37, and 65 °C. P(MEO_2MA_{38}-*co*-$OEGMA_{10}$) was used as macro-CTA to polymerize **NG1**~**NG4**. **NG1** and **NG2** were prepared at same condition except mixture ratios of polymerization solutions (water/2-propanol = 1/4 or 4/1 v/v). Diameters of **NG1** were 166 ± 7, 193 ± 6, and 137 ± 2 nm at 15, 37, and 65 °C, respectively. On the other hand, NG2 was observed to precipitate during the polymerization. Diameters of **NG3** and **NG4** with 5 mol% of cross-linker were also affected by the water/2-propanol mixed ratios. In TEM images, interestingly, nanoparticles (< 10 nm) were observed at **NG1** and **NG3** (Figure 2(a) and 2(b)). Typically, nanomaterials show aggregative properties with decreasing the diameters for their high surface areas.[26] The Large diameters measured by DLS might be occurring from

aggregation of the small nanoparticles. Moreover, the presence of water in the polymerization solution may help in the formation of uniform NG structures. Therefore, a mixture ratio of water/2-propanol (1/4) was used in the nanogels (**NG5**~**NG11**) synthesis. Ratio of [macro-CTA]/[VA-044] was also changed from 2.5 to 3 for preparation of **NG5**~**NG11** because of long chain length of the macro-CTAs. **NG5**~**NG8** were prepared using P(MEO_2MA_{66}-*co*-$OEGMA_{17}$) as macro-CTA at different core amounts and cross-liker contents. The diameters of **NG5**~**NG8** were 204 ± 2 (PDI 0.16), 117 ± 24 (0.344), 211 ± 3 (0.211), and 104 ± 2 (0.11) nm, respectively at 15 °C. In TEM images of **NG5** and **NG7**, nanoparticles and the aggregation were observed (Figure 2(c) and 2(e)). **NG6** was observed to form nanoparticles by TEM image (Figure 2(d)). However, it was also found to have small amount of aggregates. The diameter of **NG6** were found to decrease from 96 ± 1 (0.184) to 73 ± 13 nm (0.247) by increasing solution temperature at 37 and 65 °C, respectively. Interestingly, although LCST of shell P(MEO_2MA_{66}-*co*-$OEGMA_{17}$) was 56.9 °C (Table 1), **NG6** was found to maintain its high stability at 65 °C. The stabilities over their LCSTs are discussed later. **NG8** was observed to have clear nanoparticles by TEM (Figure 2(f)). Size of polymeric materials measured by dry state is usually small as compared to that of swollen state in solution.[1d] The diameters of **NG8** were found to increase from 139 ± 1 (0.138) and 182 ± 6 (0.163) by

increasing solution temperature from 37 to 65 °C, respectively. The aggregation at 37 °C (below LCST of shell) suggests amount or chain length of the hydrophilic P(MEO_2MA_{66}-*co*-$OEGMA_{17}$) at shell is not enough to stabilize the hydrophobic core. **NG9**~**NG11**, were therefore, polymerized using P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) with longer chain length. **NG10** showed large PDI (0.388) at 15 °C. On the other hand, the diameters of **NG9** and **NG11** were 280 ± 5 (0.096) and 264 ± 5 (0.121), respectively with relatively narrow PDI at 15 °C and their structures were spherical as shown in Figure 2(g) and 2(h). Moreover, the diameter of **NG9** was found to decrease to 256 ± 2 (0.090) with narrow PDI at 37 °C (Figure S2). This result suggests that the hydrophobic core of the NG is completely covered with hydrophilic P(MEO_2MA_{92}-*co*-$OEGMA_{24}$), and the diameter reduction at 37 °C is expected to be due to dehydration of the core part. The calculated swelling ratio of **NG9** between 15 and 37 °C was 1.1, which is close to what other researchers found.[17] At 65 °C, the diameter of **NG9** decreases with increasing PDI (244 ± 18 nm (0.173)), and the non-single peak of DLS suggested aggregation (data not shown). The **NG9**, however, was found to keep its high stability visually in water at 65 °C with no precipitation. When the shell was composed of P(MEO_2MA_{66}-*co*-$OEGMA_{17}$) with lower CL content (10 mol%) spherical NG structures was obtained. For P(MEO_2MA_{92}-*co*-$OEGMA_{24}$), on the other hand, 20 mol% of CL was found to be

optimal in the formation of NGs. It was noted, however, that increasing the chain length of macro-CTAs, core compositions, and CL contents tends to lead NGs with large diameters. Polymerization time of macro-CTA is also important factor to in controlling the NGs structures. All macro-CTAs of P(MEO_2MA-*co*-OEGMA)s used were polymerized for 6h. As a control experiment, a P(MEO_2MA-*co*-OEGMA) (M_n = 31,200 g/mol, M_w/M_n = 1.34, OEGMA content = 20.4 mol%) was polymerized for 24 h and was used to prepare NGs at different conditions. However, all NGs were observed to aggregate at 37 °C, which shows that the hydrophobic core was not completely covered with the P(MEO_2MA-*co*-OEGMA). There may be loss of the terminal RAFT agent at the end of the polymer chain due to the long reaction time.[16] These results could explain why the **NG9** showed high stability at temperature above the LCST of the shell.

(Table 2, and Figure 2)

Temperature responsive properties of NG9

Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most studied temperature responsive polymer has a LCST around 32 °C.[27] PNIPAAm shows generally strong dehydration as compared to that of P(MEO_2MA-*co*-OEGMA)s having similar LCST.[28] Sugihara *et al.* prepared poly(2-methoxyethyl vinyl ether)

(PMOVE)-*b*-PNIPAAm block copolymer and their ΔH was measured by differential scanning calorimetry (DSC) were 27.0 (PNIPAAm block) and 9.7 J/g (PMOVE block), respectively.[29] The weak dehydration of (ethylene glycol)-based polymers leads their slight hysteresis at heating-cooling cycle in aqueous solution.[30] In this study, P(MEO_2MA-*co*-OEGMA)s, however, showed sensitive transmittance change due to the dehydration with increasing solution temperature (Figure S1). Therefore, NGs consisting of P(MEO_2MA-*co*-OEGMA)s as shell ought to aggregate with each other over their LCST. Although LCSTs of P(MEO_2MA-*co*-OEGMA)s were thought to be relatively insensitive to polymer concentration, the latter was still an important factor.[30] LCSTs of temperature responsive polymers are usually increased with decreasing concentration.[31] In the case of the modification of substrates with high polymer density, however, the LCSTs were found to decrease in contrast to that of the free polymers with same concentration by their topical high concentration. Xu *et al.* prepared dual-temperature responsive block copolymer from hyperbranched polyester (HBP-PNIPAAm-*b*-poly(2-(dimethylamino)ethyl methacrylate) (PDMA, LCST 40~50 °C)).[10c] The dehydrations of PNIPAAm and PDMA on HBP were noted due to a diameter change and the decrease in size was found to start from 20 to 35 °C, and the LCSTs are lower than LCSTs of the individual homopolymers. Figure 3 shows transmittance change of **NG9** as a function of temperature at 0.1 and

0.3 wt% in water. By the suspension of the **NG9**, the initial transmittance was found to be 78.03% at 0.1 wt% and 35.05% at 0.3 wt% (*i.e.* Tyndall phenomenon). At 0.1 wt%, transmittance was slightly decreased between 20~35 °C due to the dehydrated core. The LCST of P(MEO_2MA) was previously reported to be approximately 28 °C.[9(b)] However, the transmittance was found to be lower at 20 °C which is lower than the LCST of P(MEO_2MA). Such behavior is probably due to the cross-linked nature of the core at relatively high concentration. Transmittance change by dehydrated shell was not observed clearly during the increase in temperature to 75 °C. On the other hand, at 0.3 wt%, another transmittance decreasing was also observed at 65 °C. The temperature was higher by approximately 10 °C than the original LCST of 55.2 °C. These results suggest that although the shell P(MEO_2MA-*co*-OEGMA)s turned their properties to hydrophobic, NGs show no (or slight) aggregation to each other. In our previous study, mixed micelles consisting of hydrophilic and hydrophobic polymers in the shell at different contents were prepared and it was found that the hydrophilic polymers prevent aggregation of the micelles.[10f,10g] Therefore, next the carboxylic acid groups on the surface of the NGs which can influence the property of the nanogels with pH are evaluated.

(Figure 3)

Multi-stimuli responsive properties of NG9

It is well known that the properties of polymers are strongly affected by the end groups. Duan *et al.* polymerized PNIPAAm ($M_{n,MS}$ = 3,000, 3,400, 4,200, and 5,000 g/mol) with hydrophobic pyrene at end of the polymer chain and the LCSTs were dramatically decreased (21.7, 24.8, 26.3, and 29.3 °C) with decreasing molecular weights.[32] The synthesized NGs have carboxylic acid groups on the surface (Scheme 1). Therefore, it is hypothesized that the presence of these carboxylic acid groups should in principle prevent the aggregation due to electrostatic repulsion. Zeta-potential of **NG9** was -27.3 ± 0.28 mV at 15 °C in 0.1 wt% milliQ (measured by ZetaPlus-Zeta Potential Analyzer (Brookhaven Instruments Corporation)). Nakayama *et al.*, prepared amphiphilic block copolymers of poly(benzyl methacrylate)- *b*-P(NIPAAm-*co*-*N*,*N*-dimethylacrylamide (DMAAm)) having different end groups were pH responsive sulfadimethoxine (SD), phenyl, and hydroxyl groups.[33] These end groups were located at the micelle surface in aqueous solution. The LCSTs of P(NIPAAm-*co*-DMAAm) shells were strongly affected by their end groups. Micelles with non-ionic phenyl and hydroxyl groups showed LCSTs at 23 and 45 °C, respectively. On the other hand, LCSTs of micelles having pH responsive SD groups were found to change from 38.6 to 22.6 °C with pHs ranging from 5.4 to 8.1. Figure 4 shows transmittance change of **NG9** in acidic solution (0.1

wt%, pH 2 $HCl_{aq.}$). Interestingly, two LCSTs at 20~35 °C and 48 °C were clearly observed. The accelerated aggregation of the NGs was found to occur with the protonated COOH groups. LCST of P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) was also measured in pH 2 at different concentrations (Figure S3). Although the LCST showed 55.2 °C in milliQ (0.5 wt%, Table 1), it was found to decrease to 50.1 °C under the same condition. Moreover, the LCSTs at pH 2 increase from 51.1 to 53.1 °C with decreasing concentrations, from 0.3 to 0.1 wt%, respectively. P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) shell in Figure 4, however, showed lower LCST at 48 °C as compared to that of free P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) (0.1wt%, 53.1 °C, Figure S3). These results suggest the lower LCST of shell in **NG9** is caused by the presence of COOH groups and the topical high concentration of P(MEO_2MA_{92}-*co*-$OEGMA_{24}$). Furthermore, the LCST of **NG9** and free P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) were measured in $NaCl_{aq.}$ at the same ionic strength as the $HCl_{aq.}$ (pH 2). It is well known that salts strongly affect LCSTs of temperature responsive polymeric materials including linear polymers, bulk gels, and micro/nanogels.[31,34] Zhang *et al.* investigated the effect of various salts (*i.e.* Hofmeister series) on temperature responsive properties of PNIPAAm.[35] Kosmotrope NaCl led to a decrease of the LCST of PNIPAAm depending on the concentrations (*i.e.* salting-out). Magnusson *et al.* prepared (ethylene glycol)-based

temperature responsive polymers at different compositions and investigated the temperature responsive properties using Hofmeister series.[10i] In kosmotrope salts, the LCSTs of (ethylene glycol)-based polymers were found to decrease with increasing salt concentrations as well as with PNIPAAm. LCST of free P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) was 52.6 °C (0.1 wt%, 10 mM $NaCl_{aq.}$), which was similar value with that of 53.1 °C in pH 2 (Figure S4). The LCST of P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) of **NG9** was around 63 °C (0.1 wt%, 10 mM $NaCl_{aq.}$) (Figure 4). Interestingly, the LCST was higher than in pH 2 of **NG9** (48 °C) and in 10 mM $NaCl_{aq.}$ of free P(MEO_2MA_{92}-*co*-$OEGMA_{24}$) (52.6 °C). These results are surmised that although salting-out effect of NaCl accelerates the dehydration of the shell of **NG9**, deprotonated carboxylic acid groups control the aggregation by the electrostatic repulsion. The diameters of NG9 in $HCl_{aq.}$ (pH 2) and $NaCl_{aq.}$ (10 mM) were also measured using DLS at 15 and 37 °C. Interestingly, at both solution conditions, aggregations were observed not only 37 °C but also 15 °C with large PDI > 0.2 (Figure S5). Both core and shell of the **NG9** should have hydrophilic property at 15 °C. At pH 2 (15 °C), the aggregation was accelerated due to the protonated carboxylic acid groups on the NG. In $NaCl_{aq.}$ (15 °C), on the other hand, the aggregation appeared to be due to the salting-out and electrostatic repulsion. The LCST of shell of **NG9** was found to decrease to 50 °C when it was measured in 50

mM $NaCl_{aq.}$ (Figure S6). Therefore, this decrease may be due to the presence of carboxylic groups on NGs surface (Figure 5).

(Figure 4, Figure 5)

Conclusions

Dual-temperature and pH responsive (ethylene glycol)-based core-shell NGs were successfully polymerized by RAFT polymerization at different compositions in aqueous solution at 37 °C. These NGs were collected by freeze-drying and showed excellent re-dispersion in aqueous solution. The core (cross-linked $PMEO_2MA$) and shell (P(MEO_2MA-*co*-OEGMA)) of NGs were observed to have LCSTs around 20~35 and 65 °C in water, respectively. The LCST of shell was strongly affected by the solution pH and salt concentration because of the carboxylic acid groups arrangements on the NGs. Therefore, using minimal starting materials, multi-functional NGs were prepared by the optimum structural design. These results are significant for the structural design of new nanomaterials for targeted applications.

Acknowledgements

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Supporting information

Preparation methods of **NG1~11**. Transmittance change of P(MEO_2MA-*co*-OEGMA)s and **NG9** at various conditions. Size distribution histogram of **NG9** at various conditions.

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FIGURE LEGENDS

Figure 1. Schematic representation of dual-temperature and pH responsive (ethylene glycol)-based nanogel.

Scheme 1. Synthesis of (A) P(MEO_2MA-*co*-OEGMA) (Macro-CTP) and (B) dual-temperature and pH responsive (ethylene glycol) nanogel by RAFT polymerization.

Figure 2. TEM images of (ethylene-glycol)-based NGs.

Figure 3. Transmittance change of 0.1 and 0.3 wt% of NG9 in water.

Figure 4. Transmittance change of 0.1 wt% NG9 in milliQ (black), pH 2 $HCl_{aq.}$(red), and 10 mM $NaCl_{aq.}$ (blue).

Figure 5. (A) Photographs of NG9 in milliQ, 10mM $NaCl_{aq.}$, and pH2 $HCl_{aq.}$ at 15, 37, and 70 ^{o}C. (B) Schematic representation of solution behavior of NG9 at several conditions.

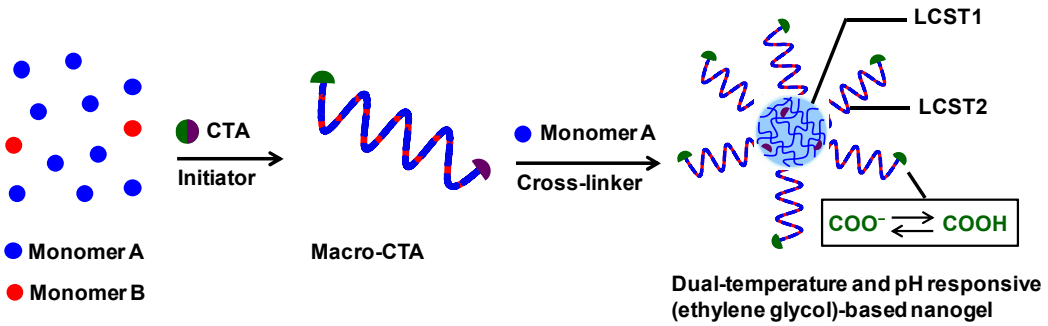
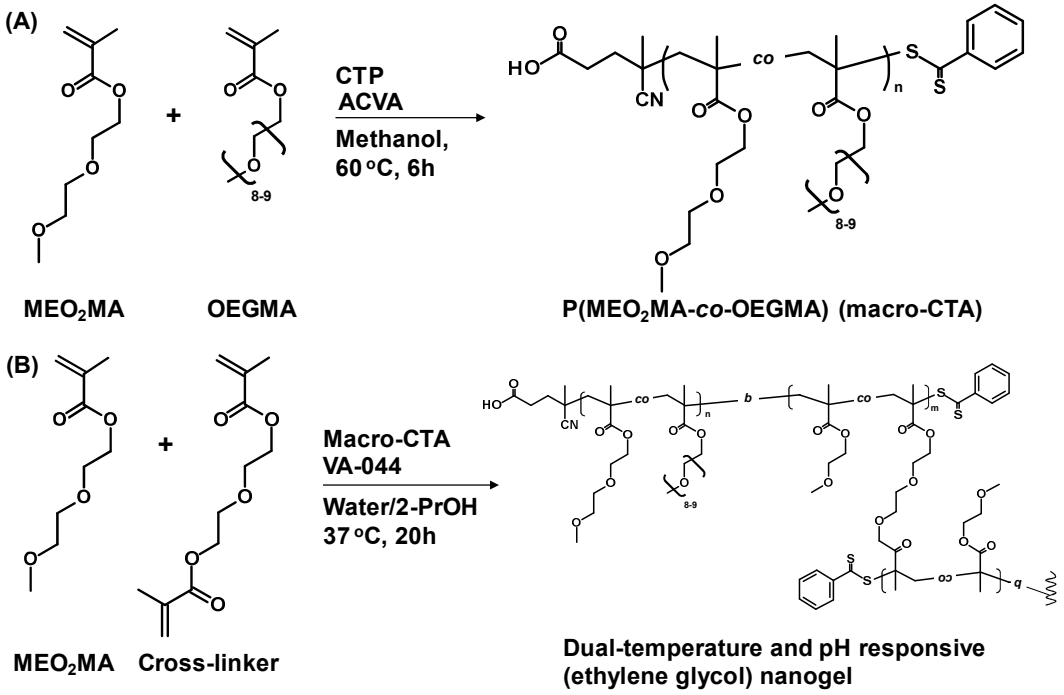


Figure 1. Schematic representation of dual-temperature and pH responsive (ethylene glycol)-based nanogel.



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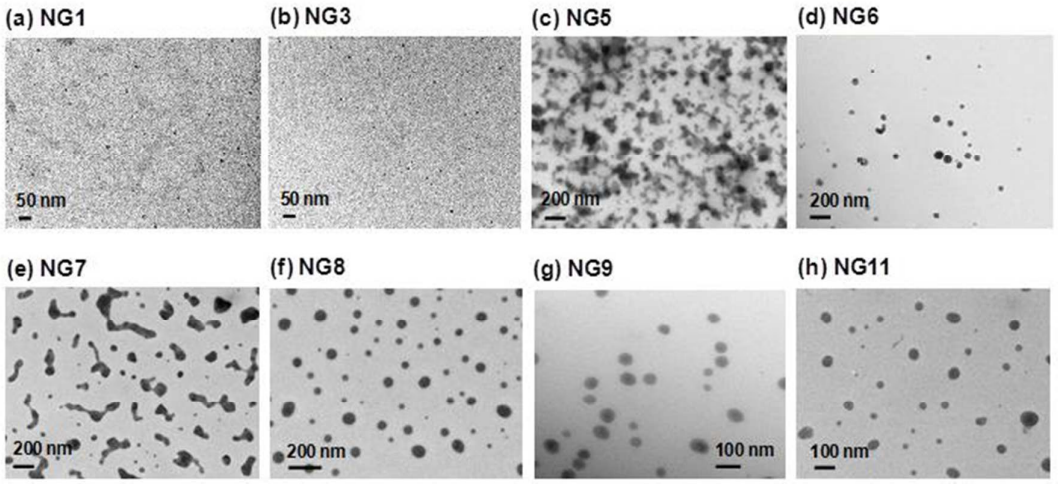


Figure 2. TEM images of (ethylene glycol)-based NGs.

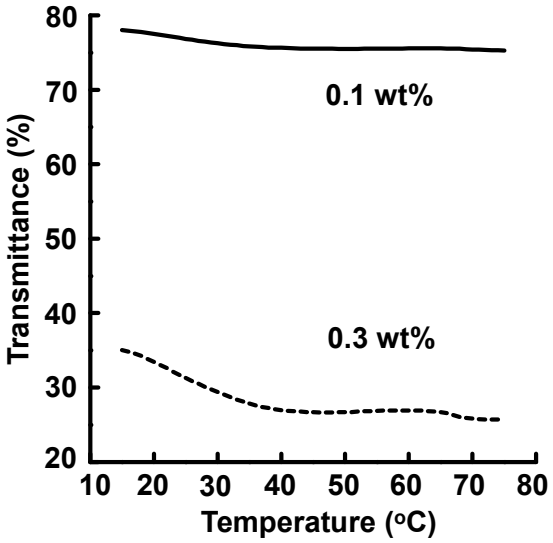
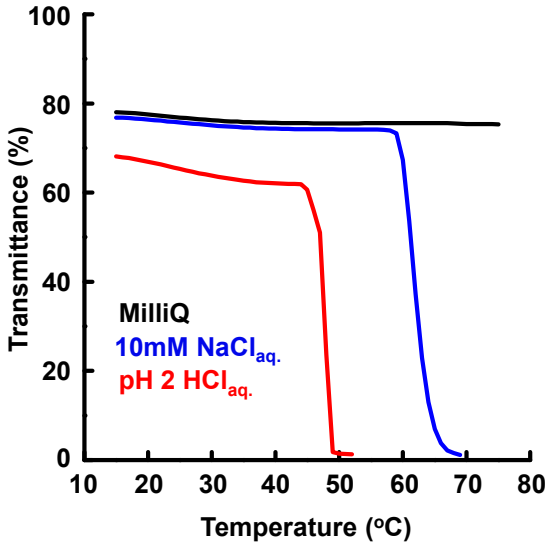


Figure 3. Transmittance change of 0.1 and 0.3 wt% of NG9 in water.



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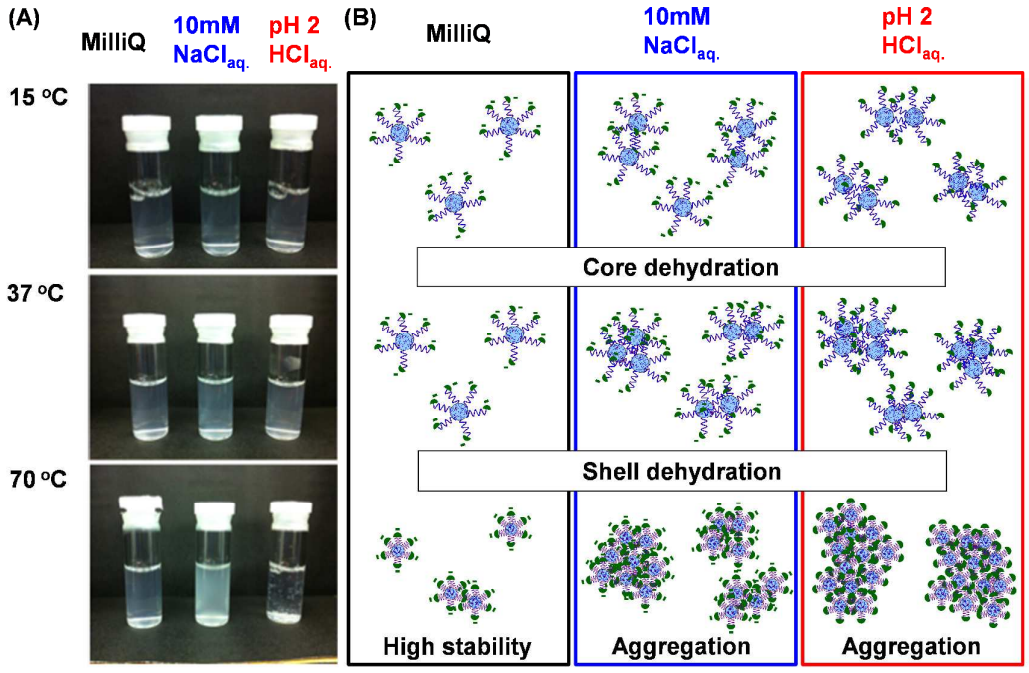

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Table 1. Characterization of P(MEO_2MA-*co*-OEGMA)s

Monomer numbers[a]	OEGMA content in feed (mol%)	OEGMA content in copolymer[b] (mol%)	M_n[c] (g/mol)	M_w/M_n[c] (-)	LCST[d] (°C)
P(MEO_2MA_{38}-*co*-$OEGMA_{10}$)	21	20.8	12,200	1.34	56.7
P(MEO_2MA_{66}-*co*-$OEGMA_{17}$)	21	20.6	20,900	1.33	56.9
P(MEO_2MA_{92}-*co*-$OEGMA_{24}$)	21	20.3	28,900	1.27	55.2

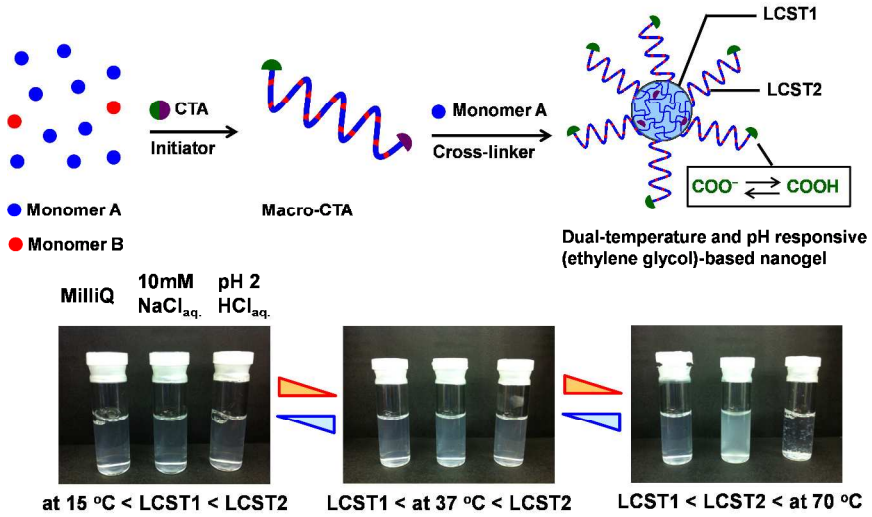
[a]The monomer contents were calculated by GPC and ^{1}H NMR. [b]Determined by ^{1}H NMR. [c]Determined by GPC using 10 mM LiBr DMF [d]LCSTs were determined at the temperature with 50% of transmittance.

Table 2. Characterization of (ethylene glycol)-based NGs

	Macro-CTA	Solvent Water/2-PrOH	Core amount[c]	CL Content[d] (mol%)	Diameter (PDI) (nm)[e] 15 °C	37 °C	65 °C	Formation[f]
NG1[a]	P(MEO_2MA_{38}-*co*-$OEGMA_{10}$)	1/4	300	20	166 ± 7 (0.192)	193 ± 6 (0.194)	137 ± 2 (0.186)	NPs < 10 nm
NG2		4/1	300	20	Precipitation	Precipitation	Precipitation	n.d.
NG3		1/4	300	5	37 ± 18 (0.322)	252 ± 2 (0.106)	212 ± 32 (0.191)	NPs < 10 nm
NG4		4/1	300	5	89 ± 2 (0.350)	41 ± 1 (0.269)	38 ± 3 (0.269)	n.d.
NG5[b]	P(MEO_2MA_{66}-*co*-$OEGMA_{17}$)	1/4	300	20	204 ± 2 (0.160)	190 ± 1 (0.172)	156 ± 25 (0.272)	NPs and aggregation
NG6			300	10	117 ± 24 (0.344)	96 ± 1 (0.184)	73 ± 13 (0.247)	NPs with small aggregation
NG7			200	20	211 ± 3 (0.211)	191 ± 1 (0.212)	361 ± 11 (0.218)	NPs and aggregation
NG8			200	10	104 ± 2 (0.11)	139 ± 1 (0.138)	182 ± 6 (0.163)	NPs
NG9	P(MEO_2MA_{92}-*co*-$OEGMA_{24}$)	1/4	300	20	280 ± 5 (0.096)	256 ± 2 (0.090)	244 ± 18 (0.173)	NPs
NG10			300	10	134 ± 5 (0.338)	87 ± 1 (0.178)	79 ± 7 (0.191)	n.d.
NG11			200	20	264 ± 5 (0.121)	235 ± 2 (0.148)	183 ± 32 (0.254)	NPs

Nanogels (NGs) were prepared by [Macro-CTA]/[VA-044] = [a](**NG1~4**: 2.5/1.0) and [b](**NG5~11**: 3.0/1.0). [c]Core amounts were calculated as ([MEO_2MA]+[Cross-linker])/[Macro-CTA]. [d]Cross-linker (CL) contents were calculated as [Cross-linker]/([MEO_2MA]+[Cross-linker])×100. [e]Determined by DLS. [f]Determined by TEM (NPs: nanoparticles, n.d.: no data).

Graphical Abstract



Dual-temperature and pH responsive (ethylene glycol)-based nanogels were synthesized. Both the core and shell of the nanogels showed lower critical solution temperature (LCST) and the LCST of the shell was strongly affected by the solution pH and salt concentration due to the presence of carboxylic acid groups at the nanogel surface.