Sulfonamide-Based pH- and Temperature-Sensitive Biodegradable Block Copolymer Hydrogels

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Novel pH- and temperature-sensitive biodegradable poly(ϵ-caprolactone-*co*-lactide)-poly(ethylene glycol) (PCLA-PEG) block copolymers were synthesized with oligomeric sulfamethazine (OSM) end groups (OSM-PCLA-PEG-PCLA-OSM). Aqueous solutions of these block copolymers have shown sol−gel transition behavior upon both temperature and pH changes under physiological conditions (37 °C, pH 7.4). The sol−gel transition of these block copolymer solutions was fine-tuned by controlling the PEG length, the hydrophobic to hydrophilic block ratio (PCLA/PEG), and the molecular weight of the sulfamethazine oligomer. Since changes in temperature do not induce gel formation in this pH- and temperature-sensitive block copolymer solution, this hydrogel can be employed as an injectable carrier using a long guide catheter into the body. In addition, the pH of the block copolymer solution showed no change following PCLA degradation over 1 month, and no indication of gel collapse was observed on addition of buffer solution. As such, these properties make the OSM-PCLA-PEG-PCLA-OSM hydrogel an ideal candidate for use as an injectable carrier for certain protein-based drugs known to denature in low-pH environments.

Introduction

Stimuli-sensitive hydrogels have attracted considerable attention as intelligent materials in the fields of biochemistry and biomedicine due to their ability to detect environmental changes and undergo structural changes by themselves such as solubility and swelling ratio.[1−4] Among the developed stimuli-sensitive materials, polymers showing a sol-to-gel transition with changing temperature have been proposed for use as injectable drug delivery systems.[5−7] In particular, block copolymer hydrogels composed of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic biodegradable polyesters, such as poly(L-lactic acid) (PLLA), poly(D,L-lactic acid) (PDLLA), and poly(D,L-lactic acid-*co*-glycolic acid) (PLGA), have been studied as controlled-release drug carriers, due to their ability to biodegrade in vivo, ruling out the need for them to be surgically removed.[8−10] Kim et al. reported on the temperature-responsive phase transition of di- and triblock copolymers composed of PEG and various aliphatic polyesters.[11−13] These hydrogels have been shown to exhibit sol-to-gel (lower) and gel-to-sol (upper) transitions with increasing temperature. The lower transition is important for drug delivery applications, because the solution flows freely at room temperature and forms a gel at body temperature.[14−16]

These hydrogels, however, have several unresolved drawbacks that limit their potential as injectable drug delivery systems. When temperature-sensitive hydrogels are injected into the body via syringe, the warmth of the body tends to cause gels to form within the needle, making them difficult to inject into the body. Also, after injection, the hydrogels tend to undergo the rapid degradation of the block copolymer, resulting in the formation of an acidic monomer such as lactic or glycolic acid. It decreases the pH of the hydrogel. Consequently, the low-pH environment associated with the hydrogel is known to be deleterious to some proteins and nucleic acids, such that the pH change that occurs within these biodegradable hydrogels is an important consideration.[17−19]

In the system considered here, the hydrophobic poly(ϵ-caprolactone-*co*-lactide) (PCLA) part of the temperature-sensitive block copolymer (PCLA-PEG-PCLA) is decidedly less biodegradable than PLGA. Moreover, this temperature-sensitive block copolymer has also been modified with pH-sensitive sulfonamide moieties in order to impart pH sensitivity (OSM-PCLA-PEG-PCLA-OSM). Copolymers bearing sulfonamide groups, such as poly(*N*,*N*-dimethylacrylamide-*co*-sulfonamide), show a critical solubility transition in a narrow pH range around pH 7.4.[20−22]

In this study, the sol−gel transition behavior of the pH- and temperature-sensitive block copolymers (OSM-PCLA-PEG-PCLA-OSM) was investigated with respect to changes in both pH and temperature. To demonstrate the sol−gel transition in conditions simulating those of the body (37 °C, pH 7.4), the sol−gel transition curve was modified precisely by altering the hydrophilic−hydrophobic block ratio, the block length, and the molecular weight of the pH-sensitive moiety.

Experimental Section

Materials. Various poly(ethylene glycol)s (PEGs) were obtained from Sigma-Aldrich (St. Louis, MO) (M_n = 1500 and 2000) and ID Biochem, Inc. (Seoul, Korea) (M_n = 1750). D,L-Lactide (LA), ϵ-caprolactone (CL), sulfamethazine (SM), methacryloyl chloride, stannous 2-ethyl hexanoate ($Sn(Oct)_2$), 3-mercaptopropionic acid (MPA), dicyclohexyl carbodiimide (DCC), 4-(dimethyl amino)pyridine (DMAP), *N*,*N*-dimethylformamide (anhydrous), tetrahydrofuran, and methylene chloride (anhydrous) were purchased from Sigma-Aldrich and used as received. 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from Junsei Co. (Tokyo, Japan) and was recrystallized from methanol twice prior

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to use. Unless specified otherwise, all chemicals were reagent grade and used as received.

Temperature-Sensitive Block Copolymer (PCLA-PEG-PCLA). The synthesis of the PCLA-PEG-PCLA block copolymer was performed through a ring-opening copolymerization reaction using PEG as an initiator and $Sn(Oct)_2$ as a catalyst. The ratios of PEG/PCLA and CL/LA were adjusted by altering the feed ratios of PEG, CL, and LA. The detailed synthesis was as follows: PEG and $Sn(Oct)_2$ were added to a two-neck round-bottom flask and were dried for 4 h under vacuum at 110 °C. After cooling the flask to room temperature, LA and CL were added under dry nitrogen, and the reactant mixture was dried for 1 h under vacuum at 60 °C. Then, the temperature was raised slowly to 130 °C, and the reaction was performed over a period of 24 h under dry nitrogen. The reactants were then cooled to room temperature, dissolved in methylene chloride (MC), and then added to excess diethyl ether, causing the products to precipitate. The precipitated block copolymer was then dried under vacuum at 40 °C over 48 h, affording a yield of over 75%.

Sulfamethazine Oligomer. The sulfamethazine monomer (SMM) was synthesized from sulfamethazine (SM) and methacryloyl chloride. First, SM (0.1 mol) and sodium hydroxide (0.1 mol) were dissolved in aqueous acetone (100 mL, 1:1 v/v), and methacryloyl chloride (0.12 mol) was then added dropwise to the solution with stirring (0 °C). The resulting mixture was then stirred for a further 3 h at 0 °C. The precipitated SMM was filtered from solution, washed with distilled water, and then dried under vacuum at room temperature for 48 h. The SMM yield was approximately 85% after drying.

The sulfamethazine oligomer (OSM) containing a carboxyl acid end group was synthesized by conventional radical polymerization with SMM, AIBN, and 3-mercaptopropionic acid (MPA). The OSM molecular weight was controlled by altering the feed ratios of AIBN and MPA. The synthetic procedure was as follows: SMM (90 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (DMF) (150 mL), after which, AIBN (9 mmol) and MPA (9 mmol) were added under dry nitrogen to afford a SMM/AIBN/MPA mole ratio of 100/10/10. The temperature of the reactants was slowly increased to 85 °C, and the reaction was carried out for 48 h. Subsequently after evaporating the solvent (DMF), the resultant was redissolved in tetrahydrofuran (THF). Slow addition of the THF solution to an excess diethyl ether resulted in the precipitation of OSM, which was filtered and dried slowly at 40 °C for 48 h. The yield (weight of the final product (OSM) to total feed amount of SMM and MPA) was 90%.

Coupling Sulfamethazine Oligomer with Temperature-Sensitive Block Copolymers. The temperature-sensitive block copolymer (PCLA-PEG-PCLA) and OSM were coupled together using 1,3-dicyclohexylcarbodiimide (DCC) and a 4-(dimethylamino)pyridine (DMAP) as an catalyst. The coupling reaction process was as follows: The PCLA-PEG-PCLA block copolymer (6 g) was weighed into a two-neck flask and dried under vacuum at 85 °C for 2 h. OSM was then added to the flask under dry nitrogen, and the reactant was dried under vacuum at 85 °C for 1 h in order to completely remove any moisture. The reactant was cooled to room temperature under dry nitrogen, and then, an anhydrous MC solution (60 mL) containing DCC and DMAP was added to the flask using a glass syringe, to afford a PCLA-PEG-PCLA/OSM/DCC/DMAP feed ratio of 1/2.4/2.8/0.28 mol. The reaction was carried out at room temperature for 48 h. Although OSM is insoluble in MC, it reacts with PCLA-PEG-PCLA due to the high solubility of the PCLA-PEG-PCLA block copolymer in MC. Over the course of the coupling reaction, the DCC was slowly converted into dicyclohexylurea (DCU). The residual DCC was also converted into DCU by the addition of two or three drops of water, and the combined DCU byproducts were precipitated and removed (0.4 μm filter paper) along with residual OSM. The final product was obtained by pouring the filtered reactant mixture into excess diethyl ether, and the resulting precipitate dried under vacuum at 40 °C over 48 h to give a final yield of over 60%.

Characterization. ^{1}H NMR spectra were recorded on a Varian Unity Inova 500NB operated at 500 MHz. These spectra were used to determine the molecular structure and composition of the block copolymers. Deuterated dimethylsulfoxide (DMSO-d^6) and chloroform ($CDCl_3$), each containing 0.03 v/v % tetramethylsilane (TMS), were used as NMR solvents. The composition and ratio of each polymer block were calculated by comparing the proton peak integration of PEG, CL, and LA from the corresponding ^{1}H NMR spectra. Gel permeation chromatography (GPC), conducted using two stryragel columns (Shodex-KF 802.5, KF 803L, Tokyo, Japan), was performed in order to measure the molecular weight and molecular weight distribution. THF was used as an eluant at a flow rate of 1 mL/min. Calibration was carried out using PEG standards with a molecular weight range 420−22 100 (Waters Co. Milford, MA). The retention volume was measured at 45 °C using a refractive-index detector (Shodex, RI-101).

Critical Micelle Concentration (cmc) and Micelle Size. The cmc was determined with a fluorescence probe technique using pyrene.[23,24] A stock solution of pyrene in THF was poured into phosphate-buffered saline (PBS) solution, and the THF was eliminated by stirring at 40 °C for 4 h. The final pyrene concentration in solution was 1×10^{-6} M. Block copolymer solutions of various concentrations ranging from 2.0×10^{-6} mg/mL to 10 mg/mL were prepared by dissolution of the polymer and dilution in the pyrene-solubilized buffer solution. The excitation spectrum of pyrene was measured using fluorescence spectrometry (AMINCO·BOWMAN Series2) at fixed emission (392 nm) wavelengths. The average size and distribution of the block copolymer micelles were measured using a Malvern PCS100 spectrogoniometer and a Brookhaven BI-9000AT digital autocorrelator at a wavelength of 633 nm. The polymer solution was prepared in PBS solution with a concentration of 0.2 mg/mL,and filtered (0.45 μm filter) prior to being transferred into light-scattering cells. The intensity autocorrelation was measured at a scattering angle of 90°. The Laplace inversion of the autocorrelation function in the CONTIN algorithms was used to obtain the size distribution of the micelles. The mean diameter of the micelles was evaluated using the Stokes−Einstein equation. The fluorescent and dynamic light scattering (DLS) measurements were performed from 10 to 40 °C, in increments of 10 °C at pH 7.4 and 8.0. The temperature was controlled using a thermostat circulator.

Phase Diagram Measurement. The block copolymers were dissolved at a given concentration in a buffer solution (in a 4 mL vial) for 1 day at 0 °C. The buffer solution was prepared using PBS tablets and NaOH (0.9 wt %). Here, the pH of the block copolymer solution was adjusted to a specific pH by adding small amounts of 5 M HCl solution at 2 °C. Each solution was kept at 4 °C for 30 min in a water bath. The vial was then slowly heated in a water bath in intervals of 2 °C. The vial was held at each temperature for 10 min to equilibrate and then laid down horizontally for a further 1 min. The sol (flow)−gel (no flow) phase-transition temperature of the block copolymer solutions was determined using this method. It was repeated three times, and each point represented an average with an accuracy of ±2 °C. Also, the viscosity changes of the block copolymer solutions were investigated by increasing the temperature with a Reologica Rheometer (REOLOGICA Instruments AB). The rheometer was operated at an oscillation frequency of 1 Hz and 1×10^{-3} strain with a 0.5 °C/min temperature ramp from 5 to 40 °C.

Degradation of Block Copolymer. The degradability of the block copolymers was determined by following the molecular weight changes over time. The block copolymer solution was prepared at 0 °C using a method similar to the phase diagram experiment and was adjusted to pH 7.4. The vials containing the block copolymer solution (0.5 g) were immersed in a shaking water bath (20 strokes/min) at 37 °C. After 5 min, PBS solution (2 mL, pH 7.4, 37 °C) was added to each vial. Samples were then taken at designated time intervals and freeze-dried. The molecular weight change was determined by GPC.

Results and Discussion

Synthesis and Characterization. Various PCLA-PEG-PCLA block copolymers were obtained from the ring-opening polym-

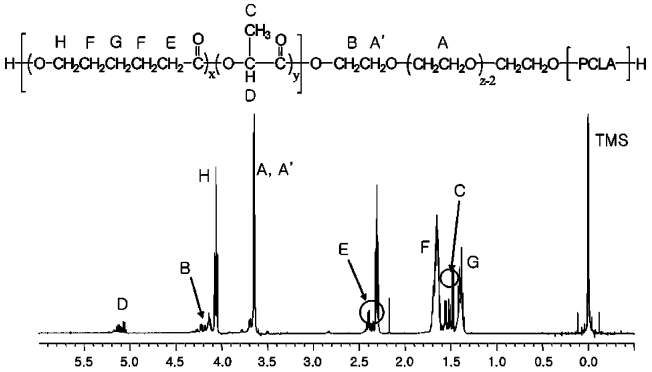

Figure 1. ^{1}H NMR spectrum of PCLA-PEG-PCLA block copolymer in $CDCl_3$ and its chemical structure.

Table 1. List of PCLA-PEG-PCLA Block Copolymers Synthesized

PCLA(CL/LA)-PEG[a]-PCLA(LA/CL) M_n[b]	PEG/PCLA (w/w)[b]	CL/LA (mol/mol)[b]	M_w/M_n[c]
1384−1500−1384 (A-1)	1/1.85	2.44/1	1.33
1554−1500−1554 (A-2)	1/2.08	2.59/1	1.35
1642−1750−1642 (B-1)	1/1.89	2.44/1	1.39
1823−1750−1823 (B-2)	1/2.08	2.49/1	1.41
1856−2000−1856 (C-1)	1/1.86	2.64/1	1.45
2104−2000−2104 (C-2)	1/2.10	2.71/1	1.49

[a] Provided by Aldrich (M_n = 1500, 2000) and ID Biochem, Inc. (M_n = 1750). [b] Determined by ^{1}H NMR. [c] Measured by GPC.

erization reaction. The number average molecular weight (M_n) of the block copolymers was calculated using the ^{1}H NMR spectrum of a PEG standard of known molecular weight. Figure 1 shows the representative ^{1}H NMR spectrum of the PCLA-PEG-PCLA block copolymer and its chemical structure. All proton signals of the block copolymer were assigned as labeled in Figure 1. Among the proton peaks, the methylene proton of the oxyethylene unit (A, A′), the methine proton of the LA unit (D), and the methylene proton (on the neighboring carbonyl group) of the CL unit (E) were used to calculate the M_n and composition of the block copolymer according to the following equations:

$$\frac{2y}{4(z-2)+4} = \frac{I_D}{I_A + I_{A'}}$$

$$\frac{4x}{4(z-2)+4} = \frac{I_E}{I_A + I_{A'}}$$

The number of oxyethylene units (x) was calculated from the known PEG molecular weight. Table 1 shows the M_n and composition of the block copolymers synthesized in this study. In addition, the GPC trace of each block copolymer is unimodal and shows a narrow molecular weight distribution (Figure 2). These results demonstrate that a block copolymer with a narrow molecular weight distribution had been well-synthesized.

The molecular structures of the synthesized SMM and OSM were confirmed by ^{1}H NMR, as shown by the corresponding spectra and peak assignments in Figure 3. The aromatic (c,d) and amine (e) protons shown at 7.65 ppm (c), 6.55 ppm (d), and 5.98 ppm (e) in the sulfamethazine (SM) spectrum (A) were observed to shift to 7.95 ppm (c), 7.85 ppm (d), and 10.08 ppm (e) in the corresponding SMM spectrum (B), respectively. In confirming the formation of OSM, the methyl signal (1.98 ppm, f) in the SMM spectrum is shifted upfield to 1.10 ppm (*f′*), while the corresponding ethylene signals (=CH_2, g,h) show a significant decrease. The peak of the hydrogen proton in the

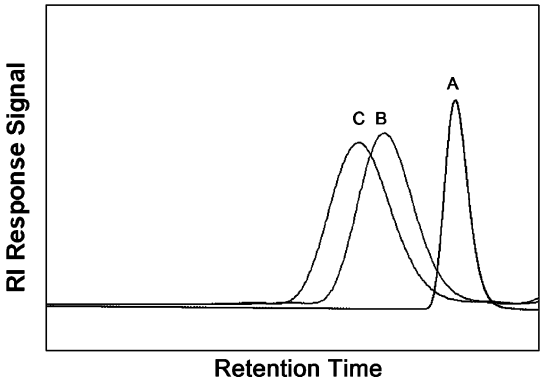

Figure 2. GPC traces. (A) PEG (M_n = 1500), (B) PCLA-PEG-PCLA (A-1), (C) OSM-PCLA-PEG-PCLA-OSM (a-1-1).

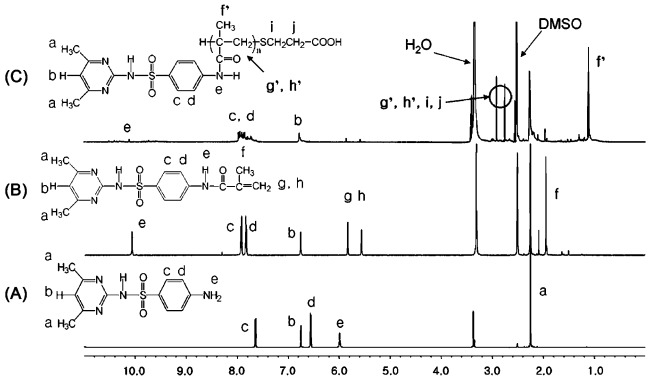

Figure 3. ^{1}H NMR spectra of sulfamethazine (A), sulfamethazine monomer (B), and sulfamethazine oligomer (C) in DMSO-d^6.

Table 2. Sulfamethazine Oligomers

feed ratio (mol ratio)[a]	M_n[b]	M_w/M_n[b]
1/0.1/0.1 (OSM_1)	1144	1.35
1/0.1/0.2 (OSM_2)	937	1.24

[a] [monomer]/[initiator]/[transfer agent]. [b] Measured by GPC relative to PEG standards.

sulfonamide group (SO_2NH) is shown at 11.6 ppm in ^{1}H NMR spectra for all the three samples. These ^{1}H NMR results show conclusively that SMM had been synthesized successfully. On the other hand, further evidence is required to confirm the molecular structure of OSM. The molecular weight of OSM was controlled by the feed ratio of the monomer, the initiator, and the transfer agent. Table 2 shows the molecular weight of OSM, as determined by GPC (relative to PEG standards).

To confirm the structural integrity of the carboxyl acid terminated OSM, the molecule was coupled with monomethoxy poly(ethylene glycol) (MPEG) using DCC and DMAP. After coupling, the presence of the methylene proton in the MPEG end unit (neighboring the ester bond formed by coupling with the carboxyl terminated OSM) was observed at 4.05 ppm in the ^{1}H NMR spectrum (Supporting Information, Figure S1), confirming successful coupling. This ^{1}H NMR evidence was further corroborated by the increase in the molecular weight of the MPEG, as determined using GPC (Supporting Information, Figure S2). These results indicate that the carboxyl acid terminated OSM was synthesized successfully.

OSM was coupled with the temperature-sensitive block copolymers (PCLA-PEG-PCLA) using DCC and DMAP. The synthesized OSM-PCLA-PEG-PCLA-OSM block copolymers were confirmed using ^{1}H NMR and GPC. The ^{1}H NMR spectrum of OSM-PCLA-PEG-PCLA-OSM shows aromatic

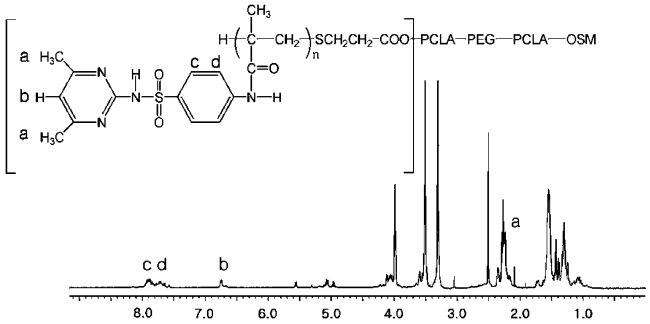

Figure 4. ^{1}H NMR spectrum of OSM-PCLA-PEG-PCLA-OSM in DMSO-d^6.

protons (7.6−8.0 ppm, c,d) and an imidazole ring proton (around 6.8 ppm, b), which are typical signals associated with OSM (Figure 4). Also, the molecular weight of OSM-PCLA-PEG-PCLA-OSM showed a significant increase compared to PCLA-PEG-PCLA (Figure 2). Table 3 shows the molecular weights of PCLA-PEG-PCLA and OSM-PCLA-PEG-PCLA-OSM, as determined by GPC.

Sol−Gel Phase Transition. The sol−gel transition phase diagrams of the block copolymer solutions were investigated under various pH and temperature conditions. The sol−gel mechanism of the pH- and temperature-sensitive block copolymer solution was discussed in detail in our previous paper.[25] Figure 5 shows the sol−gel transition phase diagrams of PCLA-PEG-PCLA (A-1, A-2) and OSM-PCLA-PEG-PCLA-OSM (a-1-1, a-2-1) block copolymers. As the hydrophobicity of the PCLA block increased with increasing temperature, the PCLA-PEG-PCLA block copolymer solution showed typical sol-to-gel transition characteristics. For temperatures higher than the gel transition phase, the block copolymer separated from the water due to the strong hydrophobicity of the block copolymer. In addition, the sol−gel diagrams of the PCLA-PEG-PCLA block copolymer solutions show similar gel temperature regions in the pH range 7.0−8.2 (Figure 5a). However, in the OSM-PCLA-PEG-PCLA-OSM block copolymer solutions, a decrease in the pH from 8.0 to 7.2 resulted in a decrease in the sol-to-gel transition temperature and a broadening of the gel temperature region (Figure 5b). At high pH (pH 8.0), the sulfonamide group (SO_2NH) of the pH-sensitive moiety (OSM) is mainly present in its ionized state, where the ionized OSM acts as a hydrophilic block in the OSM-PCLA-PEG-PCLA-OSM block copolymer. Therefore, the OSM-PCLA-PEG-PCLA-OSM block copolymer solution is unable to form gels in high pH conditions, despite increases in temperature (Figure 5b,c). In addition, the viscosity of the solution increases only very slightly, because the hydrophilicity of the ionized species weakens the hydrophobic interactions between the PCLA blocks at high temperature. At low pH (pH 7.4), however, the degree of OSM ionization decreases, and the non-ionized OSM acts as a hydrophobic block in the OSM-PCLA-PEG-PCLA-OSM block copolymer. The OSM-PCLA-PEG-PCLA-OSM block copolymer solution forms a gel in low-pH conditions as the temperature increases (Figure 5b,c). As the PCLA block length increases, the gel regions of the block copolymer solutions become larger due to the presence of stronger hydrophobic interactions.

Effect of OSM Block Length. Figure 6 shows sol−gel diagrams of the OSM-PCLA-PEG-PCLA-OSM block copolymer solution with respect to the molecular weight of the OSM component. It is found that OSM, which is present mainly in an ionized state in the high-pH range, is present in the sol state regardless of its molecular weight. However, it can be seen that a decrease in pH results in the formation of a non-ionized OSM, which thus acts as a hydrophobic block. Also, particularly in the low-pH range, the hydrophobicity of the block copolymer increases with increases in the OSM molecular weight, resulting in an overall increase in the temperature range at the point where the gel forms.

Effect of PEG Molecular Weight. Figure 7 shows the changes in the sol−gel diagrams that occur with increases in the molecular weights of PEG and the block copolymer, in a state where the molecular weight ratios of the hydrophilic (PEG) and hydrophobic (PCLA) components were fixed at 1/1.9 and 1/2.1, respectively. It was found that the sol−gel phase diagram of the block copolymer moved toward higher temperatures with increasing block copolymer molecular weight at the same PEG/PCLA ratio, yet revealed little or no change in the temperature range at the point where the block copolymer formed a gel. This suggests that, when the length of the block copolymer is increased with a constant ratio of hydrophobic to hydrophilic blocks, gel formation by the block copolymer becomes possible due to stronger hydrophobic conditions (i.e., strong hydrophobic interactions at high temperature). It was also found that the temperature range at which gels formed was affected mainly by the ratio of hydrophobic to hydrophilic blocks. In addition, it was shown that, in the low pH range, the temperature range at which gels formed decreased with increasing block copolymer molecular weight. This occurs because, regardless of the PEG and PCLA lengths in various pH- and temperature-sensitive block copolymers, the OSM (with constant molecular weight) is present in a non-ionized state at low pH and thus acts as a hydrophobic block, so that the ratio of the hydrophobic (PCLA-OSM) to hydrophilic (PEG) blocks is decreased with increases in the PEG molecular weight. Accordingly, it could be found that, at low pH, the temperature range at which a gel is formed is slightly decreased with increases in the total molecular weight of the block copolymer.

Concentration Dependence. Figure 8 shows phase diagrams of the OSM-PCLA-PEG-PCLA-OSM block copolymer solution resulting from changes in pH and temperature at various concentrations. Above 10 wt %, the block copolymer solution formed a gel as the temperature increased in the low-pH region (below pH 7.4). Here, as the concentration was increased, the sol-to-gel transition temperature decreased, and the gel region became wider. At low concentrations, gelling of the block copolymer solution became possible due to the stronger hydrophobic conditions, i.e., the stronger hydrophobic PCLA present at high temperatures and the mostly non-ionized OSM present at low pH. On the contrary, at high concentration, gelling of the block copolymer solution was found to occur easily in the weaker hydrophobic conditions, although it was difficult to solubilize the block copolymer above 25 wt % in the buffer solution. At high concentrations (above 25 wt %), the block copolymer solution formed gels even at 0 °C and showed no evidence of a sol state at low temperatures in the pH range used in these experiments (pH 7.2−8.0).

Properties of Block Copolymer Solution. The sol−gel transition of the OSM-PCLA-PEG-PCLA-OSM block copolymer solution was confirmed by viscosity measurements. Figure 9 shows the viscosity changes associated with the OSM-PCLA-PEG-PCLA-OSM block copolymer solution as the temperature was increased at pH conditions of pH 7.4 and 8.0, respectively. At pH 7.4, the viscosity showed a significant increase from 60 to 18 000 poise as the temperature of the block copolymer solution was increased. Moreover, this increase was particularly rapid in the sol-to-gel transition region. At pH 8.0, however, the viscosity was found to increase only slightly from 5 to 550

Table 3. Physical Parameters of PCLA-PEG-PCLA and OSM-PCLA-PEG-PCLA-OSM Block Copolymers

PCLA-PEG-PCLA	PCLA-PEG-PCLA M_p[b]	OSM-PCLA-PEG-PCLA-OSM[a]	OSM-PCLA-PEG-PCLA-OSM M_p[b]	OSM-PCLA-PEG-PCLA-OSM M_w/M_n[b]
A-1	3895	a-1−1	5044	1.45
A-2	4347	a-2−1	5500	1.48
		a-2−2	5046	1.46
B-1	4844	b-1−1	5978	1.50
B-2	5102	b-2−1	6205	1.53
C-1	5458	c-1−1	6525	1.54
C-2	5896	c-2−1	7024	1.56

[a] All PCLA-PEG-PCLA block copolymers were coupled with OSM_1 except for a-2−2, which was synthesized with A-2 and OSM_2. [b] The molecular weight of peak maximum in the GPC trace (relative to PEG standards).

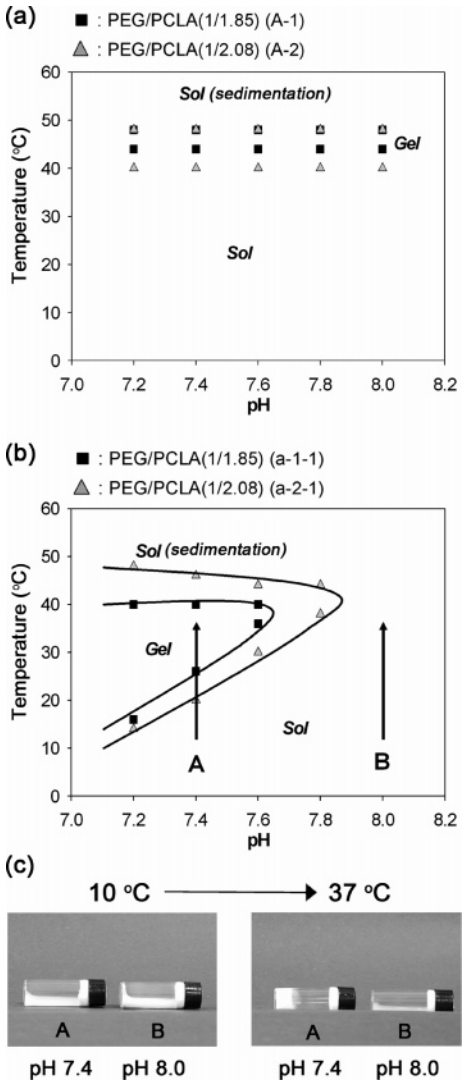

Figure 5. Sol−gel phase diagrams of block copolymers in buffer solutions. M_n of PEG = 1500; concentration = 15%. (a) PCLA-PEG-PCLA (A-1, A-2), (b) OSM-PCLA-PEG-PCLA-OSM (a-1-1, a-2-1), (c) Sol−gel transition of OSM-PCLA-PEG-PCLA-OSM (a-2-1) solution.

poise with increasing temperature. The viscosity increase with temperature is explained by the increasing hydrophobicity of the PCLA block. Also, at the same temperature, because the hydrophobicity of the block copolymer increased due to the non-ionized state of the OSM at pH 7.4, the solution showed higher viscosities at pH 7.4 than at pH 8.0.

To verify the sol−gel transition of the pH- and temperature-sensitive block copolymer solution at high concentrations, the cmc and micelle size were determined by increasing the temperature at pH 7.4 and 8.0 for low concentrations. Figure

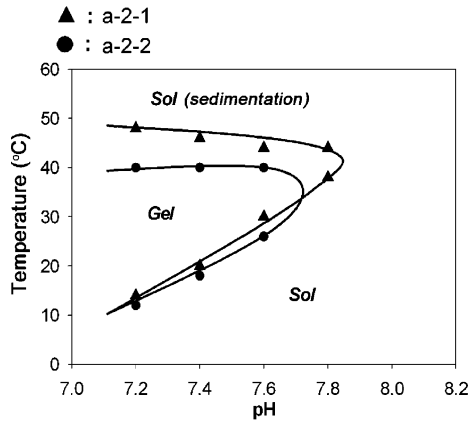

Figure 6. Sol−gel phase diagrams of OSM-PCLA-PEG-PCLA-OSM (a-2-1, a-2-2) block copolymer solutions with different molecular weight of sulfamethazine oligomers. M_n of PEG = 1500; PEG/PCLA = 1/2.08 (w/w); concentration = 15%.

10 show the cmc of the OSM-PCLA-PEG-PCLA-OSM block copolymer deduced from the excitation spectra. As the OSM is mainly present in its non-ionized state at pH 7.4 and its ionized state at pH 8.0, the hydrophobicity of the block copolymer is found to be stronger at pH 7.4 than at pH 8.0. Therefore, the cmc at pH 7.4 was determined to be lower than that at pH 8.0 (Figure 10a). Moreover, the cmc's decreased with increasing temperature (Figure 10b). These results are due to the increasing hydrophobicity of the block copolymer.

The micelle size and distribution of the block copolymer were measured by dynamic light scattering (DLS). Figure 11 shows the micelle size and distribution at various temperatures at pH 7.4 and 8.0. The micelle sizes at both pH 7.4 and 8.0, which were approximately 100 and 200 nm, respectively, were observed to decrease slightly with increasing temperature; this can be attributed to the reduction in micelle volume resulting from dehydration at elevated temperatures. It should be noted that the relative intensity of micelle aggregates over 1000 nm increased gradually at pH 7.4, due to intermicellar association with increasing temperature. Also, the size of the micelle aggregates was found to increase gradually with increasing temperature. However, an increase in temperature at pH 8.0 revealed no evidence of the formation of aggregates, because the ionized OSMs disturb intermicelle association. These cmc and micelle size results provide strong evidence to support the sol−gel transition of the block copolymer at high concentrations with changes in pH and temperature.

Degradation of Block Copolymer. The degradation of the OSM-PCLA-PEG-PCLA-OSM block copolymer was investigated in buffer solution (37 °C, pH 7.4). Figure 12 shows the changes in the GPC trace (a) and molecular weight (b) of the block copolymer at peak maximum during degradation. In

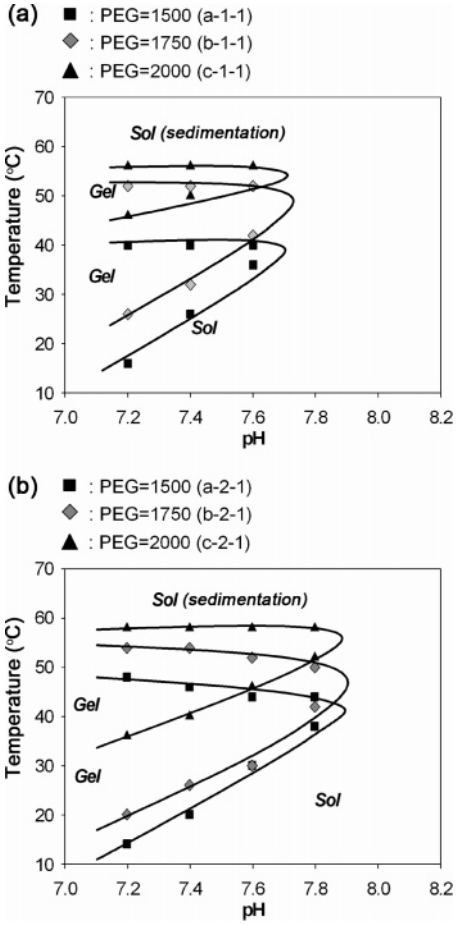

Figure 7. Sol−gel phase diagrams of OSM-PCLA-PEG-PCLA-OSM block copolymer solutions with different PEG molecular weights, similar PEG/PCLA ratios, and the same sulfamethazine oligomer. Concentration = 15%; (a) PEG/PCLA ≈ 1/1.9; (b) PEG/PCLA ≈ 1/2.1.

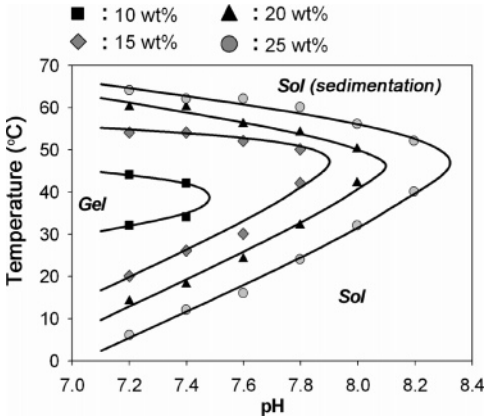

Figure 8. Sol−gel phase diagrams of OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer solutions at various concentrations.

Figure 12a, the retention time at peak maximum was found to increase, indicating a decrease in the block copolymer molecular weight during degradation. The broadening of the peaks was deemed to be the result of an increase in the polydispersity of the block copolymers. In Figure 12b, the molecular weight showed a decrease from 5900 to 3900 during the course of 1 month. Although degradation of the CL and LA units in the block copolymer resulted in the formation of 6-hydroxyhexanoic acid and lactic acid, the pH of the gel remained constant at pH 7.4. But, in the degradation of the PCLA-PEG-PCLA block copolymer, the pH decreased from 7.4 to 6.0 during the course

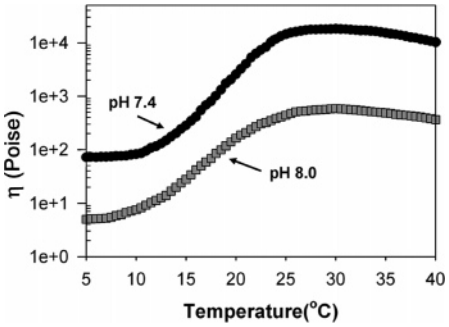

Figure 9. Viscosity change of OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer solution. Concentration = 15%.

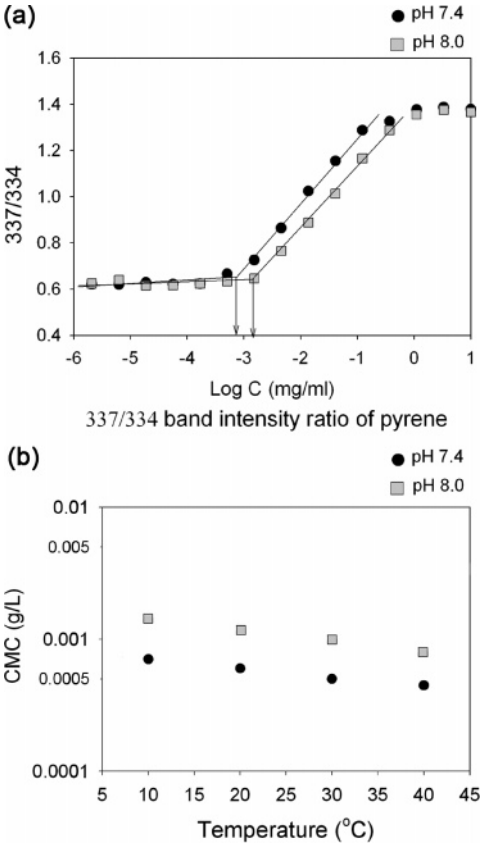

Figure 10. (a) The intensity ratios (337 nm/332 nm) in the excitation spectra as a function of the OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer concentration at 10 °C. (b) CMC dependence of the OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer as a function of temperature.

of 1 month. The reason that the pH of the OSM-PCLA-PEG-PCLA-OSM block copolymer hydrogel is constant, unlike the PCLA-PEG-PCLA, seems to be that the presence of the partially ionized OSM acts as a proton sponge in the block copolymer hydrogel. For this reason, the hydrophobic interaction between the PCLA-OSM blocks increased due to a decrease in the degree of ionization of the OSM, resulting in the formation of a stable gel, which remained in the buffer solution for more than 1 month. This persistent and constant nature of the gel pH could be advantageous for the long-term sustained release of both hydrophobic drugs and protein-based drugs.

Conclusion

pH- and temperature-sensitive block copolymers of the form OSM-PCLA-PEG-PCLA-OSM, with varying PEG lengths,

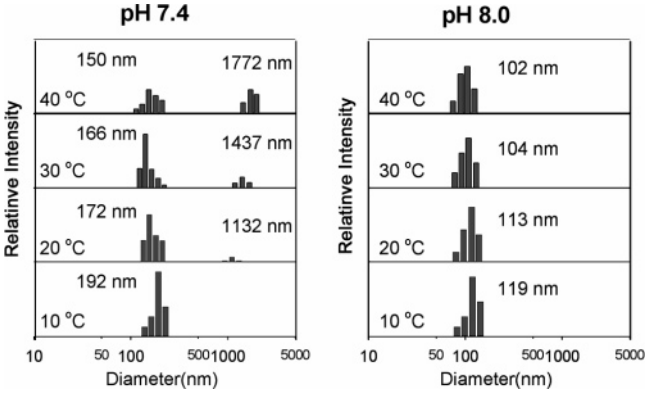

Figure 11. Micelle size and its distribution of OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer solution as a function of temperature at pH 7.4 and 8.0. Concentration = 0.2 mg/mL.

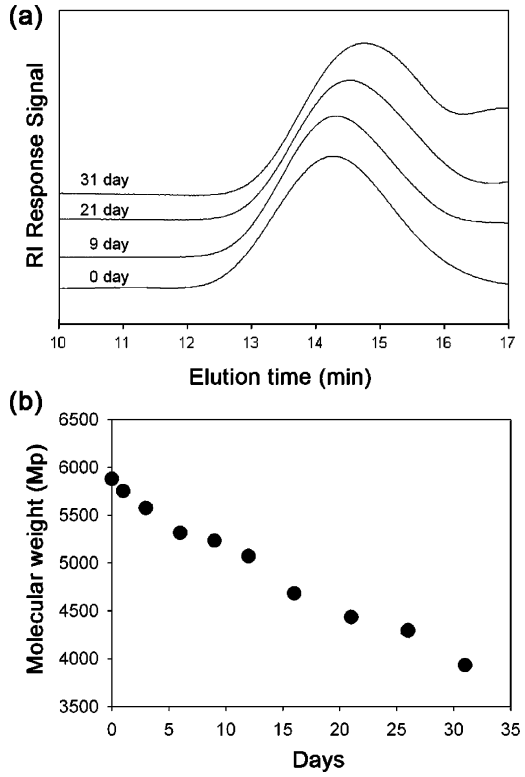

Figure 12. Changes in molecular weight during the degradation of OSM-PCLA-PEG-PCLA-OSM (b-2-1) block copolymer. (a) GPC trace; (b) change of M_p (M_w of peak maximum) (relative to PEG standards).

PEG/PCLA ratios, and OSM molecular weights, were synthesized through a combination of ring-opening reactions and DCC coupling. Their aqueous solutions show a sensitive, reversible sol-to-gel transition over a small pH range (pH 7.2−8.0) and temperature range (10−50 °C), representative of conditions found in the body. The sol−gel phase diagrams in the block copolymer solutions were controlled deliberately by altering the ratio of hydrophobic to hydrophilic blocks within the block copolymer, the PEG length, or the molecular weight of the OSM component. Accordingly, it could be confirmed that the temperature and pH ranges at which the sol−gel transition occurs can be adjusted depending on the molecular weight and composition ratio of the block copolymer. The aqueous block copolymer solution composed of PEG = 1750, PEG/PCLA ratio = 1/2.08, and OSM = 1144 formed the most stable gel in simulated in vivo conditions (37 °C, pH 7.4). Here, increasing the temperature during the sol-to-gel transition at pH 7.4 resulted in a rapid increase in the block copolymer viscosity, consistent with the formation of a gel. At pH 8.0, however, the viscosity of the block copolymer solution increased only very slightly with increasing temperature, with no obvious gel formation, implying that this pH- and temperature-sensitive block copolymer solution can be easily injected into the body. Moreover, since the injected block copolymer solution is able to form a gel by the small pH change from pH 8.0 to pH 7.4 in vivo, this hydrogel has potential as an injectable carrier for sustained-release drug delivery systems.

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Supporting Information Available. Figure S1, ^{1}H NMR spectrum of MPEG-OSM in DMSO-d^6 (500 MHz), and Figure S2, GPC traces of MPEG and MPEG-OSM. This material is available free of charge via the Internet at http://pubs.acs.org.

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