

Novel pH and Temperature-Sensitive Block Copolymers: Poly(ethylene glycol)-*b*-poly(ϵ -caprolactone)-*b*-poly(β -amino ester)

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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.¹⁻³ Among the developed stimuli-sensitive materials, polymers showing a sol-gel transition with changing temperature have been proposed for use as injectable drug delivery systems.⁴⁻⁶ However, the temperature-induced sol-gel transition is a suboptimal condition for applying hydrogels for deep anatomical sites in the body due to thermal equilibration during the delivery process of the solution through a narrow conduit, resulting in premature gelation inside the conduits or tubings. Here, we report a tailored polymeric material of which aqueous solutions undergo sol-gel transition by pH change, as well as by temperature change.

To make a hydrogel that shows sol-gel transition by pH change, poly(β -amino ester) (PAE) was introduced to thermo-sensitive block copolymer. PAE has been reported to be a pH-responsive biodegradable polymer. The solubility of PAE in aqueous solution rapidly increases when the pH is lowered to under 6.5. Moreover, at pH values in the physiological range (pH 7.4), this polymer interacted electrostatically with plasmid DNA and formed nanometer-scale polymer/DNA complexes. PAE is noncytotoxic and degrades into

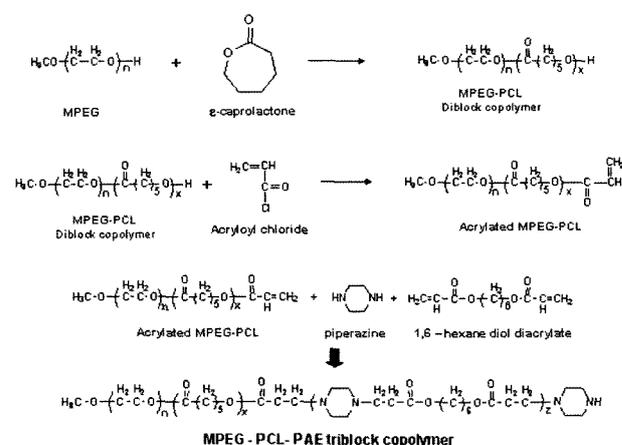
nontoxic small molecule byproducts.⁷⁻¹⁰

Here, we report a polymeric material of which aqueous solutions undergo sol-gel transition by pH change, as well as by temperature change. To construct optimized polymers for the desired property of pH/temperature induced gelation, we have coupled the PAE as a pH sensitive moiety with temperature sensitive block copolymer (methoxy polyethylene glycol-poly(ϵ -caprolactone)) (MPEG-PCL). On the basis of these backgrounds, we synthesized PAE-modified block copolymer (MPEG-PCL-PAE), which can be responsive to both temperature and pH, and evaluated its sol-gel transition behavior upon pH and temperature.

Experimental

Materials. Methoxy poly(ethylene glycol) (MPEG) (M_n = 2k, 5k), ϵ -caprolactone, triethyl amine, anhydrous methylene chloride (MC), stannous-2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$), piperazine and 1,6-hexane diol diacrylate (HDD) were purchased from Sigma-Aldrich Korea. MPEG and ϵ -caprolactone (CL) were both dried under vacuum at 60°C for over 24 h before use. All other chemicals were reagent grade, and used as received. *n*-Hexane, chloroform, and tetrahydrofuran were obtained from Samchun Chemical Co. (Korea) and were used as received.

Synthesis of MPEG-PCL Diblock Copolymers. Hydroxy terminated MPEG-PCL amphiphilic block copolymers were synthesized by a ring-opening polymerization of PCL in the presence of MPEG homopolymer and $\text{Sn}(\text{Oct})_2$, as shown in Scheme I. Molecular weight was controlled by feed ratio and then synthesized diblock copolymers are shown in Table I. Before initiating the reaction, MPEG and $\text{Sn}(\text{Oct})_2$ were first dried under vacuum at 85°C. Then, CL was added to the flask at 65°C under N_2 atmosphere, and



Scheme I. Synthesis of MPEG-PCL-PAE triblock copolymer.

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the resulting mixture was dried under vacuum at 65 °C for 1 h and then stirred at 135 °C for 24 h under N₂ atmosphere. The resulting MPEG-PCL diblock copolymers were precipitated in *n*-hexane to remove unreacted CL monomer, and then dried under vacuum at 40 °C for 24 h.

Acrylation of MPEG-PCL Diblock Copolymer. Acrylated MPEG-PCL diblock copolymer (MPEG-PCL-A) was obtained as shown in Scheme I. First, the synthesized MPEG-PCL diblock copolymer (1 eq.) was dried under vacuum and dissolved in anhydrous methylene chloride at 10% solids concentration in a two-necked round bottom flask protected from moisture by a CaCl₂ guard tube. Triethylamine (2 eq.) was added and the content was cooled to 0 °C, followed by the dropwise addition of acryloyl chloride (1.5 eq.). The mixture was stirred at 0 °C for 2 h, allowed to attain room temperature and then stirred for a further 24 h, after which MC was evaporated by rotary evaporator. The dried product was dissolved in THF and the resulting solution was filtered to remove salts. The filtrate was precipitated in *n*-hexane, and the precipitated product was dried under vacuum at room temperature.

Synthesis of MPEG-PCL-PAE Triblock Copolymers. MPEG-PCL-PAE triblock copolymers were synthesized by Michael reaction of MPEG-PCL-A, piperazine and HDD. The molecular weight of PAE in MPEG-PCL-PAE block copolymers was controlled by feed ratio.

First, MPEG-PCL-A, HDD and piperazine were dissolved in chloroform (CHCl₃). The polymerization was then carried out at 50 °C for 48 h, after which the product was obtained by precipitation in *n*-hexane. Finally, the precipitated product was dried under vacuum at room temperature.

Buffer Preparation. Borax buffer solutions (1 L) with pH range 4.0 to 9.0 were prepared. Potassium dihydrogenphosphate (0.05 mol, 6.8 g) and potassium tetraborate tetrahydrate (0.05 mol, 15.275 g) were dissolved in 1 L of deionized water. For each buffer solution, 5 M HCl (NaOH) and 1 M HCl (NaOH) solutions were used to adjust the pH.

Characterization. The extent of reaction was confirmed by ¹H-NMR spectroscopy (500 MHz JNM-LA FT-NMR). The molecular weight and polydispersity index (PDI) were measured using gel-permeation chromatography (GPC,

Shodex-KF 802.5, KF 803L), with THF as the eluent and a flow rate of 1 mL/min.

Sample Preparation for Sol-Gel Transition. The sol-gel phase transition temperature of the MPEG-PCL-PAE block copolymer in buffer solution was recorded using the inverting test method with a 4 mL vial test tube at a temperature interval of 2 °C. Each sample at a given concentration (30 wt%) was dissolved in pH 2 buffer solution for 2 h at 60 °C. After complete dissolution, At 50 °C, pH of the solutions was changed to the desired pH in the range of 5.0 to 7.5 (pH intervals of 0.5) using 5 M HCl and 5 M NaOH solutions. The sol-gel transition was determined by tilting the vial horizontally after it had been kept for 10 min at constant temperature to achieve an equilibrium state with decreasing temperature by 2 °C from 50 °C.

Results and Discussion

GPC and ¹H-NMR Characterization. Table I summarizes the result of polymerization. Hydrophilic block and hydrophobic block were controlled by feed ratio. All polymers have the PAE block with 600~800 g/mol.

The MPEG-PCL diblock were obtained in almost quantitative yield. The molecular weight of the PCL in MPEG-PCL could be controlled by feed ratio of CL to MPEG.

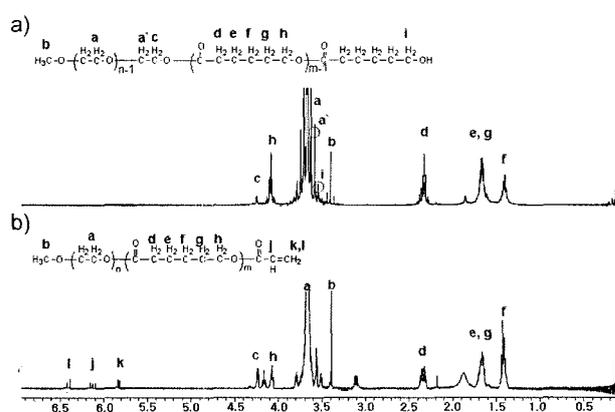


Figure 1. ¹H-NMR spectra of a) MPEG-PCL and b) MPEG-PCL-A.

Table I. The Molecular Weight of Synthesized Polymers

Sample Name	MPEG ^a	MPEG-PCL ^b	MPEG-PCL-PAE ^c	MPEG-PCL-PAE (wt/wt/wt)	PDI ^c	Sol-Gel Phase Transition
A1	2000	2898	3495	1/0.4/0.3	1.2	X
A2		3124	3751	1/0.6/0.3	1.2	O
A3		3467	3991	1/0.7/0.3	1.3	O
A4		3893	4451	1/0.9/0.3	1.3	X
B1	5000	6328	7175	1/0.3/0.2	1.3	O

^aDetermined by supplier. ^b¹H-NMR. ^cGPC.

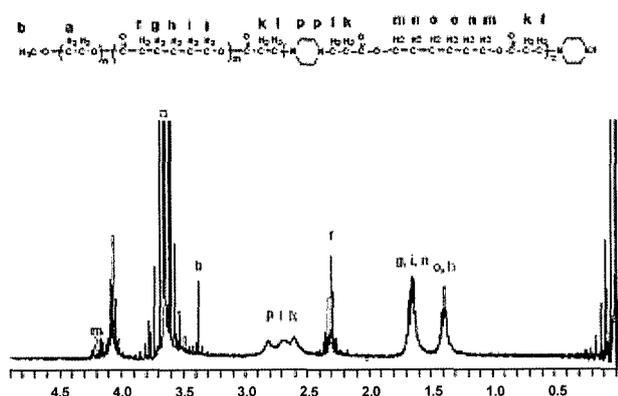


Figure 2. $^1\text{H-NMR}$ spectrum of MPEG-PCL-PAE.

Figure 1 shows the $^1\text{H-NMR}$ spectrum of MPEG-PCL and MPEG-PCL-A. The proton peaks of alken could be found at 5.81, 6.14 and 6.41 ppm.

The structure of MPEG-PCL-PAE was confirmed by H-NMR. The alken proton peaks (5.81, 6.14 and 6.41 ppm) of 1,6-hexane diol diacrylate and acrylated MPEG-PCL diblock copolymers were shifted to left (2.62, 2.70 and 2.83 ppm). Figure 2 shows $^1\text{H-NMR}$ spectrum of MPEG-PCL-PAE.

Sol-Gel Phase Diagram. The gelation mechanism of thermo-responsive hydrogel was explained very well in previous studies.¹¹⁻¹³ The basic concept of gelation is the packing of micelles that are formed by increasing the temperature. The mechanism of pH-responsive hydrogel is similar to that of thermo-responsive hydrogel. In this experiment, the triblock copolymers composed of a hydrophilic block (MPEG), a hydrophobic block (PCL) and a pH-sensitive block (PAE) were synthesized. PAE is an important factor in the gelation of the block copolymer. The pK_b of PAE is about 6.5 at room temperature.¹⁰ Below this pH, PAE is the ionic state and it is the deionized state above this pH. The combined hydrophobic blocks of PCL and the deionized PAE can form a micelle and form a gel at room temperature. When the PAE is an ionic state, it is hydrophilic and the copolymer is soluble in PBS solution even at high temperature around 50°C. Thus this block copolymer shows the pH responsive sol-gel transition.

Figure 3 shows a sol-gel phase diagram of block copolymers without (a) and with (b) pH-sensitive block. MPEG-PCL block copolymer does not show a pH-responsive gelation but shows a thermo-responsive gelation. At lower temperature, this diblock copolymer forms a gel by micelle packing. At higher temperature, the core of micelles is so hydrophobic that the micelle size decreases due to diffusing out of the water from the core. The packing fraction of micelle changed to below the maximum packing fraction due to the small size. Thus the polymer solution is a sol at high temperature.

In the case of MPEG-PCL-PAE block copolymer, A2

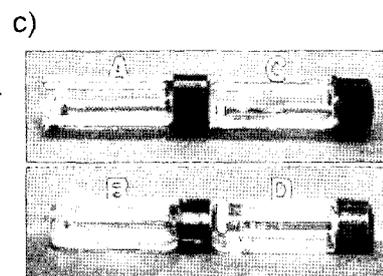
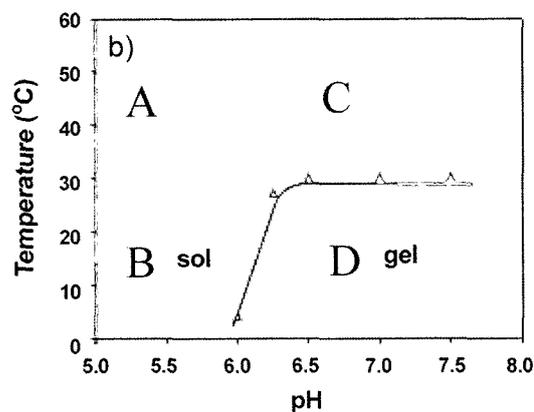
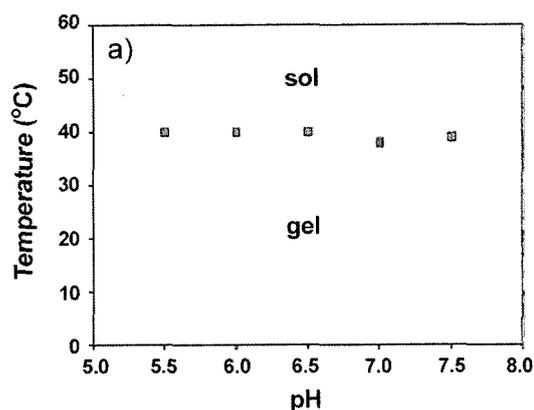


Figure 3. Sol-gel diagram; the effect of PAE block on pH-sensitive gelation. a) MPEG-PCL (1/0.6); b) sample A2 (MPEG-PCL-PAE (1/0.6/0.3)); c) photograph of sol-gel state.

shows a temperature-responsive and pH-responsive gelation property due to the ionization of PAE block. Polymer solution does not form a hydrogel with decreasing temperature below pH 6.4, and on the other hand, it shows a sol-gel transition with decreasing temperature above pH 6.4 (Figure 3(b)). Figure 3(c) shows a flowing state at each state (A, B, C, D). Except for state D, all solutions could flow. This result originates from the ionization of PAE block. Because PAE block has many tertiary amine groups, it can be ionized at low pH. A tendency that whole block is solubilized more easily at low pH than at high pH affects the micelle forming.

The sol-gel transition was measured by changing the molecular parameters of MPEG-PCL-PAE triblock copolymers,

such as the PCL length and MPEG length. Sol to gel temperature was increased as the PCL block length was increased from A2 to A3 with fixed MPEG-PAE length (Figure 4). This shows that the sol-gel temperature of longer hydrophobic block copolymer has higher transition temperature compared to the block copolymer having shorter hydrophobic block. Because A3 having longer hydrophobic block has a bigger core than A2, its micelle size is bigger. Then it will form a hydrogel more easily. On the other hand, A1 and A4 do not have a sol-gel transition. Because A1 is soluble in water and it is too hydrophilic to form a micelle by changing the temperature due to insufficient hydrophobic block length. A4 is insoluble at any pH due to an immoderate chain length of hydrophobic block. This indicates that the fine tuning of hydrophobic block length is needed for the sol-gel transition.

On increasing MPEG block length of MPEG-PCL-PAE triblock copolymers from 2,000 to 5,000 (Figure 4), the phase diagram shifted to high temperature and low pH. Similar to the result of increasing hydrophobic length, higher-molecular-weight MPEG can form a bigger corona of micelle. Accordingly B1 having MPEG5k shows broader gelation region than block copolymers having MPEG2k. Similarly the micelle size of B1 is bigger than that of MPEG2k series; therefore it can show gelation even at low pH. The micelle size increase by PEG length change and the molecular weight increase of PAE¹⁰ make the phase diagram to shift to lower pH region.

From these results, MPEG-PCL-PAE triblock copolymers hydrogel can be optimized. The human body maintains 37°C and pH 7.4, therefore the triblock copolymer like B1 could be applicable to injectable hydrogel system.

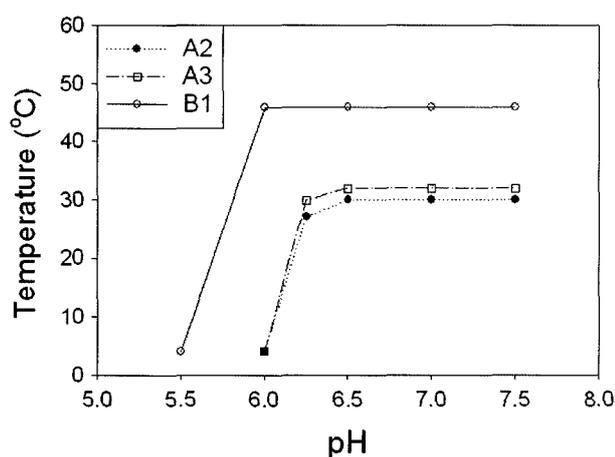


Figure 4. Sol-gel diagram; the effect of MPEG and PCL block length on pH-sensitive gelation.

Conclusions

Block copolymers, composed of MPEG, PCL and PAE, were synthesized through a combination of ring opening reaction and Michael reaction. Their aqueous solutions show a sensitive sol-gel transition by pH and temperature within a small pH range. The sol-gel phase diagrams of the block copolymer solutions were controlled deliberately by altering either the ratio of hydrophobic to hydrophilic blocks within the block copolymer or the PEG length. The aqueous block copolymer solution composed of MPEG=5,000, PEG/PCL ratio=1/0.3 and PAE=847 had a gel region including the *in vivo* conditions (37 °C, pH 7.4). This polymer solution could be formulated with various drugs at pH 5~6 buffer solution for the injection to the human body. This could be one of the candidate of the injectable hydrogel for the sustained release of drug.

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References

- (1) Z. Ding, R. B. Fong, C. J. Long, P. S. Stayton, and A. S. Hoffman, *Nature*, **411**, 59 (2001).
- (2) R. Yoshida, K. Sakai, T. Okano, and Y. Sakurai, *J. Biomater. Sci. Polym. Ed.*, **6**, 585 (1994).
- (3) C. M. Hassan, F. J. Doyle III, and N. A. Peppas, *Macromolecules*, **30**, 6166 (1997).
- (4) Z. Yang, S. Pickard, N. J. Deng, R. J. Barlow, D. Attwood, and C. Booth, *Macromolecules*, **27**, 2371 (1994).
- (5) M. Malmsten and B. Lindman, *Macromolecules*, **25**, 5440 (1992).
- (6) G. Chen and A. S. Hoffman, *Nature*, **373**, 49 (1995).
- (7) D. M. Lynn and R. Langer, *J. Am. Chem. Soc.*, **122**, 10761 (2000).
- (8) D. M. Lynn, M. M. Amiji, and R. Langer, *Angew. Chem. Int. Ed.*, **40**, 1707 (2001).
- (9) A. Potineni, D. M. Lynn, and R. Langer, *J. Control. Release*, **86**, 223 (2003).
- (10) M. S. Kim, E.-K. Choi, H.-J. Park, J.-S. Kim, and D. S. Lee, *Macromol. Res.*, **13**, 147 (2005).
- (11) B. Jeong, D. S. Lee, Y. H. Bae, and S. W. Kim, *Nature*, **388**, 860 (1997).
- (12) B. Jeong, D. S. Lee, J. I. Shon, Y. H. Bae, and S. W. Kim, *J. Polym. Sci.; Part A: Polym. Chem.*, **37**, 751 (1999).
- (13) M. J. Song, D. S. Lee, J. H. Ahn, D. J. Kim, and S. C. Kim, *J. Polym. Sci.; Part A: Polym. Chem.*, **42**, 772 (2004).