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Molecular design of novel pH/temperature-sensitive hydrogels

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ABSTRACT

A series of poly(β-amino ester)–poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone)–poly(β-amino ester) pentablock copolymers (PAE–PCL–PEG–PCL–PAE) were designed and prepared to examine factors affecting sol–gel phase transition behavior. First, the composition of a series of PCL–PEG–PCL copolymers was controlled by changing the feed ratios of PCL/PEG and the molecular weight of PEG. Second, the composition of pentablock copolymers was varied using different PCL–PEG–PCL copolymers and several feed ratios of PAE monomers. The physicochemical properties of triblock and pentablock copolymers were characterized by ^{1}H NMR and gel permeation spectroscopy. The PAE–PCL–PEG–PCL–PAE copolymers in aqueous solution (20–30 wt%) underwent sol–gel transitions with changes in both pH change and temperature. With increasing molecular weight of PAE, the sol–gel transition zone became narrower because the hydrophobic character of the copolymers decreased. Also, with increases in PCL/PEG ratio and PEG molecular weight, changes in the hydrophobic/hydrophilic balance within copolymers resulted in alterations in sol–gel phase transitions.

1. Introduction

Intelligent polymers and hydrogels responding to both temperature and pH changes have attracted increasing interest as they offer unique advantages compared with polymers that respond to only a single stimulus [1–5]. Hydrogels bearing cationic groups have received considerable attention because they can bind macromolecules such as proteins through ionic interactions [2–5]. An example of a cationic copolymer is poly(2-(dimethylamino)ethyl methacrylate)-pluronic-poly(2-(dimethylamino)ethyl methacrylate) [2]. An aqueous solution of this pentablock copolymer can change to a gel at about pH 7.4 and 37 °C. Unfortunately, this polymer cannot be degraded in aqueous solution.

Recently, our group has developed another kind of cationic multiblock copolymer hydrogels based on poly(amino urethane) (PAU) [6,7]. These copolymers were synthesized using condensation polymerization of 1,6-diisocyanato hexamethylene (HDI) with bis-1,4-(hydroxyethyl)piperazine (HEP) and hydroxyl groups of poly(ethylene glycol) (PEG) to create $(PAU–PEG–PAU)_n$ or hydroxyl groups of poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL–PEG–PCL) to create $(PAU–PCL–PEG–PCL–PAU)_m$. It was difficult to control the molecular weight of these copolymers, thus multiblock copolymers were formed.

Poly(β-amino ester) (PAE) is a cationic, non-cytotoxic, and biodegradable polymer [8]. PAE has been used for gene delivery [9,10], paclitaxel administration [11], to mediate cellular uptake of heparin prior to cancer cell death [12], and in tissue engineering [13]. In our previous paper, PAE served as a bifunctional unit in the design of a pH/temperature-sensitive pentablock copolymer [3]. The copolymer was synthesized by conjugating PAE to both sides of thermo-sensitive poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) to create poly(β-amino ester)–poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone)–poly(β-amino ester) pentablock copolymers (PAE–PCL–PEG–PCL–PAE). This material exhibited many advantages including the possibility of ionic link formation involving the positive charges of PAE and the negative charges of drugs/proteins. The copolymer solution could be injected with no surgical procedure, no clogging during injection, straightforward drug loading to the polymer solution, no initial burst of release, and long sustained release. The release of anionic drugs or proteins was controlled principally by the PAE degradation rate.

In addition, the polymer/protein solution showed a shift in sol–gel transition compared to that of the polymer solution alone [3]. Control of such a shift by pH and temperature near physiological values is important in practical applications. To obtain phase shifts under physiological conditions, it is thus necessary to understand factors affecting the sol–gel transition.

In this study, a series of PAE-based pentablock copolymers (PAE–PCL–PEG–PCL–PAE) were synthesized using PEGs of various molecular weights, and by varying the feed ratios of both PCL/PEG and PAE monomers, to examine factors affecting sol–gel phase transition behavior of copolymers in solution. The sol–gel transition window can be precisely tuned by varying PEG molecular weight, the hydrophobic/

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hydrophilic block ratio (PCL/PEG), the molecular weight of the pH-sensitive block (PAE), and the polymer concentration. In addition, the effects of storage conditions on copolymer degradation were examined.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol)s (PEGs) (M_n 1500 and 1650) were purchased from Sigma–Aldrich Co. (St. Louis, MO) and ID Biochem (Seoul, Korea) (M_n 1750). The PEGs were recrystallized from *n*-hexane and dried under vacuum for 3 days prior to use. The chemical ε-caprolactone (CL) and phosphate-buffered saline (PBS) were obtained from Sigma–Aldrich. Stannous octoate [$Sn(Oct)_2$] was obtained from Sigma–Aldrich and was dried for 24 h under vacuum at ambient temperature prior to use. Acryloyl chloride (AC), triethylamine (TEA), 1,4-butanediol diacrylate (BDA), and 4,4′-trimethylene dipiperidine (TMDP) were purchased from Sigma–Aldrich. HCl (37%, w/v), sodium chloride, chloroform, dichloromethane (DCM), tetrahydrofuran (THF) and diethyl ether were all products of Samchun Co. (Korea). All other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of PCL–PEG–PCL triblock copolymers

A series of PCL–PEG–PCL triblock copolymers were synthesized from CL and PEG by ring opening polymerization using $Sn(Oct)_2$ as a catalyst. PEG of various molecular weights (1500, 1650, and 1750) was used to synthesize the copolymers. The composition and molecular weights of the copolymers were adjusted to control the hydrophobicity/hydrophilicity balance using PEG of any particular molecular weight. The synthesis procedure of a triblock copolymer, composed of PCL/PEG at a weight ratio of 1.8 and PEG of M_n 1500, commenced with drying of 4 g PEG and 0.04 g $Sn(Oct)_2$ in a two-neck round-bottom flask at 110 °C for 2 h under vacuum. After cooling to 60 °C, 7.6 g CL was added under a dry nitrogen atmosphere. The reaction mixture was dried for 1 h under vacuum at 60 °C, and the temperature was then increased slowly to 130 °C (over 30 min). After 18 h, the temperature was decreased to room temperature, and chloroform was added to dissolve the product. The precipitated product obtained by dropping the polymer solution into excess diethyl ether was dried under vacuum at ambient temperature for 48 h. The yield of triblock copolymer was over 85%.

2.3. Synthesis of triblock copolymer diacrylate

TEA was used as a catalyst to conjugate AC to the hydroxyl groups of a PCL–PEG–PCL copolymer. Reactant amounts were calculated based on the molar ratios of the triblock copolymer, AC, and TEA. Each triblock was acrylated at the same feed ratio:

$$\text{Triblock/AC/TEA} = 1/3.2/2(\text{molar ratio}) \quad (1)$$

Based on equation (1), the quantities of reactants for acrylation were 4 g of triblock PCL–PEG–PCL (with PEG M_n 1500 and a PCL/PEG ratio of 1.8 [w/w]), 0.26 mL TEA, and 0.25 mL AC (96%, v/v). The PCL–PEG–PCL copolymer was dried for 2 h under vacuum at 80 °C in a two-neck round-bottom flask. Next, anhydrous chloroform was used to dissolve the copolymer at room temperature to obtain a solution of 20 wt%. After addition of TEA and AC, reaction proceeded at 10 °C under nitrogen for 48 h. The chloroform was evaporated at room temperature and the dry product dissolved in DCM. The precipitate obtained by dropping the DCM polymer solution into excess diethyl ether was dried under vacuum at ambient temperature for 48 h.

2.4. Synthesis of pH/temperature-sensitive PAE–PCL–PEG–PCL–PAE pentablock copolymers

PAE was conjugated to the PCL–PEG–PCL copolymer by Michael reaction addition polymerization employing the vinyl group at the end of the acrylated triblock and BDA, with active hydrogen supplied by the amine groups of TMDP. The molar ratio of BDA/TMDP was 1/1. The molecular weight of PAE could be controlled by varying the feed amounts of BDA and TMDP.

To prepare a pentablock copolymer with PEG of M_n 1500, a PCL/PEG ratio of 1.8 (w/w), and PAE of molecular weight ~1.3 k, 4 g acrylated triblock, 1.89 mL BDA, and 1.96 g TMDP were employed. The acrylated triblock copolymer, BDA, and TMDP were dissolved in 40 mL DCM in a round-bottom flask at ambient temperature. The reaction proceeded for 48 h at 50 °C using a refluxing condenser. Next, DCM was removed by evaporation at 40 °C and the dry residue dissolved in THF. The pentablock copolymer solution was filtered through filter paper (5C 100 circles; Toyo Roshi Kaisha, Japan). THF was evaporated at 50 °C and the dried copolymer was dissolved in DCM and purified by precipitation into diethyl ether. The precipitated product was dried under vacuum at room temperature for 48 h. The yield of pentablock copolymer was over 72% after drying. The synthetic route is shown in Scheme 1.

2.5. ^1H NMR analysis

The molecular structure and composition of copolymers were determined by analysis of 500 MHz ^1H NMR (Unity Inova 500NB; Varian) spectra. Chloroform with 0.03 (v/v) tetramethylsilane (TMS) was used as solvent. The composition of each block was calculated on the basis of typical proton peak integration for PEG and CL [12].

2.6. Gel permeation chromatography (GPC) analysis

The molecular weights and distributions of copolymers were measured by gel permeation chromatography (GPC) (Model 410; Waters) equipped with a refractive-index detector (RI-101; Shodex), using two 4 mm Styragel columns from 500 to 10 Å in series. Tetrahydrofuran (THF) was used as eluting solvent. Poly(ethylene glycol)s (Waters) of molecular weights 420–22,100 were used as standards. The flow rate was 1 mL/min at a temperature of 36 °C.

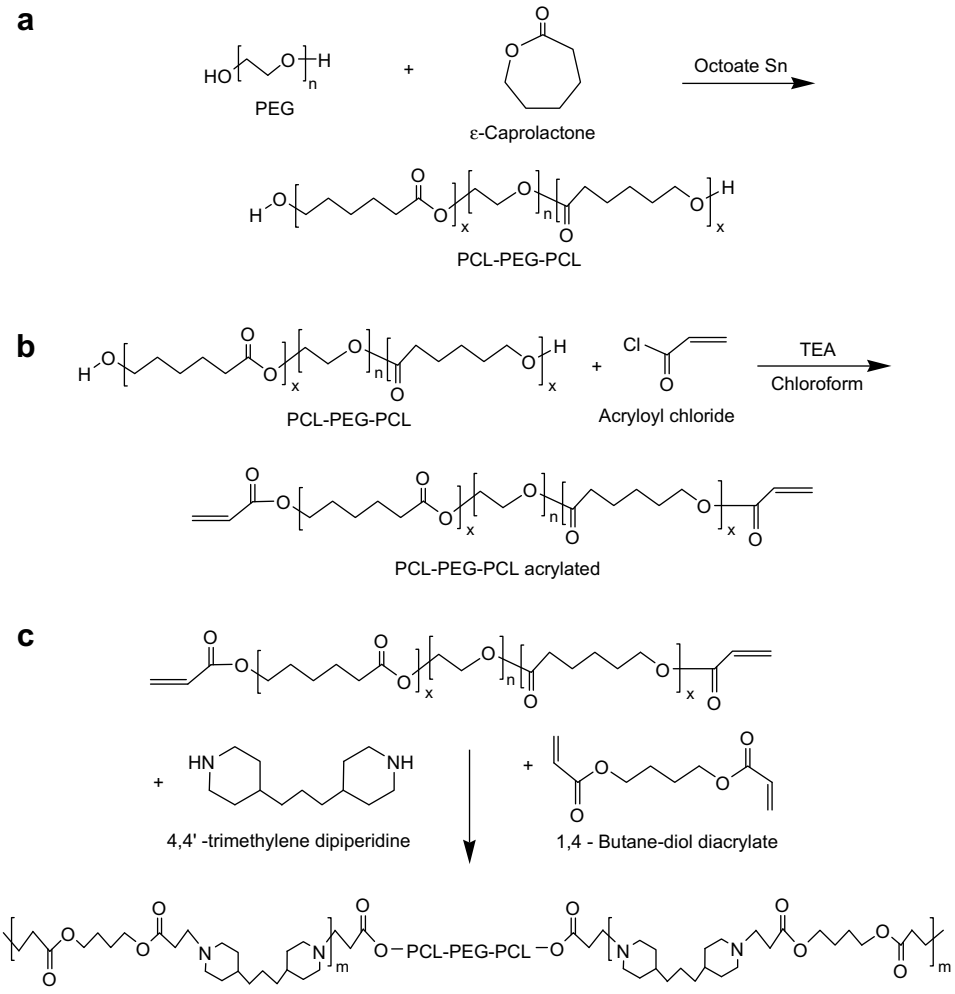
2.7. Sol–gel phase transition measurement in vitro

The tube inversion method was used to determine phase transitions of copolymers in aqueous media at temperature intervals of 1 °C. Copolymer solutions were prepared by dissolving triblock and pentablock copolymers in PBS buffer (with 2% [v/v] of 37% [v/v] HCl) at 2 °C. The pH of pentablock copolymer solutions was about 6.0 after dissolution. The pH was adjusted with sodium hydroxide (5 M) and HCl (5 M) and pH values were measured by pH meter (IQ 204 instrument). The sol–gel transition at each temperature was determined by angling the vial horizontally after holding at a constant temperature for 10 min [14].

2.8. Gel integrity in vivo

To study the gel integrity of aqueous copolymer solutions in vivo, male Sprague–Dawley (SD) rats (Hanlim Experimental Animal Laboratory, Seoul, Korea) were used. Rats (5–6 weeks old, average body weight 200 g) were handled in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication 85-23, revised 1985).

PAE–PCL–PEG–PCL–PAE (PEG M_n 1650, PCL/PEG ratio 1.8 [w/w], PAE of 1.25 k) was dissolved in water to 20 wt% and 200 μL amounts



Scheme 1. Synthesis of pH/temperature-sensitive PAE–PCL–PEG–PCL–PAE block copolymer. (a) Synthesis of PCL–PEG–PCL triblock copolymer, (b) synthesis of acrylated triblock copolymer, (c) synthesis of PAE–PCL–PEG–PCL–PAE pentablock copolymer.

(at 10 °C and pH 6.6) were subcutaneously injected into the sides of the back. After 15 min and 1 week, gel integrity and biocompatibility were evaluated by operation.

2.9. Evaluation of storage stability of PAE–PCL–PEG–PCL–PAE

Stored samples were prepared by two methods. In Method 1, pentablock copolymer samples were kept as dry powders in a refrigerator at 0 °C. In Method 2, samples were kept as copolymer solutions (20 wt%, pH 6.6) also at 0 °C. Samples were taken at various times. Samples of copolymer stored by Method 2 were freeze-dried to obtain dried polymer. All samples were dissolved in THF and filtered. The degradation rate of a polymer was measured by analysis of molecular weight change over time as determined by GPC (eluent: THF; 36 °C; PEG standards were employed).

3. Results and discussion

3.1. Synthesis and characterization of block copolymers

The PCL–PEG–PCL triblock and PAE–PCL–PEG–PCL–PAE pentablock copolymers were analyzed by ^{1}H NMR.

As shown in Fig. 1a, characteristic signals at 3.58 and 2.21 ppm may be assigned to the methylene hydrogen of the EO unit and the commencement of the CL unit, respectively. The characteristic signal at 4.0 ppm is from the methylene hydrogen at the end of the PEG unit and the end of the CL unit. The characteristic signals at 1.23 and 1.25 ppm may be assigned to the other methylene hydrogens of CL. Compositions were obtained by calculating peak areas [14]. With the acrylated triblock copolymer, the characteristic signals at 5.80 ppm and 6.39 ppm are from the two β-hydrogens of the acrylate unit, and the signal at 6.11 ppm may be assigned to the α-hydrogen of the acrylate unit. Based on the ^{1}H NMR spectra, the number of repeat units of PCL and the molecular weights of triblocks can be calculated by equation (2) and are shown in Table 1:

$$x = C^*(n-1)/(A+A') \quad (2)$$

Here, the number of repeat units (n) of PEG is provided by the suppliers and x is the number of repeat units of PCL (Fig. 1a).

In Fig. 1b, the peaks assigned to the β-hydrogen of the acrylate unit are not observed in the ^{1}H NMR spectrum of PAE–PCL–PEG–PCL–PAE. In addition, new peaks at 2.41, 2.59, and 2.80 ppm may be assigned to the methylene hydrogen unit of C2 in piperidine, and acrylate. The signals appearing at 1.56 and 4.10 ppm are from the methylene hydrogen units of butanediol. Moreover, there is no signal showing crosslinking between acrylate groups, indicating that no radical initial reaction of these groups occurred during the synthesis of PAE.

In addition, PCL–PEG–PCL and PAE–PCL–PEG–PCL–PAE molecular weights and distributions were analyzed by GPC. As shown in Fig. 2, the GPC traces were very smooth and narrow, indicating that the copolymers had narrow molecular weight distributions. The

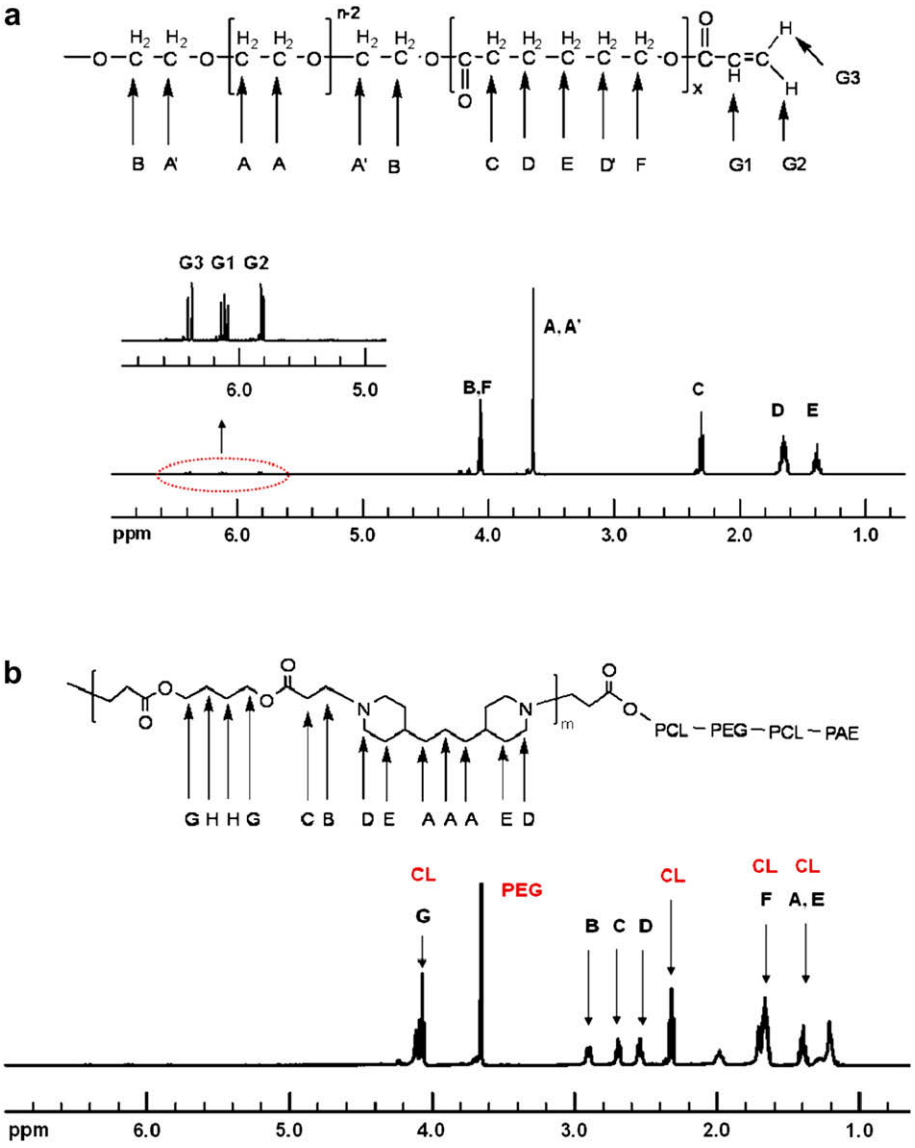


Fig. 1. [1]H NMR spectra of copolymers at composition: PEG 1650, a PCL/PEG of ratio 1.8 (w/w); PAE of 1.25 k. (a) PCL–PEG–PCL acrylated, (b) PAE–PCL–PEG–PCL–PAE.

molecular weights and distributions of PCL–PEG–PCL and PAE–PCL–PEG–PCL–PAE copolymers, obtained by GPC, are listed in Table 1.

3.2. Sol–gel phase transition diagrams

The sol–gel transitions of PAE–PCL–PEG–PCL–PAE copolymer solutions were determined by the tube inversion method using PBS buffer at various pH values and temperatures. Four copolymer sol–gel states, seen at high copolymer concentrations (20–30 wt%), are shown in Fig. 3. At low temperature (5 °C) and pH (pH 5.5), the PAE blocks were ionized and thus hydrophilic [3] whereas the hydrophobicity of PCL blocks was weak. This led to weak association of PCL–PAE blocks [3] and the copolymer solution remained a sol (in the "A" state). At a higher temperature and low pH (such as 37 °C and pH 5.5), the PCL blocks became more hydrophobic and micelles tended to grow because of hydrophobic interactions between these blocks. However,

Table 1
Molecular weight and polydispersity of PCL–PEG–PCL triblock and PAE–PCL–PEG–PCL–PAE pentablock copolymers.

PCL–PEG–PCL (M_n)[a]	PEG M_n[b]	PCL/PEG (w/w)[a]	PAE–PCL–PEG–PCL–PAE[c]	M_w/M_n[c]
984–1500–984	1500	1.3	1285–984–1500–984–1285	1.43
1110–1500–1110	1500	1.5	1301–1110–1500–1110–1301	1.46
1364–1500–1364	1500	1.8	1225–1364–1500–1364–1225	1.45
1364–1500–1364	1500	1.8	1925–1364–1500–1364–1925	1.52
1364–1500–1364	1500	1.8	2345–1364–1500–1364–2345	1.58
1262–1650–1262	1650	1.5	1287–1262–1650–1262–1287	1.43
1572–1650–1572	1650	1.8	1258–1572–1650–1572–1258	1.41
1310–1750–1310	1750	1.5	1254–1310–1750–1310–1254	1.43

[a] PCL–PEG–PCL number-average molecular weights were calculated from [1]H NMR.
[b] Provided by Aldrich.
[c] Measured by GPC.

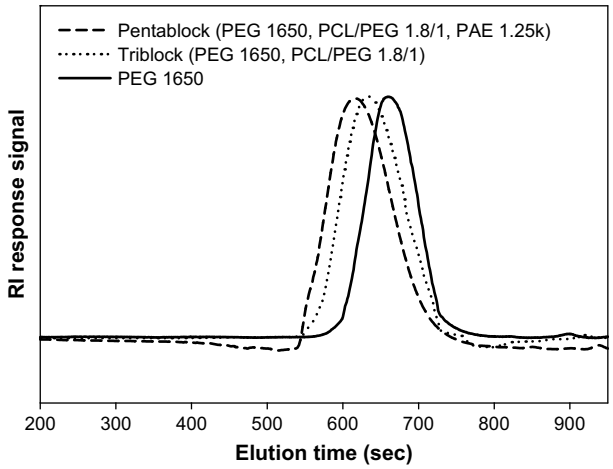


Fig. 2. GPC traces of PEG, PCL–PEG–PCL, and PAE–PCL–PEG–PCL–PAE.

the polymer solution could not gel because ionized PAE blocks obstructed strong micellar interactions (in the "B" state). Similarly, at a high pH and a low temperature (in the "C" state, 5 °C and pH 7.4), the PAE blocks became hydrophobic because of deionization resulting from increasing pH, leading to micellar aggregation. However, PCL is a rather weak hydrophobic block at low temperature. Therefore, the copolymer solution assumed the sol state [15]. However, in the "D" state (37 °C and pH 7.4), a strong micellar aggregation is formed by hydrophobic interactions between PCL–PAE blocks.

3.3. Effect of PAE molecular weight on phase transitions

Fig. 4 shows phase diagrams of the PCL–PEG–PCL and PAE–PCL–PEG–PCL–PAE copolymers with PEG M_n 1500, all at the same PCL/PEG 1.8 (w/w) ratio, and using PAE of various molecular weights. A 20 wt% preparation of PCL–PEG–PCL exhibited a sol–gel transition as the temperature increased. In the pH range 5.5–7.6, the sol–gel transition showed only temperature dependence, and not pH dependence. Three regions corresponding to different states are shown; these are the sol state at temperatures below 30.5 °C, the gel region between 30.5 °C and 51 °C, and another sol (sedimentation) region when the temperature was higher than 51 °C. After conjugation of PAE to triblock copolymers, the pentablock copolymers in solution showed sol–gel transitions responsive to both temperature and pH. As the molecular weight of PAE increased, the hydrophobicity of

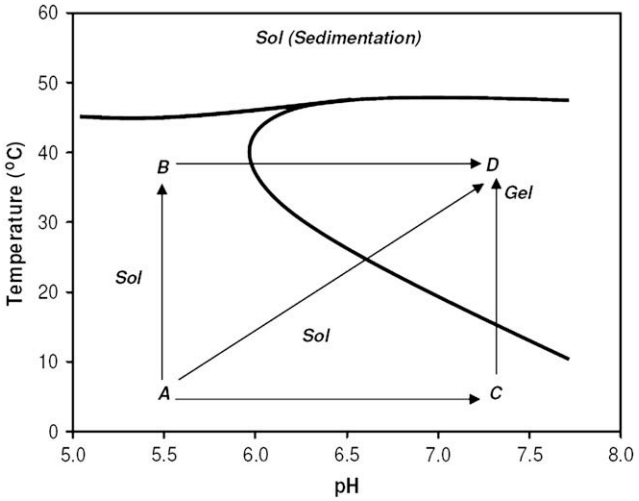


Fig. 3. Sol–gel transition of PAE–PCL–PEG–PCL–PAE.

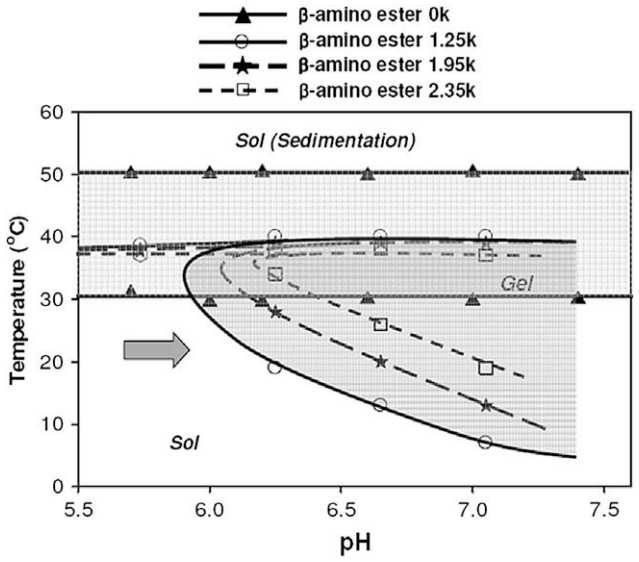


Fig. 4. Sol–gel phase transitions of PAE–PCL–PEG–PCL–PAE (PEG M_n 1500 and PCL/PEG ratio of 1.8 [w/w]) block copolymer solutions made using PAE of varying molecular weights.

a pentablock copolymer decreased at pH values below pK_b because of ionization of PAE [3,16]. Thus, the PAE–PCL block became more hydrophilic and the copolymer solution existed as a sol at low pH.

The temperatures of the sol-to-gel transitions became higher, whereas the gel-to-sol transition temperatures remained similar, when PAE block length increased from 1.25 k to 2.35 k. This resulted in narrowing of the phase diagram gel region. With increases in the sizes of PAE blocks, the net hydrophilicity of PAE–PCL–PEG–PCL–PAE copolymers increased. To assist in gel formation, therefore, not only did a pH increase induce more PAE block deionization, but a temperature increase also induced more PCL block hydrophobicity.

3.4. Effect of PEG molecular weight on phase transitions

Fig. 5 shows the phase diagrams of PAE–PCL–PEG–PCL–PAE transitions of copolymers containing PEG of various molecular weights, with the PCL/PEG weight ratio fixed at 1.5/1, and using PCL of 1.3 k. The sol–gel diagrams show a shift toward higher transition temperatures

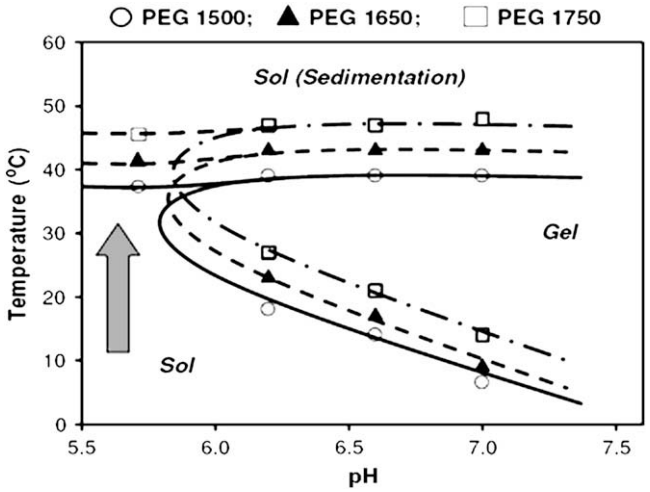


Fig. 5. Sol–gel phase transitions of PAE–PCL–PEG–PCL–PAE (PCL/PEG ratio of 1.5 [w/w] and PAE of about 1.3 k) block copolymer solutions made using PEG of varying molecular weights at a concentration 20 wt% under various pH and temperatures.

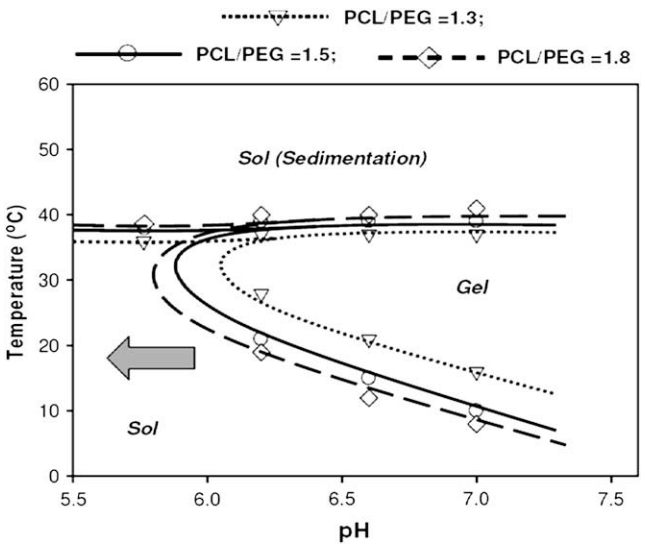


Fig. 6. Sol–gel phase transitions of PAE–PCL–PEG–PCL–PAE (PEG M_n 1500 and PAE of about 1.3k) block copolymer solutions with different PCL/PEG ratios at a concentration 20 wt% under various pH and temperatures.

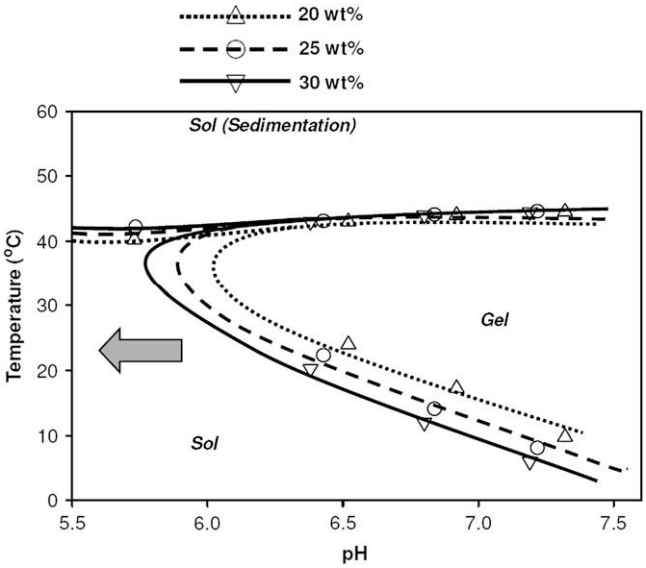


Fig. 7. Sol–gel transition diagrams of PAE–PCL–PEG–PCL–PAE (PEG M_n 1650, PCL/PEG ratio of 1.8, and PAE of 1.25 k) solutions with different concentrations.

when the molecular weight of PEG increased from 1500 to 1750. PAE is in the de-ionized state at high pH and thus acts as a hydrophobic block. Therefore, at a fixed ratio of PCL/PEG and a fixed PAE block length, the molar ratio of hydrophobic blocks (PCL–PAE) to hydrophilic blocks (PEG) decreases with increasing PEG molecular weight. Hence, it is necessary to increase temperature to induce stronger hydrophobicity of PCL–PAE [17,18].

In addition, when a pH/temperature-sensitive block copolymer changes from a sol to a gel, the material remains homogeneous. However, when the copolymer changes from a gel to a sol at the gel-to-sol transition temperature, the copolymer is heterogeneous (composed of gel and squeezed-out water). When the temperature is higher than the gel-to-sol temperature, the hydrogen bonding between polymer and water is weakened. Therefore, water is squeezed out from the gel matrix. The suspension-forming mechanism was explained in a previous paper [17].

3.5. Effect of hydrophobic/hydrophilic ratio on phase transitions

The sol–gel transition diagrams of pentablock copolymers with varying hydrophobic/hydrophilic ratios, and containing a fixed level of PAE blocks, over the pH range pH 5.5–7.6, are presented in Fig. 6. As the PCL/PEG weight ratio increased from 1.3 to 1.8, the pH intervals during which the materials existed as gels became broader and the sol-to-gel temperature decreased. This is because longer PCL blocks lead to stronger hydrophobic interactions at any given temperature [1,16].

3.6. Effect of copolymer concentration on phase transitions

Fig. 7 shows phase diagrams of pentablock copolymers of varying copolymer concentrations. As the copolymer concentration increased, the sol-to-gel temperature decreased, and the gel temperature window became broader. At low concentration (20 wt%), the gelation of polymer solution became possible if more hydrophobic interactions (PCL–PAE) occurred. It was meant that PAE blocks were more de-ionized at high pH and PCL blocks were stronger hydrophobic at high temperature. On the contrary, as the concentration of the polymer solution increases, the gelation of the polymer solution was easily formed at weaker hydrophobic interactions at lower pH and temperature. At high concentration (30 wt%), the viscosity rises due to an increase in the number of micelles and micellar association thus occurs at progressively lower temperatures.

3.7. Gel integrity in vivo

In order to examine the gel integrity in vivo, a polymer solution (20 wt%, pH 6.6, 10 °C) was subcutaneously injected into the sides

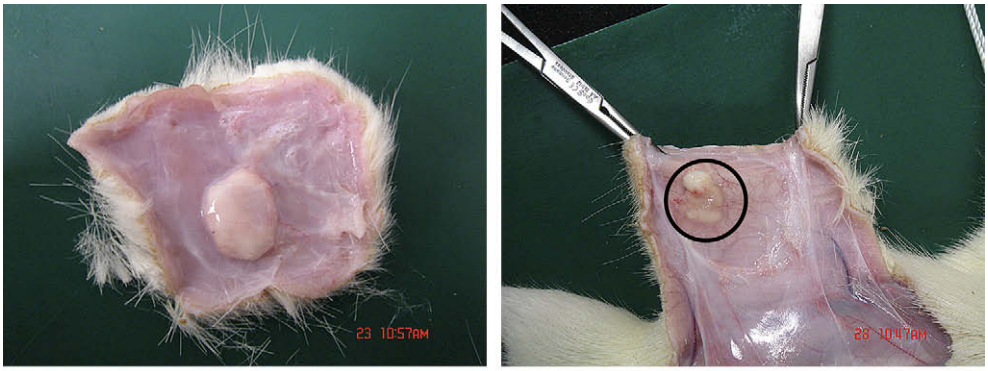


Fig. 8. Gel integrity in vivo of PAE–PCL–PEG–PCL–PAE (PEG M_n 1650, PCL/PEG ratio of 1.8 and PAE of 1.25 k) on SD rats.

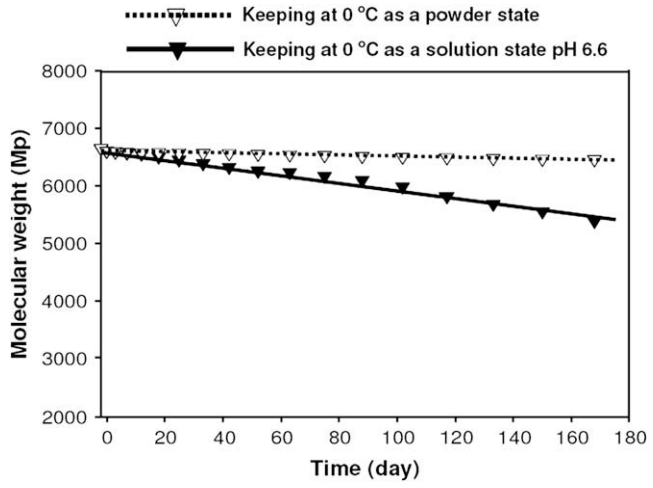


Fig. 9. Molecular weight change with time of PAE–PCL–PEG–PCL–PAE (PEG M_n 1500; PCL/PEG ratio of 1.8; PAE of 1.25 k) at storage conditions.

of the rat's back. After 15 min injection, the rat was scarified to determine the gel integrity. As shown in Fig. 8, the copolymer solution changed to a gel at the injection site as a result of an increase in the pH and temperature caused by body's condition. Fig. 8 also shows the gel integrity after 6 days. It is found that no inflammation was observed around the gel site, indicating that this material is compatible with tissue.

3.8. Storage stability

Fig. 9 shows degradation of the pentablock copolymer under two storage conditions. Degradation in solution (pH 6.6) was faster than in the powder state. After storage in the refrigerator at 0 °C for 6 months, the molecular weight of pentablock in the powdered state decreased only slightly, from 6732 to 6593. However, the molecular weight fell from 6732 to 5376 when the polymer was stored as a solution at pH 6.6. These data are not surprising. It should be noted, however, that most temperature-sensitive injectable block copolymer hydrogels are provided as solutions because reconstitution can be difficult. But PAE–PCL–PEG–PCL–PAE pentablock copolymers can be easily dissolved, within minutes, at about pH 6. Therefore, such copolymers can be supplied as powders. This is important in commercialization.

4. Conclusions

A series of novel pH/temperature-sensitive injectable hydrogels of PAE–PCL–PEG–PCL–PAE pentablock copolymers were prepared to examine sol–gel transitions in aqueous solution. The tendencies of sol phases to change to gel phases increased with rises in both temperature and pH. The sol–gel window of pH/temperature-sensitive block copolymers could be controlled by varying the hydrophobicity/hydrophilicity ratio (PCL/PEG), the molecular weight of PAE, the chain length of PEG, and polymer concentration. The phase diagram of a copolymer with PEG M_n 1650, PCL/PEG weight ratio of 1.8/1, and PAE of 1.25 k showed transitions at physiological values of pH and temperature, as required for biomedical applications. Also, after subcutaneous injection of a copolymer solution into rats, a hydrogel formed within a short time and no inflammation was observed around the hydrogel site. This novel material enlarges applications of injectable hydrogels as new templates for anionic drug, protein, and DNA delivery.

Acknowledgment

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References

[1] Shim WS, Yoo JS, Bae YH, Lee DS. Biomacromolecules 2005;6:2930–4.
[2] Determan MD, Cox JP, Mallapragada SK. J Biomed Mater Res 2007;81A:326–33.
[3] Huynh DP, Nguyen MK, Pi BS, Kim MS, Chae SY, Lee KC, et al. Biomaterials 2008;29:2527–36.
[4] Suh JM, Bae SJ, Jeong B. Adv Mater 2005;17:118–20.
[5] Sy Park, Lee Y, Bae KH, Ahn CH, Park TG. Macromol Rapid Commun 2007;28:1172–6.
[6] Dayananda K, He C, Lee DS. Polymer 2008;49:4620–5.
[7] Dayananda K, He C, Park DK, Park TG, Lee DS. Polymer 2008;49:4968–73.
[8] Lynn DM, Langer R. J Am Chem Soc 2000;122:10761–8.
[9] Anderson DG, Lynn DM, Langer R. Angew Chem Int Ed 2003;42:3153–8.
[10] Akinc A, Anderson DG, Lynn DM, Langer R. Bioconjug Chem 2003;14:979–82.
[11] Potineni A, Lynn DM, Langer R, Amiji MM. J Control Release 2003;86:223–34.
[12] Berry D, Lynn DM, Sasisekharan R, Langer R. Chem Biol 2004;11:487–98.
[13] Ferruti P, Bianchi S, Ranucci E, Chiellini F, Caruso V. Macromol Biosci 2005;5:613–22.
[14] Jeong BM, Lee DS, Shon J, Bae YH, Kim SW. J Polym Sci Part A Polym Chem 1999;37:751–60.
[15] Bae SJ, Syh JM, Bae YH, Kim SW, Jeong B. Macromolecules 2005;38:5260–5.
[16] Kim MS, Lee DS, Choi EK, Park HJ, Kim JS. Macromol Res 2005;13:147–51.
[17] Shim MS, Lee HT, Shim WS, Park I, Lee H, Chang TS, et al. J Biomed Mater Res 2002;61:188–200.
[18] Shim WS, Sung WK, Lee DS. Biomacromolecules 2006;7:1935–41.