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Synthesis and characterization of pH/temperature-sensitive block copolymers via atom transfer radical polymerization

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Abstract

In order to prepare well-defined pH-sensitive block copolymers with a narrow molecular weight distribution (MWD), we synthesized a pH-sensitive block copolymer via atom transfer radical polymerization (ATRP) of sulfamethazine methacrylate monomer (SM) and amphiphilic diblock copolymers by the ring-opening polymerization of D,L-lactide/ε-caprolactone (LA/CL), and their sol–gel phase transition was investigated. SM, which is a derivative of sulfonamide, was used as a pH responsive moiety, while PCLA–PEG–PCLA was used as a biodegradable, as well as a temperature sensitive one, amphiphilic triblock copolymer. The pentablock copolymer, OSM–PCLA–PEG–PCLA–OSM, was synthesized using Br–PCLA–PEG–PCLA-Br as an ATRP macroinitiator. The number average molecular weights of SM were controlled by adjusting the monomer/initiator feed ratio. The macroinitiator was synthesized by the coupling of 2-bromoisobutyryl bromide with PCLA–PEG–PCLA in the presence of triethyl amine catalyst in dichloromethane. The resultant block copolymer shows a narrow polydispersity. The block copolymer solution shows a sol–gel transition in response to a slight pH change in the range of 7.2–8.0. Gel permeation chromatography (GPC) and NMR were used for the characterization of the polymers that were synthesized.

Keywords: pH/temperature-sensitive block copolymers; Amphiphilic block copolymer; Atom transfer radical polymerization

1. Introduction

Stimuli-sensitive hydrogels are studied for their widespread applications in the pharmaceutical, biochemistry, biomedical and related fields [1–5]. In recent years, polymers containing pendant groups have received considerable attention. Particular interest in this field has been attached to sol–gels exhibiting reversible volume phase transitions in response to small changes in the external environment, such as in the temperature [6], ionic strength, pH [7] or electric field [8,9]. In addition, the use of block copolymer hydrogels consisting of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic biodegradable polyesters, such as poly(D,L-lactic acid) (PDLLA), poly(D,L-lactic acid-co-glycolic acid) (PLGA) and poly(lactic acid) (PLLA) has been widely studied as controlled release drug carriers, due to their biocompatibility and ability to biodegrade in vivo, thus providing a potential method of avoiding the use of surgical procedures [10,11]. Recently, there have been a few reports on the temperature-responsive phase transition of di, tri and star-shaped block copolymers composed of poly(ethylene glycol) and various aliphatic polymers [12–17]. These hydrogels have been shown to exhibit sol–gel (lower) and gel–sol (upper) transitions with increasing temperature. The lower temperature transition is important for drug delivery applications, because the solution flows freely at room temperature and forms a gel at physical body temperature [18–21].

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The disadvantage of these hydrogels, in their potential biomedical application as injectable drug delivery systems, is that when the temperature-sensitive hydrogels are injected into the body via a syringe, the heat transfer of the body tends to cause gels to form within the needle, making them difficult to inject into the body. In order to overcome these problems, the temperature-sensitive block copolymers were modified by introducing a pH-sensitive moiety. Block copolymers bearing sulfonamide groups, such as poly(*N*,*N*-dimethylacrylamide-co-sulfonamide), show a critical solubility transition in a narrow pH range of around 7.4 [22–24]. In our two recent communications [25,26], an amphiphilic block copolymer containing the hydrophilic poly(ethylene glycol) and hydrophobic poly(ε-caprolactone-co-lactide) (PCLA) moieties as part of the temperature-sensitive block copolymer (PCLA–PEG–PCLA) was reported. However, this temperature-sensitive block copolymer has also been modified by introducing pH-sensitive sulfonamide moieties, in order to impart pH sensitivity. The polymers were synthesized by conventional method, and the resulting polymers show mixture of triblock and pentablock copolymers.

In this study, in order to prepare well-defined block copolymers with a narrow molecular weight distribution, we synthesized a pentablock copolymer (OSM–PCLA–PEG–PCLA–OSM) via atom transfer radical polymerization (ATRP). This is one of the important types of controlled living radical polymerizations that allow well-defined polymers to be synthesized [27,28]. The macroinitiator, Br–PLCA–PEG–PCLA–Br, was synthesized by the coupling of 2-bromoisobutyryl bromide with PCLA–PEG–PCLA in the presence of triethyl amine base catalyst, followed by the polymerization of sulfamethazine methacrylate monomer using the $NiBr_2(PPh_3)_2$ catalyst system in DMF as the solvent at 80 °C. The resulting polymers were characterized by ^{1}H NMR and GPC.

2. Experimental

2.1. Materials and methods

Poly(ethylene glycol) (PEG) ($M_n = 2000$), D,L-lactide(LA), ε-caprolactone (CL), stannous 2-ethylhexanoate ($Sn(Oct)_2$), *N*,*N*-dimethyl formamide (DMF) (anhydrous), dichloromethane (anhydrous), methacryloyl chloride, 2-bromoisobutyryl bromide, triethyl amine, sulfamethazine (SM), and $NiBr_2(PPh_3)_2$ were purchased from Sigma–Aldrich, Korea. Sulfamethazine methacrylate monomer was synthesized according to a previous report [24] and PEG was dried in a vacuum at 110 °C for over 4 h before use. All other chemicals were reagent grade and used as received. Diethyl ether, chloroform, and tetrahydrofuran were obtained from Samchun Chemical Co. (Korea) and were used as received.

2.2. Synthesis of PCLA–PEG–PCLA triblock copolymer

The synthesis of the PCLA–PEG–PCLA triblock 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 varying the feed ratios of PEG, CL, and LA. The detailed procedure was as follows: PEG and $Sn(Oct)_2$ were added to a two-neck round-bottom flask and placed in an oil bath at 110 °C and dried for 4 h in a vacuum. After cooling the flask to room temperature, LA and CL were added under dry nitrogen, and the resulting reaction mixture was dried for 1 h in a 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 dichloromethane (DCM), and the resulting product was precipitated in excess diethyl ether. The precipitated block copolymer was dried in a vacuum at 40 °C for over 48 h, affording a yield of over 70%.

2.3. Synthesis of macroinitiator Br–PCLA–PEG–PCLA–Br

To a dry 250 mL round-bottom flask equipped with a magnetic stir bar, PCLA–PEG–PCLA triblock copolymer having a hydroxyl end group was added. The flask was placed in an oil bath at 80 °C under vacuum, maintained at this temperature for 2 h and then allowed to cool to room temperature. The block copolymer was dissolved in DCM and then an excess amount of triethyl amine catalyst was added five times. The resulting reaction mixture was cooled in an ice bath and the temperature maintained in the range of 0–10 °C, followed by the addition of an excess amount of 2-bromoisobutyryl bromide five times in a dropwise manner. After the completion of the addition, the reaction mixture warmed up to room temperature and continuous stirring was applied for 24 h. After this, the resulting solution was concentrated under vacuum, redissolved in THF, the residue of the $Et_3N{\cdot}HBr$ salt was removed by filtering and then the solution was concentrated, followed by precipitation in cold ether to obtain the macroinitiator, Br–PCLA–PEG–PCLA–Br, which was dried under vacuum at room temperature for 48 h.

2.4. Synthesis of OSM–PCLA–PEG–PCLA–OSM pentablock copolymer

To a dry Schlenk tube equipped with a magnetic stir bar, the macroinitiator Br–PCLA–PEG–PCLA–Br (1.0 mmol) was added. The Schlenk tube was then fitted with a rubber septum, evacuated twice, and filled with dry nitrogen. Following this, degassed DMF (6 mL) and sulfamethazine methacrylate (3.46 g, 10 mmol) were added and the reaction mixture was stirred well for 20 min under nitrogen. Finally the catalyst, $NiBr_2(PPh_3)_2$ (0.743 g, 1.0 mmol), was added to the reaction mixture and the Schlenk tube was placed in an oil bath at 80 °C and maintained at this temperature for 12 h with continuous stirring. After this, the resulting solution was precipitated in methanol and then dried. For the sample used in the GPC analysis, the catalyst residue was removed by passing the solution through a neutral alumina column.

2.5. Sol–gel phase diagram

The sol (flow)–gel (non-flow) phase transition temperature of the OSM–PCLA–PEG–PCLA–OSM copolymer in buffer solution was recorded using the inverting test method with a 4 mL vial test tube at temperature intervals of 2 °C for 10 min. Each sample at a given concentration (15 wt%) was dissolved in phosphate buffered saline solution (PBS) containing 1.2 wt% NaOH and kept for 1 day at 2 °C. Then, the pH of the block copolymer solution was adjusted to a certain pH (e.g., pH 8.0) by adding a small amount of 5 M HCl solution at 2 °C in an ice bath.

Characterization: The resulting polymers were characterized by ^{1}H 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. The molecular weights were calculated against low polydispersity PEG standards.

3. Results and discussion

We investigate the synthesis of the pentablock copolymer, OSM–PCLA–PEG–PCLA–OSM, using the macroinitiator, Br–PLCA–PEG–PCLA–Br, via the atom transfer radical polymerization (ATRP) of SM, and the resulting polymers were characterized by ^{1}H NMR and GPC. The pH/temperature dependence of the sol–gel phase transition was studied in 1.2 wt% NaOH PBS buffer. The triblock copolymer, PCLA–PEG–PCLA, was synthesized according to our previously reported procedure [26]. The ^{1}H NMR spectrum of the triblock copolymer is shown in Fig. 1(a). The peaks at 2.2, 3.6 and 5.0 ppm corresponding to the methylene protons of the caprolactone ($COC\mathbf{H}_2CH_2CH_2CH_2CH_2$), the ethylene protons of the ethylene glycol ($C\mathbf{H}_2C\mathbf{H}_2O$) and the methyne protons of the lactide ($-OCOC\mathbf{H}CH_3O$) moieties, respectively, confirm the formation of the block copolymer. In order to synthesize the pH-sensitive moiety, sulfamethazine (SM), via ATRP, an initiating site was introduced by the coupling of 2-bromoisobutyryl bromide with the triblock copolymer (PCLA–PEG–PCLA) in the presence of triethyl amine as a catalyst in dichloromethane at 0–10 °C [29]. The resulting product was characterized by ^{1}H NMR (500 MHz, $CDCl_3$) spectroscopy, as shown in Fig. 1(b). The ^{1}H NMR spectrum showed a new peak at 1.9 ppm corresponding to the $-C(C\mathbf{H}_3)_2-Br$ methyl protons along with the triblock copolymer peaks, confirming the attachment of the initiating group. Recently, Hong et al. reported the ATRP of a sulfonamide derivative using CuBr/Me_6TREN in DMF [30]. In our investigation, the polymerization was carried out with the $NiBr_2(PPh_3)_2$ catalyst system in DMF. Scheme 1 illustrates the ATRP, initiated by the macroinitiator, of the sulfamethazine methacrylate block, resulting in the pentablock copolymer with polydispersities in the range of 1.3–1.5. The results for these polymers are summarized in Table 1. Because polymers synthesized via the controlled living radical method have a relatively narrow polydispersity, one can control the number average molecular weight by varying the initiator to monomer feed ratio, in contrast to polymers synthesized by the conventional method [26]. ^{1}H NMR spectrum (in DMSO-d_6) of the synthesized pentablock copolymer is shown in Fig. 2. It showed new peaks at 6.4 ppm (imidazole ring protons) and 7.6–8.0 ppm (aromatic protons) corresponding to the sulfamethazine moiety along with the macroinitiator peaks, confirming the synthesis of the pentablock copolymer, OSM–PCLA–PEG–PCLA–OSM. The synthesis of the pentablock copolymer was further confirmed by gel permeation chromatography in THF using PEG standard traces, as shown in Fig. 3. The gel

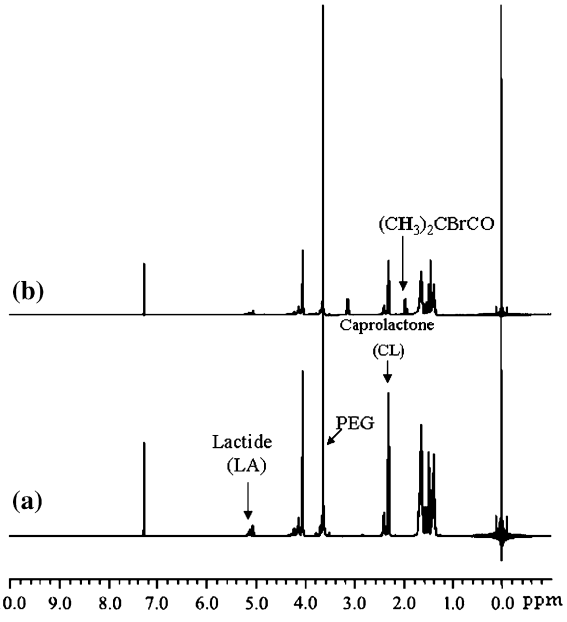
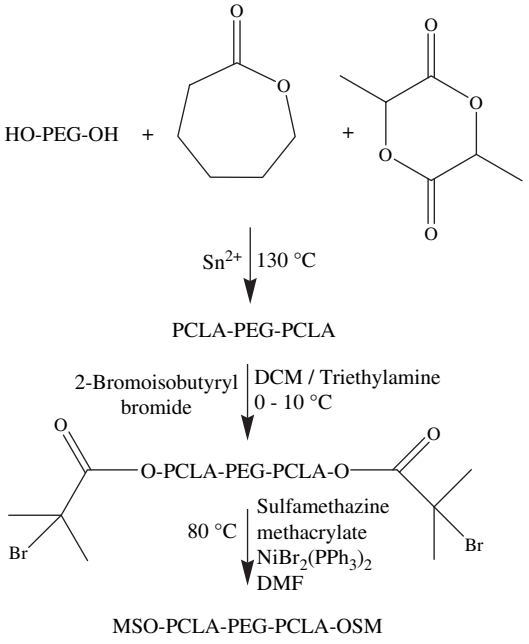


Fig. 1. ^{1}H NMR spectra of (a) PCLA–PEG–PCLA and (b) macroinitiator (Br–PCLA–PEG–PCLA–Br).



Scheme 1. Synthesis of PCLA–PEG–PCLA and OSM–PCLA–PEG–PCLA–OSM from macroinitiator.

Table 1
Molecular characteristics of tri- and pentablock copolymers

Sample code	PCLA–PEG–PCLA M_n (PDI)	OSM–PCLA–PEG–PCLA–OSM M_n (PDI)	Oligomer SM M_n (n)
A	4450 (1.34)	5557 (1.38)	1040 (~3)
B	4746 (1.38)	7760 (1.43)	3000 (~9)
C	5400 (1.30)	6800 (1.42)	1400 (~4)

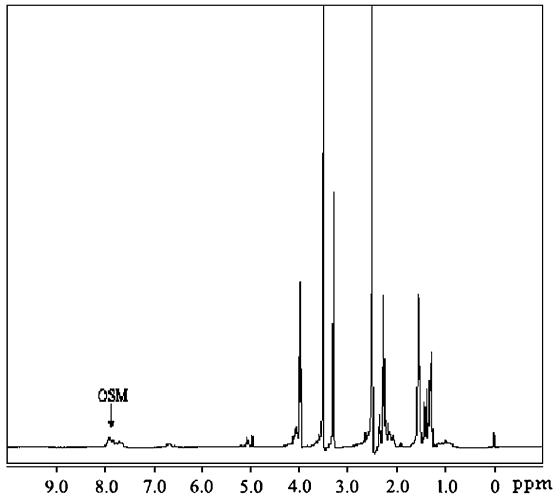


Fig. 2. ^{1}H NMR spectrum of pentablock copolymer (OSM–PCLA–PEG–PCLA–OSM).

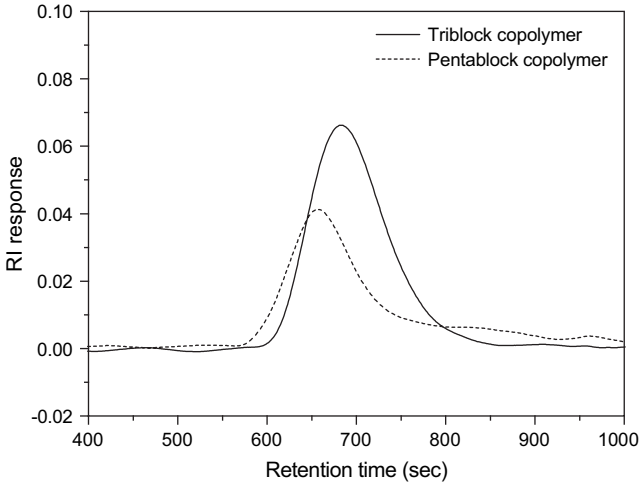


Fig. 3. Gel permeation chromatogram traces of block copolymers.

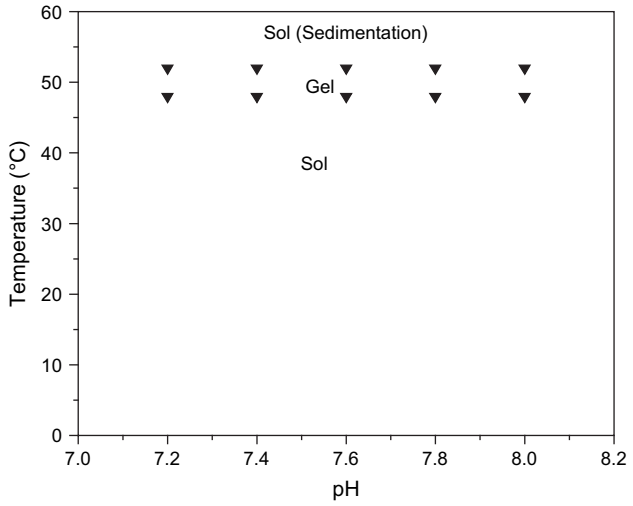


Fig. 4. Sol–gel phase transition diagram of triblock copolymer.

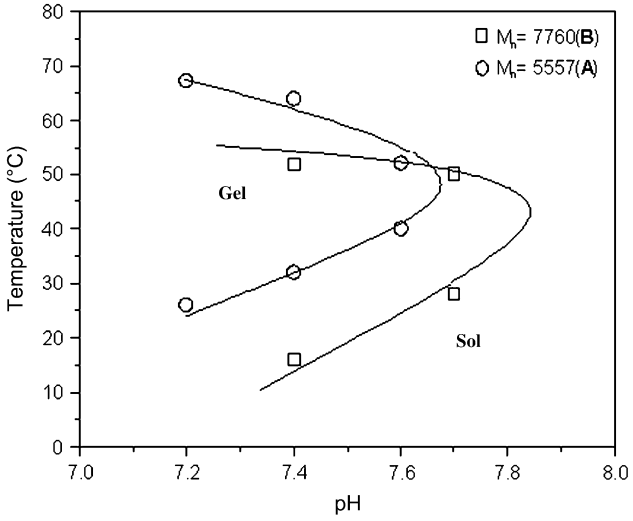


Fig. 5. Sol–gel phase transition diagram of pentablock copolymers. (A) $M_n = 5557$ and (B) $M_n = 7760$.

permeation chromatogram of the pentablock copolymer showed an increase in the number average molecular weight from 4700 to 7800, confirming the formation of the block copolymer. The chromatogram of the pentablock copolymer, however, indicates the presence of a small low molecular weight tail. This could be due to the consumption of less than 100% of the macroinitiator.

3.1. Sol–gel phase diagrams

The gelation mechanism of the temperature-sensitive triblock copolymer hydrogel was well explained in previous studies [10–15,19]. The gelation mechanism of the pH-sensitive hydrogel is similar to that of temperature-sensitive hydrogels [25,26]. The sol–gel phase diagrams of the block copolymers were investigated under various pH and temperature conditions. Figs. 4 and 5 show the sol–gel phase transition diagrams of the triblock copolymer, PCLA–PEG–PCLA, and pentablock copolymer, OSM–PCLA–PEG–PCLA–OSM, respectively. As the hydrophobicity of the PCLA blocks increased with increasing temperature, the PCLA–PEG–PCLA block copolymer solution showed sol–gel phase transition characteristics. When the temperature was increased

above the gel transition temperature, the block copolymer separated out from the aqueous phase, 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 temperature regions for the entire pH range, as shown in Fig. 4. However, in the solution of the end functional modified pentablock copolymer (**A**), OSM–PCLA–PEG–PCLA–OSM, the sol–gel phase transition temperature decreased and the gel temperature region widened when the pH was decreased, as shown in Fig. 5. At high pH, ca. 8.0, the sulfonamide functional group of the pH-sensitive moiety (OSM) is in its ionized state and the ionized OSM functioned as a hydrophilic block in the pentablock copolymer. Therefore, the pentablock copolymer does not form a gel at high pH and the viscosity change of the solution is only slight, because of the stronger hydrophobic interactions between the PLCA blocks at higher temperatures. In contrast, at low pH, ca. 7.4, the degree of ionization of the OSM decreased and the non-ionized OSM functioned as a hydrophobic block in the pentablock copolymer. The sol–gel phase transition was studied for the pentablock copolymer (**B**) having OSM blocks with a slightly higher molecular weight, as shown in Fig. 5. It was shown that the OSM, which is in an ionized state in the high pH range, is present in the sol state, regardless of its molecular weight. However, at low pH, the OSM is in the non-ionized state and thus functions as a hydrophobic block and, particularly in the low pH range, the hydrophobicity of the block copolymer increases with increasing molecular weight of the OSM block, which results in a decrease in the temperature range in which the gel is formed.

4. Conclusions

The pentablock copolymer, OSM–PCLA–PEG–PCLA–OSM, having a narrow polydispersity was prepared in three steps: first, the amphiphilic block copolymer, PCLA–PEG–PEG was synthesized by the ring-opening polymerization of LA/CL in the presence of PEG macroinitiator. Secondly, an ATRP initiating site was anchored by the coupling of 2-bromoisobutyryl bromide with PCLA–PEG–PCLA in the presence of triethyl amine catalyst. Finally, a pH-sensitive moiety was introduced by the atom transfer radical polymerization of sulfamethazine methacrylate monomer using $NiBr_2(PPh_3)_2$ catalyst in DMF at 80 °C. The resultant block copolymers show a narrow polydispersity compared to block copolymers synthesized by the conventional polymerization method and a sol–gel phase transition in the pH range of 7.4–8.0. These block copolymers are expected to find applications in various biomedical fields, especially in the area of drug delivery.

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