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Polymer 47 (2006) 7918–7926
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pH/temperature sensitive poly(ethylene glycol)-based biodegradable polyester block copolymer hydrogels

Dai Phu Huynh [a], Woo Sun Shim [a], Ji Heung Kim [b], Doo Sung Lee [a,*]

[a] Department of Polymer Science and Engineering, Sungkyunkwan University, Chonchondong 300, Jangan-ku, Suwon, Gyeonggi 440-746, Republic of Korea
[b] Department of Chemical Engineering, Sungkyunkwan University, Suwon, Gyeonggi 440-746, Republic of Korea

Received 19 May 2006; received in revised form 5 September 2006; accepted 7 September 2006
Available online 10 October 2006

Abstract

Novel pH and temperature sensitive biodegradable block copolymers composed of poly(ethylene glycol) (PEG), polyglycolide (GA), ε-caprolactone (CL) and sulfamethazine oligomers (OSMs) were synthesized by ring opening polymerization and 1,3-dicyclohexyl-carbodiimide (DCC) mediated coupling reactions. Their physicochemical properties in aqueous media were characterized by ^{1}H NMR spectroscopy and gel permeation spectroscopy. The sol–gel phase transition behavior of OSM–PCGA–PEG–PCGA–OSM block copolymers was investigated both in solution and injection to PBS buffer at pH 7.4 and 37 °C. Aqueous solutions of OSM–PCGA–PEG–PCGA–OSM changed from a sol to a gel phase with increasing temperature and decreasing pH. The sol–gel transition properties of these block copolymers are influenced by the hydrophobic/hydrophilic balance of the copolymers, block length, hydrophobicity, stereoregularity of the hydrophobic components within the block copolymer, and the ionization of the pH functional groups in the copolymer, which depends on the environmental pH. Degradation of the triblock and pentablock copolymers at 37 °C (pH 7.4), and at 0 °C and 5 °C both at pH 8.0, was investigated. It was demonstrated here using the in vitro test method, that the anticancer agent paclitaxel (PTX) could be loaded and released by the pH and temperature sensitive OSM–PCGA–PEG–PCGA–OSM block copolymer, such that this could be used as a suitable matrix for subcutaneous injection in drug delivery systems.

Keywords: Block copolymer; Biodegradable polymer; Hydrogel

1. Introduction

Hydrogels that exhibit both liquid-like and solid-like behaviors exhibit a wide variety of functional properties (i.e. swelling, mechanical, permeation, surface and optical), and these have provided many potential applications for hydrogels in fields such as medicine, agriculture, and biotechnology [1–5]. The stimuli-sensitive hydrogels are intelligent materials that can change their structure in response to environmental stimuli. Various stimuli-sensitive hydrogels that respond to pH [6], temperature [7,8–10], electric fields [11,12], and other stimuli have been studied both experimentally and theoretically [13,14]. Over the past few years, a number of stimuli-sensitive polymer hydrogels, especially thermo-reversible and pH-reversible gels, have been developed for use as polymeric drug carriers, implants, and other medical devices [15–20]. Aqueous solutions of triblock or diblock copolymers of poly(ethylene oxide) and poly(butylene oxide) (PBO), which undergo a sol–gel transition in response to changing temperature, are typical examples of thermosensitive polymer hydrogels [21,22].

The thermo-reversible hydrogels of poly(ethylene oxide)-*b*-poly(LL-lactide-*co*-glycolide acid) [PEO–P(LLA/GA)] and poly(ethylene oxide)-*b*-poly(DL-lactide-*co*-glycolide acid) [PEO–P(DLLA/GA)] [23,24] in aqueous solution are all biodegradable copolymers. The upper transition temperature (gel to sol transition temperature) of the PEO–P(LLA/GA) diblock copolymers with fixed PGA block length is lower than that of the PEO–PLLA diblock copolymers with similar PLLA block

* Corresponding author. Tel.: +82 31 290 7282; fax: +82 31 292 8790.
E-mail address: dslee@skku.edu (D.S. Lee).

0032-3861/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2006.09.021

length, due to a decrease in hydrophobicity and stereoregularity. However, these hydrogels have several unresolved drawbacks that limit their application in injectable drug delivery systems. When temperature sensitive hydrogels are injected into the body via syringe, they tend to form a gel as the needle becomes warm by the body temperature, making injection difficult [25].

Kim et al. used two crosslinked copolymers, poly(methacrylic acid-*co*-methacryloxyethyl glucoside) [P(MMA-*co*-MEG)] and poly(methacrylic acid-*g*-ethylene glycol) [P(MMA-*g*-EG)], to determine the mechanism of penetrant transport through anionic pH sensitive hydrogels [26]. They observed that the water transport mechanism was significantly dependent on the pH of the swelling medium. At high pH (higher than the pK_a of the gel), the water transport was controlled more by polymer relaxation rather than by penetrant diffusion. For both P(MMA-*co*-MEG) and P(MMA-*g*-EG) hydrogels, the swelling mechanism exhibited little dependence on the copolymer compositions of the hydrogels at the same pH. As such, the characteristics of these systems for drug delivery applications were investigated [27]. It was found that the mesh size of these hydrogels changed from small (18–35 Å) in the collapsed state at pH 2.2 to very large (70–111 Å) at pH 7.0, and increased between two and six times during the swelling process, demonstrating some potential disadvantages for use as drug delivery systems by the subcutaneous injection method as it was very difficult to control the amount of drug which was loaded into the polymer.

In a previous paper, we synthesized a pH/temperature sensitive pentablock copolymer from the pH sensitive poly(ε-caprolactone-*co*-lactide) (PCLA) and PEG block copolymer (PCLA–PEG–PCLA), capped either side with the temperature sensitive sulfamethazine oligomers (OSMs) to form OSM–PCLA–PEG–PCLA–OSM. This block copolymer solution showed a reversible sol–gel transition as a result of both a small pH change in the range of pH 7.4–8.0, and a temperature change around body temperature [28]. These properties made this polymer a potential candidate for use as a carrier in the loading and releasing of drugs or proteins, in particular for the delivery of cationic proteins. However, the degradation period of this polymer was found to be far too long. Thus, in the present study, temperature sensitive chains (i.e. the ABA block copolymer poly(ethylene glycol)–poly(glycolide-*co*-ε-caprolactone) (PCGA–PEG–PCGA)) and graft copolymers of pH sensitive chains (sulfamethazine oligomer (OSM)) were combined to design a new pH/temperature sensitive polymer hydrogel (OSM–PCGA–PEG–PCGA–OSM) (PNDSP). In this work, it was determined that the degradation time of OSM–PCGA–PEG–PCGA–OSM is shorter than OSM–PCLA–PEG–PCLA–OSM, and that this hydrogel has some advantages for application in drug delivery systems.

2. Experimental section

2.1. Materials

Poly(ethylene glycol) (PEG) was purchased from Sigma-Aldrich (St. Louis, MO) ($M_n = 1000$, 1500 and 2000) and ID Biochem, Inc. (Seoul, Korea) ($M_n = 1750$), and were recrystallized in *n*-hexane and dried in vacuum for 3 days prior to use. Glycolide (GA) obtained from Polyscience Boehringer Ingelheim was purified by recrystallization in diethyl acetate. ε-Caprolactone (CL) was obtained from Sigma. Stannous octoate [$Sn(Oct)_2$] was obtained from Sigma and was dried for 24 h under vacuum at ambient temperature prior to use. Sulfamethazine was obtained from Sigma and used as received. Methacryloyl chloride, 4-dimethylamino pyridine (4-DMAP), 3-mercapto propionic acid (MPA), 1,3-dicyclohexyl-carbodiimide (DCC) and *N*,*N*-dimethyl formamide (DMF) were obtained from Aldrich. 2,2′-Azobisisobutyronitrile (AIBN, Junsei) was recrystallized twice in methanol (Samchun). Chloroform ($CDCl_3$), methylene chloride (MC), and diethyl ether were all obtained from Samchun, while paclitaxel (PTX) was purchased from Samyang Genex Corporation. All other reagents were of analytical grade and used without further purification.

2.2. Synthesis of PCGA–PEG–PCGA temperature sensitive block copolymer

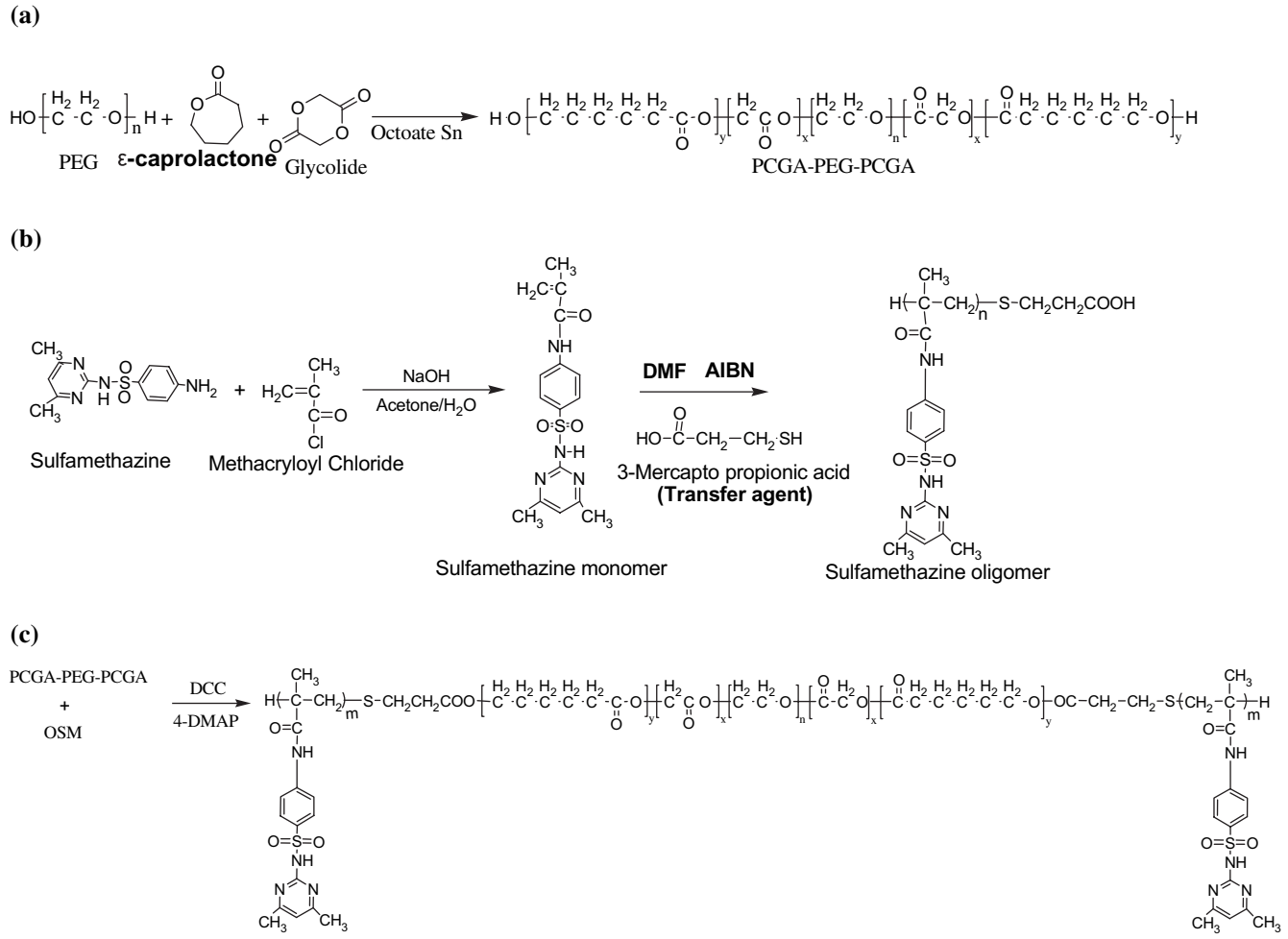
PCGA–PEG–PCGA triblock copolymers were synthesized by the ring opening polymerization of GA, and CL initiated by the hydroxyl group of PEG in the presence of the stannous octoate catalyst. To control the balance of hydrophobic/hydrophilic PEG/PCGA and GA/CL components, the composition and molecular weight of the triblock copolymer were adjusted by the feed ratios of PEG, GA and CL. The detailed synthesis for PCGA–PEG–PCGA = 1590–1500–1590 (M_n) is as follows: 4 g of PEG (1500) and 0.04 g of stannous octoate were placed in a dried two-neck round-bottom flask dried for 2 h under vacuum at 110 °C. After cooling to room temperature, 5.74 g of CL and 1.46 g of GA were added to the flask under a dry nitrogen atmosphere. The reaction mixture was first dried for 1 h under vacuum at 60 °C, and then the temperature was raised slowly to 130 °C. The ring opening reaction was carried out for 18 h. Subsequently, the reactants were cooled to room temperature, dissolved in methylene chloride (MC), and then precipitated in excess diethyl ether. The precipitated products were dried under vacuum at room temperature for 48 h. The overall yield of these triblock copolymers was over 70% after drying.

2.3. Sulfamethazine oligomer

The sulfamethazine monomer (SMM) and sulfamethazine oligomer (OSM) were synthesized from sulfamethazine (SM) and methacryloyl chloride by the process followed in the previous paper [28].

2.4. Coupling OSM with PCGA–PEG–PCGA block copolymer

The temperature sensitive block copolymer (PCGA–PEG–PCGA) and pH sensitive block (OSM) were coupled in the presence of DCC and DMAP as a catalyst. The detailed coupling reaction process is as follows (Scheme 1): 4 g



Scheme 1. Synthesis of pH/temperature sensitive copolymer. (a) Synthesis of triblock copolymer (PCGA–PEG–PCGA), (b) synthesis of sulfamethazine oligomer, (c) synthesis of pentablock copolymer (OSM–PCGA–PEG–PCGA–OSM).

of PCGA–PEG–PCGA (1590–1500–1590 (M_n), CL/GA = 2.39/1 (mole ratio)) and 3.29 g of OSM were weighed in a two-neck round-bottom flask and dried under vacuum at 85 °C for 2 h. At the same time, 0.5 g of DCC and 0.3 g of 4-DMAP (calculated by mole ratio: triblock/OSM/DCC/DMAP = 1/2.2/2.8/0.28) were dissolved in 60 ml anhydrous MC. This solution was added into the flask at 20 °C, and the reaction was carried out at room temperature under nitrogen for 48 h. After filtration of the resulting copolymers with filter paper (0.4 μm) to remove the residual OSM, the solvent was reduced in volume and the copolymer was precipitated in diethyl ether. The precipitated products were dried under vacuum at room temperature for 48 h. The yield of OSM–PCGA–PEG–PCGA–OSM was 87%.

2.5. Characterization

The number-average molecular weight (M_n) and molecular distribution (MWD) of the as-synthesized block copolymers were determined by gel permeation chromatography (GPC) on a Waters Model 410, equipped with 4 μm styragel columns from 500 to 10 Å in series, at a flow rate of 1.0 ml/min (eluent: THF, 36 °C, PEG as standard). ^{1}H NMR measurements were performed on a Varian Unity Inova 500 instrument (500 MHz) to determine the molecular structures and compositions of PEG, CL, and GA [28,29].

2.6. Phase diagram measurement

The sol (flow) to gel (non-flow) phase transition behavior of the triblock copolymers in aqueous media was determined using the inverting test method with a 4-ml (10 mm diameter) vial test tube at a temperature interval of 1 °C. The block copolymers were dissolved in buffer solution at a given concentration for 2–4 days at 0 °C. The pH of these samples was adjusted with sodium hydroxide (5 M) and the solutions were maintained at 0 °C for 1–2 days. The sol–gel transition at each temperature was determined by angling the vial horizontally after keeping it at a constant temperature for 10 min, as described in our previous paper [28,29].

On the other hand, the sol–gel phase transition of the pentablock copolymers in aqueous media during injection was determined by using the injection test method with a 4-ml test cylinder at a temperature interval of 1 °C and a 40 ml vial test

tube. Each sample was dissolved in buffer solution at a given concentration for 2 days at 0 °C. The pH of these samples was adjusted using sodium hydroxide (5 M) and HCl (5 M) and the solutions were maintained at 0 °C for 1–2 days. The pH of a water (20 ml) sample in a 40 ml vial test tube was adjusted accordingly and this was maintained at constant temperature for 30 min. The sample, which was housed in a test cylinder at different temperatures, was then injected into the 40 ml vial. After holding the vial under these conditions for 5 min, it was shaken for a further 5 min. The phase diagrams reveal that if the sample is soluble in water then it is a sol, if not, then the sample is considered a gel.

2.7. Degradation experiment

Degradation at 37 °C and pH 7.4: 0.5 g of triblock and pentablock solutions at 20 wt% in water (pH 7.4) were placed in a 4 ml vial and incubated at 37 °C for 30 min. PBS buffer (3 ml) at pH 7.4 and 37 °C was added to the solution. At a given time, the sample vial was removed and then freeze-dried. The degradation of the polymers was determined by GPC.

2.8. Drug loading and releasing experiment

The drug paclitaxel (PTX) was loaded into the pentablock solutions (20 wt% in water at pH 8.0) at 0 °C over a period of 1 day. The sample pH was adjusted to 7.4 with sodium hydroxide (5 M) and HCl (5 M) and then maintained at 0 °C for 12 h. Subsequently, 0.5 g of the mixture was placed in a 4 ml vial and then incubated at 37 °C for 30 min. Fresh serum (3 ml, 2.4 wt% Tween 80, 4 wt% Cremophor EL in PBS buffer at pH 7.4) at 37 °C was added to the vial samples. At a given time, 1.5 ml of the serum (releasing sample) was extracted from the vial sample, and 1.5 ml of fresh serum was added to the vial sample. H_2O in the releasing sample was freeze-dried to get solid PTX, and then the PTX was dissolved by ACN and determined by HPLC (Column: C18, 250 × 4.0 mm, 5.0 μm; mobile phase: ACN/H_2O = 2/8; flow rate: 0.5 ml/min; detector: UV at 254 nm).

3. Results and discussion

Various PCGA–PEG–PCGA block copolymers were obtained from the ring opening polymerization. The number-average molecular weight (M_n) of the block copolymers can be calculated by comparison of the peak ratios of CL and GA with those of PEG (of known molecular weight) in the ^{1}H NMR spectrum. Fig. 1 shows a representative ^{1}H NMR spectrum of the PCGA–PEG–PCGA block copolymer and its chemical structure. The characteristic signal appearing at 3.6 ppm was assigned to the methylene protons of the EO units and those at the ends of the CL units, while the signals at 4.68 ppm and 2.35 ppm correspond specifically to the methylene protons of the GA unit and those at the beginning of the CL unit. The molecular compositions of the synthesized block copolymers were obtained by calculating the corresponding peak areas, as described previously [29].

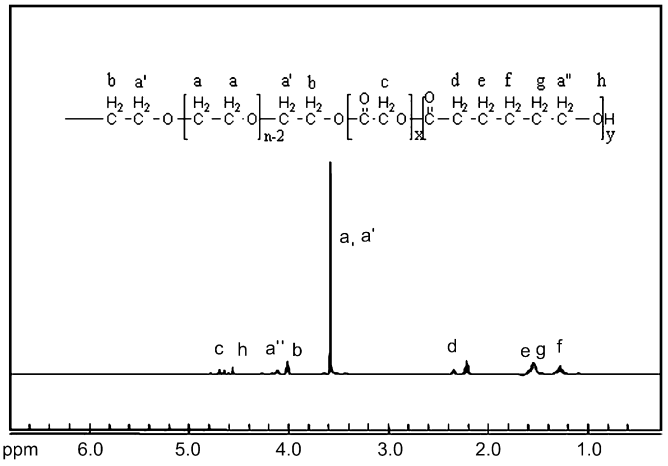


Fig. 1. ^{1}H NMR spectrum of PCGA–PEG–PCGA.

The synthesized SMM and OSM components were confirmed by ^{1}H NMR (Fig. 2). The characteristic signal appearing at 2.1 ppm (Fig. 2a and b) was assigned to the methylene protons of the sulfamethazine, SMM, and OSM units. The characteristic signals appearing at 6.0 ppm and 10.1 ppm (Fig. 2a and b) were assigned to the amine hydrogen proton of sulfamethazine and the amide hydrogen protons of SMM and OSM, respectively. The characteristic signals appearing at 5.6 ppm and 5.8 ppm, 2.7 ppm and 2.9 ppm (Fig. 2b) assigned to the methylene hydrogen double bond protons of SMM, and methylene hydrogen single bond proton of OSM. In addition, following the conversion of SMM to OSM, the signal assigned

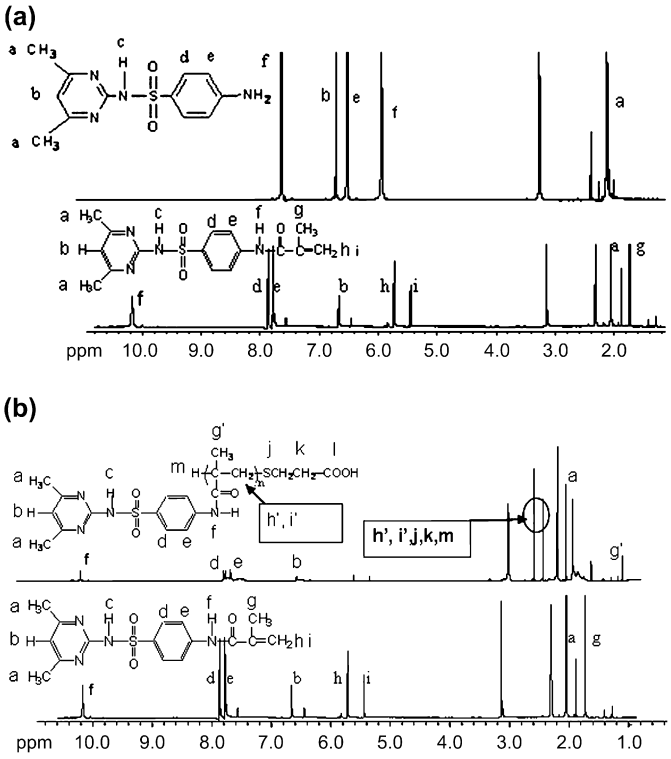


Fig. 2. ^{1}H NMR spectrum of sulfamethazine, SMM, and OSM. (a) Sulfamethazine and SMM; (b) SMM and OSM.

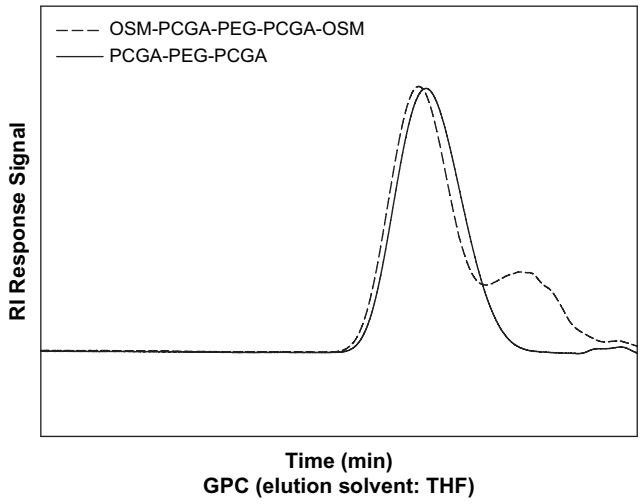


Fig. 3. GPC of triblock and pentablock copolymer.

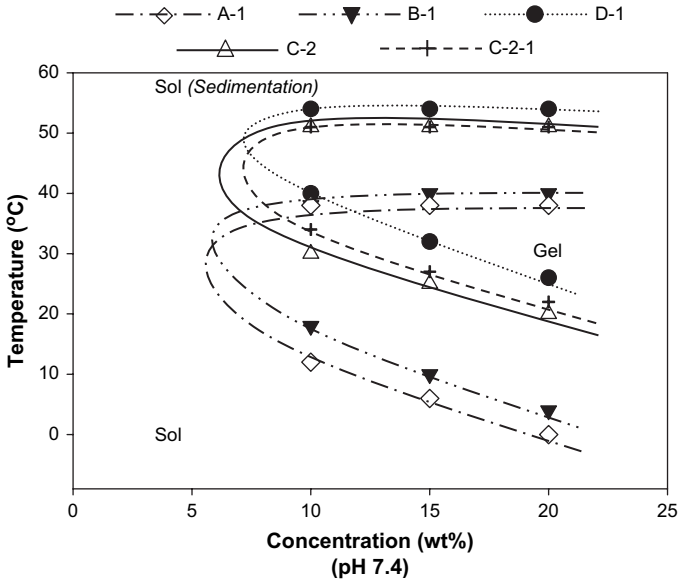


Fig. 4. Sol–gel transition phase diagrams of OSM–PCGA–PEG–PCGA–OSM with various PEG molecular weights and concentrations at pH 7.4.

to the methyl hydrogen protons of the methacrylate group showed an upfield shift from 1.9 ppm to 1.0 ppm.

On the other hand, as can be seen in Fig. 3, the GPC curves of OSM and PCGA–PEG–PCGA both showed a unimodal peak with a narrow molecular weight distribution of M_w/M_n, as listed in Table 1.

3.1. Phase diagrams of sol–gel transition

Fig. 4 shows the phase diagrams of various OSM–PCGA–PEG–PCGA–OSM block copolymers synthesized using PEGs with different molecular weights and concentrations at pH 7.4. As can be seen in Fig. 4, the critical gel concentration (CGC) and the upper (gel to sol transition) and lower (sol to gel transition) critical gel temperature (CGT) curves are shown. The CGC of each composition did not change significantly (if the PCGA/PEG ratios are maintained), despite changes in the PEG molecular weight from 1000 to 2000, and a slight change in the CL/GA ratio. However, the CGT did show a change over a large range. When the PEG molecular weight was increased from 1000 to 2000, the lower CGT at a concentration of 10 wt% and pH 7.4 increased from 10 °C (806–1091–1000–1091–806) (A-1) to 39 °C (806–2474–2000–2474–806) (D-1), and the upper CGT increased from 38 °C to 54 °C. These results indicate that the CGC values of the OSM–PCGA–PEG–PCGA–OSM block copolymers with the same hydrophobic/hydrophilic length ratios are not particularly influenced by the PEG molecular weight, although increases in the CGT values of OSM–PCGA–PEG–PCGA–OSM occur with increasing PEG chain length.

Fig. 5 shows the phase diagrams of the OSM–PCGA–PEG–PCGA–OSM block copolymers with various PEG molecular weights and pH values at a concentration of 10 wt%. In this figure, the critical gel pH (CGpH) and the lower (sol to gel transition) and upper (gel to sol transition) critical gel temperature (CGT) curves are shown. The laws of changing of CGT are similar to the phase diagrams shown in Fig. 4. The phase diagrams in Figs. 4 and 5 show that when the PEG chain length in the block copolymer increases, the CGT increases and the range between the lower and upper CGT curves decreases accordingly. At a concentration of 10 wt% and pH 7.4, the range of the two CGT curves of samples A-1, B-1,

Table 1
Physical parameters of OSM–PCGA–PEG–PCGA–OSM

OSM–PCGA–PEG–PCGA–OSM	PEG M_a [b]	PEG/PCGA (wt/wt)[a]	CL/GA (mol/mol)	OSM M_n [c]	M_w/M_n [c]
806–1091–1000–1091–806 (A-1)	1000	1/2.18	2.34/1	806	1.30
806–1590–1500–1590–806 (B-1)	1500	1/2.12	2.35/1	806	1.34
806–2474–2000–2474–806 (D-1)	2000	1/2.47	2.37/1	806	1.50
806–1881–1750–1881–806 (C-2.1)	1750	1/2.15	2.32/1	806	1.35
904–1461–1750–1461–904 (C-1)	1750	1/1.67	2.26/1	904	1.35
904–1881–1750–1881–904 (C-2)	1750	1/2.15	2.32/1	904	1.35
904–2117.5–1750–2117.5–904 (C-3)	1750	1/2.42	2.26/1	904	1.36
904–2336.2–1750–1336.2–904 (C-4)	1750	1/2.67	2.29/1	904	1.36
904–2494–1750–2494–904 (C-5)	1750	1/2.85	2.28/1	904	1.35

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

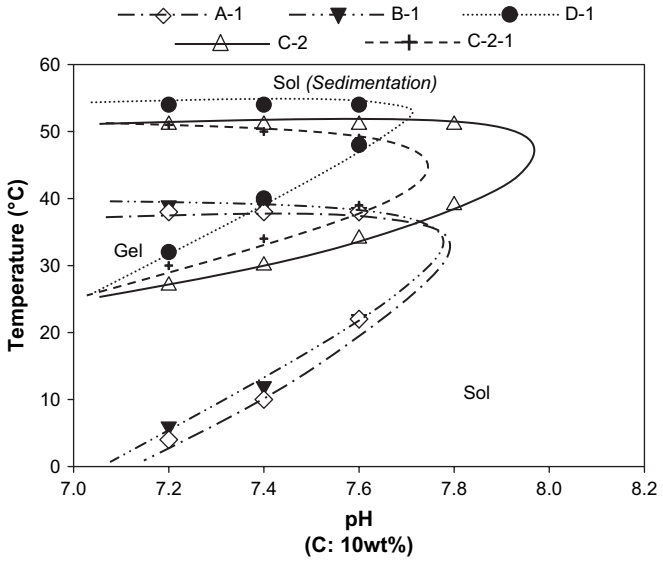


Fig. 5. Sol–gel transition phase diagrams of OSM–PCGA–PEG–PCGA–OSM with various PEG molecular weights and pH values at concentration 10 wt%.

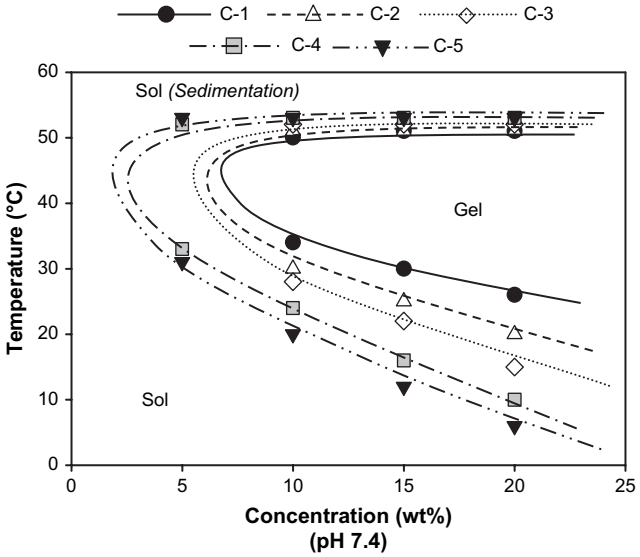


Fig. 6. Sol–gel transition phase diagrams of OSM–PCGA–PEG–PCGA–OSM with various hydrophobic/hydrophilic chain lengths and concentrations at pH 7.4.

C-2.1 and D-1 is 29 °C, 27 °C, 18 °C and 15 °C (calculated from the data of Fig. 4), respectively. When the chain length of the pH sensitive block is increased from 806 to 904 (Table 1), at the same pH, the ionic interactions between the micelles forming the semi-interpenetrating network structure [30,31] are not particularly different, so that the CGC and CGT of C-2.1 (806–1881–1750–1881–806) and C-2 (904–1881–1750–1881–904) (Fig. 4) are similar. However, when the pH changes in accordance with the use of a longer pH sensitive chain block, the response to pH stimuli is stronger and more ionic links are formed between the micelles and the composing gel matrix. As a result, the CGpH in the phase diagram of C-2 (904–1881–1750–1881–904) is higher than that of C-2.1 (806–1881–1750–1881–806) (Fig. 5). With almost every sample, when the temperature is increased from below the lower CGT to above it, the solution of the pH/temperature sensitive block copolymer transforms from the sol to the gel phase in a single stage. However, when temperature increased from below the upper CGT to above it, the gel–sol transition and the suspension phase occur concurrently. When the temperature is greater than the upper CGT, the enthalpy of H_2O at this temperature is too high, such that the water is effectively liberated from the gel matrix. An explanation of the mechanism of the suspension was given in our previous paper [24].

Figs. 6–8 show the phase diagrams for the OSM–PCGA–PEG–PCGA–OSM block copolymers with various hydrophobic/hydrophilic chain lengths and concentrations at pH 7.4, and various hydrophobic/hydrophilic chain lengths and pH values at concentrations of 10 wt% and 20 wt%. As the hydrophobic/hydrophilic chain length ratio is increased from 1.67 (composition C-1) to 2.85 (composition C-5) (Table 1), the CGC of OSM–PCGA–PEG–PCGA–OSM decreases in a proportional manner, the CGpH increases, and the range between the lower CGT and upper CGT increases from 15 °C to 35 °C at the concentration of 10 wt% and pH 7.4 (calculated from the data of Figs. 6 and 7). As the PCGA/PEG ratio rises, the hydrophobic chain length becomes longer, and so the hydrophobicity of the copolymer becomes greater, such that the resulting micelles are generally formed with fewer associated chains [32]. As more bridging connections are formed between the micelles, more grouped micelles are produced, and hence the sol to gel and gel to sol phase transitions occur at lower and higher CGTs, respectively [28]. From Figs. 7 and 8, it is noted that the solution concentration has a direct influence on CGC, CGpH and CGT, such that an increase in concentration causes a decrease in CGC, to and concomitant increases in CGpH and the range of the gel temperature. When the concentration of compositions C-1 and C-5 is increased from

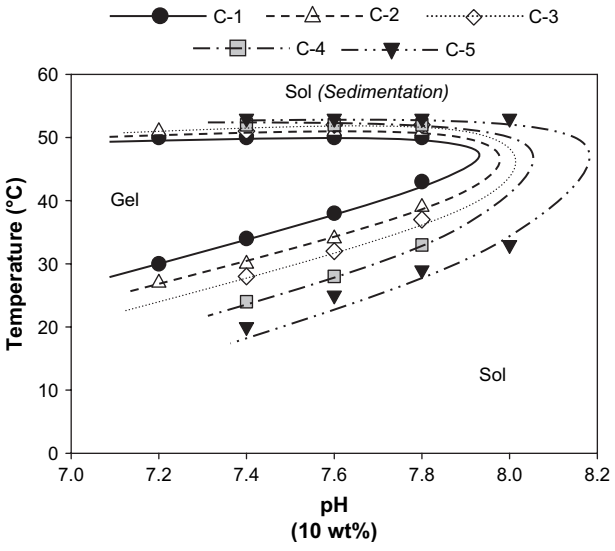


Fig. 7. Sol–gel transition phase diagrams of OSM–PCGA–PEG–PCGA–OSM with various hydrophobic/hydrophilic chain lengths and pH values at concentration 10 wt%.

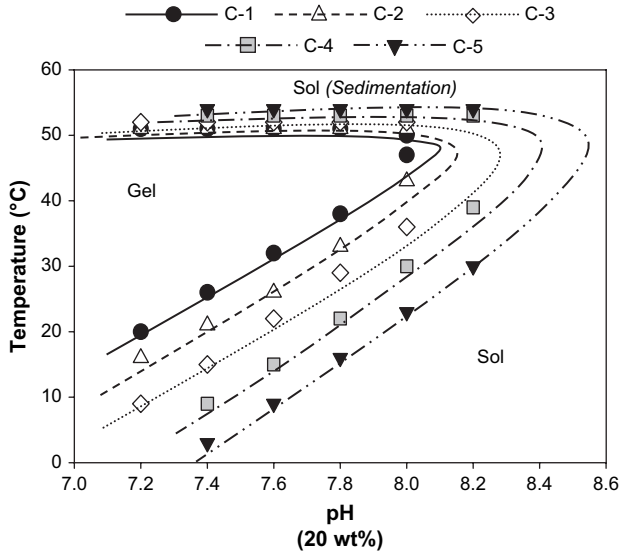


Fig. 8. Sol–gel transition phase diagrams of OSM–PCGA–PEG–PCGA–OSM with various hydrophobic/hydrophilic chain lengths and pH values at concentration 20 wt%.

10 wt% to 20 wt%, the range of the gel temperature is raised from 14 °C to 35 °C (calculated from the data of Figs. 7 and 8). As a result, the gel phase areas in the phase diagrams of OSM–PCGA–PEG–PCGA–OSM can be controlled by changing the hydrophobic/hydrophilic length ratios and the concentration of the solution in water. Thus, the increasing gel phase zone is dependent on the increases in hydrophobic change length and concentration of the solution of the pH/temperature sensitive copolymers.

3.2. The sol–gel transition by injection

Table 2 shows the sol–gel transition behavior of the pentablock copolymer aqueous solution obtained during the injection experiments. Based on Figs. 4 and 8, the points at 37 °C and pH 8.0 for compositions A-1 (806–1091–1000–1091–806), B-1 (806–1590–1500–1590–806), D-1 (806–2474–2000–2474–806), C-1 (904–1461–1750–1461–904), C-2 (904–1881–1750–1881–904), C-2.1 (806–1881–1750–1881–806) (Table 1), and the point at 37 °C and pH 7.4 of A-1 (806–1091–1000–1091–806), all appear outside the gel stage area of the phase diagrams, indicating that these compositions cannot transform into the gel phase following injection under these conditions. The points at 37 °C and pH 7.4 for compositions B-1 (806–1590–1500–1590–806), D-1 (806–2474–2000–2474–806), and for C-3 (904–2117.5–1750–2117.5–904), C-4 (904–2336.2–1750–1336.2–904), and C-5 (904–2494–1750–2494–904) (Table 1), are all within the gel stage areas of their respective phase diagrams, and so are able to form gels when injected under these conditions. However, since all of these points fall well within the border of the phase diagrams, the long-term stability of the corresponding gel stages under these conditions is not good (the gel stages of B-1, D-1, C-3, C-4, and C-5 are stable for 20 days, 5 days, 1 day, 5 days, and 5 days, respectively (Table 2)). The points

Table 2
Sol–gel transitions obtained during injection testing

Time remaining		5 min	1 day	5 days	10 days	20 days	30 days
A-1	pH 8.0	S	S	S	S	S	S
	pH 7.4	S	S	S	S	S	S
B-1	pH 8.0	S	S	S	S	S	S
	pH 7.4	G	G	G	G	G	S
D-1	pH 8.0	S	S	S	S	S	S
	pH 7.4	G	G	G	S	S	S
C-2.1	pH 8.0	S	S	S	S	S	S
	pH 7.4	G	G	G	G	G	G
C-1	pH 8.0	S	S	S	S	S	S
	pH 7.4	G	G	G	G	G	G
C-2	pH 8.0	S	S	S	S	S	S
	pH 7.4	G	G	G	G	G	G
C-3	pH 8.0	G	G	S	S	S	S
	pH 7.4	G	G	G	G	G	G
C-4	pH 8.0	G	G	G	S	S	S
	pH 7.4	G	G	G	G	G	G
C-5	pH 8.0	G	G	G	S	S	S
	pH 7.4	G	G	G	G	G	G

The pH of the environment is 8.0 and 7.4 at 37 °C; the pH of the OSM–PCGA–PEG–PCGA–OSM solutions in water (concentration 20 wt%) is 8.0 at 0 °C. S: the sample is dissolved in the environment, unable to form a gel. G: the sample is the gel stage in the environment.

at 37 °C and pH 7.4 for compositions C-2.1 (806–1881–1750–1881–806), C-1 (904–1461–1750–1461–904), C-2 (904–1881–1750–1881–904), C-3 (904–2117.5–1750–2117.5–904), C-4 (904–2336.2–1750–1336.2–904), and C-5 (904–2494–1750–2494–904) (Table 1) are all located at the center of the phase diagrams, so the corresponding gel stages are stable under these conditions over at least 30 days (Table 2).

3.3. Degradation of triblock and pentablock copolymers

Fig. 9 shows the degradation behaviors of the PCGA–PEG–PCGA and OSM–PCGA–PEG–PCGA–OSM block copolymer solutions compared to those of PCLA–PEG–PCLA and OSM–PCLA–PEG–PCLA–OSM at 37 °C and pH 7.4, as well as and the degradation of OSM–PCGA–PEG–PCGA–OSM in aqueous solution at 0 °C and 5 °C for a specified period. As can be seen in Fig. 9a, the degradation slopes for PCGA–PEG–PCGA, PCLA–PEG–PCLA and OSM–PCLA–PEG–PCLA–OSM are similar. With OSM–PCGA–PEG–PCGA–OSM, the rate of degradation is particularly fast in the early stages, as represented by the steep gradient. After 18 days, however, the rate of degradation in aqueous solution is observed to be slow. Interestingly, the molecular weight of these polymers was also observed to decrease after 32 days' degradation. PCGA–PEG–PCGA and OSM–PCGA–PEG–PCGA–OSM demonstrated losses of 101 and 2323, respectively, compared to PCLA–PEG–PCLA and OSM–PCGA–PEG–PCGA–OSM, which showed losses of 934 and 998, respectively (calculated from Fig. 9a). These results indicate that OSM–PCGA–PEG–PCGA–OSM degrades faster than OSM–PCGA–PEG–PCGA–OSM. As such, it is expected that drugs will be released faster when loaded in OSM–PCGA–PEG–PCGA–OSM compared

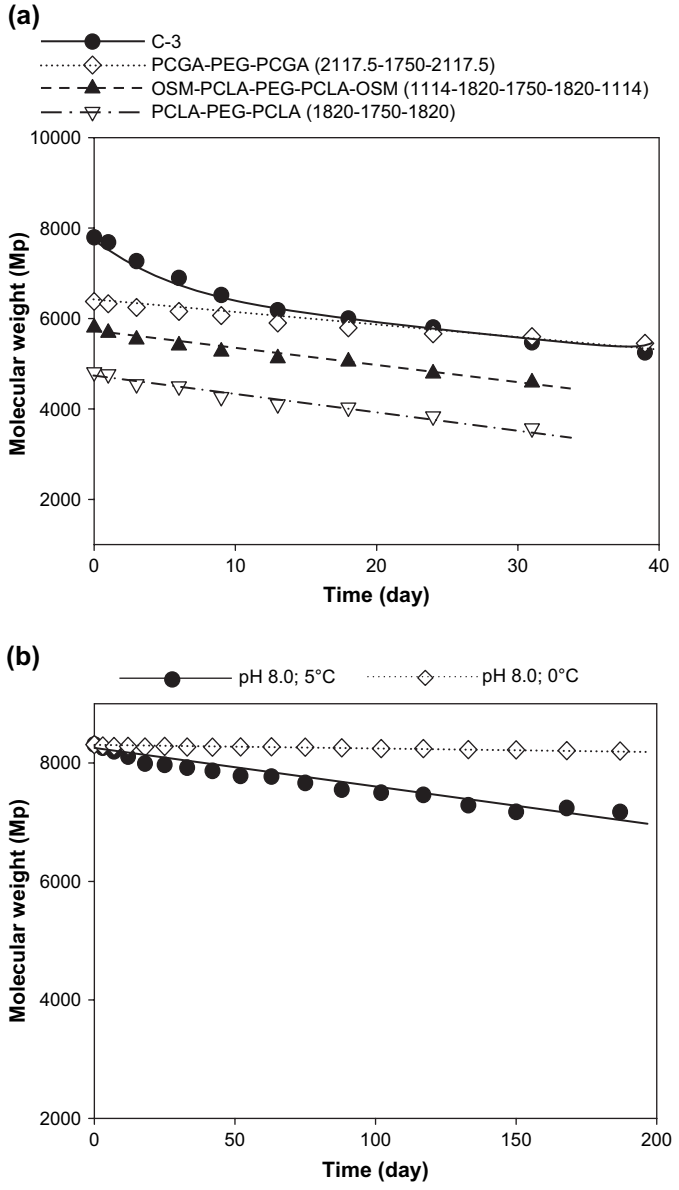


Fig. 9. Degradation of triblock and pentablock copolymers. (a) Degradation of triblock and pentablock copolymers in aqueous solution at 37 °C and pH 7.4. (b) Degradation of OSM–PCGA–PEG–PCGA–OSM (PEG: 1750, PEG/PCLA: 1/1.89) at 5 °C and 0 °C over specified time periods.

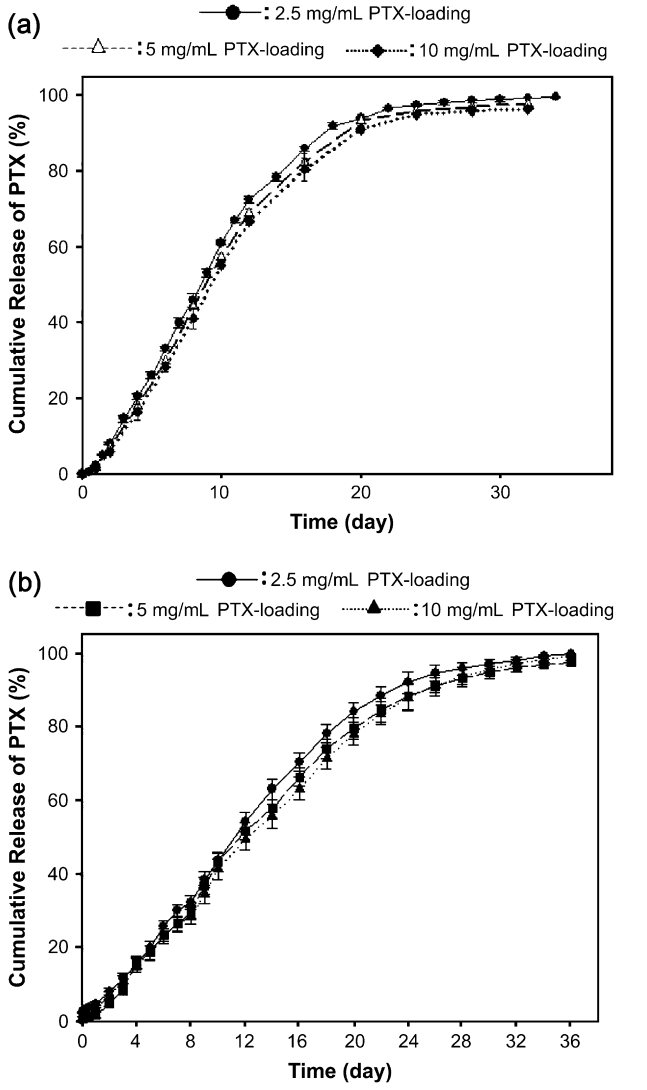


Fig. 10. Observed Genexol (PTX) drug release from the hydrogel. (a) PTX release from OSM–PCGA–PEG–PCGA–OSM (C-3) matrix during in vitro testing. (b) PTX release from OSM–PCLA–PEG–PCLA–OSM (PEG: 1750, PEG/PCLA: 1/1.89) matrix during in vitro testing.

to OSM–PCLA–PEG–PCLA–OSM. The degradation of OSM–PCGA–PEG–PCGA–OSM in aqueous solution at 5 °C is faster than at 0 °C (the corresponding molecular weight loss is 109 and 1147, respectively, after 7 months calculated from Fig. 9b). It is believed that the molecular weight of OSM–PCGA–PEG–PCGA–OSM remains constant over this entire 7-month period in aqueous solution.

3.4. Drug loading and release

Fig. 10 shows the results for the release into the environment of the drug paclitaxel (PTX), which was loaded into the OSM–PCGA–PEG–PCGA–OSM and OSM–PCLA–PEG–PCLA–OSM matrices, obtained by the in vitro test method (the drug content in the polymer aqueous solution was weight percent compared to the matrix). As can be seen in Fig. 10a, more than 90% of the PTX was released from the matrix after 20 days. When a higher concentration of drug was loaded into the matrix, the amount of drug remaining in the matrix after its release was found to be higher. The amount of drug remaining in the matrix 32 days after the drug release date was 0.8% for the sample with a 2.5 wt% PTX loading, 2.8% for a 5 wt% PTX loading, and 3.5% for the sample with a 10 wt% PTX loading (calculated from Fig. 10a).

PTX was loaded into hydrogel and it was contained in the hydrophobic core of OSM and PCGA or PCLA. The drug was released by the degradation/corrosion of the copolymers. Such, the release rate of the drug is depending on the degradation of copolymers. Because the degradation of OSM–PCLA–PEG–PCLA–OSM is faster than OSM–PCGA–PEG–PCGA–OSM (Fig. 9a), the releasing time of drug which was loaded into

OSM–PCGA–PEG–PCGA–OSM should be shorter than that in OSM–PCLA–PEG–PCLA–OSM. The releasing time of 90% PTX in OSM–PCGA–PEG–PCGA–OSM is more than 20 days (Fig. 10a), while it takes more than 28 days to release 90% PTX in OSM–PCLA–PEG–PCLA–OSM (Fig. 10b).

4. Conclusions

OSM–PCGA–PEG–PCGA–OSM triblock copolymers of varying PEG molecular weight and PCGA/PEG ratios were synthesized, and their pH- and thermo-reversible phase transition behaviors immediately following injection were investigated. The typical phase diagrams exhibited a CGC, CGpH and two CGT curves. The CGC and CGpH of the pH/temperature sensitive block copolymers can be controlled by varying the ratio of hydrophobic/hydrophilic chain lengths (PCGA/PEG), the concentration of the copolymer in solution, and the molecular weight of the pH sensitive component. The CGT and the range between the lower CGT and the upper CGT can both be determined by the length of the hydrophilic segment (PEG) and by the concentration of copolymer in solution. In other words, the gel phase zones of OSM–PCGA–PEG–PCGA–OSM can be adjusted by varying the PEG molecular weight, the PCGA/PEG ratio and the concentration of the pH sensitive block. For injection purposes, the best condition for the excited gel stage of the pH/temperature sensitive block copolymer solutions is positioned directly in the center of the gel phase area of the phase diagrams. The degradation of OSM–PCGA–PEG–PCGA–OSM is notably faster than that of OSM–PCLA–PEG–PCLA–OSM, indicating its potential for drug delivery. This polymer can be kept in aqueous solution at 0 °C for more than 7 months without degradation. Following the loading of OSM–PCLA–PEG–PCLA–OSM with the anticancer drug paclitaxel, more than 90% of the drug was released from this matrix after 20–22 days. The results of this investigation demonstrate the potential usefulness of this pH/temperature sensitive block copolymer for application in drug delivery systems.

Acknowledgement

This work was supported by Korea Research Foundation Grant (KRF-2003-005-D00009 and KRF-2004-005-D00070).

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