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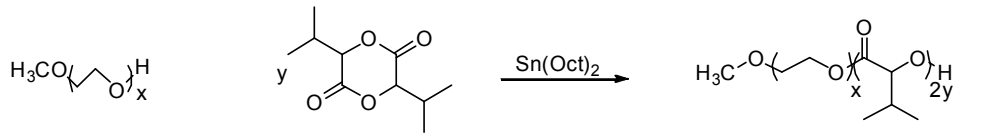
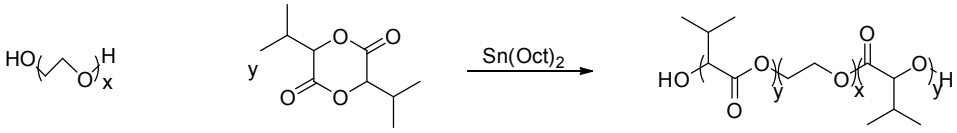


Poly(ethylene glycol) methyl ether (MePEG) (4)

L-diisopropyl glycolide (L-DIPG) (3)

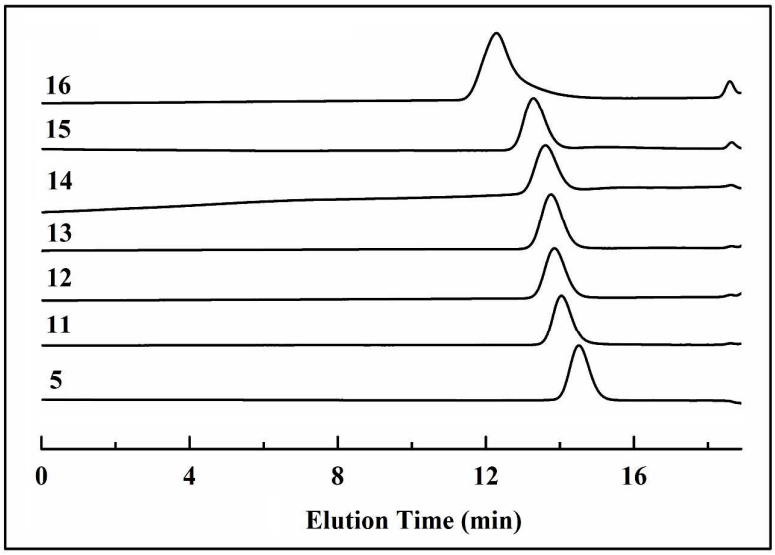
MePEG-PDIPG diblock copolymers (6, 7, 8, 9, 10)



Poly(ethylene glycol) (PEG) (5)

L-diisopropyl glycolide (L-DIPG) (3)

PDIPG-PEG-PDIPG triblock copolymers (11, 12, 13, 14, 15, 16)



Synthesis and Properties of Novel Isopropyl-Functionalized Polyglycolide-PEG Copolymers

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Abstract

Because of the importance of glycolide-based polymers in materials and medical applications, we synthesized alternative novel thermosensitive biomaterials to be possible candidates to those well-known polymers. After the synthesis of *l*-diisopropyl glycolide monomer was performed in two steps, novel PEG based poly(diisopropyl glycolide) diblock and triblock copolymers (MePEG-PDIPG, PDIPG-PEG-PDIPG) were synthesized with high conversions by ring opening polymerization. The molar mass distributions of copolymers were very narrow, and NMR measurements confirmed no racemization in polymer synthesis even at 180 °C. Gel-sol experiments were studied after each component's length in MePEG-PDIPG and PDIPG-PEG-PDIPG was adjusted with care during the synthesis. The polymer showed a sol property at 42-45 °C, which is suitable for the injection and a gel property at body temperature. After loading paclitaxel into gels effectively, the anticancer drug showed prolonged release (60 days). SEM analysis confirmed that when release time proceeded, gels turned into more porous structure due to drug release.

Keywords: Poly(diisopropyl glycolide), Thermosensitivity, Drug Delivery Systems

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1. INTRODUCTION

Drug delivery can be simply expressed as the delivery of a various properties of drugs to humans or animals using proper formulations. Drug delivery systems have now reached at a multi-billion dollar industry with versatile applications of many interdisciplinary sciences.[1-2] Also, it will undoubtedly continue to be one of the important research areas in future life. Thermosensitive biodegradable polymers for the use of drug delivery systems are one of the most preferred polymers in the biomedical field in last decade because they can easily be subjected to a physical change with an external heat due to their features of thermosensitivity, and no effort is needed to remove them from the body due to their characteristics of biodegradability.[3-5] PEG based copolymers of poly(lactide), poly(glycolide), and poly(lactide-co-glycolide) (PLGA) are one of the most preferred thermosensitive biodegradable polymers in biomedical field.[6-9] In contrast, there have been a few reports describing the use of symmetric glycolic acid derivatives as monomers or comonomers for the preparation of glycolide family polymers.[10-17] Novel versatile biomaterials are needed day after day for growing pharmaceutical and biomedical industry. Especially, poly(diisopropyl glycolide) may be considered as highly promising material for those industries in future due to their enhanced properties. There are only a few studies related to homopolymer of diisopropyl glycolide in literature.[18-22] Synthesis and the conformational aspects of poly(diisopropyl glycolide) homopolymer were performed, and the conformational aspects were evaluated by means of optical rotatory dispersion (ORD) and circular dichroism (CD).[18-19] Also, homopolymerization of poly(diisopropyl glycolide) under the conditions of various

catalysts, temperature, and time was examined, and the optical purity of the polymers was investigated.[20-21] The polymerization kinetics, spectroscopic and thermal properties of poly(diisopropyl glycolide) were also studied.[22] In the present work, novel PEG-based diblock and triblock copolymers of poly(diisopropyl glycolide) were synthesized in order to gain thermosensitive property to these PDIPG homopolymers for the application of drug delivery systems. Then, the gel-sol transition temperature was adjusted by changing the length of each component. Aqueous solutions of these copolymers were sols at around 42-45 °C, and then they were loaded with bioactive molecules. After fast cooling to body temperature, the loaded copolymer formed a gel that could serve as a sustained-release matrix for medicines. Homogenous drug loading and sustained release of paclitaxel over 60 days were observed successfully.

2. EXPERIMENTAL SECTION

Materials

L-valine (98%), sodium nitrite ($NaNO_2$), *p*-toluene sulfonic acid monohydrate (PTSA) (98.5%), diethyl ether (99.5%), toluene (99.7%), dichloromethane (99%), and methanol (99.9%) were purchased from Sigma-Aldrich (Germany) and used without further purification except when mentioned specifically. MePEG2000 homopolymer (Fluka), PEG2000 homopolymer (Sigma), and tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) (Aldrich, 95%) were used in syntheses of di- and triblock copolymers. Paclitaxel anticancer drug (Alfa Aesar 99.5%) were used as received. Acetonitrile (Sigma-Aldrich, 99.9%) mobile phase was filtered out with a filtration system prior to use.

Characterization

^{1}H- and ^{13}C-NMR experiments were carried out with Bruker Avance DPX 400 and Bruker Avance III 500 MHz for the structural analysis and the determination of number average molecular weight of the copolymers. ATR-FTIR spectra of samples were recorded using an ATR Bruker-Tensor 27 spectrometer between 600 and 4000 cm^{-1}. The elemental analysis was carried out on a Costech Elemental Combustion System (ECS 4010) elemental analyzer. Analysis of copolymers was carried out with gel permeation chromatography (GPC) at 30 °C on a Shimadzu prominence GPC system equipped with a RID-10A refractive index detector, a LC-20AD solvent delivery unit, a CTO-10AS column oven and a set of two columns, PSS SDV 5 µL 1000 A° and PSS SDV 5 µL 50 A°. THF (HPLC grade) was used as a mobile phase at 1.0 mL/min. The sample concentration was adjusted to 2 mg/mL, and the injection volume was set at 50 µL. The calibration curve was made with seven polystyrene standards having the average molecular weight range from 162 to 34,300 Da. HPLC spectra were recorded on 1260 Infinity Agilent with a UV detector. Data was analyzed with the Chem Station software program. ZORBAX SB-C18 4.6 x 150 mm, 3.5 µm HPLC column was used for the analysis of paclitaxel anticancer drug. Scanning electron microscope (Jeol 6060) was used in microstructural investigations of copolymer gels. The samples collected were frozen in liquid nitrogen and dried by freeze-drying (Labconco). Thermogravimetric analyses (TGA) were performed on a Perkin Elmer TGA 4000 under nitrogen atmosphere between 20 and 600 °C at a heating rate of 40 °C/min. Differential scanning calorimeter (DSC) analyses were performed using Mettler Toledo DSC Star System or Perkin Elmer DSC 4000 in nitrogen atmosphere between -65 and 220 °C in a heating rate of 10 °C/min (or 40

°C/min) with double run for determination of melting point and glass transition temperatures of copolymers.

Synthesis of *l*-2-hydroxy-3-methylbutanoic acid (2)

L-2-hyroxy-3-methylbutanoic acid was prepared with a modified protocol.[23] A solution of sodium nitrite (28.8 g, 0.42 mol) in water (200 mL) was added dropwise into a solution of *l*-valine (12 g, 0.1 mol) in 1.0 M H_2SO_4 (200 mL) over 2h in an ice-bath, and the reaction mixture was stirred overnight at 0 °C. The proceeding of the reaction was followed by thin layer chromatography (TLC, silica gel, distilled water). Then, the TLC plate was treated with ninhydrin solution that only stained *l*-valine. The reaction mixture was saturated with NaCl, extracted with diethyl ether (6 x 60 mL), and the combined organic extracts were dried over Na_2SO_4. After removing the ether under reduced pressure, the residue was recrystallized from toluene to afford pure *l*-2-hydroxy-3-methylbutanoic acid as a white solid (50%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.92 (3H, d), 1.04 (3H, d), 2.09-2.2 (1H, m), 4.15 (1H, d), 6.2-8.6 (2H, b). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16.1, 18.9, 32.1, 75, 179.2. ATR-FTIR (ν_{max}/cm^{-1}): 3413 (OH), 2972, 2935, 2880 (CH), 1702 (C=O).

Synthesis of *l*-3,6-diisopropyl-1,4-dioxane-2,5-dione (L-DIPG) (3)

L-3,6-diisopropyl-1,4-dioxane-2,5-dione was synthesized by modifying the method in literature.[10] A mixture of *l*-2-hydroxy-3-methylbutanoic acid (25 g, 0.21 mol) and *p*-toluene sulfonic acid monohydrate (0.5 g, 2.5 mmol) in toluene (200 mL) was refluxed for five days in order to remove water with a Dean-Stark apparatus. After the mixture was cooled, toluene was removed by rotary evaporation until the small volume of solvent was remained. The toluene solution was left to crystallize at 4 °C for 3-4 hours and the resulting crystals were separated by decantation. The second

crystallization was performed in order to eliminate impurities. A small volume of toluene was added into the residue. Then, it was heated at 55 °C to solve the crystals. The solution was kept at 4 °C for a while. The resulting crystals was collected by cold filtration and dried under vacuum to give 8.5 g (40%) of *l*-3,6-diisopropyl-1,4-dioxane-2,5-dione. Elemental analysis for $C_{10}H_{16}O_4$ (200.23 g/mol): Calc. C 59.98, H 8.05%; found: C 59.97, H 7.75%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05 (6H, d), 1.15 (6H, d), 2.44-2.55 (2H, m), 4.73 (2H, d). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16, 18.7, 29.6, 79.8, 166.6. ATR-FTIR (ν_{max}/cm^{-1}): 2969, 2939, 2878 (CH), 1748 (C=O).

Synthesis of MePEG-PDIPG diblock copolymers

MePEG-PDIPG diblock copolymers were synthesized in the melt via ring opening polymerization. 240 mg of MePEG2000 (0.12 mmol), 400 mg of L-DIPG (2 mmol), and 20 mg of $Sn(Oct)_2$ (0.05 mmol) were added to the polymerization tube. The reaction was stirred at 120 °C for 3 hours under nitrogen atmosphere. Purification of the copolymer **9** can be performed with following method: Synthesized diblock copolymer **9** was dissolved in a minimum volume of dichloromethane (4 mL) before it was precipitated with an excess volume of cold methanol (40 mL). Then the product **9** was dried under vacuum overnight. In similar way, various molecular weights of diblock copolymers (**6**, **7**, **8**, **10**) could be obtained by changing the feed ratio of L-DIPG at a constant mole of MePEG. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05-1.09 (6H, t), 2.15-2.59 (1H, m), 3.66 (4H, s), 4.99 (1H, d). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17, 18.6, 30.3, 70.6, 77, 168.8. ATR-FTIR (ν_{max}/cm^{-1}): 2970, 2935, 2877 (CH), 1753 (CO).

Synthesis of PDIPG-PEG-PDIPG triblock copolymers

In order to perform the synthesis of PDIPG-PEG-PDIPG triblock copolymers, PEG homopolymer containing hydroxyl groups at both ends was used instead of MePEG homopolymer having a methoxy group on one end and a hydroxyl group on the other end. A series of triblock copolymers were obtained by changing the mole of L-DIPG at constant mole of PEG.

Determination of thermosensitivity properties of copolymers

Thermosensitivity of copolymers was determined by inverting vial in different temperatures using a controlled water bath.[4] Firstly, various amounts of copolymers were mixed with distilled water to prepare a series of suspensions in 1.5 mL vials. All suspensions were vortexed to get homogenous mixture if possible. After the homogenous mixtures were kept at 4 °C for 30 min, they were immersed in a temperature controlled water bath. The gel-sol transition temperatures of copolymers were examined between 4 and 80 °C with 2 °C increments. The vials were kept in water baths for 2 min at each temperature before inverting. The critical gel-sol transition temperature was determined as the temperature at which the gel turned into sol form immediately after inverting the tube.

Preparation of phosphate buffer solution (PBS)

2 g of NaCl, 0.05 g of KCl, 0.36 g of Na_2HPO_4, and 0.06 g of KH_2PO_4 were dissolved in distilled water. Then, the medium of pH was adjusted to 7.4 with diluted HCl, and the volume of mixture was brought to 250 mL with distilled water.

Paclitaxel loading into copolymer gel

Paclitaxel was used as an anticancer drug to determine the release behavior of synthesized copolymers. Paclitaxel was loaded into produced copolymer gels effectively at 1.0%. One of the drug loaded copolymer gel was prepared with following method: 2 mg paclitaxel, 200 mg PDIPG-PEG-PDIPG **12** triblock copolymer, and 300 μL distilled water were added to the vial, and the sample was vortexed for 2 min at room temperature to obtain a drug loaded gel. Other drug-loaded gels were prepared in a similar method.

Drug release studies

1 mL of 2.0% Tween 80 in PBS at pH = 7.4 was added to the upper side of drug loaded gels for the drug release experiments. These samples were inserted into an incubator, and they were shaken at a constant speed of 200 rpm and at 37 °C. At different time intervals, 1 mL of release medium was taken from the vials and replaced with 1 mL of fresh one. The amount of paclitaxel released into the medium was measured using HPLC with UV detector.

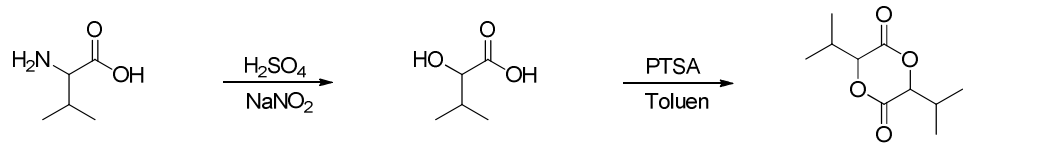
RESULT AND DISCUSSION

General Aspects to Polyglycolide family polymers. The most widely investigated polymers in the controlled release drug delivery of therapeutic agents are aliphatic polyesters such as poly(D,L-lactide) (PLA) and poly(D,L-lactide-coglycolide) (PLGA) because of their properties of predictable degradation kinetics, biocompatibility, ease of fabrication and regulatory approval by the Food and Drug Administration (FDA). These two polymers are relatively hydrophobic polyesters, not stable in damp medium and biodegradable to non- toxic by-products (lactic acid,

glycolic acid, CO_2, and H_2O). Substituent in glycolide monomer drastically changes its physiochemical property. When hydrogen substituent (glycolide) is replaced with methyl group (lactide) in cyclic dimer monomer, bio-applications are considerably affected due to the change in hydrophobicity, crystallinity, biodegradation, mechanic and thermal properties. Here we especially examined the physicochemical properties when one hydrogen substituent was changed with an isopropyl group in cyclic dimer. Especially it is very practical way in terms of industrial perspective to synthesize peg based poly(*l*-diisopropyl glycolide) polymer in bulk starting from valine, which is basic and cheap amino acid. More hydrophobic polymer due to isopropyl group may increase hydrophobic drug loading for the application of various drug delivery systems. On the other hand, it is also very convenient to prepare thermosensitive PDIPG copolymer with hydrophilic PEG to control hydrophobic/hydrophilic balance. Thus, the solution of PDIPG-PEG block copolymer displayed as a liquid at around 42-45 ^{o}C for the injection and a gel at body temperature for the release of bioactive drug into its surroundings. These controlled release biocompatible hydrogel systems provide particularly with an advantage for local drug delivery; e.g., around the solid tumor site. These novel biodegradable injectable controlled release systems could be good candidates for the treatment of solid brain tumors or solid tumors just under skin. The reason for brain tumors is that the blood barrier limits drugs and/or excess drugs.[4] Also, side effects can be greatly diminished with local therapy of drug loaded gels like PDIPG-PEG due to no systematic circulation of the drug in body.[4]

Monomer Synthesis. L-2-hydroxy-3-methylbutanoic acid **2** was synthesized from *l*-2-amino-3-methylbutanoic acid **1** by using sodium nitrite in the presence of sulfuric acid for 24 hours at 0 °C.[23] Conversion of free amine to alcohol group in compound **2** was easily confirmed by the appearance of a new -OH peak at 3413 cm^{-1} in ATR-

FTIR spectrum. Then, the synthesis of *l*-3,6-diisopropyl-1,4-dioxane-2,5-dione **3** was carried out from the condensation of compound **2** at reflux temperature for 5-days in the presence of *p*-toluenesulfonic acid monohydrate in toluene (Scheme 1).[10] It was found that the synthesis of *l*-diisopropyl glycolide monomer was importantly time dependent reaction. GPC and FTIR analyses confirmed that there was still *l*-2-hydroxy-3-methylbutanoic acid **2**, starting material, in 24 hours while the monomer started to turn into oligomeric species in longer reaction time, especially more than 5 days. The formation of *l*-diisopropyl glycolide monomer was proved by shifting carbonyl stretching of the carboxylic acid group in the compound **2** from 1702 cm^{-1} to 1748 cm^{-1} in cyclic monomer. Also, the complete disappearance of OH peak at 3413 cm^{-1} in the ATR-FTIR spectrum proved the synthesis of ring-closed compound. Similar results were observed by proton and carbon NMR, as well. CH proton at compound **2** shifted from 4.15 ppm to 4.73 ppm in monomer. Ester formation was also observed in ^{13}C-NMR by the shifting from 179.2 ppm (acid carbonyl group) to 166.6 ppm (ester carbonyl group). Here, we presented the detailed synthesis and characterization analysis of the L-DIPG monomer, which differs from literature. [18-22] All spectra for the characterizations of compound **2** and **3** can be found in supporting info section.



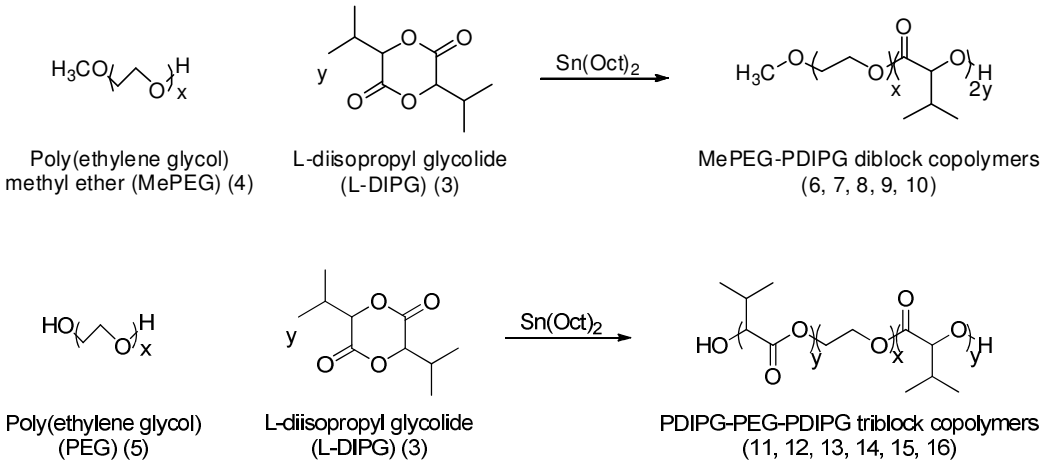
L-2-amino-3-methyl-butanoic acid (1) | L-2-hydroxy-3-methyl-butanoic acid (2) | L-3,6-diisopropyl-1,4-dioxane-2,5-dione (L-DIPG) (3)

Scheme 1. Synthesis of *l*-3,6-diisopropyl-1,4-dioxane-2,5-dione

Polymer Synthesis. MePEG-PDIPG diblock and PDIPG-PEG-PDIPG triblock copolymers were obtained from ring-opening polymerization of diisopropyl glycolide

monomer with poly(ethylene glycol) methyl ether (MePEG2000) or poly(ethylene glycol) (PEG2000) under nitrogen atmosphere (Scheme 2). All polymerization reactions were performed in a bulk in 120 ^{o}C under melt conditions except for copolymers **10** and **16**. Both of these copolymers needed higher temperature (180 ^{o}C) to melt due to the high mole of hydrophobic component (Table 1). Stannous octoate ($Sn(Oct)_2$) was selected as catalyst due to almost complete conversions and low toxicity compared to other heavy metal salts.[24-25] The molecular weight of MePEG was purposely selected as 2000 g/mol to synthesize all copolymers because higher molecular weight of PEG (above ~10k) is inappropriate for filtration through membrane of human kidney due to being large hydrodynamic radius of the PEG in aqueous phase.[3]

Also, hydrophobic PDIPG length was adjusted by changing mole of diisopropyl glycolide (0.25-10.0 mmol) at a constant mole of hydrophilic MePEG (0.12 mmol), as seen in Table 1. Also, [DIPG] / [cat] ratio was kept between 5 and 200 for the synthesis of di- and triblock copolymers. The determination of number average molecular weight of copolymers was performed from the ratio of CH protons of monomer and CH_2 protons of MePEG or PEG in ^{1}H-NMR. The number average molecular weights estimated by ^{1}H-NMR were close to the ones determined by GPC.



Scheme 2. Synthesis of MePEG-PDIPG and PDIPG-PEG-PDIPG diblock and triblock copolymers

Table 1. Conditions and characterization of diblok and triblock copolymers

Conditions for synthesis of copolymers							Characterization of diblock and triblock copolymers							
ID	Copolymer	PEG (mmol)	L-DIPG (mmol)	$Sn(Oct)_2$ (mmol)	Time (hour)	Temperature (°C)	M_w[a] (g/mol)	M_n[a] (g/mol)	M_n[b] (g/mol)	Theoretical	M_w/M_n[a]	% [b] Conv.	RU[b] of DIPG	L-DIPG/ $Sn(Oct)_2$
6	MePEG-PDIPG	0.12	0.25	0.05	3	120	3050	2930	2670	2420	1.04	97.3	7	5
7			0.75		3	120	3880	3670	3120	3250	1.06	97.8	11	15
8			1.5		3	120	4740	4380	4380	4500	1.08	99.9	24	30
9			2		3	120	5100	4730	5060	5330	1.08	99.5	30	40
10			10		8	180	18,900	14,430	16,370	18,670	1.31	95.6	144	200
11	PDIPG-PEG-PDIPG	0.12	0.6	0.05	3	120	3960	3800	3310	3000	1.04	96.5	13	12
12			0.8		3	120	4300	4100	3470	3330	1.05	97.4	15	16
13			1		3	120	4690	4450	3710	3670	1.05	97.7	17	20
14			1.5		3	120	5470	5190	4650	4500	1.05	99.6	27	30
15			3		3	120	7190	6800	6760	7000	1.06	94.1	48	60
16			10		8	180	19,390	16,000	15,800	18,670	1.21	97	138	200

[a] Determined by GPC, [b] Determined by ^{1}H-NMR spectrum (The conversion was calculated using the signal from the CH (δ 4.7 ppm) of the unreacted monomer, and the CH (δ 4.9 ppm) of the polymer), RU: Repeating unit.

Polymer Characterization. GPC was performed to evaluate molar mass distributions of the various copolymers. Figure 1 shows the GPC curves of PEG2000 and PEG2000-initiated triblock copolymers **11** to **16**. The peak of PEG2000 **5** appeared at an elution time of 14.5 min, with a polydispersity index of 1.03. On the GPC curves of the copolymers, the peak corresponding to PEG was not monitored, indicating that copolymers were effectively synthesized with no residual PEG homopolymer. Very high (>95%) conversions were observed even before purification steps in many syntheses. The molar mass distributions of many diblock and triblock copolymers were very narrow, with polydispersity indices in the 1.04 to 1.08 range except for copolymers **10** (1.31) and **16** (1.21). Moreover, the elution time shifted to 13.9 min for copolymer **11**, 13.7 min for copolymer **13**, and 13.3 min for copolymer **15**, with corresponding M_n values of 3800, 4450, and 6800 $g.mol^{-1}$, respectively. Similar behavior was observed for other diblock copolymers. Molecular weight of copolymers was purposely kept between 3kDa and 7kDa for gel-sol experiments. Copolymers **10** and **16** were synthesized to show that higher molar mass copolymers could be obtained for any other applications if desired.

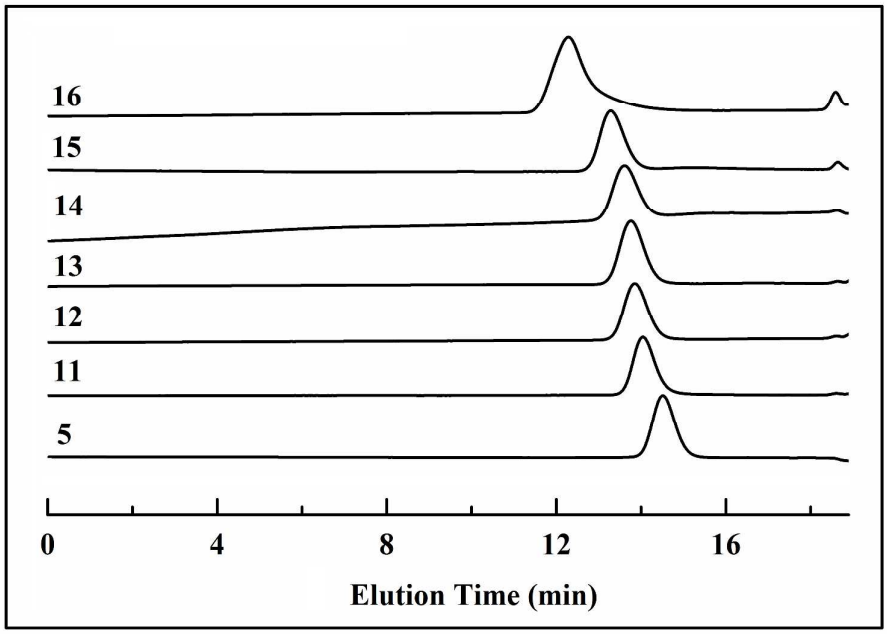


Figure 1. GPC curves of PDIPG-PEG-PDIPG triblock copolymers

The structure of MePEG-PDIPG diblock copolymers was analyzed with ^{1}H- and ^{13}C-NMR (Figure 2). For ^{1}H-NMR spectrum of MePEG-PDIPG **9**, resonances in the 4.99 ppm (C*H*) (f), 2.48-2.26 ppm range (C*H*) (b), and in 1.18-1.01 ppm range (CH_3) (a) belong to PDIPG blocks. The methylene protons (d) in CH_2 group of MePEG were recorded at around 3.66 ppm, and the slight singlet peak at 3.41 ppm can be attributed to methoxy protons (-O-CH_3) (c) at the end of the MePEG blocks. The α-methylene protons of PDIPG-connecting EO units (PDIPG-COO-CH_2-CH_2-) (e), confirming copolymer formation, appear as a multiplet in the 4.38-4.23 ppm range, together with the C*H* protons of the hydroxylated diisopropyl end units (Figure 2a). Figure 2b shows the ^{13}C-NMR spectrum of the copolymer $MePEG_{45}/PDIPG_{30}$ **9** in $CDCl_3$. The resonances of carbon atoms in the copolymer were assigned as -*C*H_2-*C*H_2 of EO units (d) at 70.6 ppm in PEG blocks, -(*C*H_3)CH(CH_3) (a), -(CH_3)CH(*C*H_3) (b), -*C*H (c), -*C*H (e), and –*C*=O (f) at 17, 18.6, 30.3, 77, and 168.8 ppm in PDIPG block units, respectively. A single sharp peak at 168.8 ppm confirmed no racemization. It was

also not noticed any racemization for all other polymer batches including copolymer **10** synthesized in 180 °C. Also, MePEG-PDIPG **9** copolymer was analyzed with ATR-FTIR spectroscopy. C-H stretching in compound **9** was assigned at peaks of 2970, 2935, and 2877 cm^{-1} while peak of carbonyl group was observed at 1753 cm^{-1} in ATR-FTIR spectrum as seen in Figure 2c. Similar results were obtained when other diblock copolymers were characterized by ^{1}H-, ^{13}C-NMR, and ATR-FTIR (supporting info). The only exception was that ^{1}H- and ^{13}C-NMR peaks of the end-repeating units of PDIPG were observed more visible when the length of PDIPG drastically decreased (For example Figure 27 or 31 in sup. info). The additional peak at 174.6 ppm in ^{13}C-NMR was the carbonyl group of hydroxylated end unit, which was more apparent if a couple of repeating units existed in copolymer (low molecular weight copolymers). On the other hand, when higher mole of DIPG monomer was used, end unit and neighboring units to the end was not so visible in NMR spectrum due to the existence of so many inner repeating units, as expected (Figure 30 or 36 in sup. info). There was only main repeating unit for PDIPG block units in NMR (i.e. carbonyl peak at 168.8 ppm). This phenomenon was well matched with literature.[27] The general features of NMR and ATR-FTIR spectra of triblock copolymers were similar with those spectra of diblock copolymers (Supp. Info, Figure 7-12, 19-24, 31-36).

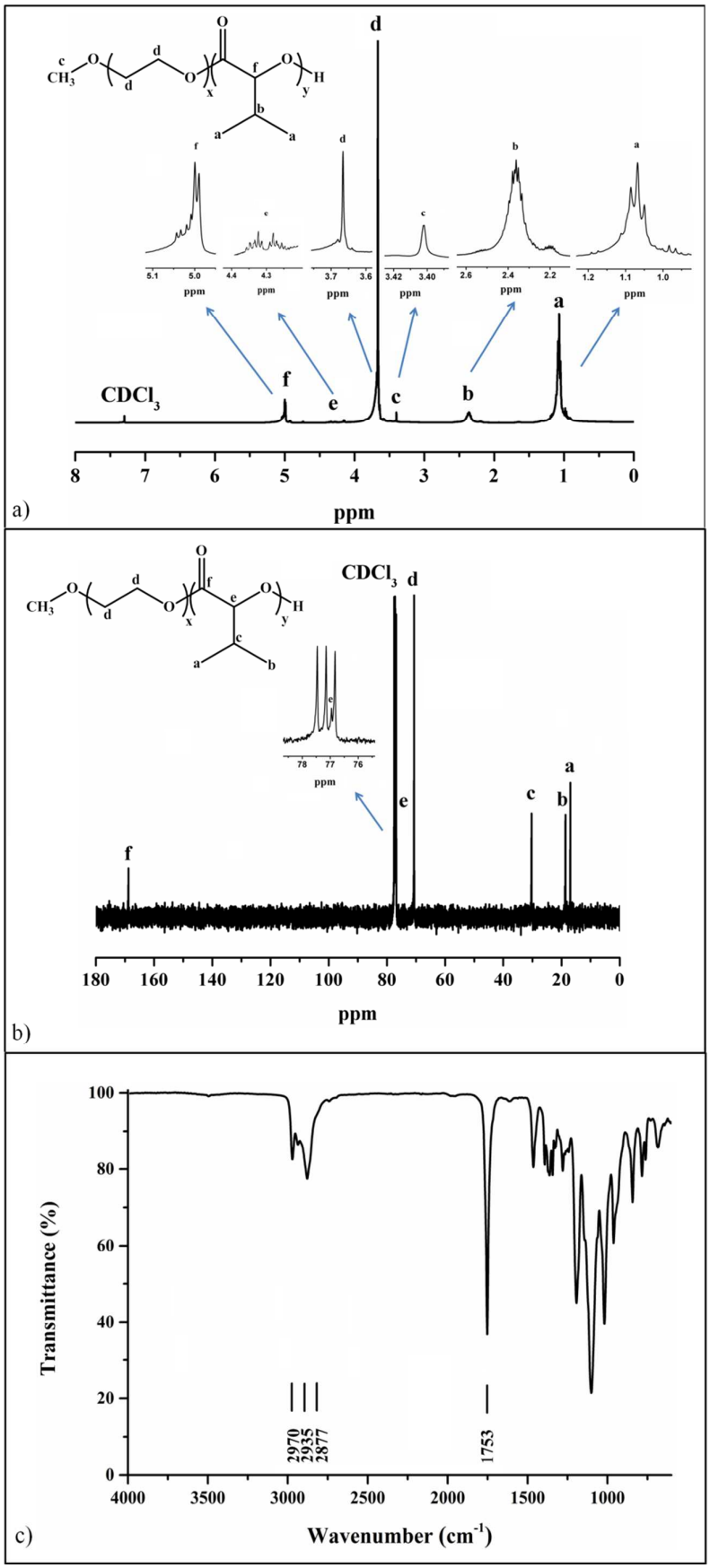


Figure 2. ^{1}H-NMR (a), ^{13}C-NMR (b), and ATR-FTIR (c) spectra of MePEG-PDIPG **9**

Gel-Sol Transition Properties. All PEG based poly(diisopropyl glycolide) diblock and triblock copolymers (i.e. **7**, **8**, **11**, **12**, and **13**) in various concentrations were tested to determine a gel-sol transition temperature. Especially, our aim was to search for appropriate copolymers that exhibited a sol behavior at around 42-45 °C, which was suitable for the injection, and then a gel with subsequent rapid cooling to body temperature. The gel-sol transition temperature was managed by changing of concentration and biodegradable block content. Diblock copolymer **8** at 25% conc. and triblock copolymer **12** at 40% conc. showed a gel about 37 °C and a sol behavior at 42-45 °C (Figure 3a, b). The upper left region of each curve represented for a sol phase and the opposite region for a gel phase. Also, it was observed that the gel-sol transition occurred at lower concentrations with increasing molecular weight of hydrophobic PDIPG block (Figure 3b). Diblock copolymers showed a steeper slope than triblock copolymers. The gel-sol transition of diblock copolymers was more sensitive to the change of concentration. Therefore, the range of gel to sol transition temperature of diblock copolymers was broad in a narrow concentration range. It was confirmed that very high diisopropyl glycolide content relative to the PEG moiety in a triblock chain did not provide any homogenous suspensions even at 5% concentration (i.e. copolymer **15**). Therefore, each component's length in MePEG-PDIPG and PDIPG-PEG-PDIPG copolymers was adjusted with a great care during syntheses. Molecular weight of copolymers was purposely kept in a specific range for the gel-sol experiments.

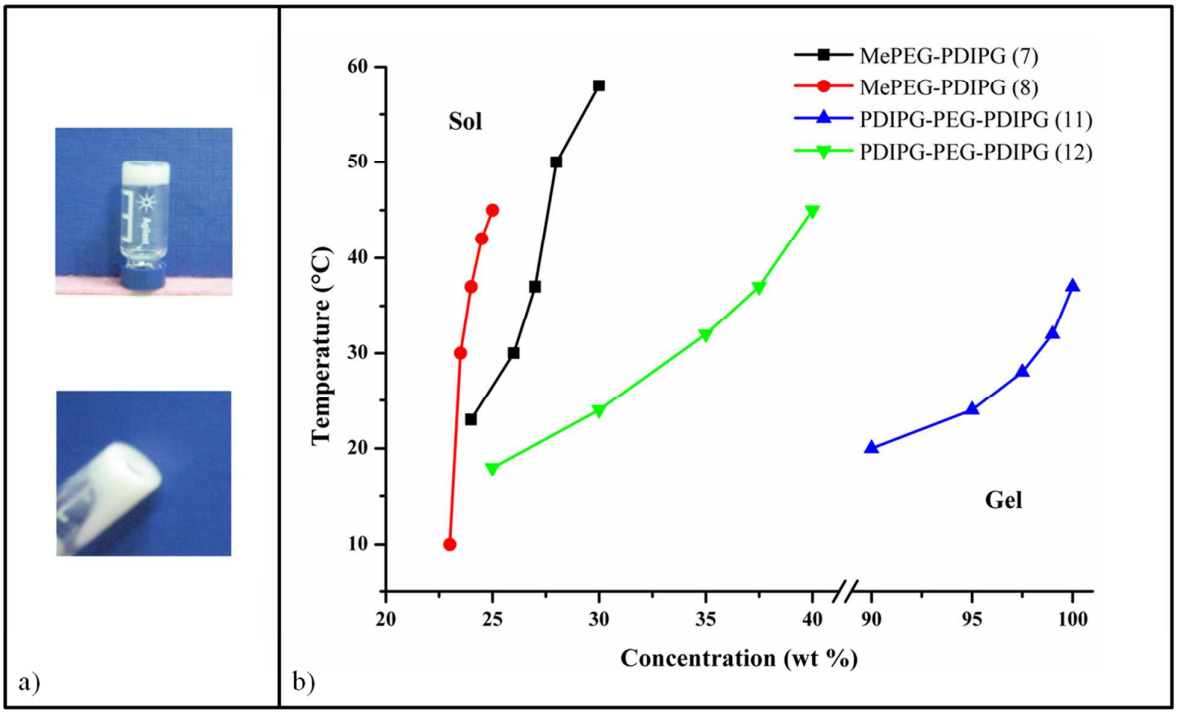


Figure 3. Image of gel and sol behavior of copolymer (a), Gel-sol curves of PDIPG-MePEG **7**, **8** and PDIPG-PEG-PDIPG **11**, **12** copolymers (b)

Release studies. The release behavior of paclitaxel anticancer drug from MePEG-PDIPG **7**, **8** and PDIPG-PEG-PDIPG **11**, **12** copolymers was examined over 60 days (Figure 4). In the end of first day, an initial burst release was about 25% and 6% for diblock copolymers **7** and **8**, respectively. It was about 12% and 8% for triblock copolymer **11** and **12**, respectively. These results confirmed that higher hydrophobic length of PDIPG caused lower burst release possibly due to the good hydrophobic-hydrophobic interaction between PDIPG block and paclitaxel. After the initial burst release, the drug was slowly released by diffusion over the next 59 days due to very low solubility of paclitaxel under in vitro condition. As seen in Figure 4, 57%, 52%, 54%, and 31% of paclitaxel were released from MePEG-PDIPG **7**, MPEG-PDIPG **8**, PDIPG-PEG-PDIPG **11**, and PDIPG-PEG-PDIPG **12** copolymer gels by the end of 60 days. The different release behavior of paclitaxel from gels is associated with

hydrophobic PDIPG block length. For example, when triblock copolymers **11** and **12** were compared each other, the increase in hydrophobic chain in copolymer **12** caused slow drug release (31 vs. 54) due to reasons mentioned above. Similar behavior but less distinct was also observed in diblock copolymers, as well.

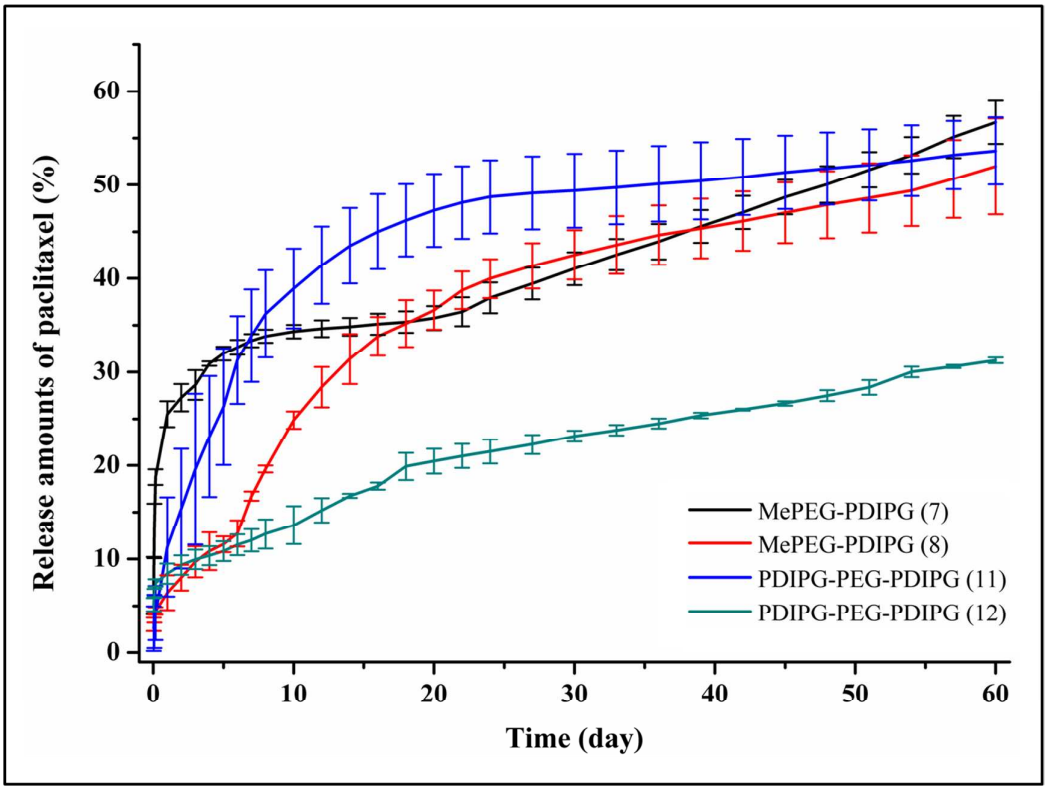


Figure 4 Paclitaxel release curves from diblock and triblock gels. Each point of plot is the result of an average of at least three independent reproductions.

SEM micrographs of free drug (Figure 5a) and drug loaded diblock copolymer **8** gel before (0 day, Figure 5b) and after releasing paclitaxel in PBS/Tween 80 (2%) at 37 °C for 1 day (Figure 5c) and 14 days (Figure 5d) were recorded. No significant differences were observed in surface morphology in the first 24 h of drug release in PBS/Tween 80 (2%) (Figure 5b vs. 5c). But, larger pore sizes and rougher surfaces were observed after the drug release in gel continued in PBS/Tween 80 (2%) up to 2

weeks (Figure 5d). Therefore, SEM analysis confirmed that when release time proceeded, gels turned into more porous structure due to drug release.

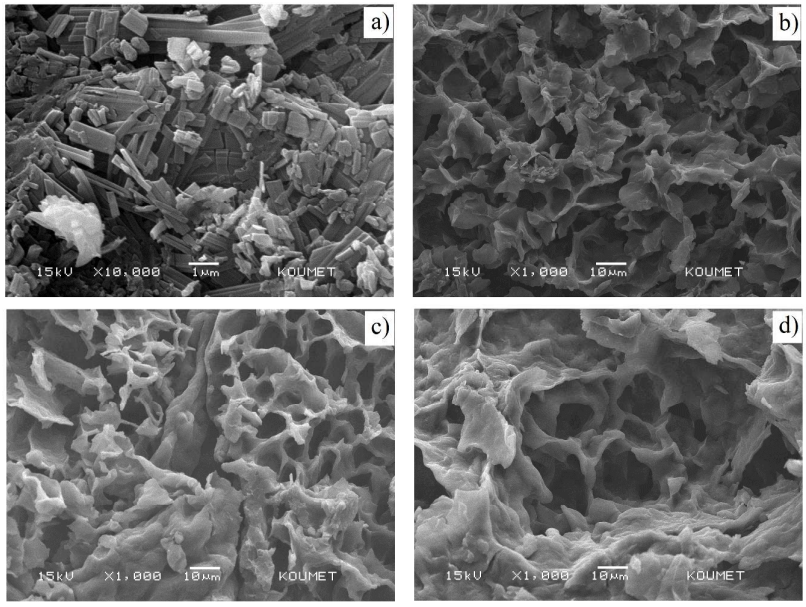


Figure 5 SEM analyses of drug (a), drug-loaded gel before release study (0 days) (b), drug-loaded gel after 1 day (c), and after 14 days (d).

We also performed the degradation test using GPC to confirm if the formation of more porous structure secondly resulted from hydrolytic degradation of polymers in buffer media in addition to drug release. GPC showed that diblock **8** and triblock **12** copolymer degraded into oligomeric species (15 %, M_n: 1300 and 500 g/mol for diblock **8**) and (26 %, M_n: 1000 g/mol, for triblock **12**) at 37^{o}C in PBS media in the end of two weeks, respectively (Figure 6). In conclusion, GPC showed the degradation of copolymer after two weeks. These newly formed oligomeric species were more soluble in buffer media, which helped the formation of more porous structure with the removal of oligomers when the buffer media was replaced with fresh one.

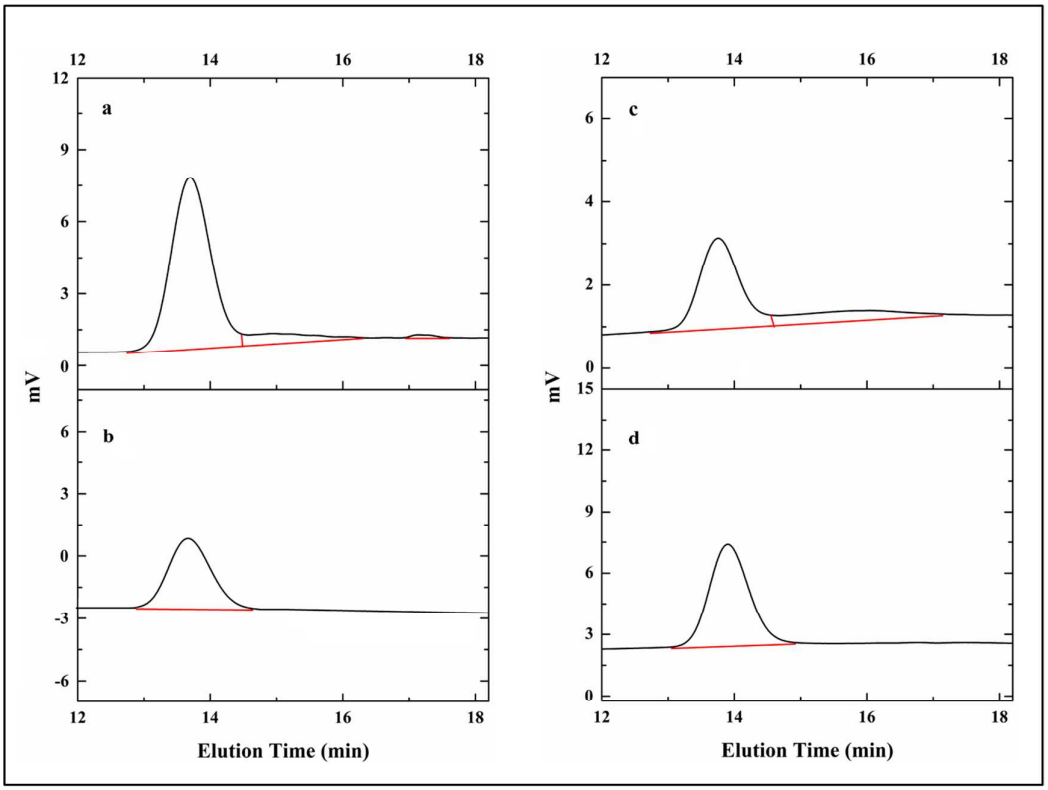


Figure 6 GPC curves of copolymer **8** and **12** after and before degradation. The diblock copolymer **8** after *2* weeks of degradation (a) and before degradation (b); the triblock copolymer **12** after 2 weeks of degradation (c) and before degradation (d)

Thermal Properties. The determination of decomposition characteristics of the copolymers was performed by thermogravimetric analysis (TGA). Also, TGA analysis of MePEG and PEG homopolymers were recorded for comparison, as seen in Figure 7. The thermal degradation profile of $MePEG_{45}$-$PDIPG_{144}$ **10** (under nitrogen flow) displayed two decomposition stages. The first one was due to decomposition of PDIPG with 88% weight loss at 337.1 °C. The second stage, appearing at higher temperatures, was due to MePEG decomposition with 10.15% weight loss at 415.6 °C (Pure MePEG: 415.1 °C). After the heating to 600 °C, a char yield of compound **10** was found as 1.85%. Similar behavior was also obtained for $PDIPG_{69}$-PEG_{45}-$PDIPG_{69}$ **16**. The thermal degradation profile of compound **16** under nitrogen flow showed two

decomposition stages, as presented in Figure 7. The decompositions for 87% weight loss at 328.2 °C and for 11.8% weight loss at 417.6 °C were closely related to PDIPG and PEG parts (Pure PEG: 420.5 °C), respectively. After the heating to 600 °C, a char yield of compound **10** was found as 1.2%. It was also confirmed from TGA curves that the weight percentage of each block was compatible with the weight percentage of components in crude feed.

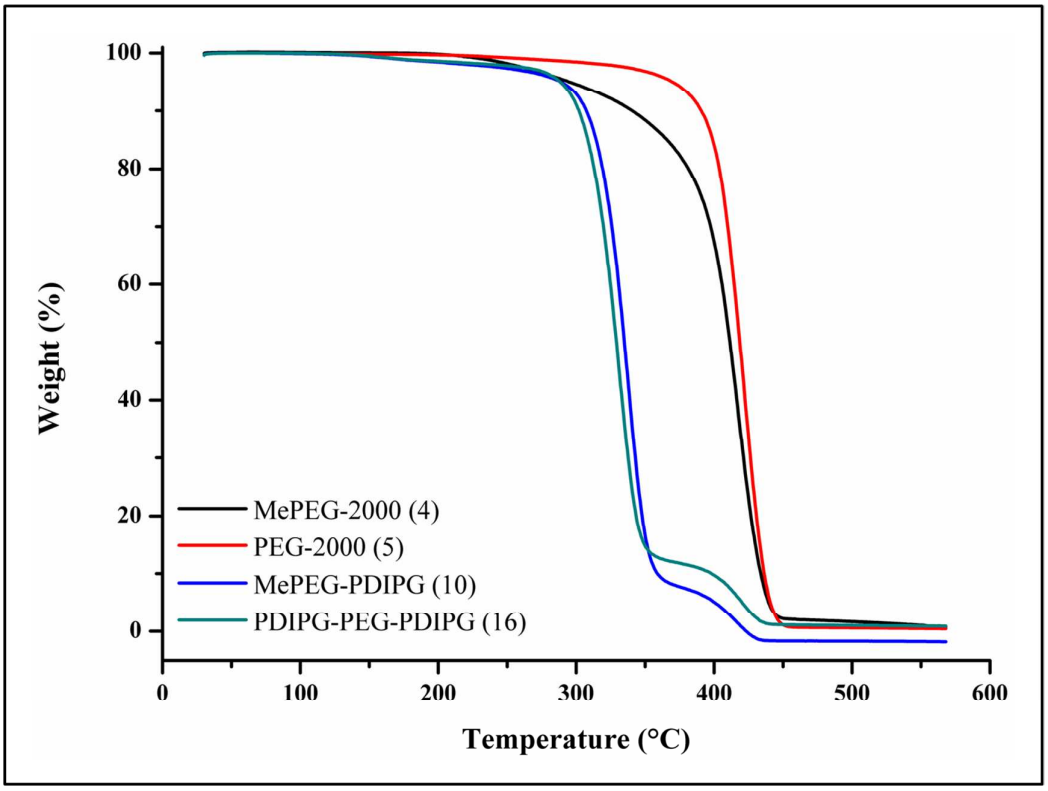


Figure 7 TGA curves of MePEG-2000 **4**, $MePEG_{45}$-$PDIPG_{144}$ **10**, PEG-2000 **5**, $PDIPG_{69}$-PEG_{45}-$PDIPG_{69}$ **16**

DSC thermograms of different copolymers revealed that higher block length of PDIPG in the copolymers caused higher melting peak of PDIPG and lower melting peak of MePEG/PEG (Figure 8). For example, the melting temperatures of MePEG and PEG2000 were around 60 °C [26] and 50 °C, [27] respectively. T_m values of MePEG

were found as 48.2 and 44.8 °C for diblock copolymer **7** and **8**, respectively, and T_m values of PEG were 36 and 30 °C for triblock copolymer **11** and **14**, respectively. Therefore, the presence of PDIPG blocks attached to PEG blocks reduced the melting temperature of corresponding PEG. Similar behavior was observed in polylactide-peg copolymers in literature.[26] This situation proved that the crystallization of each components were remarkably affected by the presence of the other component.

In the cases of diblock copolymer **10** and triblock copolymer **16**, which had higher repeating unit of PDIPG, T_m values of MePEG and PEG were not exactly detected due to the low content of PEG. In contrast, the copolymer of $MePEG_{45}$-$PDIPG_{24}$ **8** exhibited two small melting peaks at 121 and 136 °C, and the copolymer of $MePEG_{45}$-$PDIPG_{144}$ **10** exhibited double melting peak at 181 and 190 °C. It was clearly understood that higher PDIPG length increased melting point of PDIPG in copolymers. T_m value of $MePEG_{45}$-$PDIPG_{11}$ **7** was not detected because of short length of PDIPG. The presences of two different crystal structures or two different thicknesses of crystal lamellae with the same type of crystal structure or the simultaneous melting-reorganization/recrystallization-remelting of the lamellae originally formed during the crystallization process could be reasons for the formation of double melting peaks in the DSC heating profiles of PDIPG component in copolymer.[26] In addition, the glass transition temperatures of the copolymers of $MePEG_{45}$-$PDIPG_{144}$ **10** and $PDIPG_{69}$-PEG_{45}-$PDIPG_{69}$ **16**, were found as -14 °C and -3 °C, respectively (data not shown).

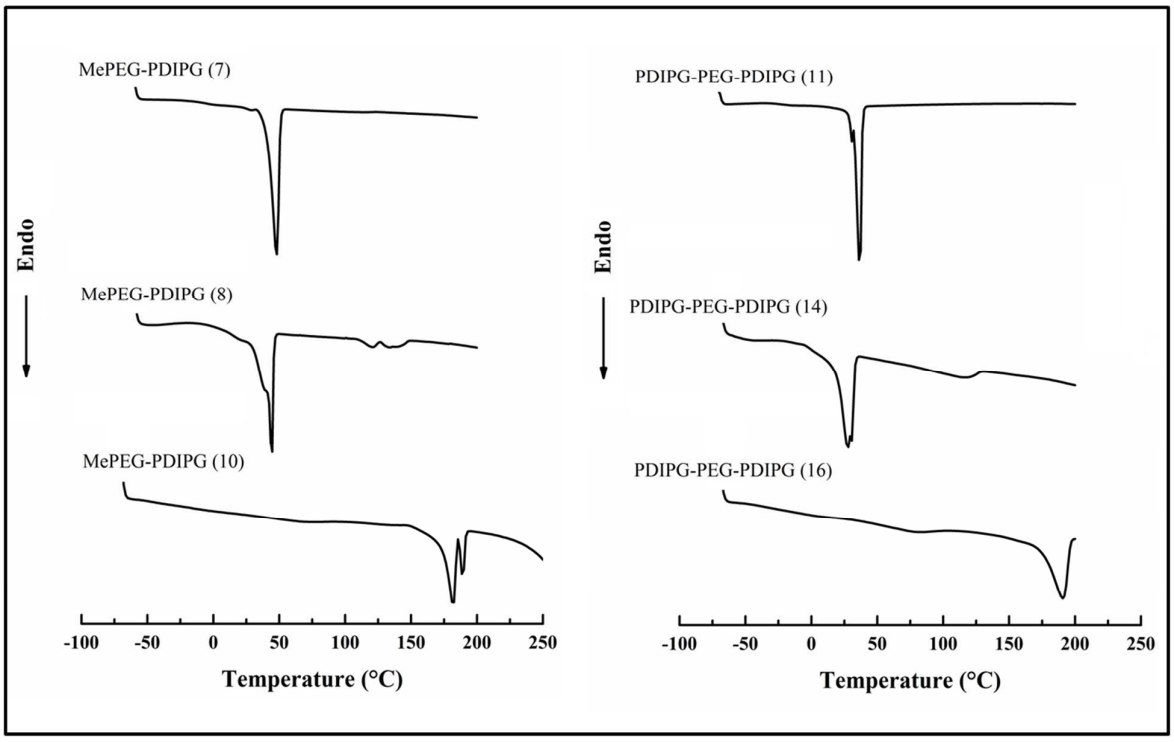


Figure 8 DSC thermograms of diblock and triblock copolymers. All samples were recorded in the second run.

Conclusion

In this work; synthesis and characterization of novel biomaterials, which could be an alternative to commonly studied polyester copolymers such as PEG based poly(lactide), poly(lactide-co-glycolide) and poly(ε-caprolactone) in literature, were performed. Then, usage of these biomaterials in controlled drug delivery systems were studied. Their characterizations were performed by thermal (DSC, TGA), spectroscopic (NMR, ATR-FTIR), chromatographic (GPC), and microscopic (SEM) methods. In the second stage, thermosensitive properties of those copolymers considering their physical changes were studied in detail. With increasing molecular weight of hydrophobic biodegradable block (PDIPG), the gel to sol transition occurred at lower concentrations. The results confirmed the relationship between gelation properties and copolymer structure, as well as presented more information for

these copolymers in drug delivery applications. The paclitaxel loaded gels of MePEG-PDIPG diblock and PDIPG-PEG-PDIPG triblock copolymers described here would be ideal for future use as an effective treatment for localized solid tumors.

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