Synthesis and Characterization of Biocompatible PEG-based Block Copolymers

by

Mei Li

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 5, 2013

Keywords: amphiphilic, ring opening polymerization, block copolymer, hydrogel

Approved by

Gisela Buschle-Diller, Chair, Professor of Polymer and Fiber Engineering
Xinyu Zhang, Assistant Professor of Polymer and Fiber Engineering
Maria Lujan Auad, Associate Professor of Polymer and Fiber Engineering

Abstract

A series of poly(ethylene glycol) (PEG)-based biocompatible tri-block copolymers, poly(ε-caprolactone-co-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-co-lactide) (PCLA-PEG-PCLA) and poly(D,L-lactide)-poly(ethylene glycol)-poly(D,L-lactide) (PLA-PEG-PLA) were synthesized via ring opening polymerization of D,L-lactide and ε-caprolactone, respectively, and reacted with PEG of different molecular weights. Aqueous solutions of these block copolymers have shown a sol-gel transition behavior at approximately body temperature (37°C). The tri-block copolymers were further coupled with sulfamethazine oligomer at both ends to create five-block copolymers that are more sensitive to external stimuli. The products were confirmed by Fourier transform infrared spectra (FT-IR), ^{1}H-NMR. Their porous morphology was observed by scanning electron microscopy (SEM). Microcrystalline cellulose was used in an attempt to improve the mechanical properties of block copolymers. The sol-gel transition behavior of gels made at different concentrations was observed and recorded within a range of different temperatures and pH values. The observed properties suggest that these block copolymers are potential candidates for use as biocompatible, and possibly injectable hydrogels in biomedical and pharmaceutical applications.

Acknowledgments

The author would like to express her heart-felt thanks to her advisor, Dr. Gisela Buschle-Diller, for giving generous support, guidance and valuable advice during her pursuit of Master's degree at Auburn University. Also the author is grateful to her committee members, Dr. Xinyu Zhang, and Dr. Maria L. Auad for their suggestions and help.

The author also wishes to appreciate the assistance of Mr. Steve Howard, Dr. Bo Yuan and Kai Wang, their worthy suggestions and help with testing. Also thanks to all faculty and staff in the Department of Polymer and Fiber Engineering, as well as the graduate students, for their genuine help during her study at Auburn University.

She would like to express her infinite thanks to her parents, for their encouragement and support to boost her confidence and impetus through out these years.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

Hydrogels are three-dimensional, cross-linked physical or chemical networks of polymers. They can absorb considerable amounts of water and still maintain their integrity. Hydrogels can be classified according to the interactions between components into physical and chemical gels. (Garcia et al., 2010) Some hydrogels, known as stimuli sensitive hydrogels (e.g. sensitivity towards temperature or pH changes, electrolyte content, electric fields, UV-light, etc.) have the ability to respond to changes in the environment. Hydrogels that are responsive to specific molecules, such as glucose or antigens can be used as biosensors as well as drug delivery systems. Light sensitive, pressure sensitive and electro-sensitive hydrogels also have the potential to be used in drug delivery and bio-separation. Temperature and pH sensitive hydrogels which can potentially be used as injectable hydrogels are probably the most typical classes of stimuli sensitive hydrogels studied in tissue engineering and for drug delivery systems. They are focused on being more effective in terms of localized drug delivery at the site of the actual need as well as in regard to decreased dosage requirement or/and frequency of dosage.

The temperature and pH sensitive hydrogels are intended to perform as either a sol or a gel in a specific range of temperature and pH. For those intended for use in tissue engineering and pharmaceutical applications of the human body, the target for the gel point should be around 37°C and pH=7.4. Thus, when injected for tissue repair, they are able to transform from sol phase into a hydrogel in order to initiate the release of drugs or act as

scaffold material for tissue repair.

The objective of this research is to synthesize a series of PEG-based temperature and pH sensitive hydrogels which could transition from sol into gel within the given parameters of body temperature and physiological pH. Tri-block copolymers, PLA-PEG-PLA and PCLA-PEG-PCLA were synthesized by ring opening polymerization reactions of D, L-lactide or/and ε-caprolactone. Tri-blocks were extended to five-block copolymers, OSM-PLA-PEG-PLA-OSM and OSM-PCLA-PEG-PCLA-OSM by coupling with sulfamethazine oligomer. Their properties were investigated and their behavior towards temperature and pH changes studied.

Chapter 2

Literature Review

2.1. Hydrogels

Hydrogels are three dimensional networks of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure. Hydrogels can be classified according to the interactions between their polymer components into physical and chemical gels. The networks of physical gels are held together by growing physically connected aggregates. The junction points may be molecular entanglements, ordered crystalline regions, phase-separated microdomains, and/or secondary forces including ionic, H-bonding, or hydrophobic forces (Figure 2.1). (Garcia et al., 2010)

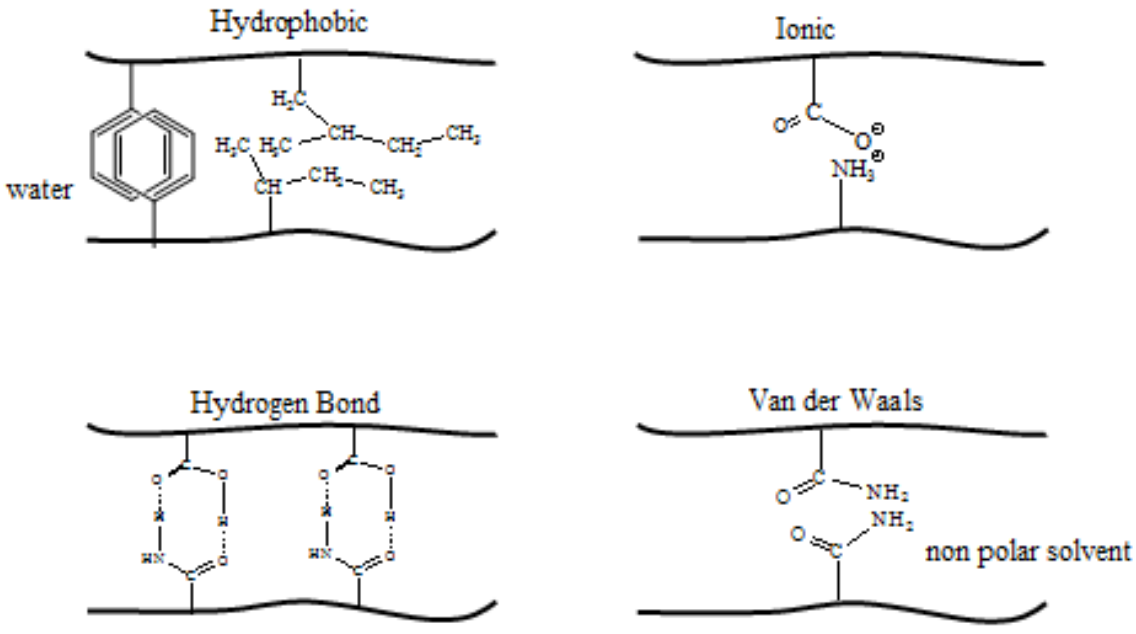


Figure 2.1. Interactions of specific functional groups in the formation of physical gels

according to García et al. (2010)

For hydrogels with predominantly hydrophobic interactions, polymers with hydrophobic segments can crosslink in aqueous environment via sol-gel transition. The gelation occurs when a gelator, which in this case is the hydrophobic segment, is coupled to a hydrophilic polymer segment within an amphiphilic polymer. These polymers are water soluble at low temperature. As the temperature rises, the hydrophobic segments aggregate to minimize the hydrophobic surface area, thus reducing the amount of water surrounding the hydrophobic segments and maximizing the solvent entropy. The gelation temperature depends on the concentration of polymer, the length of the hydrophobic segment, the chemical structure of the polymer and other parameters.

Chemically bonded hydrogels are very common and could be obtained by chemical crosslinking of hydrophilic molecules to the network. They usually contain regions of high crosslink density with low water swelling which is called “clusters” which are dispersed in the regions of low crosslink density with high swelling capabilities.

Polymers with very different chemical structures to the above discussed two types, such as branched polymers, graft polymers, dendrimers, dendronized polymers, block copolymers, and polymer stars have also been used for the preparation of biodegradable hydrogels for drug delivery system (see Figure 2.2). (Duncan, 2003)

Hydrogels can be prepared with natural and synthetic polymers. In general, natural polymers are inherent biocompatibility and biodegradability that support cellular activities. However, they usually do not provide enough mechanical properties. While synthetic hydrogels, present well defined structures which can be manipulated to obtain biodegradability and a specific functionality.

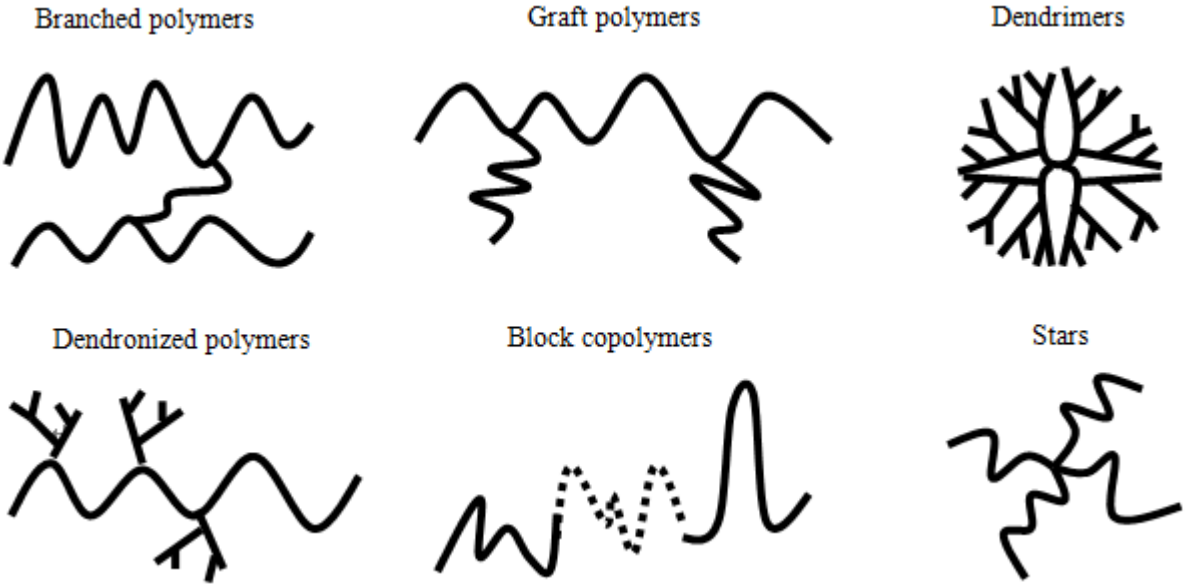


Figure 2.2. Polymers structures reported for the synthesis of hydrogels for drug delivery (Duncan, 2003)

Hydrogels can resemble natural living tissue more than any other class of synthetic biomaterials due to the high water content. Further more the high water content of materials contributes to their biocompatibility. In this regard, the phase change polymers which may trigger drug release in response to external stimuli are most investigated.

Stimuli sensitive hydrogels have been used extensively in the development of drug delivery systems. Hydrogels providing such “sensor” properties can undergo reversible volume phase transitions or sol-gel phase transitions upon changes under environmental conditions. (Park, et al., 1999) Many physical and chemical stimuli have been applied to induce responses of hydrogel systems. Physical stimuli include temperature, electric fields, light, pressure, sound and magnetic fields, and chemical or biochemical stimuli include pH, ions and specific molecular recognition events. (Hoffman, 1997; Bae, 1997) Stimuli sensitive hydrogels are ideal candidates for developing self-regulated drug delivery systems.

2.2. Biocompatible and biodegradable hydrogels

Applications of various types of hydrogels are found in cell culture, tissue engineering, drug delivery systems, medical sensors and many more. Biodegradable polymers, and especially aliphatic polyesters, such as polylactide (PLA), polyglycolide (PGA), and poly (ε-caprolactone) (PCL), have been investigated worldwide as biomaterials because of their biocompatibility and degradability under physiological conditions. (Li and Vert, 1999; Dunn, 1995; Li et al., 1999) Biocompatibility, biodegradability and safety of the polymer as well as all its decomposition products are extremely important in addition to its physicochemical properties.

Since both PLA, PCL and PEG are highly biocompatible and bioresorbable, the formation of block copolymers with hydrophilic and hydrophobic segments could open routes to more tailored applications. A number of PLA-PEG block copolymers were reported to provide materials as temporary devices for clinical and pharmaceutical purposes. For example, Zhu et al. first prepared an A-B type di-block copolymer from poly (D,L-lactide)-polyethylene glycol (PDLLA- PEG) for use as drug carrier. (Zhu et al., 1986, 1989, 1990) Zhang et al. created PLA-PEG di-block copolymers that had the capability to release loaded drugs upon exposure to ultrasound. (Zhang et al., 2009) Cohn and Younes synthesized a family of PLA-PEG block copolymers to provide biodegradable elastomers for cardiovascular implants. (Cohn and Younes, 1988) A series of amphiphilic A-B-A and B-A-B tri-block polymers from PLA and PEG were synthesized by He et al. (2006). The goal of this study was to incorporate hydrophobic drugs in form of nanomicellar particles. The researchers found that there was a significant difference of drug release kinetics depending on the block sequence.

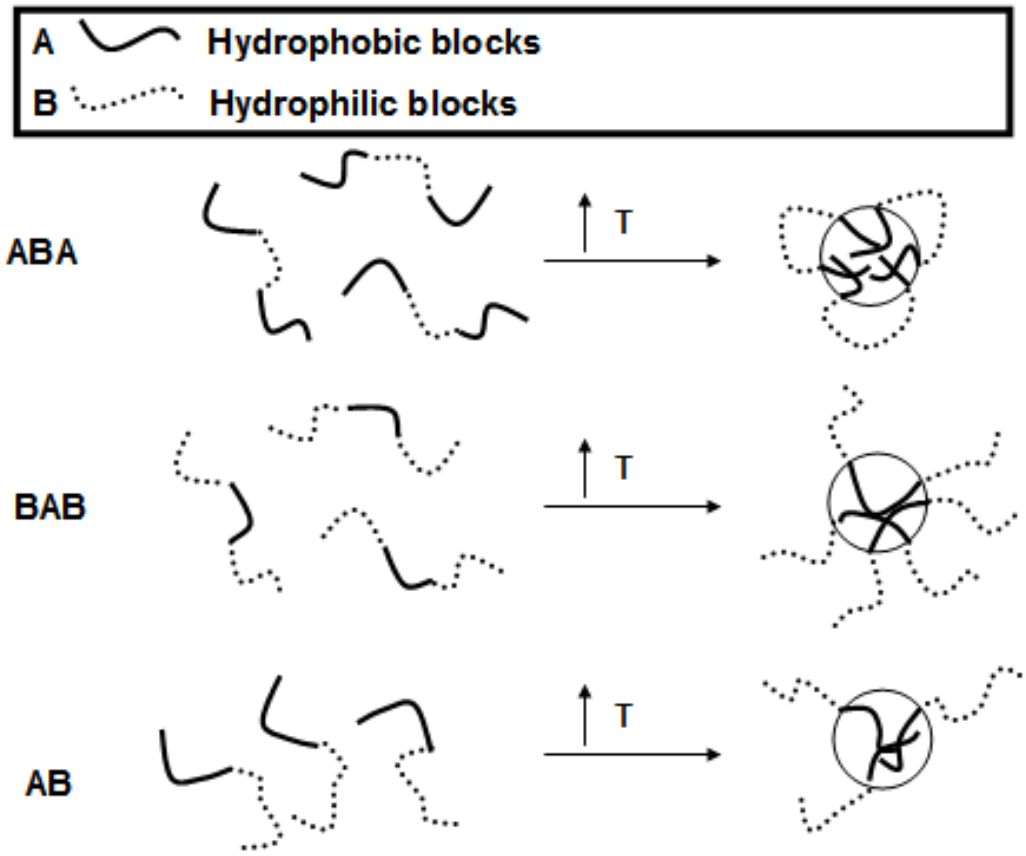


Figure 2.3. Typical polymer structures and schematic of the micelles in aqueous medium. according to Fujiwara et al.

Kim et al. demonstrated the preparation of injectable micro-particles for drug delivery systems from a B-A-B type tri-block copolymer made from polyethylene-poly(L-lactide) -polyethylene glycol (PEG-PLLA-PEG). (Jeong et al., 1997) The aqueous suspensions containing these micro-particles are characterized by the temperature-dependent sol-gel transition occurring around body temperature (37°C). Mukose et al. reported the novel temperature sensitive formation of a similar hydrogel from an enantiomeric mixture of an A-B-A type tri-block copolymer, synthesized based on poly(L-lactide)-polyethylene glycol-poly(L-lactide) (PLLA-PEG-PLLA) and poly(D-lactide)-polyethylene glycol - poly(L-lactide) (PDLA-PEG-PDLA). The driving force for gel formation in this case was attributed to stereo-complexation of PLLA and PDLA segments. (Mukose et al., 2004)

2.3. Temperature and pH sensitive hydrogels

Much attention has been paid on injectable hydrogels based on various biopolymers due to their minimal invasiveness into the physiological system. (Wang et al., 2009; Tan et al., 2012; Fu et al., 2012) Usually, they are applied as viscous aqueous solutions before administration, and after injection they rapidly form gels under physiological conditions. Temperature and/or pH sensitive hydrogels showing a sol-to-gel transition with changing the temperature and/or pH are the most popular candidates for injectable hydrogels.

In particular, block copolymers hydrogels composed of hydrophilic poly (ethylene glycol) (PEG) and hydrophobic biodegradable polyesters, such as poly(L-lactic acid) (PLLA), poly (D,L-lactic acid) (PDLLA), and poly (D,L-lactic acid-co-glycolic acid) (PLGA), have been studied as controlled drug release carriers. (Chen and Hoffman, 1995; Jeong et al., 1997; Zentner et al., 2001) Kim et al. reported research on temperature sensitive phase transition of di- and tri-block copolymers composed of PEG and various aliphatic polyesters. (Jeong et al., 1999; Jeong et, al., 1999; Zhong et al., 2002) However, these hydrogels show several unresolved shortcomings that limit their potential applications in drug delivery system. When temperature sensitive hydrogels are injected into human body via a syringe, the body warmth tends to create the sol inside the syringe which blocks the injection. In this case, pH becomes another important factor on the synthesis of injectable hydrogels.

All pH sensitive polymers contain pendant acidic (e.g. carboxylic and sulfonic acids) or basic (e.g. ammonium salts) groups that either accept or release protons in response to changes in environmental pH. The polymers containing a large number of ionizable groups are known as polyelectrolytes. Figure 2.4 shows the structure of examples of anionic and cationic polyelectrolytes and their pH dependent ionization. Poly (acrylic acid) (PAA) becomes ionized at a high pH, while poly (N,N'- diethylaminoethyl methacrylate) (PDEAEM)

becomes ionized at low pH. As shown in Figure 2.4, cationic polyelectrolytes, for example PDEAEM, dissolve more or swell more if crosslinked at low pH due to ionization. On the other hand, polyanions, such as PAA, dissolve more at high pH. (Qiu and Park, 2001)

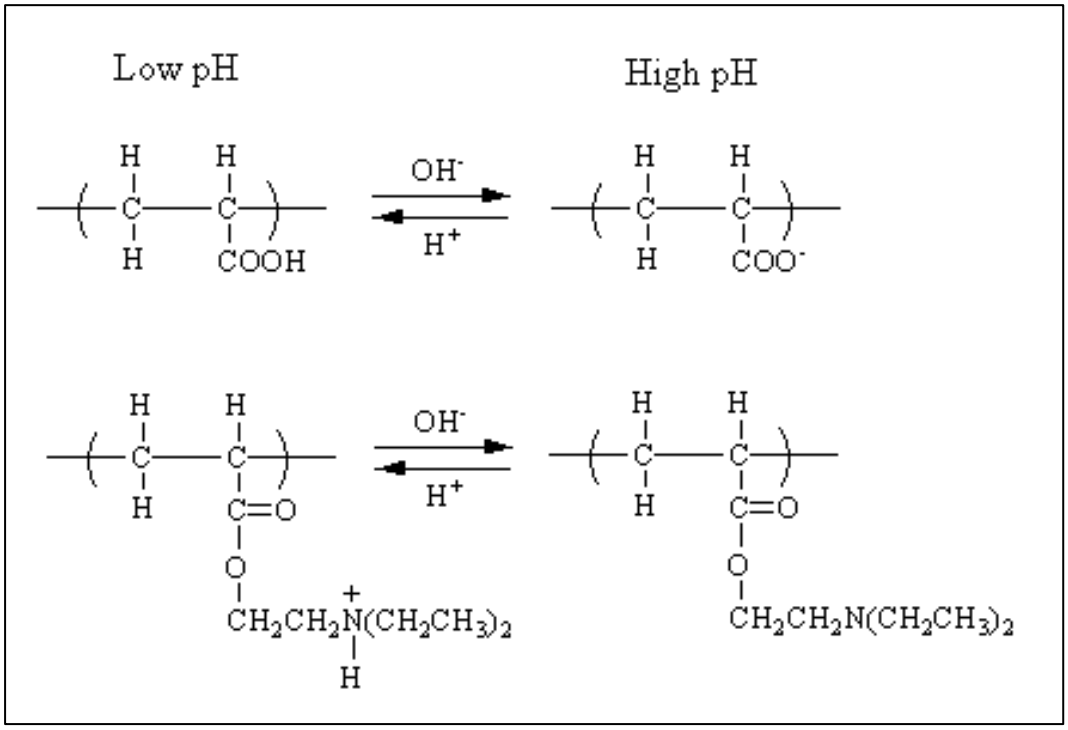


Figure 2.4. pH dependent ionization of polyelectrolytes. Poly (acrylic acid) (top) and poly (N,N'- diethylaminoethyl methacrylate) (bottom)

Hydrogels made of polyanions (e.g. PAA) crosslinked with azoaromatic crosslinkers were developed for colon-specific drug delivery. Swelling of such hydrogels in the stomach is minimal and thus the drug release is minimal. The extent of swelling increases as the hydrogel passes down the intestinal tract due to pH increase which leads to ionization of the carboxylic groups. However, the azoaromatic cross-links of the hydrogels can only in the colon be degraded by azoreductase produced by the microbial flora of the colon (Ghandehari et al., 1997), as shown in Figure 2.5.

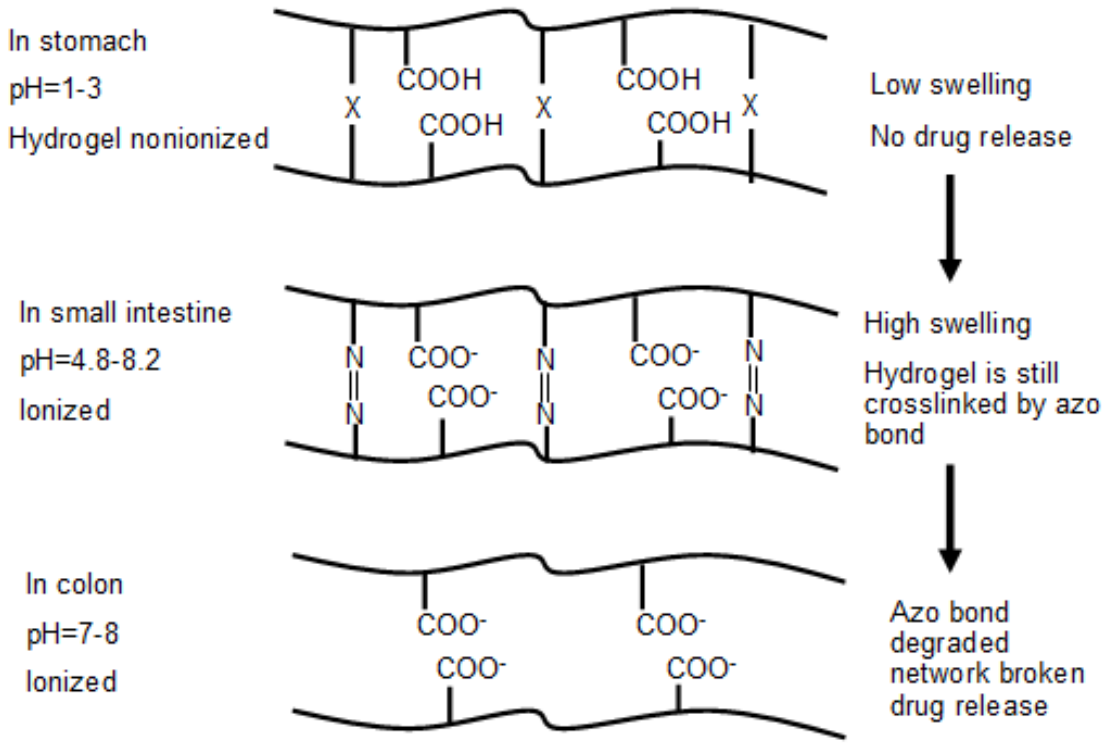


Figure 2.5. Schematic illustration of oral colon-specific drug delivery using biodegradable and pH sensitive hydrogels. The azoaromatic moieties in the cross-links are designated by –N=N– according to Ghandehari et al. (1997)

Combined with the influence of temperature, the pH value acts as a stimulus and controls the range of gel formation by simply incorporating ionizable and hydrophobic (inverse thermosensitive) fuctional groups. A pH sensitive segment that at high pH is mainly present in its ionized state acts as the hydrophilic block in the block copolymer could be transformed to a less ionized state by a change in pH. The copolymer, unable to form gels in high pH values, will gel at a lower pH values, such as at the physiological pH 7.4. Here, the influence and amount of pH sensitive (ionized) segments in the di-block polymer decreases, and the non-ionized segments can act as a hydrophobic block enabling the formation of a gel. For example, acrylic acid, is incorporated in a thermo-reversible polymer, the LCST (lower critical solution temperature) of the hydrogel depends on the ionization of the pendant

carboxyl groups, such as the pH of the medium. As the pH of the medium increases above the pK_a of the carboxyl groups of polyanions, LCST shifts to higher temperature due to the increased hydrophilicity and charge repulsion.

Chapter 3

Materials and Methods

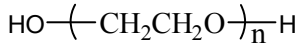
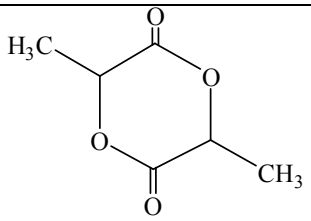
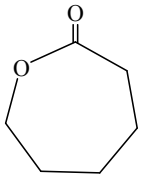
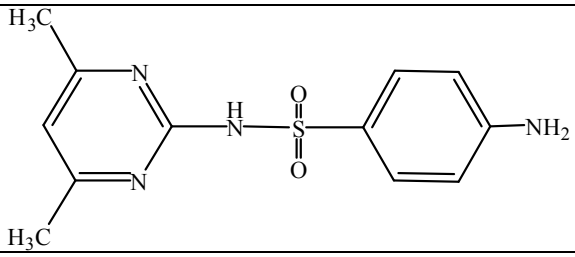
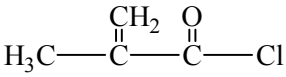
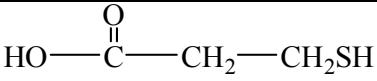
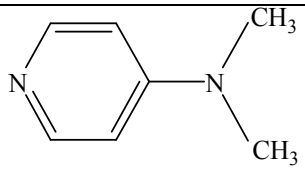
3.1. Synthesis of PEG-based block copolymers

3.1.1. Materials used

Poly (ethylene glycol) (PEG) with molecular weights of 1,500 and 10,000 was obtained from Alfa Aesar, D,L-lactide (LA, 99%) and ε-caprolactone (ε-CL, 99%) from Alfa Aesar. Diethyl ether (99+%), dichloromethane (CH_2Cl_2, 99.5+%), stannous octoate ($Sn(Oct)_2$), NaOH, acetone, 2,2-azobisisobutyronitrile (AIBN), Sulfamethazine (SM, 99%), Methacryloyl Chloride (99%), tetrahydrofuran (THF, 99%) all from Alfa Aesar, N,N-Dimethylformamide (DMF, 99+%) from Fisher chemicals, 3-Mercapto propionic acid (MPA, 99+%), N,N-Dicyclodexycarbdiiomide (DCC), 4-(dimethyl-amino) pyridine (DMAP) from Sigma Aldrich, were used as received. A sample of microcrystalline cellulose was obtained from the U.S. Department of Agriculture, Forest Products Laboratory, Madison, WI.

The chemical structures of some monomers and solvents used to synthesize the PEG-based block copolymers are listed in Table 1.

Table 1. The chemical structures of the monomers and solvents

Chemical name	Chemical structure
Poly(ethylene glycol) (PEG), Mw=1500, 10000	
D,L-Lactide,	
ε-caprolactone (ε-CL)	
Sulfamethazine (SM)	
Methacryloyl Chloride	
3-Mercaptopropionic acid (MPA)	
4-(dimethyl-amino) pyridine (DMAP)	

3.1.2. Synthesis of PLA-PEG-PLA three-block copolymers

The synthesis of PLA-PEG-PLA was performed through a ring-opening copolymerization with PEG as an initiator. $Sn(Oct)_2$ was used as the catalyst. The detailed

process was as follows: A 250 mL three-neck round-bottom flask was heated in an oven to remove the moisture. After cooling to room temperature, PEG (MW 1,500) was added to remove moisture in an oven at 105°C for 4 h. D.L-Lactide was dried separately at 40°C for 2 h. $Sn(Oct)_2$, D,L-Lactide were added into the flask with PEG under argon at room temperature. The temperature was raised slowly to 130°C under argon. The reaction was carried out for 24 h. Figure 4.1 shows the reaction scheme. After the reaction was completed, the reaction products were cooled to room temperature and dissolved in dichloromethane. The solution was precipitated in excess diethyl ether and filtered by vacuum filtration. Finally, the product was dried in an oven at 40°C for 48 h. The yield of the block copolymer was 40% on average. The reaction was repeated with PEG (Mw 10,000). The yield was 68% on average.

3.1.3. Synthesis of PCLA-PEG-PCLA three-block copolymers.

The synthesis procedure to produce PCLA-PEG-PCLA was similar with the one of PLA-PEG-PLA described above. ε-caprolactone was dried with 3A molecular sieve under stirring over night and added to the flask containing PEG (MW 1,500 or 10,000), D,L-Lactide and $Sn(Oct)_2$. The temperature was raised slowly to 130°C under argon. The reaction was carried out for 24 h (see Figure 4.5). After the reaction was completed, the reactants were cooled to room temperature and dissolved in dichloromethane. Then the solution was precipitated in excess diethyl ether and filtered by vacuum filtration. The product was dried in oven at 40°C for 48 h. The yield of the block copolymer was 65% on average.

3.2. Incorporation of cellulose microcrystals into D,L-Lactide and ε-caprolactone

copolymers

Cellulose microcrystals were first dried in oven at 40°C for 2 h and then dispersed by stirring in dried ε-caprolactone overnight. D,L-Lactide, $Sn(Oct)_2$ together with the dispersed solution were mixed in a pre-dried three-neck round-bottom flask. The temperature was raised slowly to 130°C under argon. The reaction was carried out for 24 h. After the reaction was completed, the reactants were cooled to room temperature and dissolved in dichloromethane. The solution was precipitated in excess diethyl ether and filtered by vacuum filtration. The product was dried in oven at 40°C for 48 h. The yield of the block copolymer containing microcrystalline cellulose was 19.2%.

3.3. Synthesis of five-block copolymers

3.3.1. Synthetic procedure of sulfamethazine oligomer

Sulfamethazine monomer (SMM) was synthesized with sulfamethazine (SM) and methacryloyl chloride. SM (0.1 mol) and sodium hydroxide (0.1 mol) were dissolved in aqueous acetone (100 mL, 1:1 v/v). Then methacryloyl chloride (0.12 mol) was dropped slowly into the solution under stirring in an ice bath. The synthesized SMM precipitated from the solution. Finally, SMM was separated by filtration, washed with distilled water and dried at room temperature for 48 h. Figure 4.10 shows the reaction scheme.

The sulfamethazine oligomer (OSM) with a carboxyl acid end-group was synthesized by conventional radical polymerization with SMM, 2,2'-azobisisobutyronitrile (AIBN) and 3-mercaptopropionic acid (MPA). SMM (0.09 mol) was dissolved in anhydrous DMF (150 mL), and AIBN (0.009 mol) and MPA (0.009 mol)

were added under argon atmosphere. The mole ratio of SMM/AIBN/MPA was 100/10/10. The temperature was raised slowly to 80°C and the reaction was carried out for 48 h. When the reaction was finished, DMF was vaporized in a rotary evaporator, and the dried reactants were dissolved again in THF. After the reactant solutions were slowly added to excess diethyl ether, the OSM could be precipitated from the solution. The product was filtered and dried at 40°C for 48 h. The average OSM yield was 83.4%. In Fig. 4.11 the reaction scheme is presented.

3.3.2. Coupling reaction of sulfamethazine oligomer with PLA-PEG-PLA and PCLA-PEG-PCLA three-block copolymers

The three-block copolymers PLA-PEG-PLA and PCLA-PEG-PCLA, respectively, and OSM were coupled using dicyclohexyl carbomide (DCC) as the coupling reagent and 4-(dimethyl amino) pyridine (DMAP) as the catalyst. First, PLA-PEG-PLA or PCLA-PEG-PCLA block copolymers were weighted into a pre-dried three-neck round bottom flask and dried in oven at 80°C for 2 h. (Considered to the melting point and the small amount of reactant, the temperature of drying was not carried out too high, e.g. above 100°C) OSM was dried in oven at 80°C for 1 h separately. The reactants were cooled to room temperature and OSM was added into the flask under argon atmosphere. Anhydrous methylene chloride solution (60 mL) containing DCC and DMAP was added to the flask. The feed ratio of PLA-PEG-PLA (or PCLA-PEG-PCLA), OSM, DCC and DMAP was 1 M: 2.4 M: 2.8 M: 0.28 M. The reaction was carried out under argon atmosphere at room temperature for 48 h. At the end of the coupling reaction, residual DCC was converted into dicyclohexylurea (DCU) by the addition of two or three drops of water, after which the DCU was precipitated. The precipitated DCU and residual

OSM were removed by vacuum filtration. The final product was precipitated by pouring the reactant mixture in excess diethyl ether. The final product was dried in an oven at 40°C for over 48 h. A yield of 22.6% was obtained. Figure 4.13 shows the reaction for PLA-PEG-PLA as the tri-block and Figure 4.14 for PCLA-PEG-PCLA.

3.4. Characterization of tri-block and five-block copolymers

3.4.1. FT-IR spectroscopic analysis

Fourier transform infrared (FT-IR) spectroscopy was conducted on a Thermo Scientific Nicolet 6700 with the wave number range set to 4000 to 400 cm^{-1} over 32 scan with a resolution of 4 cm^{-1}. FT-IR spectra were obtained for sulfamethazine (SM), sulfamethazine monomer (SMM), sulfamethazine oligomer (OSM); as well as for all tri-block and five-block copolymers: PLA-PEG-PLA, OSM-PLA-PEG-PLA-OSM, PCLA-PEG-PCLA, and OSM-PCLA-PEG-PCLA-OSM.

3.4.2. ^{1}H-NMR spectroscopic analysis

Nuclear magnetic resonance spectra (^{1}H-NMR) studies were carried out on a Bruker 400MHz instrument. Usually, parameters for the proton spectra were a 15 s pulse delay, an acquisition time of 3 s, and a spectral width of 20.68 ppm. For all samples, 16 scans were obtained. All PEG-based block polymer samples were dissolved in dimethyl sulfoxide-d6 (DMSO-d6, Acros Organic, 99.9 atom% D).

3.4.3. Scanning electron microscopic (SEM) analysis

An EMS 550X Sputter Coating Device was used to coat PEG-based block copolymers with gold. A Zeiss Evo 50VP Scanning Electron Microscope (SEM) was used to observe the

micro structure of the samples at 20 kV scanning voltage.

Chapter 4

Results and Discussion

4.1. Tri-block co-polymers PLA-PEG-PLA

A series of tri-block polymers of the type A-B-A were synthesized and characterized. Tri-block copolymers of poly(D,L-lactide)-poly(ethylene glycol)-poly(D,L-lactide) (PLA-PEG-PLA) were formed according to the reaction scheme shown in Figure 4.1.

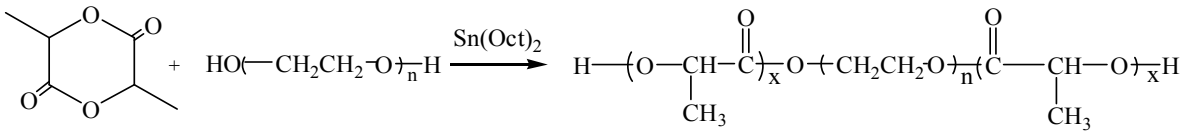


Figure 4.1. Formation of PLA-PEG-PLA tri-block copolymers.

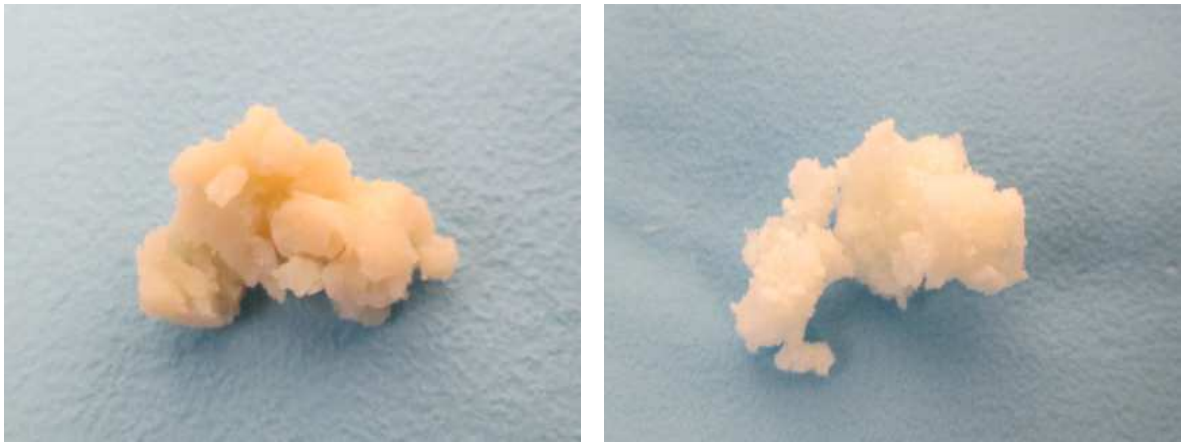


Figure 4.2. Appearance of PLA-PEG-PLA with PEG MW of 1,500 (left) and 10,000 (right)

After precipitation from diethyl ether, white to yellowish products were obtained. PLA-PEG-PLA with PEG of MW 10,000 appeared lighter in color and its water solubility was better than PLA-PEG-PLA with PEG with MW of 1,500. Figure 4.2 shows that the products had a somewhat flaky appearance with overall bigger pieces formed with PEG 1,500 (left-hand side). The yield of the product with the longer PEG segment (approx. 68%) was clearly higher than with the shorter PEG segment (40% on the average).

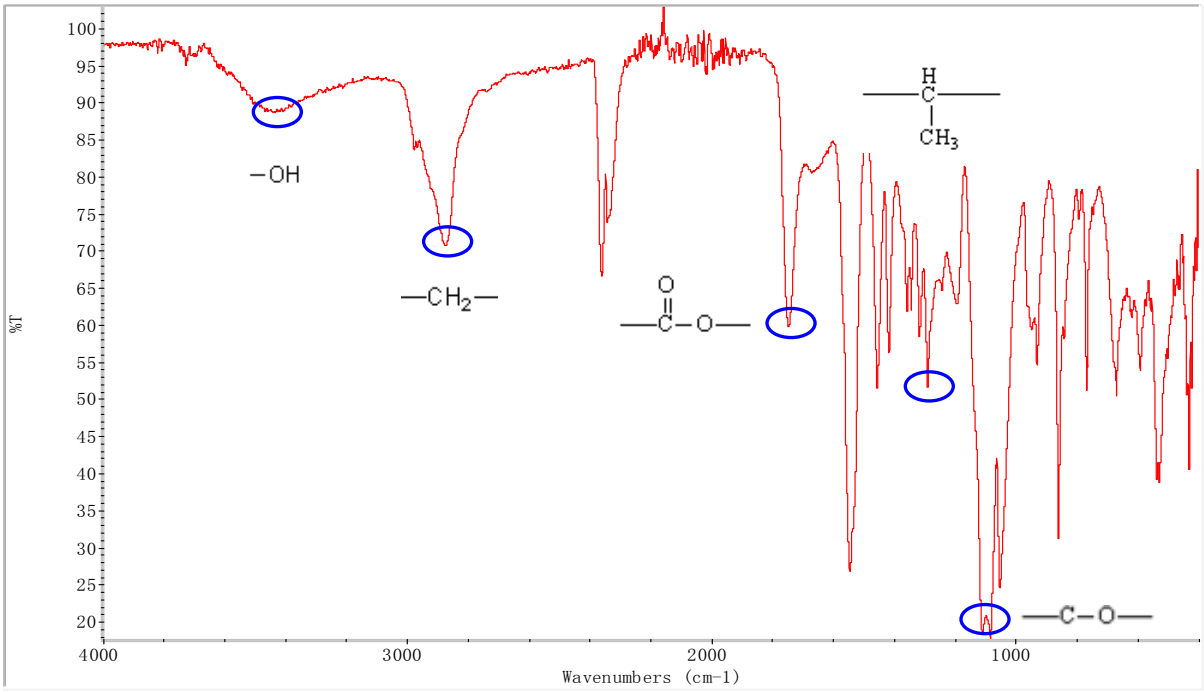


Figure 4.3. FTIR spectrum of PLA-PEG-PLA

Both tri-block PLA-PEG-PLA copolymer products were characterized by FT-IR spectroscopy. As can be seen in Figure 4.3, a peak at around 3430 cm^{-1} appeared that indicates the existence of –OH groups. In addition, the peak at around 1780 cm^{-1} points to carboxylic acid group absorption. The absorption peaks at around 2870 cm^{-1} confirm saturated C-H stretching vibration, and at 1280 cm^{-1} and 1090 cm^{-1} symmetric bending

vibration of >CH-CH_3 and C-O stretching vibration, respectively. The position of peaks of functional groups are similar to those reported by Hao et al., 2008.

The chemical composition was further confirmed by ^{1}H-NMR studies. Figure 4.4 shows a ^{1}H-NMR spectrum of PLA-PEG-PLA in DMSO-d6.

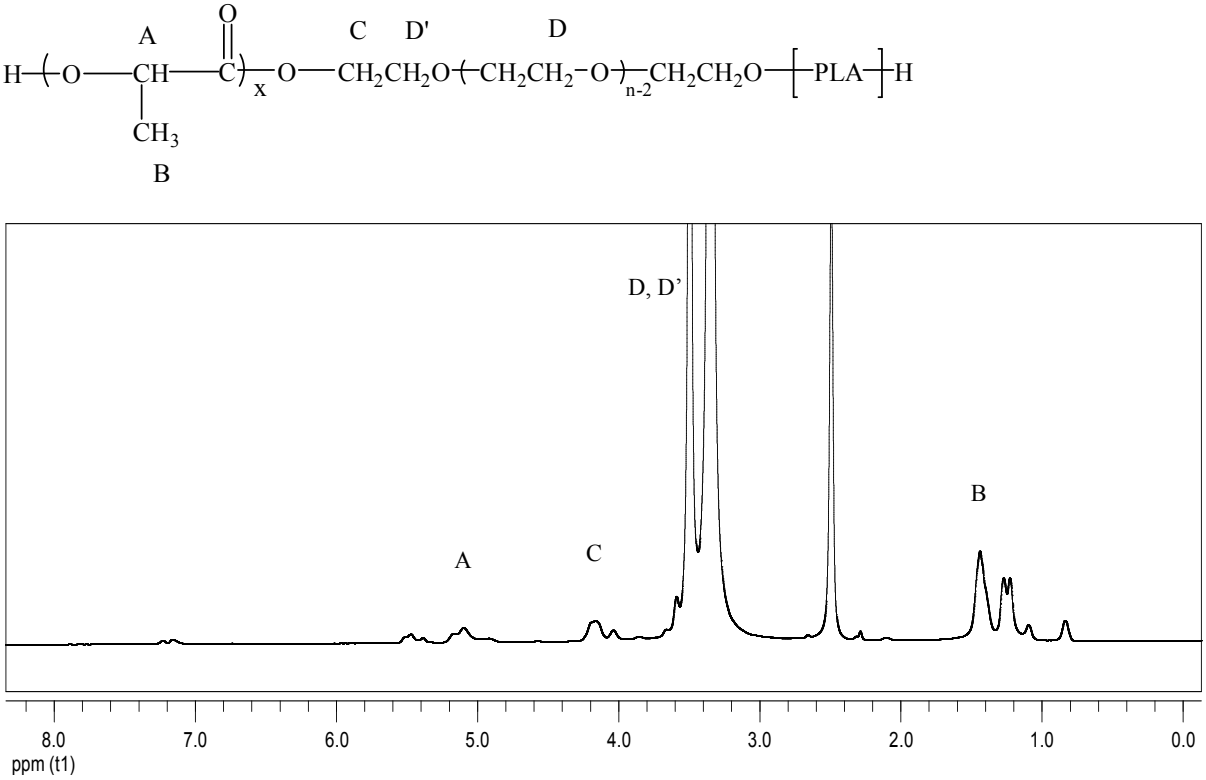


Figure 4.4. Structural analysis of PLA-PEG-PLA block polymer by ^{1}H-NMR spectrum in DMSO-d6.

The presence of methine (CH) and methyl (CH_3) protons in PLA was observed around 5.1 ppm (A) and 1.42 ppm (B). The methylene protons (CH_2) group of PEG was around 3.45 ppm (D,D'). The peaks at 4.18 ppm represent the methylene protons in (CH_2) in the PEG segments bonded to the PLA segments. The chemical shift of the peaks are similar to those reported by Shim et al., 2006.

The chemical shifts of PLA-PEG-PLA with MW of 1,500 and 10,000 are the same,

yet the peak D,D' (-CH_2CH_2- of PEG) of PLA-PEG-PLA with PEG (MW 10,000) was higher than that of PEG with MW 1,500 which means the repeat unit of –CH_2CH_2– in PEG-10,000 is longer than in PEG-1,500.

4.2. Tri-block copolymers PCLA-PEG-PLCA

A second series of tri-block copolymers was prepared based on PCLA-PEG-PCLA (poly(ε-caprolactone-co-D,L-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-co-D,L-lactide)). The reaction scheme for this reaction is shown in Fig. 5.5.

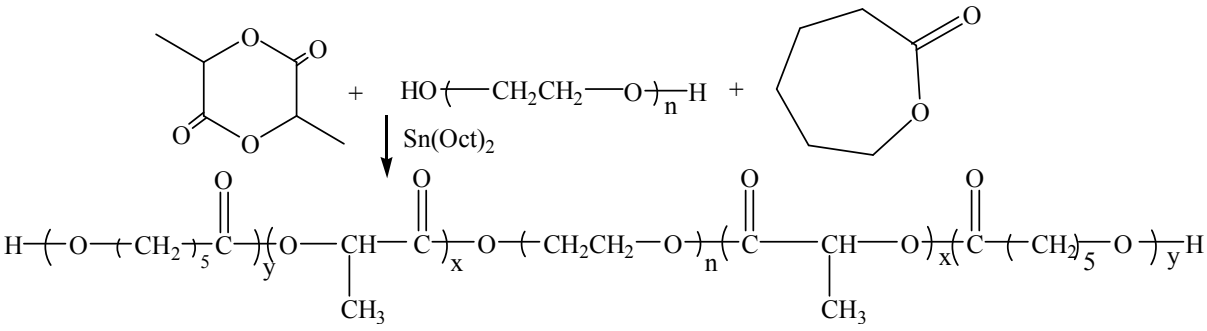


Figure 4.5. Reaction scheme for the synthesis of PCLA-PEG-PCLA tri-block copolymer.

The copolymers PCLA-PEG-PCLA with PEG of MW 1,500 and 10,000 were easier to synthesize than PLA-PEG-PLA and the yield was higher. (65% on average) Meanwhile PCLA-PEG-PCLA with the shorter PEG segment was more viscous than PLA-PEG-PLA with the longer PEG block. Both products were slightly yellowish in color (Figure 4.6).

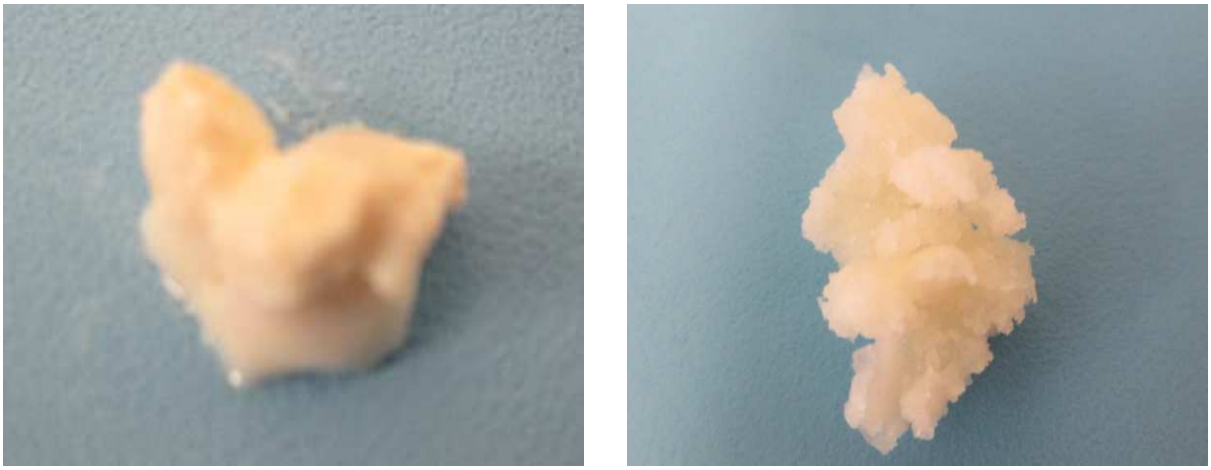


Figure 4.6. Appearance of PCLA-PEG-PCLA with PEG MW 1,500 (left) and 10,000 (right)

The tri-block PCLA-PEG-PCLA with the shorter PEG segment was tacky while the one with longer PEG segments was clearly flakier. FT-IR analysis of the products was performed and the results are shown in Figure 4.7.

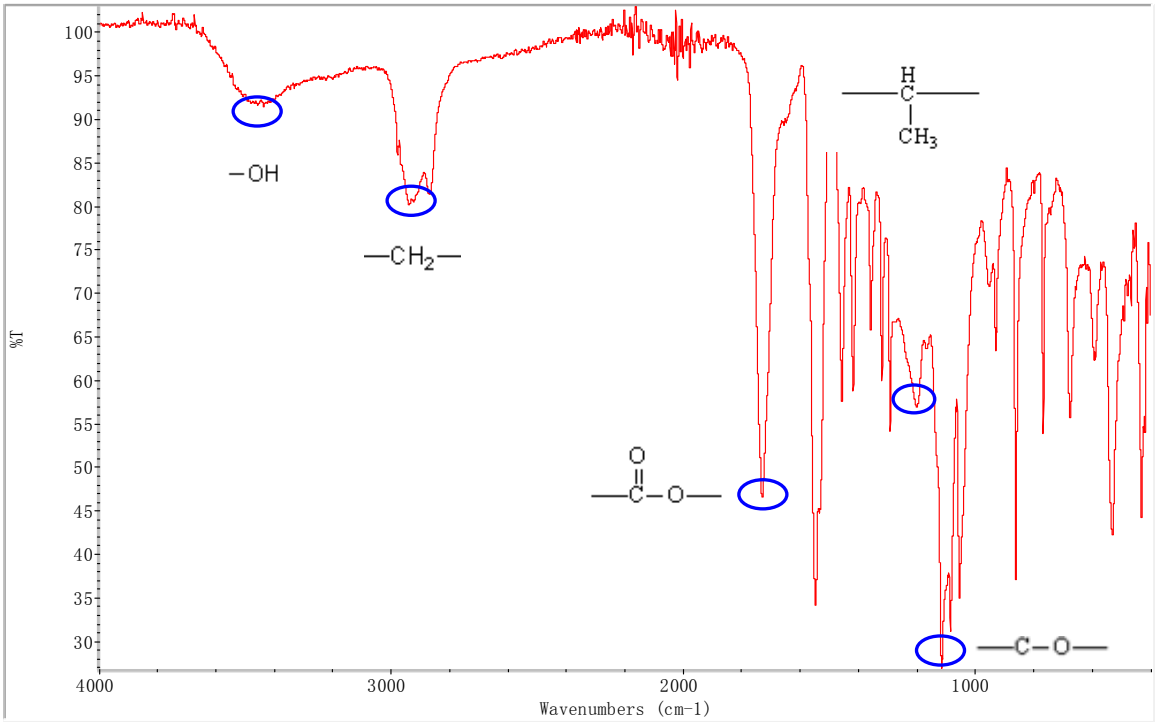
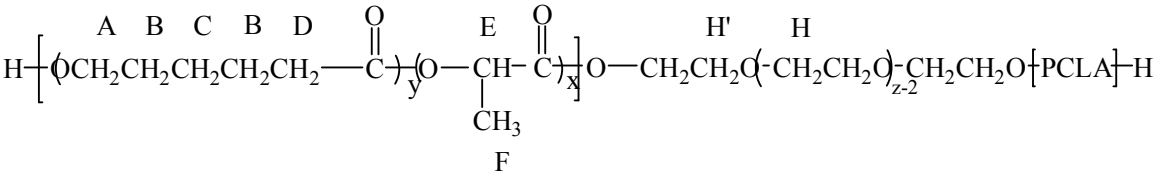


Figure 4.7. FTIR spectrum of PCLA-PEG-PCLA

The absorption peak at around 3490 cm^{-1} shows that –OH groups are present, and the peak at around 1680 cm^{-1} reveals the carboxylic acid group absorption (Figure 4.7). The absorption peaks at around 2900 cm^{-1} indicate the existence of saturated C-H stretching vibration, and at 1190 cm^{-1} and 1100cm^{-1} symmetric bending vibration of >CH-CH_3 and C-O stretching vibration, respectively. Not surprisingly the spectrum is very similar to that of PLA-PEG-PLA (Figure 4.3). (Hao et al., 2008)

The ^{1}H-NMR spectra of PCLA-PEG-PCLA is shown in Figure 4.8. All proton signals of PCLA-PEG-PCLA are labeled. The proton peaks, the methylene proton of the oxyethylene unit (H, H') appear around 3.5 ppm. The methylene proton of the lactide (LA) unit (F) and the methylene proton (on the neighboring carbonyl group) of CL unit (D) are around 5.38 ppm and 2.27 ppm, respectively. (Shim et al., 2006)



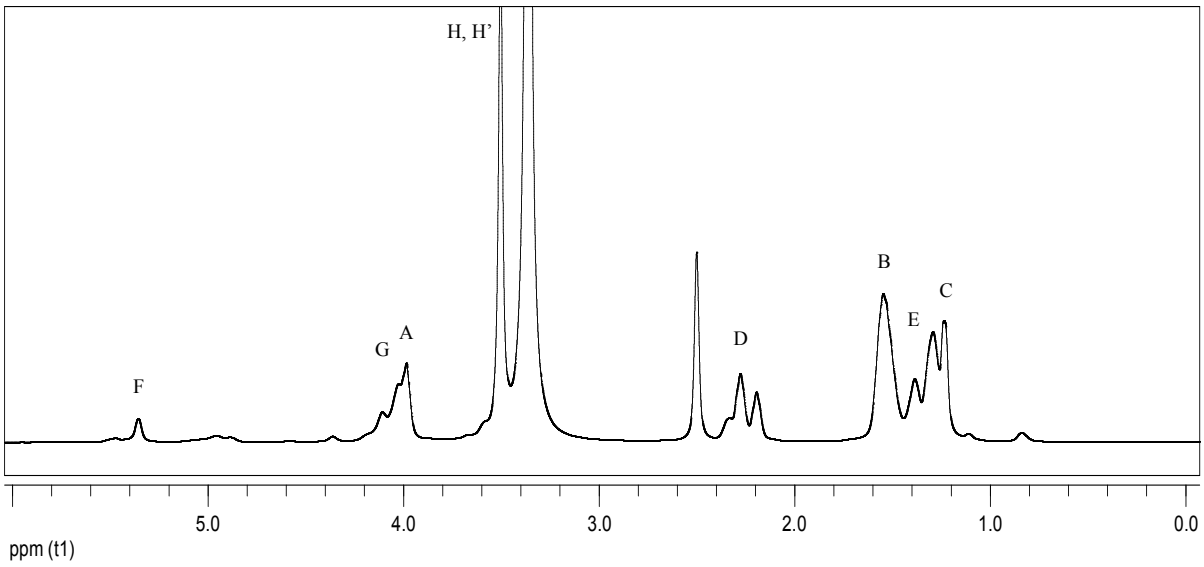


Figure 4.8. ^{1}H-NMR spectrum of PCLA-PEG-PCLA block polymer in DMSO-d6

Also, cellulose microcrystals (Figure 4.9 (left)) were used to react in the ring opening polymerization as an attempt to introduce more available hydroxyl groups, and obtain higher reaction efficiency in the ring opening polymerization of D,L-lactide and ε-caprolactone. The product of the incorporation of cellulose microcrystals into D,L-lactide and ε-caprolactone copolymers (Figure 4.9 (right)) turned out to be more rigid and dried than PLA-PEG-PLA and PCLA-PEG-PCLA.

Figure 4.9. Appearance of cellulose microcrystals (left) and incorporation of cellulose microcrystals into D,L-lactide and ε-caprolactone copolymers (right)

4.3. Five-block copolymers

Tri-block copolymers PLA-PEG-PLA and PCLA-PEG-PCLA were further coupled to form five-block copolymers with a sulfamethazine oligomer (OSM) segment. The formation of the OSM segment from the sulfamethazine monomer (SMM) is shown in Figures 4.10 and 4.11. The OSM segment was obtained in good yield (83.4%) FT-IR spectroscopy was performed on the starting materials, SMM and OSM before coupling to the tri-block copolymers was attempted. The FT-IR spectra are shown in Figure 4.12.

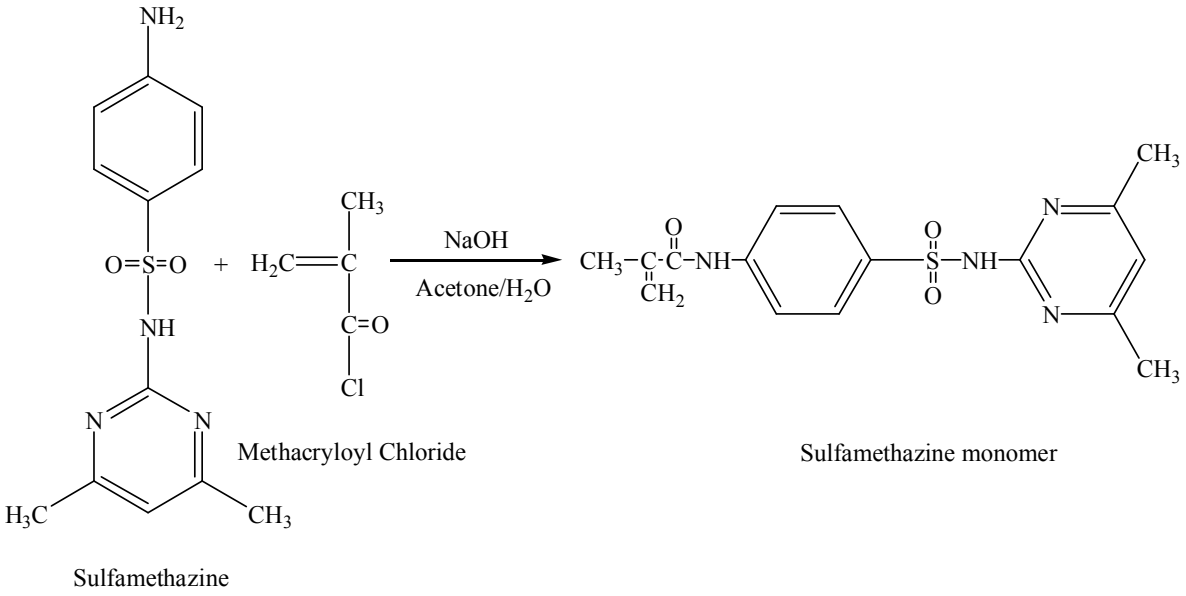


Figure 4.10. Formation of the sulfamethazine (SMM) monomer.

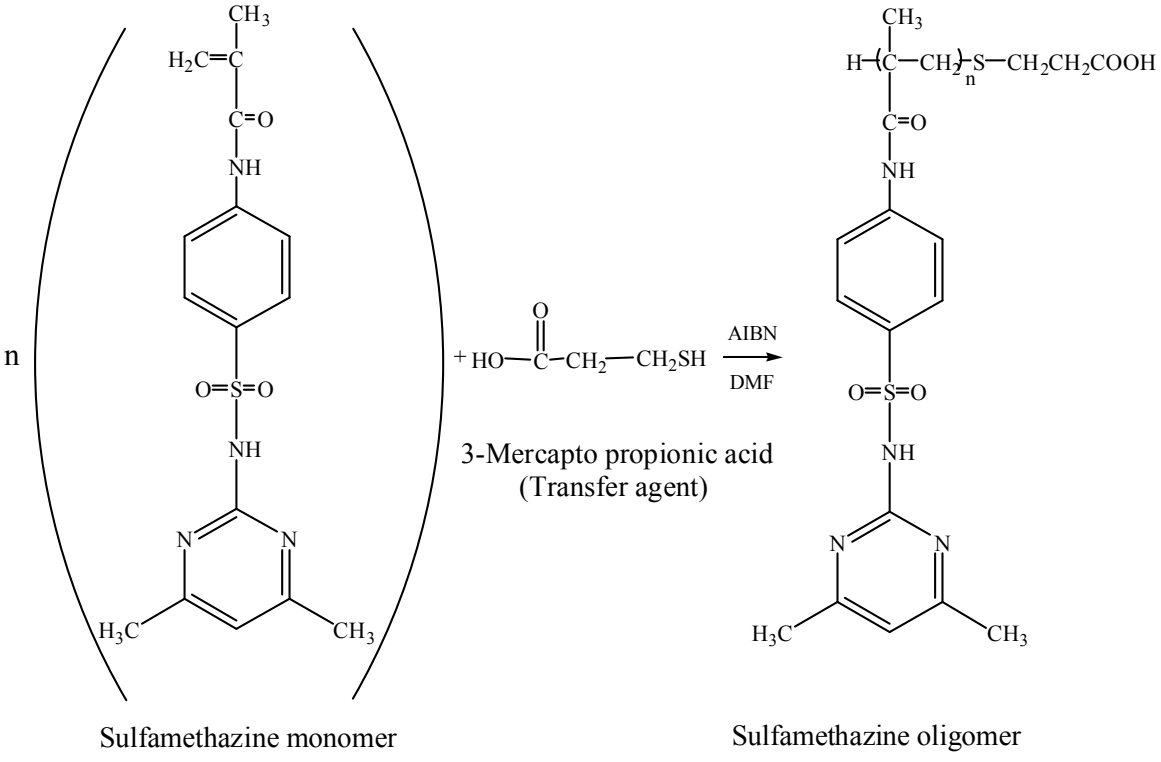


Figure 4.11. Formation of the sulfamethazine oligomer (OSM)

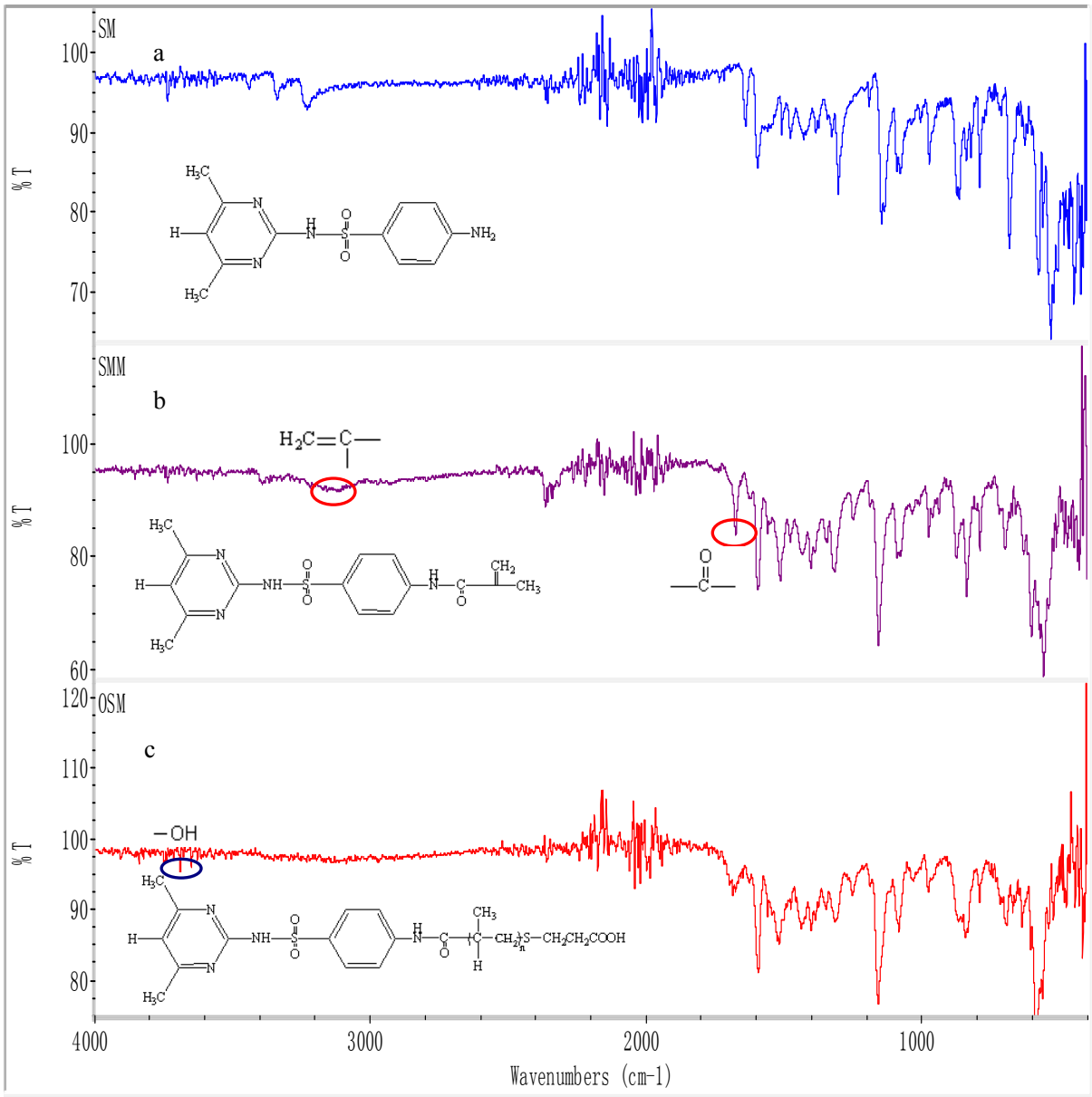


Figure 4.12. FT-IR spectra of SM (a), SMM (b), OSM(c)

When comparing the FTIR spectrum of SMM with SM, a peak for unsaturated CH_2= occurred around 3060 cm^{-1}, and a band for —C(=O)—NH— stretching vibration absorption at around 1680 cm^{-1}. (Hao et al., 2008)

The oligomer segment was then coupled to the tri-block copolymers as depicted in the reaction schemes in Figures 4.13 and 4.14.

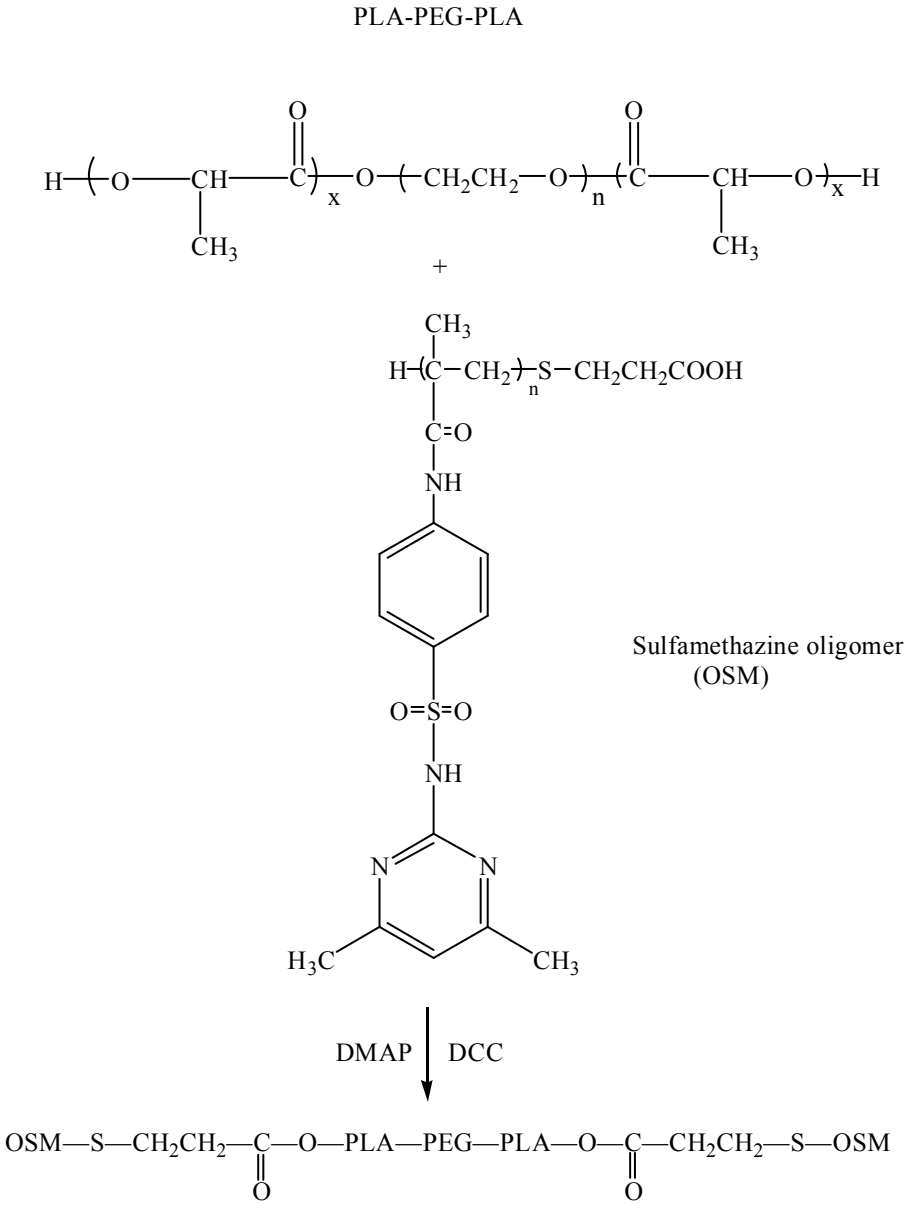


Figure 4.13. Synthesis of the five-block copolymer OSM-PLA-PEG-PLA-OSM

PCLA-PEG-PCLA

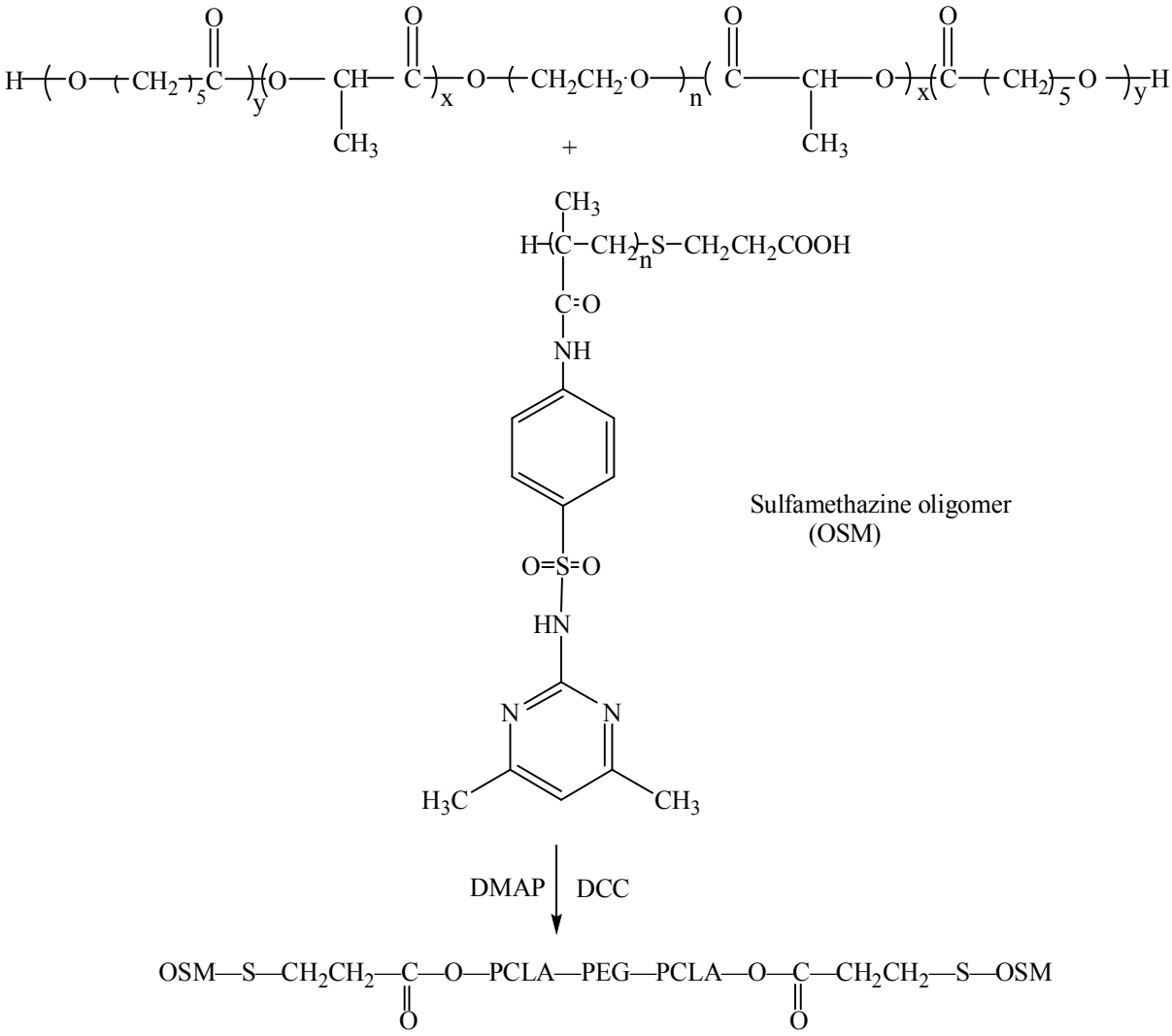


Figure 4.14. Synthesis of the five-block copolymer OSM-PCLA-PEG-PCLA-OSM.

These five-block copolymers were yellowish in appearance like their parent tri-blocks. The yield in both cases was approximately 22.6%. The products had less water solubility and seemed overall softer than their tri-block parent copolymers.

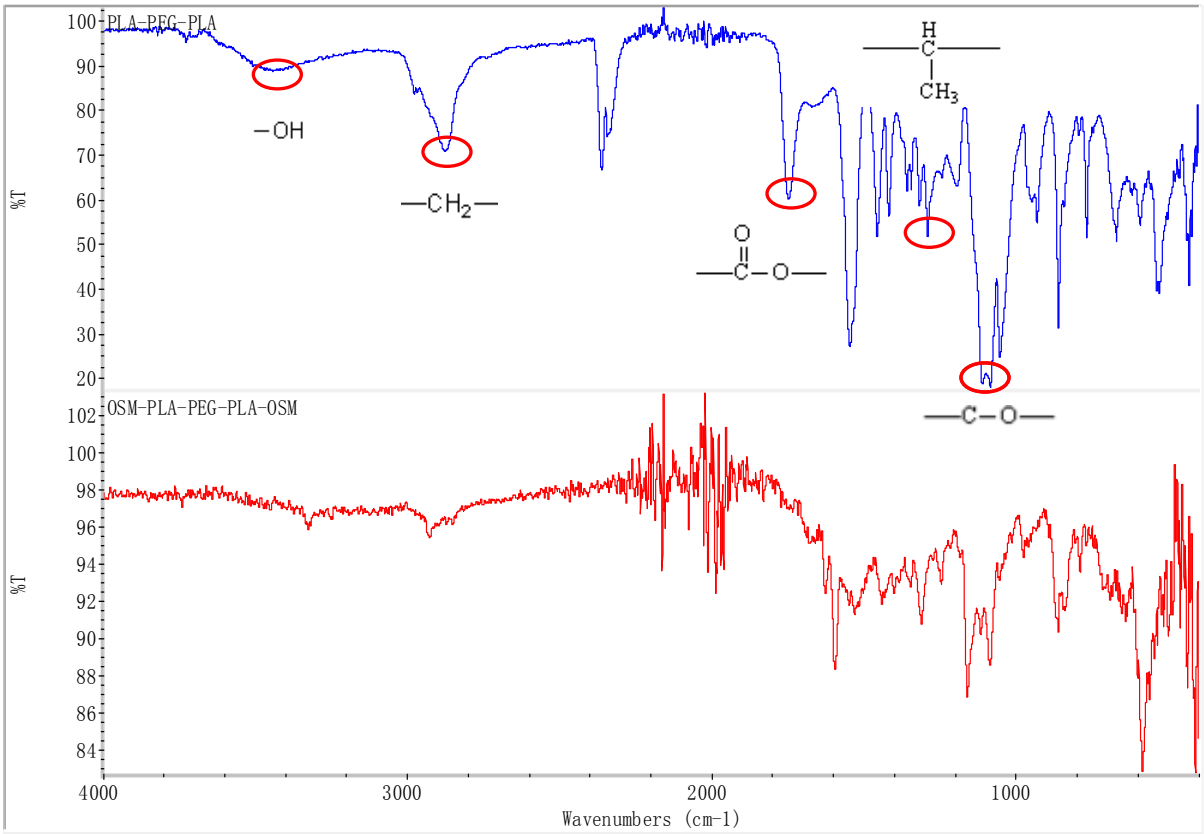


Figure 4.15. FT-IR spectra of PLA-PEG-PLA (top) and OSM-PLA-PEG-PLA-OSM (bottom)

Figures 4.15 and 4.16 show a comparison of the FT-IR spectroscopic analysis of PLA-PEG-PLA and OSM-PLA-PEG-PLA-OSM, PCLA-PEG-PCLA and OSM-PCLA-PEG-PCLA-OSM, respectively. In the spectra of the five-block copolymers the absorption peak of –OH at around 3400 cm^{-1} had disappeared which can be interpreted as successful coupling between –COOH of OSM and –OH of the two heads of PLA.

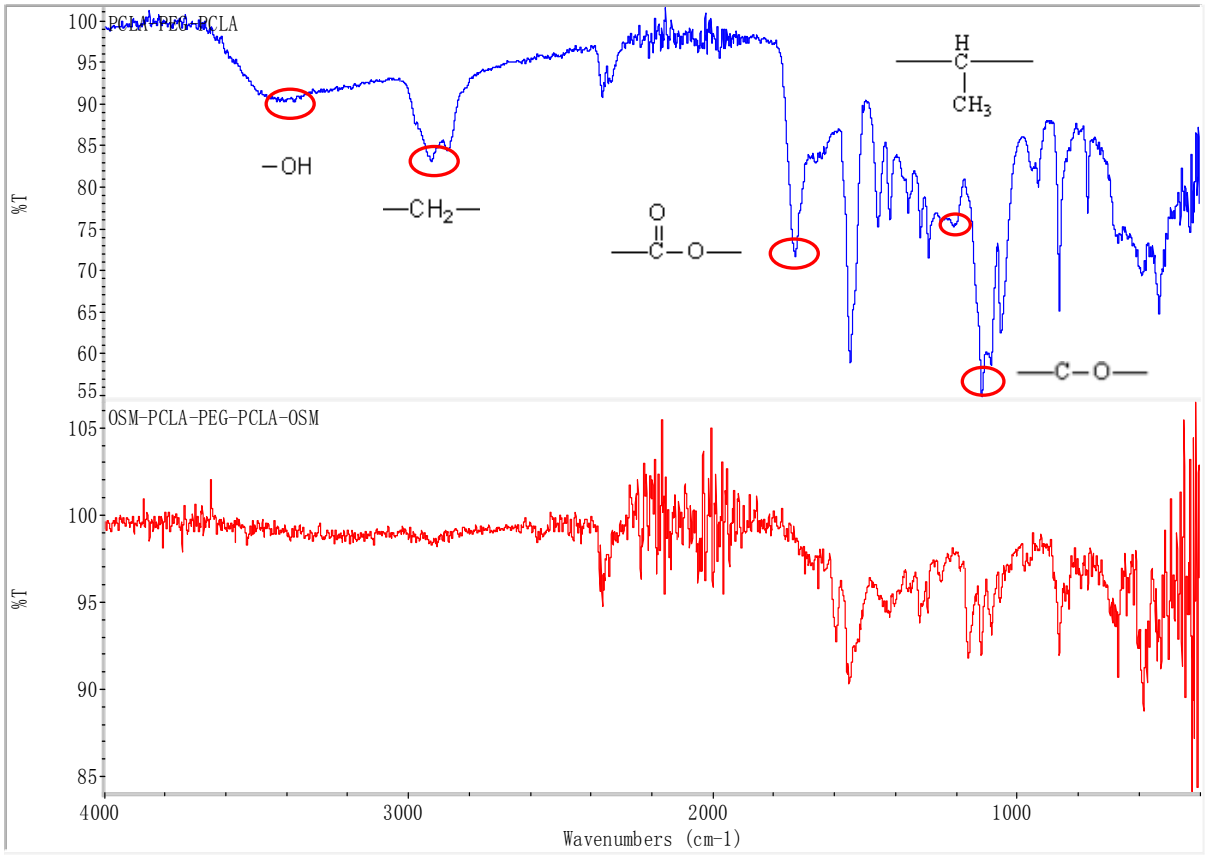


Figure 4.16. FT-IR spectra of parent PCLA-PEG-PCLA (top) and five-block OSM-PCLA-PEG-PCLA-OSM (bottom)

The molecular structures of the starting compound SM, and the synthesized SMM and OSM were confirmed by ^{1}H-NMR (Figures 4.17, 4.18 and 4.19). The aromatic (c, d) and amine protons (e) as they appeared at 7.65 ppm (c), 6.55 ppm (d) and 5.95 ppm (e) in the sulfamethazine (SM) spectrum (Figure 4.17) were shifted to 7.95 ppm (c), 7.85 ppm (d) and 10.2 ppm (e) in the corresponding SMM spectrum (Figure 4.18). Also, as the SMM was synthesized to OSM, the methyl proton peaks 1.98 ppm (f) in the SMM spectrum shift to 1.10 ppm (f') and the ethylene protons (=CH_2, g, h) in the SMM spectrum reduce and almost disappear from the OSM spectrum (Figure 4.19). These ^{1}H-NMR spectra indicated that SMM and OSM were synthesized successfully. (Shim et al., 2006)

The five-block copolymers were also characterized by ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra of the starting compounds are shown in Figure 4.19 and those of the five-block copolymer products in Figures 4.20 and 4.21.

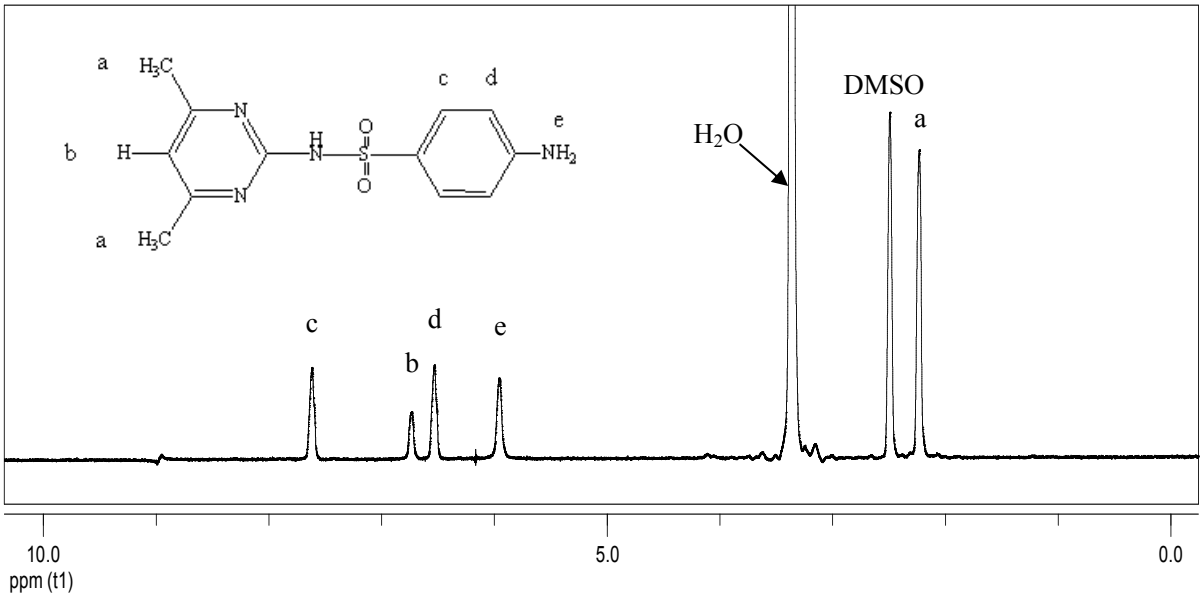


Figure 4.17. ^{1}H-NMR spectrum of sulfamethazine (SM) in DMSO-d6.

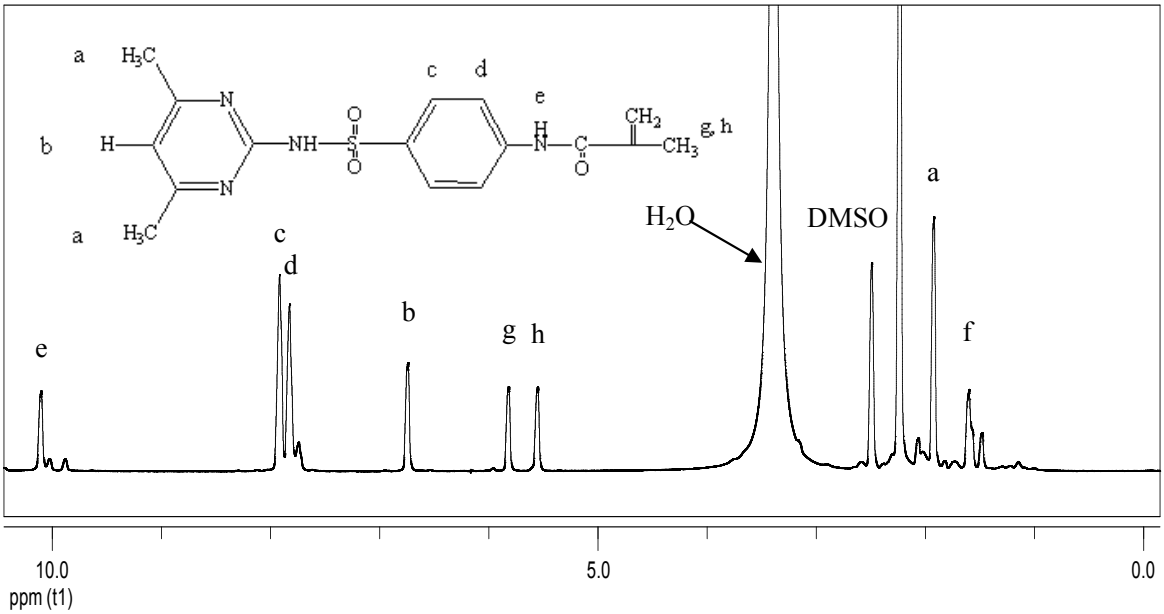


Figure 4.18. ^{1}H-NMR spectrum of sulfamethazine monomer (SMM) in DMSO-d6

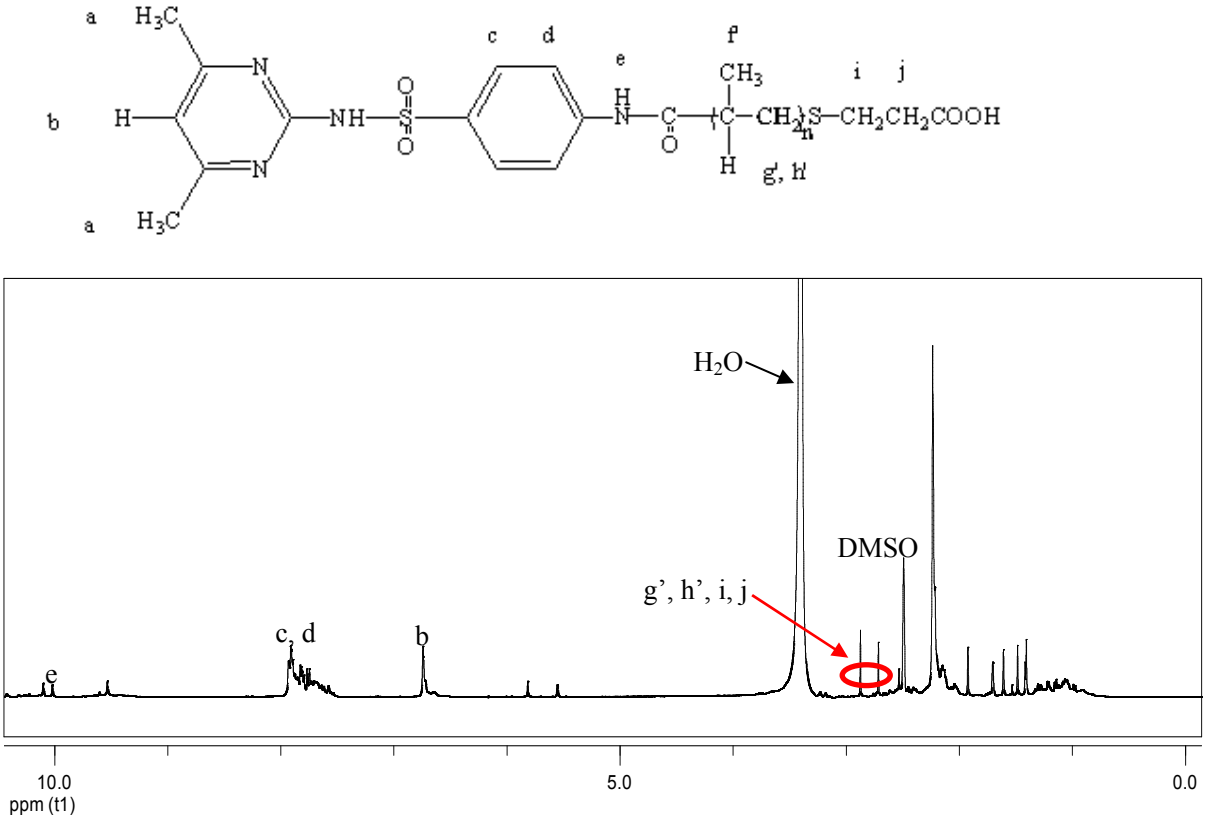


Figure 4.19. [1]H-NMR spectrum of sulfamethazine oligomer (OSM) in DMSO-d6

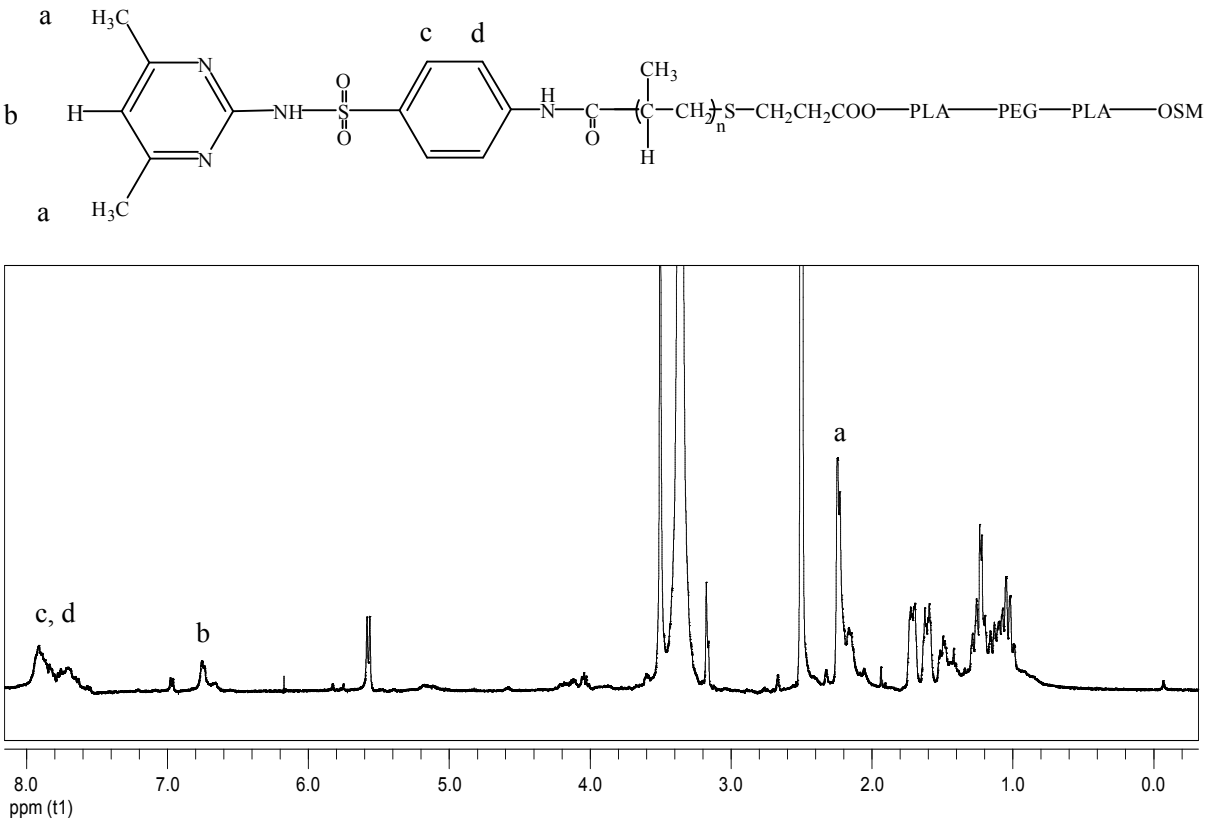


Figure 4.20. [1]H-NMR spectrum of OSM-PLA-PEG-PLA-OSM in DMSO-d6

The [1]H-NMR spectrum of OSM-PLA-PEG-PLA-OSM block copolymer shows aromatic protons (7.6- 8.0 ppm, (c), (d)) and an imidazole ring proton (around 6.75 ppm, (b)) based on PLA-PEG-PLA. This suggests that the coupling of OSM to PLA-PEG-PLA was achieved successfully. (Shim et al., 2006)

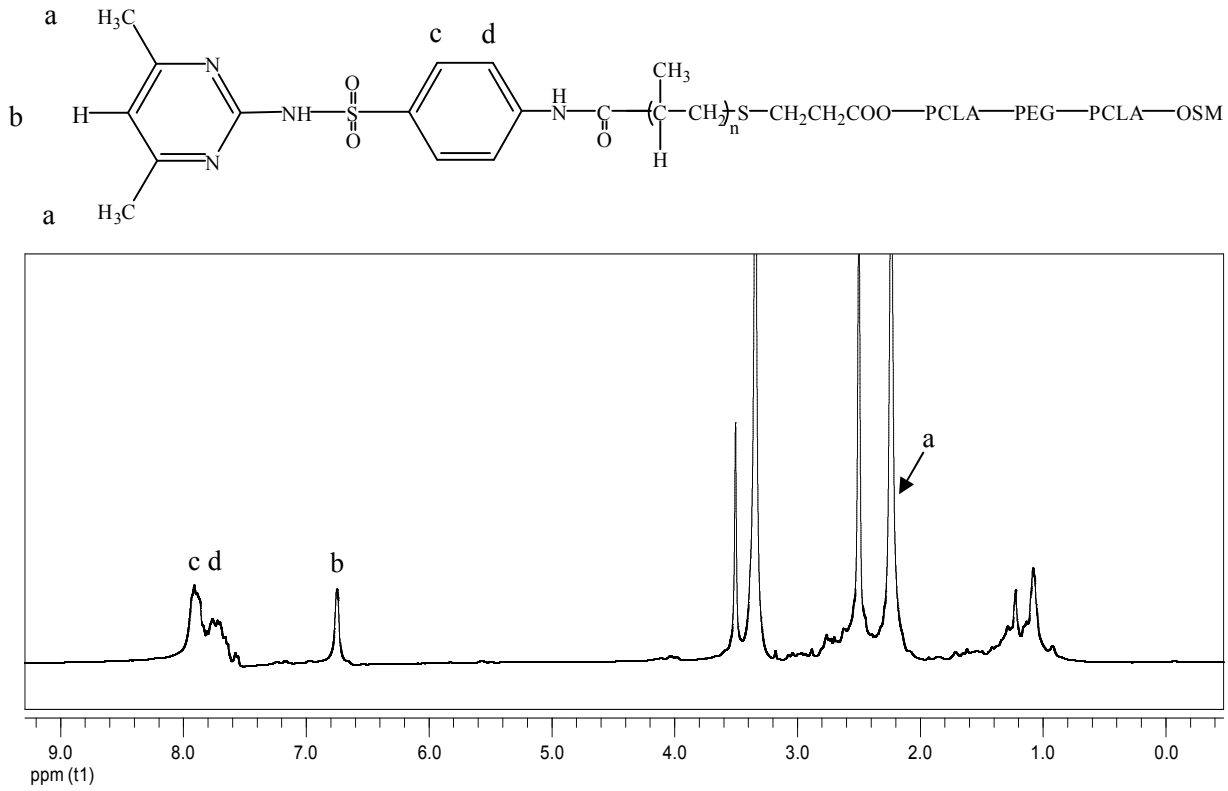


Figure 4.21. [1]H-NMR spectrum of OSM-PCLA-PEG-PCLA-OSM in DMSO-d6

The [1]H-NMR spectrum of OSM-PCLA-PEG-PCLA-OSM block copolymer shows basically the same aromatic protons (7.6- 8.0 ppm, (c), (d)) and an imidazole ring proton (around 6.75 ppm, (b)) as OSM-PLA-PEG-PLA-OSM, but in this case the parent tri-block is PCLA-PEG-PCLA. Therefore, it could be concluded that the coupling of OSM to PCLA-PEG-PCLA was also successful. In the case of the five-block copolymers differences in molecular weight of PEG segment could not be detected spectroscopically.

4.4. Thermoresponsive behavior of tri-block copolymers

The original idea had been to develop hydrogels that would display thermo-sensitivity. Ideally, the synthesized co-polymers would show a sol-gel transition in the range of 37-40°C.

PCLA-PEG-PCLA (MW of PEG is 10000) was weighed and dissolved into distilled water to prepare aqueous solutions of four concentrations, 20%, 25%, 30%, 35%, and heated to 37° C, respectively. (Figure 4.22)

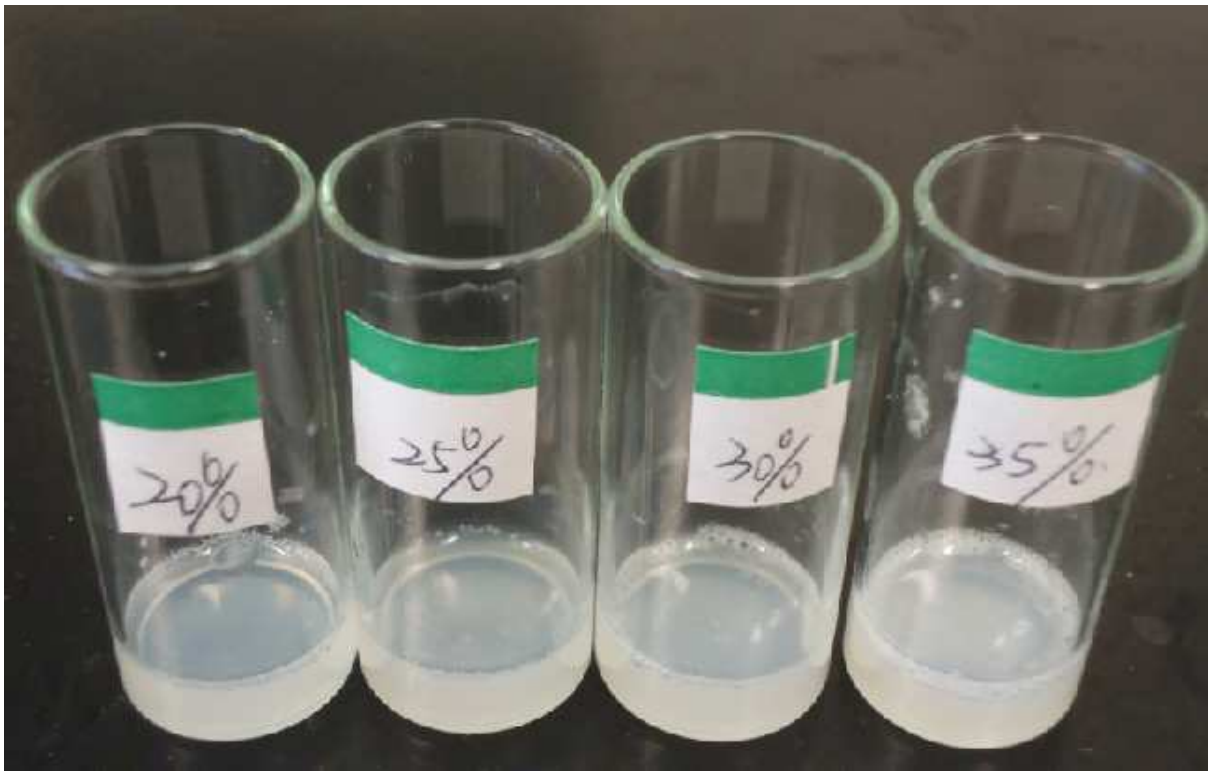


Figure 4.22. Four concentrations of PCLA-PEG-PCLA (Mw of PEG is 10,000) form opaque aqueous solutions at 37°C.

They showed slight different viscosity with difference in concentration as could be expected: the higher concentration, the higher the observed viscosity as can be seen in Figure 4.23.

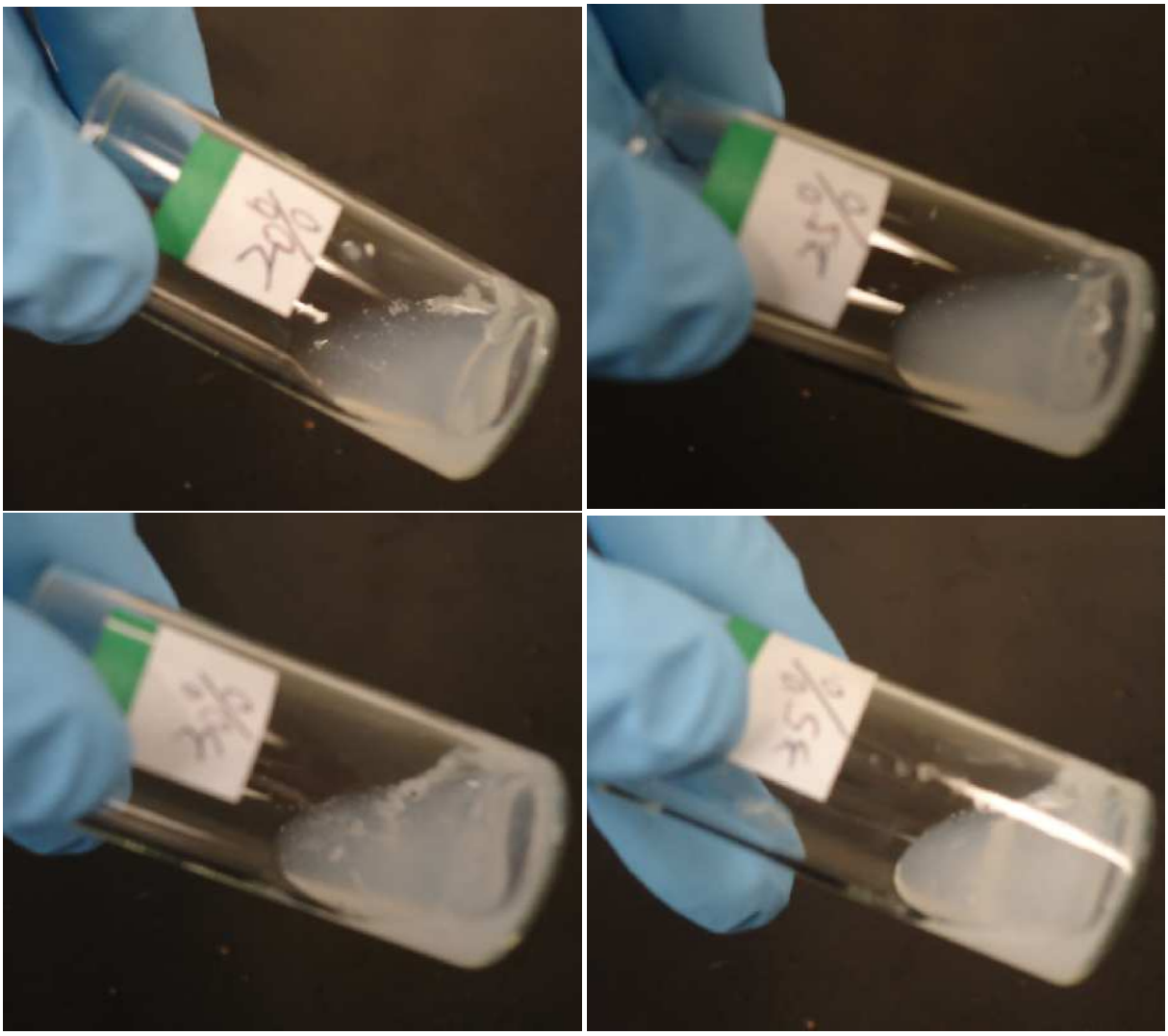


Figure 4.23. Samples of PCLA-PEG-PCLA (Mw of PEG is 10,000) in aqueous solution at 37°C show slight differences in viscosity.

However, the concentration of PCLA-PEG-PCLA (MW of PEG is 10,000) of 35% at 37°C still did not form a stable gel. Therefore, higher concentrations of PCLA-PEG-PCLA (MW of PEG 10,000) (45% and 50%) were applied to further investigate the gelling behavior (Figure 4.24). The increase in concentration to 60%, however, did not significantly change the behavior of the tri-block copolymers. At higher concentration (70%), the copolymers could only be partially dissolved in the aqueous solution which indicates that the gel concentration is in the range of 45% to approximately 65%.

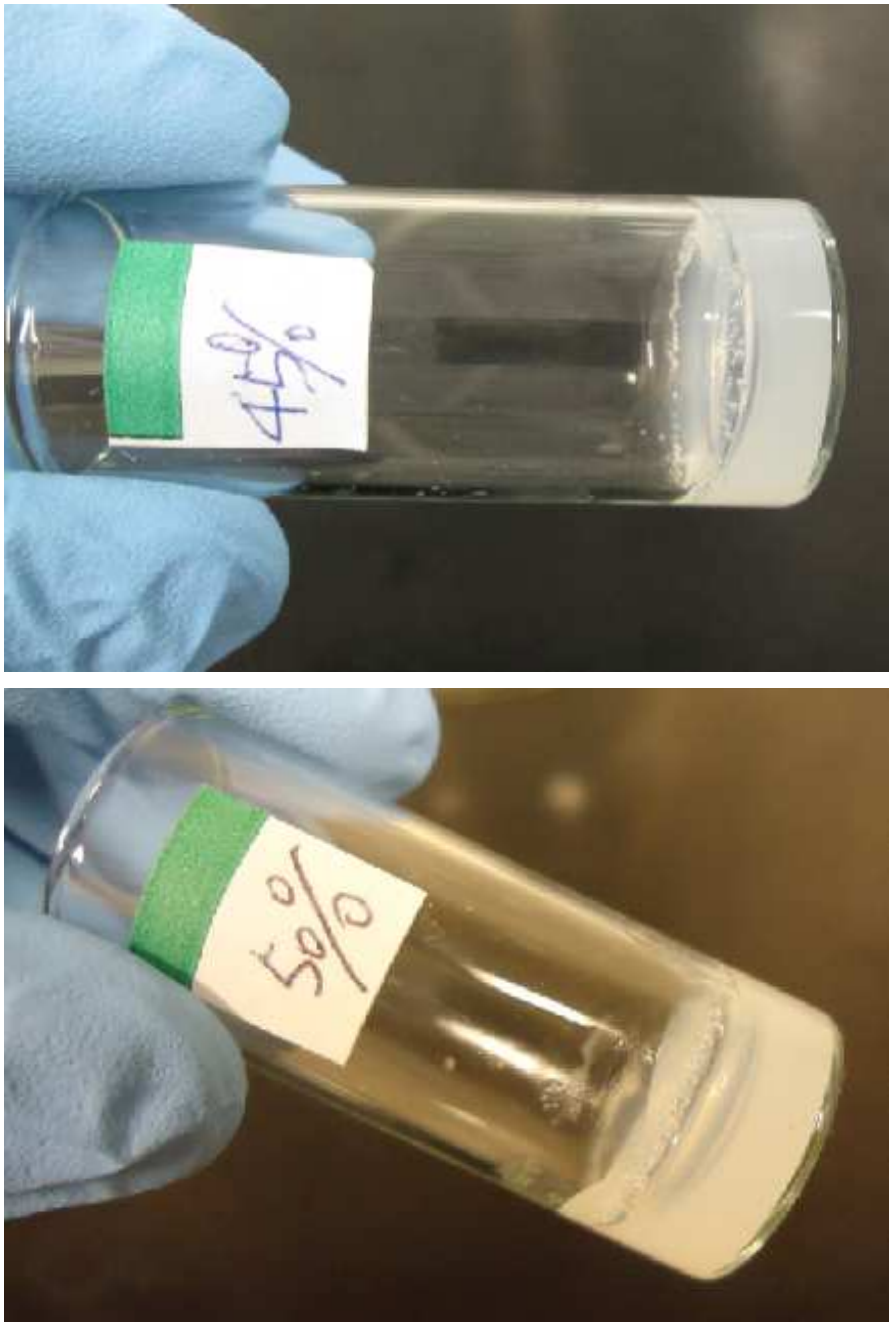


Figure 4.24. Gel of PCLA-PEG-PCLA (MW of PEG is 10,000) tri-block copolymer in aqueous solution at 37°C

When higher temperatures were applied, the viscosity of the five samples with different concentrations of PCLA-PEG-PCLA (MW of PEG is 10,000) decreased and behaved like a fluid. Fig. 4.25 shows the five concentrations of the tri-block copolymers heated to 45°C. The

gels were clearly less viscous.

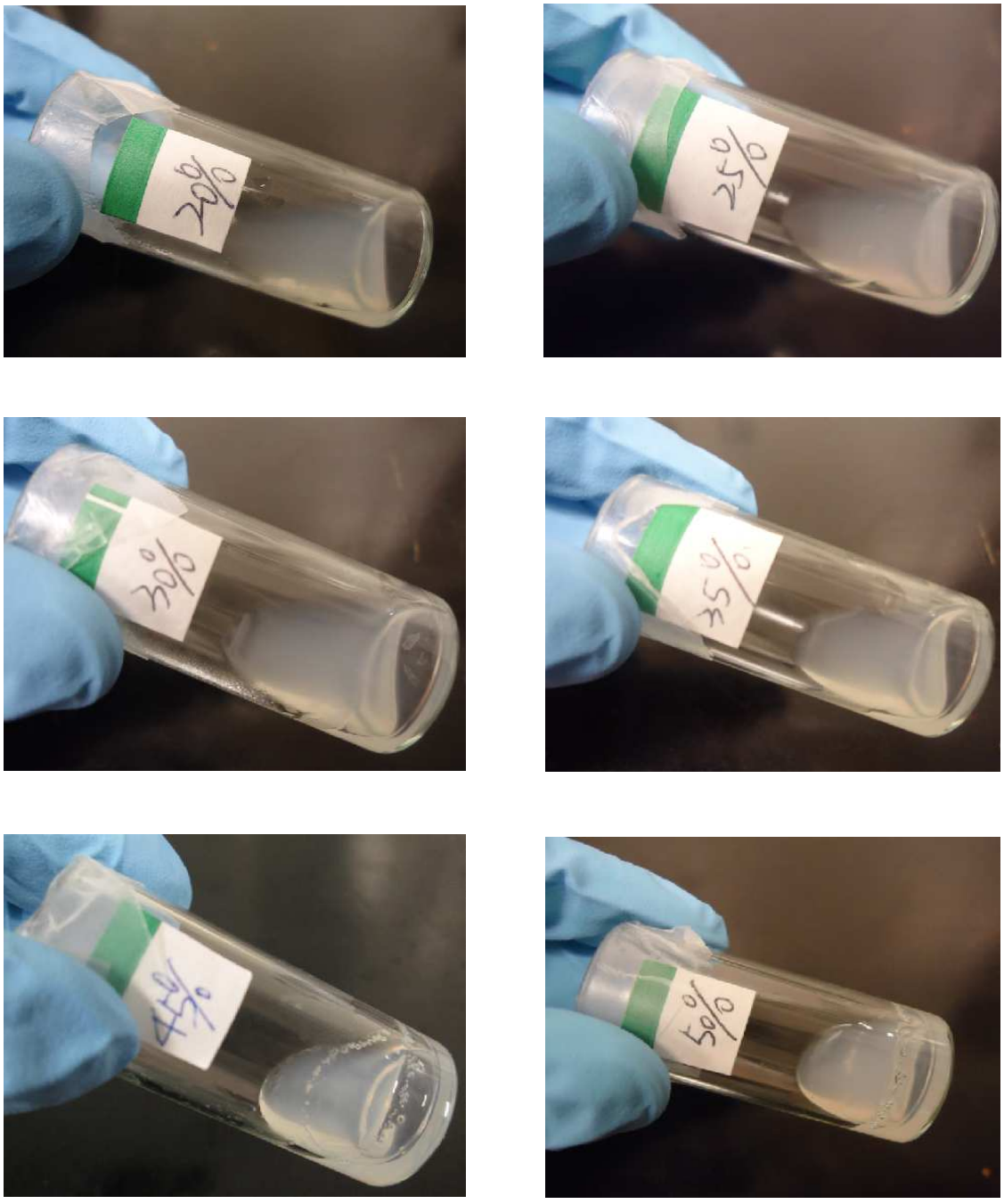


Figure 4.25. Samples of PCLA-PEG-PCLA (MW of PEG is 10,000) in aqueous solution at 45°C

The samples with 45% and 50% concentration of PCLA-PEG-PCLA (MW of PEG

10,000) showed an obvious gel-to-sol transition at the increase of temperature from 37°C to 45°C. This indicates that PCLA-PEG-PCLA with MW of PEG of 10,000 has the potential to form gels at a high concentration and to go through a gel-to-sol transition at approximately 45°C.

Another tri-block copolymer PLA-PEG-PLA (MW of PEG is 10,000) underwent similar thermo-behavior with PCLA-PEG-PCLA (MW of PEG is 10,000). The gel-sol transition may occur at lower concentrations; however, there might still have been residual water in D,L-lactide and ε-caprolactone even after drying. As the molecular weight of PLA and PCLA were calculated and predicted based on the peak area ratios of PLA, PCLA, respectively, and of PEG in the ^{1}H-NMR spectra (Shim et al. 2006), trace amounts of water might have affected the reaction efficiency to a greater extent than expected. Therefore, improvement in regard to reactant efficiency of producing tri-block copolymers and coupling efficiency of five-block copolymers to enlarged hydrophobic block length can be achieved by removing trace moisture at a higher temperature and during an extended period of drying in the oven. The pH sensitivity of the OSM segment of the five-block copolymers could then be expected to exhibit a more pronounced pH response. When optimized, more –COOH groups would then be exposed to the solvent.

4.5. SEM spectra of tri-block copolymers

Electron microscopic pictures were taken to study the morphology of the tri-block copolymers. PLA-PEG-PLA showed fairly large bubble-like pores and a surprisingly rough surface (Figure 4.26, top micrograph), while PCLA-PEG-PCLA overall had a smoother morphology with some large and some very small pores Figure 4.26, bottom). In both cases it could be speculated that the pores were formed by escaping solvent. However, in both cases

D,L-lactide and ε-caprolactone acted as reactant and as solvent at the same time.

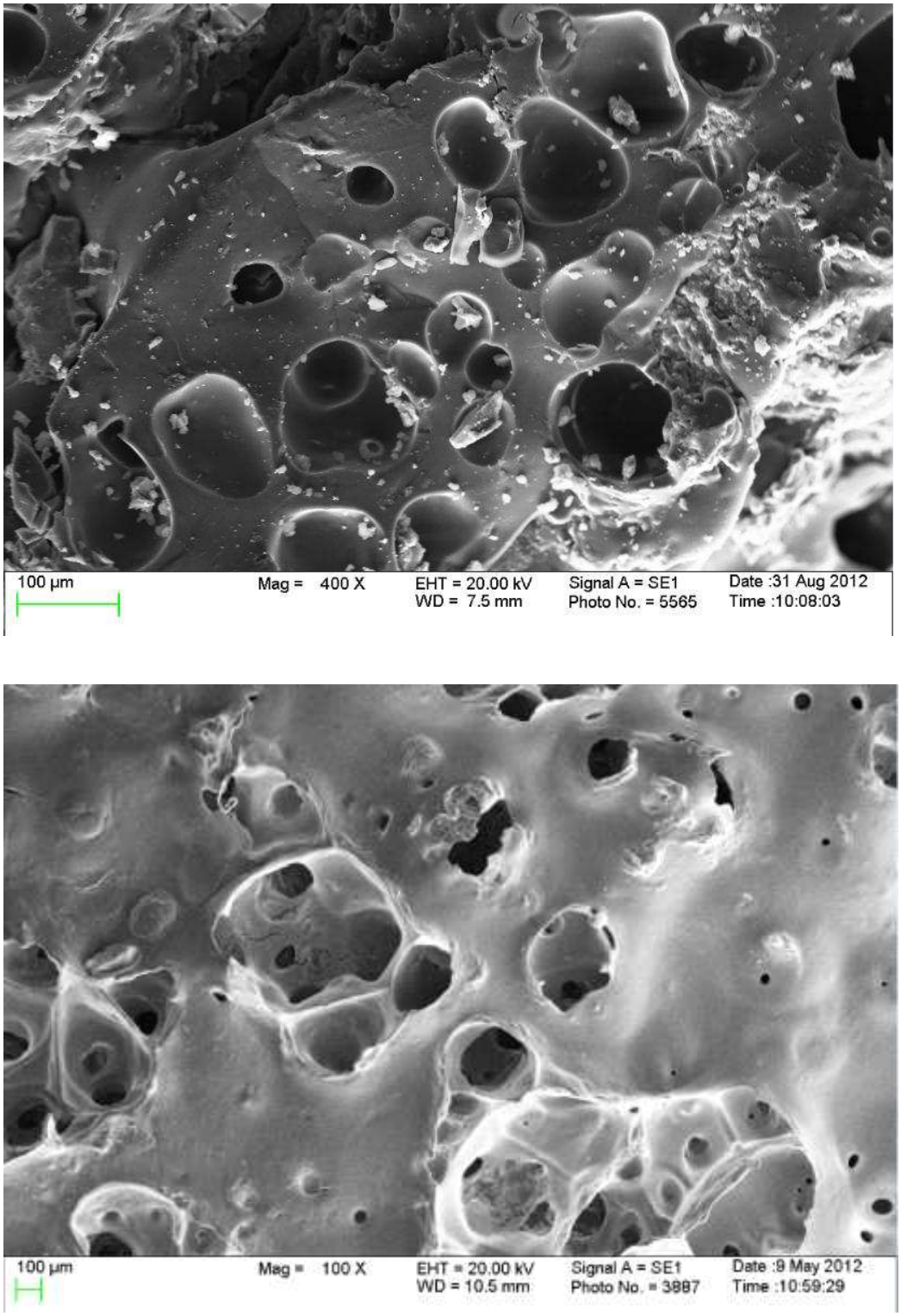


Figure 4.26. SEM spectra of PLA-PEG-PLA (top) and PCLA-PEG-PCLA (bottom)

Chapter 5

Conclusions

A series of tri-block copolymers with hydrophobic and hydrophilic segments were synthesized based on D,L-lactide or ε-caprolactone, and poly(ethylene glycol) as the starting materials. The molecular weight of poly(ethylene glycol) was 1,500 and 10,000. Cellulose microcrystals were in incorporated into D,L-lactide and ε-caprolactone copolymers, with the goal to form a more rigid and stronger product compared to the tri-block copolymers without reinforcement.

The formed tri-blocks were coupled with sulfamethazine oligomer to create five-block copolymers. The structure of the all tri-block and five-block compounds was confirmed by FT-IR and ^{1}H-NMR spectroscopy and their appearance was studied. The products were more or less tacky. At lower temperatures (below 37°C) they were gel-like. All products had a potential to form a gel with the thermal transition happening at the increase of temperature. At approximately 45°C, the viscosity of all samples decreased and samples with 45-65% concentration of PCLA-PEG-PCLA (MW of PEG 10,000) underwent a gel-to-sol transition. More experiments will be necessary to lower the transition temperature to 37°C if the gel was to be used in biomedical applications.

.

References

Bae, Y. H., Stimuli-Sensitive Drug Delivery, K. Park (Ed.), Controlled Drug Delivery: Challenge and Strategies, American Chemical Society, Washington, DC, **1997**, 147-160

Chen, G., Hoffman, A. S. Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH; Nature **1995**, 373, 49-52.

Cohn, D., Younes, H., Biodegradable PEO/PLA block copolymers; J. Biomed. Mater. Res. **1988**, 22, 993-1009.

Duncan, R. The dawning era of polymer therapeutics; Nature Reviews Drug Discovery **2003**, 2, 347-360

Dunn, R. L., In Biomedical Applications of Synthetic Biodegradable Polymers; Hollinger, J. O., Ed; CRC Press, Boca Raton, FL, **1995**; 17-31.

Fu, S.Z., Ni, P.Y., Wang, B.Y., Chu, B.Y., Zheng, L. Luo, F., Luo, J.C. Qian, Z.Y.; Injectable and thermo-sensitive PEG-PCL-PEG copolymer/collagen/n-HA hydrogel composite for guided bone regeneration; Biomaterials, 33(19), **2012**, 4801-4809.

Fujiwara, T.; Yamaoka, T.; Kimura, Y., Thermo-Responsive Biodegradable Hydrogels from Stereocomplexed Poly(lactide)s, Biomedical Applications of Hydrogels Handbook **2010**, 157-177

García, L., Aguilar, M. R., Román, J. S., Biodegradable Hydrogels for Controlled Drug Release; Biomedical Applications of Hydrogels Handbook **2010**, 147-155.

Ghandehari, H.; Kopeckova, P.; Kopecek, J., In vitro degradable of pH-sensitive hydrogels containing aromatic azo bonds, Biomaterials, 18, **1997**, 861-872

Hao, T. N.; Qiao, M. X.; Gu, J. C.; Li, Z.; Hu, H. Y.; Zhao, X. L.; Chen, D. W., Synthesis

of OSM_1-PCLA-PEG-PCLA-OSM_1 block copolymer and its pH and temperature sensitivity, Chemical Journal of Chinese Universities, 10, **2008**. 2101-2106.

He, G., Ma, L.L., Pan, J., Venkatraman, S., ABA and BAB type triblock copolymers of PEG and PLA: A comparative study of drug release properties and "stealth" particle characteristics; Intern. J. Pharma., 334(1–2), **2007**, 48-55.

Hoffman, A. S., Intelligent Polymers, K. Park (Ed.), Controlled Drug Delivery: Challenge and Strategies, American Chemical Society, Washington, DC, 1997, 485-497

Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W., Biodegradable block copolymers as injectable drug-delivery systems, Nature **1997**, 388, 860

Jeong, B.; Lee, D. S.; Shon, J.; Bae, Y. H.; Kim, S. W., Thermoreversible gelation of poly (ethylene oxide) biodegradable polyester block copolymers; J. Polym. Sci., Part A **1999**, 37, 6, 751-760

Jeong, B.; Bae, Y. H.; Kim, S. W., Thermoreversible gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions; Macromolecules **1999**, 32, 7064-7069.

Li, S.; Vert, M. "Biodegradable polymers; polyesters", in the encyclopedia of controlled drug delivery; Mathiowitz, E., ed.; John Wiley& Sons: New York, **1999**; 71-93.

Li, S.; Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids, J. Biomed. Mater. Res. **1999**, 48, 342-353.

Mukose, T.; Fujiwara, T.; Nakano, J.; Taniguchi, I.; Miyamoto, M.; Kimura, Y.; Teraoka, I.; Lee, C. W, Hydrogel Formation between Enantiomeric B-A-B-Type Block Copolymers of Polylactides (PLLA or PDLA: A) and polyoxyethylene (PEG: B); PEG-PLLA-PEG and PEG-PDLA-PEG, Macromol. Biosci., 2004, 4, 361-367

Park, K.; Park, H., Smart Hydrogels, J. C. Salamone (Ed.), Concise Polymeric Material Encyclopedia, CRC Press, Boca Raton, **1999**, 1476-1478.

Qiu, Y.; Park, K., Environment-sensitive hydrogels for drug delivery, Advanced Drug

Delivery Reviews, 53, **2001**, 321-339

Shim, W. S.; Kim, S. W.; Lee, D. S., Sulfonamide-Based pH- and Temperature-Sensitive Biodegradable Block Copolymer Hydrogels; Biomacromolecules, **2006**, 7, 1935-1941.

Tan, R.; She, Z.; Wang, M.; Fang, Z.; Liu, Y.; Feng, Q., Thermo-sensitive alginate-based injectable hydrogel for tissue engineering; Carbohydrate Polymers, 87(2), **2012**, 1515-1521.

Wang, C.; Adrianus, G. N.; Sheng, N.; Toh, S.; Gong, Y.; Wang, D. A., In vitro performance of an injectable hydrogel/microsphere based immunocyte delivery system for localised anti-tumour activity; Biomaterials, 30(36), **2009**, 6986-6995.

Zentner, G. M.; Rathi, R.; Shih, C.; McRea, J. C.; Seo, M. H.; Oh, H.; Rhee, B. G.; Mestecky, J.; Moldoveanu, Z.; Morgan, M.; Weitman, S., Biodegradable block copolymers for delivery of proteins and water- insoluble drugs; J. Controlled Release **2001**, 72, 203-215

Zhang, H.; Xia, H.; Wang, J.; Li, Y., High intensity focused ultrasound-responsive release behavior of PLA-b-PEG copolymer micelles Journal of Controlled Release; 139(1), **2009**, 31-39.

Zhong, Z. J.; Dijkstra, P. J.; Jan, F. J.; Kwon, Y. M.; Bae, Y. H.; Kim, S. W., Synthesis and aqueous phase behavior of thermoresponsive biodegradable poly (D,L-3- methylglycolide)-block- poly (ethylene glycol)- block- poly (D,L-3- methylglycolide) triblock copolymers; Macromol. Chem. Phys. **2002**, 203, 12, 1797-1803

Zhu, K. J.; Lin, X. Z.; Yang, S. L., Preparation and properties of D, L-lactide and ethylene oxide copolymer: A modifying biodegradable polymetic material; J. Polym. Sci., Part C: Polym. Lett. **1986**, 24, 7, 331-337.

Zhu, K. J.; Song, B.; Yang, S. L.; J. Polym. Sci., Part A: Polym. Chem. **1989**, 27, 1951.

Zhu, K.J.; Lin, X. Z.,Yang, S. L., Preparation, characterization, and properties of polylactide (PLA)-poly(ethylene glycol) (PEG) copolymers: A potential drug carrier; J. Appl. Polym. Sci. **1990**, 39, 1, 1-9