Project SEED 2008

Research Report:

Thermo-Responsive Hydrogels

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7-25-2008

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Introduction

This project focuses on the synthesis of PLLA-PEG-PLLA and PDLA-PEG-PDLA triblock copolymers and studying their properties as hydrogels. Hydrogels are superabsorbent materials made up of cross linked polymers. They have tissue like qualities due to the fact that they can contain over 99% of water, in which makes them flexible. Hydrogels are used in many common items such as contacts, diapers, and sanitary napkins. Hydrogels are used greatly in Tissue Engineering as scaffolds to repair tissue because human cells can be conjugated in them. [1] Hydrogels can be used for drug delivery in the body because they are thermo-responsive and biodegradable. They are liquid at room temperature (23 °C) while at body temperature (37 °C) they become gels, therefore they can be administered to the body through injection using a syringe. Once in the body, they change into gel with the medicine encapsulated inside. The mechanism of release of the encapsulated drug at a given time, rate and target has attracted much attention. [2] The application benefit of hydrogels are vast, one immediate and beneficial application is in the treatment of cancer. Chemotherapy has been the primary and effective method of treatment for cancer. Chemotherapy introduces harmful drugs to the body which have adverse side effects, such as attacking healthy cells and organs. In the contrary if chemotherapy drugs are encapsulated in a hydrogel and placed near a cancer cell most of the adverse side effects can be eliminated.

Experimental

Materials:

PEG (MW 10,000 and 4,600) and Tin (II) 2-ethylhexanoate were purchased from Sigma Aldrich. Poly(d-lactide) and Poly(L-lactide) were purchased from Purac. Tetrahydrofuran (THF), and toluene (extra dry, water <30ppm) were purchased from Acros. Hexanes and methylene chloride were purchased from Fischer Scientific. Diethyl ether was obtained from chemical supply department at the University of Memphis.

Block Copolymer Synthesis

PLLA-PEG-PLLA and PDLA-PEG-PDLA triblock copolymers were synthesized using the ring opening polymerization method. In a reaction flask either PLLA or PDLA was mixed with PEG. Tables 1-4 show the measurements for each synthesis. The reflux apparatus was assembled and N_2 was allowed to pass through the system for ~30 minutes at room temperature. The temperature was increased to 60 °C and then to 80 °C. A catalytic amount of stannous octoate was added using a syringe. The temperature was increased to 120 °C; the reaction mixture became clear and the viscosity decreased with increasing temperature. The polymerization process was left over night. After cooling the reaction mixture to room temperature, 10-15 mL of methylene chloride was added to dissolve it. Using 1:1 ether/hexane mixture 10 times the volume of the reaction mixture, the polymer was precipitated. With varying PLA: PEG ratio the polymer precipitated differently, therefore different techniques were used to recover the polymer. Table-5 describes the amount of ether/hexane used for precipitation, state of the polymer as precipitant and technique used to recover the polymer. The polymer was then dried at 40 °C for several hours using a vacuum oven. Sample was prepared for NMR analysis.

Table 1- Synthesis of PLLA-PEG-PLLA and PDLA-PEG-PDLA (CC060208)			
Compounds	MW	Amount	Mole
Poly(L-lactide)	4,000	0.8 grams	$2x10^{-4}$mol
Poly(D-lactide)	4,000	0.8 grams	$2x10^{-4}$mol
Polyethylene glycol	10,000	2.0 grams	0.2mmol
Tin(II) 2-ethlyhexonoate	405.11	0.081 grams	$2x10^{-5}$mol
Toluene	92.14	0.81 mL	

Table 2- Synthesis of PLLA-PEG-PLLA and PDLA-PEG-PDLA (CC061908)			
Compounds	MW	Amount	Mole
Poly(L-lactide)	6,000	1.2 grams	$2x10^{-4}$mol
Poly(D-lactide)	6,000	1.2 grams	$2x10^{-4}$ mol
Polyethylene glycol	10,000	2.0 grams	0.2mmol
Tin(II) 2-ethlyhexonoate	405.11	0.081 grams	$2x10^{-5}$mol
Toluene	92.14	0.81 mL	

Table 3- Synthesis of PLLA-PEG-PLLA and PDLA-PEG-PDLA (CC062308)			
Compounds	MW	Amount	Mole
Poly(L-lactide)	2,000	2.0 grams	1.0 mmol
Poly(D-lactide)	2,000	2.0 grams	1.0 mmol
Polyethylene glycol	2,000	2.0 grams	1.0 mmol
Tin(II) 2-ethlyhexonoate	405.11	0.0405 grams	$1x10^{-4}$mol
Toluene	92.14	0.405 mL	

Table 4- Synthesis of PDLA-PEG-PDLA (CC071708)			
Compounds	MW	Amount	Mole
Poly(D-lactide)	1,300	1.3 grams	$4.346 x 10^{-4}$ mol

Polyethylene glycol	4,600	2.0 grams	0.4348 mmol
Tin(II) 2-ethlyhexonoate	405.11	0.0176 grams	4.348×10^{-5} mol
Toluene	92.14	0.176 mL	

Table-5 Block copolymer synthesis, precipitation and polymer recovery.

Block Copolymer	Theoretical Block Length	% Yield	Amount of Ether: Hexane Used for precipitation	Observation during precipitation	Polymer recovered through
PLLA-PEG-PLLA	2,000-10,000-2,000	88%	100 mL Ether & 100 mL Hexane	Solid, white precipitant	Vacuum Filtration
PLLA-PEG-PLLA	3,000-10,000-3,000	86.0%	100 mL Ether & 100 mL Hexane	Solid, white precipitant	Vacuum Filtration
PLLA-PEG-PLLA	1,000-2,000-1,000	88.3%	100 mL Ether & 100 mL Hexane	Viscous liquid	Decantation and Evaporation
PDLA-PEG-PDLA	2,000-10,000-2,000	88.3%	100 mL Ether & 100 mL Hexane	Solid, white precipitant	Vacuum Filtration
PDLA-PEG-PDLA	3,000-10,000-3,000	88%	100 mL Ether & 100 mL Hexane	Solid, white precipitant	Vacuum Filtration
PDLA-PEG-PDLA	1,000-2,000-1,000	78%	100 mL Ether & 100 mL Hexane	Viscous liquid	Decantation and Evaporation
PDLA-PEG-PDLA	1,300-4,600-1,300	81%	100 mL Ether & 100 mL Hexane	Viscous liquid	Decantation and Evaporation

Micelle Formation

Five PLLA-PEG-PLLA samples were weighed in ratios of 5:8:10:15:20 (see Table-2). Similarly, this was done for PDLA-PEG-PDLA. Placed the five samples of PLLA-PEG-PLLA and the five samples of PDLA-PEG-PDLA in ten 19 mm x 51 mm vials and labeled the vials according to the relative weight and type of lactide (PLLA or PDLA). Tetrahydrofuran was added to the vials to dissolve the polymers. Tables 6-7 describe the amounts of tetrahydrofuran used in the dissipating of the polymer. Two milliliters of deionized water was added to ten 19 mm x 55 mm vials and the water line was marked off. The water line was marked off so I would know if the water evaporated and if it did I would know how much to put back in there to have 2 mL. Next, an 800 mL flask was filled with ice and placed a thermometer in it. A vial with 2 mL of deionized water was suspended in the ice bath with a sonicator in the middle. While the sonicator was turned on, one of the PLLA samples was added drop by drop to the vial suspended in the ice bath. This step was repeated for all samples for PLLA and PDLA. After all the samples of PLLA and PDLA were mixed in the 2 mL of deionized water in the 800 mL flask filled with ice, small magnetic stirrers were inserted in the vials. Then, the vials were placed on a magnetic stirrer until the tetrahydrofuran evaporated from the vials.

Table-6 Micelle formation of PLLA-PEG-PLLA in 2 mL of H_2O

Relative Weight	Actual Weight	Amount of THF used
5%	0.123 g	1 mL
8%	0.197 g	1 mL
10%	0.246 g	1.2 mL
15%	0.369 g	1.5 mL
20%	0.492 g	1.8 mL

Table-7 Micelle formation of PDLA-PEG-PDLA in 2 mL of H_2O		
Relative Weight	Actual Weight	Amount of THF used
5%	0.123 g	1 mL
8%	0.200 g	1 mL
10%	0.250 g	1.5 mL
15%	0.375 g	1.7 mL
20%	0.501 g	2.0 mL

Hydrogel Study

Five 19 mm x 55 mm vials were labeled (one 5%, 8%, 10%, 15%, and 20%) and the empty vial labeled 5% was placed in an 800 mL flask was filled with ice. A sonicator was placed in the middle of the empty 5% vial. Using a micropipette, 1 mL of the 5% PLLA micelles was inserted in to the empty vial. The sonicator was turned on and 1 mL of the 5% PDLA micelles was added to the vial. The vial was removed from the 800 mL flask and this step was repeated for the rest of the samples. The mixed micelles vials were placed in a bowl filled with ice. All the samples for PLLA were added to the ice bath to compare the PLLA-PDLA micelles and PLLA micelles together as the temperature was increase and decrease. After positioning the vials in the ice bath for 30 min., the temperature was increased by using a heating bath and then temperature was decreased. Table-8 describes the temperature recorded.

Table-8 Recorded Temperature for Hydrogel Study			
Temperature recorded during heating °C	Time	Temperature recorded during cooling °C	Time
0 ºC	30 min	0 ºC	30 min
10 ºC	43 min	10 ºC	30 min
25 ºC	34 min	25 ºC	30 min
32 ºC	30 min	37 ºC	30 min
37 ºC	35 min		
42 ºC	46 min		
60 ºC	40 min		
80 ºC	30 min		

Results and Discussion

PLA-PEG-PLA triblock copolymer synthesis study

Figure-1 gives the structure of the triblock copolymer and NMR spectrum for the 2000-10000-2000 block copolymer in $CDCl_3$ with TMS as reference. Tables 9-10 list the peak assignment and coupling of the remaining block copolymer synthesized.

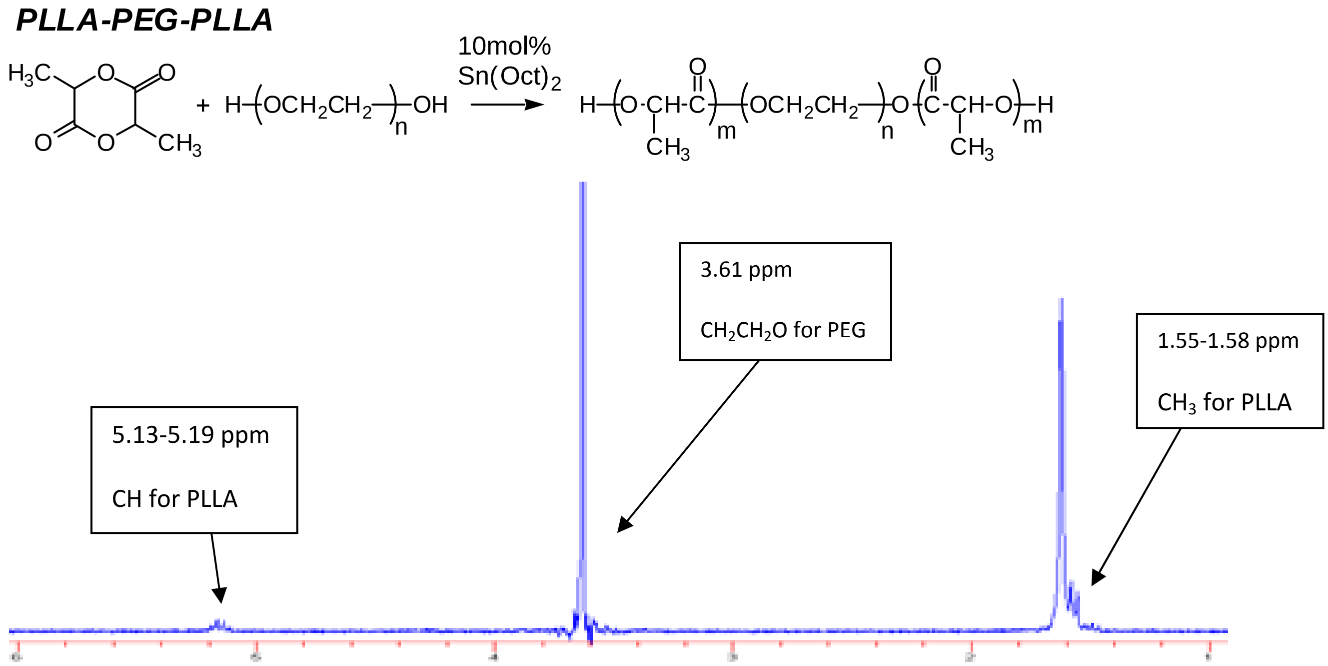

Figure 1- Structure of PLLA-PEG-PLLA and NMR spectrum for 2000-10,000-2000

The CH for PLLA shows a quartet at 5.13-5.19 ppm. Following the N+1 rule and the neighboring CH_3 has three spin-coupled protons the resulting quartet is indicative of the CH group of PLLA. The peak at 3.61 ppm is for the CH_2CH_2O group of PEG. Since both CH_2 groups of PEG are attached to Oxygen attached to Poly (lactide acid), the resulting peak is a singlet. The peak at 1.55-1.58 ppm is for the CH_3 group of PLLA. The methyl group is attached to the CH group which has one spin-coupled proton; therefore following the N+1 rule the resulting peak is a doublet.

Table-9: Peak Assignment and Coupling					
PLLA-PEG-PLLA	2000-10,000-2000		PLLA-PEG-PLLA	3000-10,000-3000	
Peak	Assignment	Coupling	Peak	Assignment	Coupling
1.5-1.8	CH3 for PLLA	Doublet	1.5-1.8	CH3 for PLLA	Doublet
3.6	CH2CH2O for PEG	Singlet	3.6	CH2CH2O for PEG	Singlet
5.13-5.19	CH for PLLA	Quartet	5.13-5.19	CH for PLLA	Quartet

Table-10: Peak Assignment and Coupling					
PDLA-PEG-PDLA	2000-10,000-2000		PDLA-PEG-PDLA	3000-10,000-3000	
Peak	Assignment	Coupling	Peak	Assignment	Coupling
1.5-1.8	CH3 for PLLA	Doublet	1.5-1.8	CH3 for PLLA	Doublet
3.6	CH2CH2O for PEG	Singlet	3.6	CH2CH2O for PEG	Singlet
5.13-5.19	CH for PLLA	Quartet	5.13-5.19	CH for PLLA	Quartet

Calculating Molecular Weight From ^{1}H NMR spectrum

$$\frac{\text{MW of PEG polymer}}{\text{MW of PEG}} = \frac{10,000}{44} = 227.2727273$$

Number of hydrogens for PEG peak = 227.2727273
227.2727273 * 4 (CH_2CH_2O) = 909.0909091

Number of hydrogens of PEG = 909.0909091
909.0909091 = 0.996 (Integration value for PEG peak)

X = 0.063755 (Integration value for PLLA peak)

X = 58.19185834 (number of PLLA hydrogens)

58.19185834 * 72 (MW of PLLA) = 4189.813801 (divide by 2 because it is a triblock polymer)

PLLA--------PEG-------PLLA
2095 10,000 2095

Table-11 Calculated Molecular Weight from ^{1}H NMR spectrum	
Polymer (theoretical molecular weight)	Block length calculated from ^{1}H NMR spectrum
PLLA-PEG-PLLA (2,000-10,000-2,000)	1360-10,000-1360
PLLA-PEG-PLLA (3,000-10,000-3,000)	2095-10,000-2095
PDLA-PEG-PDLA (2,000-10,000-2,000)	1242-10,000-1242
PDLA-PEG-PDLA (3,000-10,000-3,000)	2336-10,000-2336

Micelle and hydrogel formation study

For micelle formation the lack of solid particles precipitating and formation of a light blue color was used as indication of micelle formation. During this process the high concentration micelles turned to gel readily while the lower concentration micelles remained solution. The different length block copolymers behaved in different ways during micelle formation. Table-12 gives a summary of the differences during PLLA-PEG-PLLA micelle formation. Table-13 gives a summary of PLLA-PDLA mixed micelles formation.

Micelles are aggregates that are built from polymeric molecules such as PLLA, PDLA, and PEG. When polymers are dissolved and mixed in water they form micelles. Polymers form micelles because the PLA is hydrophobic and PEG is hydrophilic. So when they are introduced to water, the PLA forms a core in the middle because of its water fearing and PEG forms an outer shell because its water loving. That's why micelles have a flower-like structure.

Table-12 Micelle formation results of PLLA-PEG-PLLA			
Conc.	Micelle at 0 °C	Conc.	Micelle at 0 °C
5	Solution	5	Solution
8	Solution	8	Gel
10	Solution	10	Gel
15	Solution	15	Gel
20	Gel	20	Gel
2000-10000-2000		3000-10000-3000	

Table-13 Micelle formation results of PLLA-PDLA (mixed micelles)			
Conc.	Micelle at 0 °C	Conc.	Micelle at 0 °C
5	Solution	5	Gel
8	Solution	8	Gel
10	Solution	10	Gel
15	Gel	15	Gel
20	Gel	20	Gel
2000-10000-2000		3000-10000-3000	

Hydrogel was prepared by mixing the prepared PLLA-PEG-PLLA and PDLA-PEG-PLLA micelles. Figure 2-3 are observations and Figure 4-7 are pictures from the temperature change in the hydrogel study. The tilt technique was used to determine the formation of a hydrogel. If a mixture can be tilted for over 20 seconds without any movement, then it is said to be a hydrogel. . Hydrogels form when the hydrophobic segment (PLLA or PDLA) of an enantiomeric ABA triblock copolymer forms a core and the hydrophilic segment (PEG) forms a shell around it. Before heating, PLLA and PDLA are isolated from each other because the core/shell interface is too strong for PLLA and PDLA to branch off to each other. By heating, the PLLA and PDLA core/shell interface is thus weakened allowing the stereocomplexation process to begin. The hydrophobic segments of the micelles are linked to each other at 37 °C and thus form a gel. As more heat is applied, the crystallization of the stereocomplex is increased and the state of physical cross linking changes due to the reorganization of the hydrophobic cores.

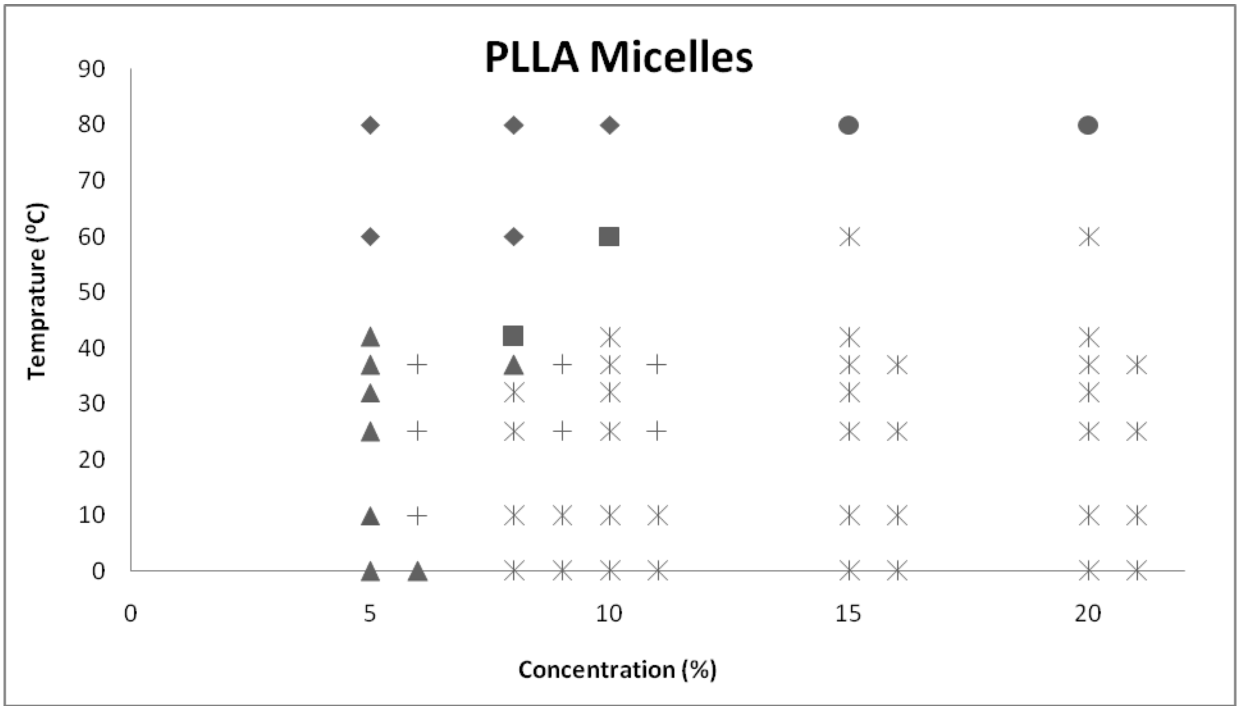

Figure 2- PLLA (3000-10,000-3000) Hydrogel response to temperature change	
Symbol	Meaning
◆	Solution, low viscosity
▲	Solution, medium viscosity
■	Solution, High viscosity
+	The bottom layer is gel, while the top layer is a solution with high viscosity
●	>90% is in gel form, while about 10% has began to flow slightly
⋇	Full Gel, when inverted or tilted no movement is seen

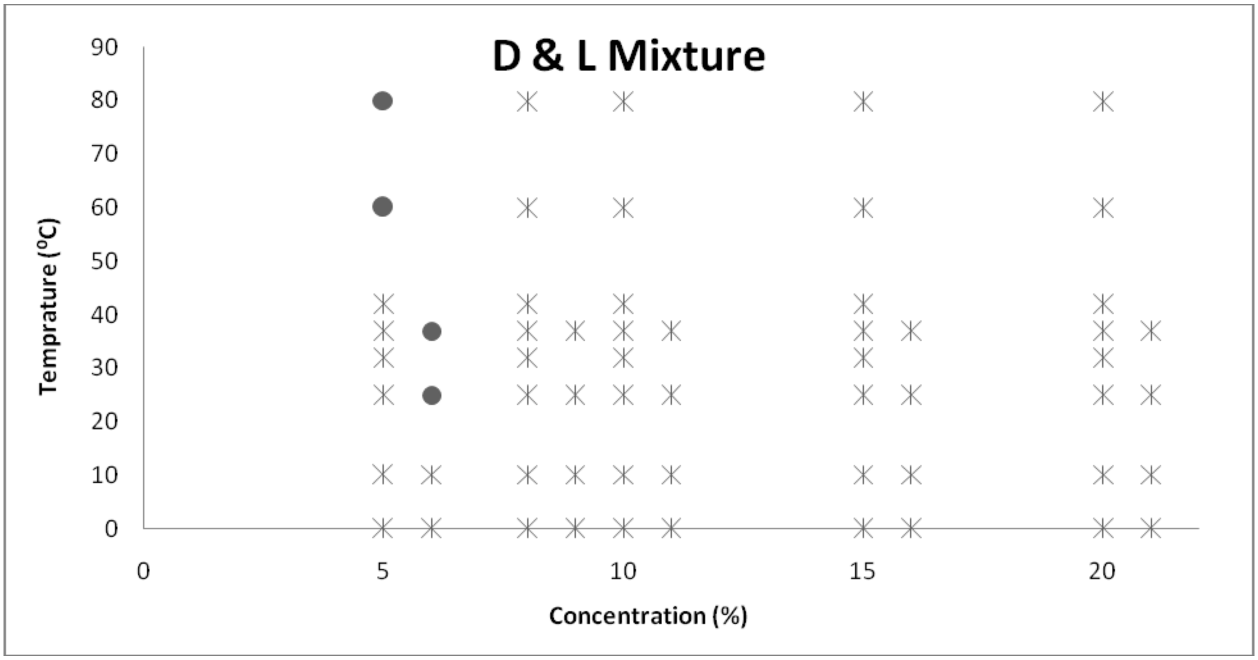

Figure 3- PLLA-PDLA (3000-10,000-3000) Hydrogel response to temperature change	
Symbol	Meaning
♦	Solution, low viscosity
▲	Solution, medium viscosity
■	Solution, High viscosity
+	The bottom layer is gel, while the top layer is a solution with high viscosity
●	>90% is in gel form, while about 10% has began to flow slightly
⋇	Full Gel, when inverted or tilted no movement is seen

Figure 4- PLLA-PDLA (3000-10,000-3000) Hydrogel at 0 °C (gel)

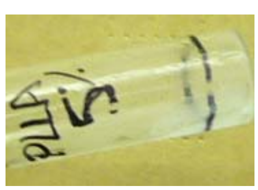

Figure 5- PLLA (3000-10,000-3000) Hydrogel at 0 °C (solution)

Figure 6- PLLA-PDLA (2000-10,000-2000) Hydrogel at 0 °C (solution)

Figure 7- PLLA (2000-10,000-2000) Hydrogel at 0 °C (solution)

Conclusion

All of my polymerizations have been a success for this summer and I didn't have to reproduce any polymers due to contamination. Also, all the polymers I synthesized produced an 78% or higher percentage yield and I was able to use all of my polymers for the formation of micelles in water. Hopefully, the data I have gathered for summer will be of some use to someone in the future.

Acknowledgment

I would like to thank Dr. Burkey for allowing me the opportunity to participate in Project SEED and I would like to thank Dr. Fujiwara for allowing me to do research in her lab for the summer of 2008. Also, I would like to thank Daniel Abebe for mentoring and aiding me this summer in my research of Thermo-Responsive Hydrogels. As an overview, I have really enjoyed myself while researching at the University of Memphis in Dr. Fujiwara's lab. This is an experience that I will remember for the rest of my life. Not only have I obtained knowledge about biochemistry and organic chemistry, I have gained experience that will improve me as a scholar as I proceed to my senior year in high school and my freshman year in college.

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