Synthesis and characterization of pH/temperature dual sensitive multiblock copolymer for drug delivery

Hyung Kyung Lee

University of Mississippi

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SYNTHESIS AND CHARACTERIZATION OF PH/TEMPERATURE DUAL SENSITIVE
MULTIBLOCK COPOLYMER FOR DRUG DELIVERY

A Thesis
presented in partial fulfillment of requirements
for the degree of Master of Science
in the Department of Pharmaceutics and Drug Delivery
The University of Mississippi

by

HYUNG KYUNG LEE

December 2017
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ABSTRACT

The objective of this study was to develop a novel multiblock copolymer (MBCP) with temperature/pH dual-sensitivity which undergoes gelation at body temperature and degradation at acidic pH for controlled drug delivery applications. The MBCP with molecular weight of 42000 Da was synthesized by polycondensation between an acid-labile ketal linker, 2,2-bis(amoethoxy)propane (BAP) and Pluroninc® P104 using L-lysine diisocyanate ethyl ester (LDI) or 1,6-Diisocyanatohexane (HDI). The gelation temperature of 20% (w/v) MBCP was in the range of 29 to 31 ºC when tested with three different techniques: rheometer, test tube inversion method, and falling ball method. The polymer showed pH-triggered degradation and drug release with nile red and irinotecan. The ketal linker connecting Pluronic® was cleaved and resulted in smaller molecular weight strain causing change in sol-gel transition temperature and critical micelle concentration. This mechanism confers the synthesized polymer with pH/temperature instigated drug release properties and thus can be used for targeted drug delivery applications.
DEDICATION

This work is dedicated to my loving family.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>2,2-bis(aminoethoxy)propane</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Chloroform-\textit{d}</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>DPH</td>
<td>1,6-diphenyl-1,3,5-hexatriene</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>HDI</td>
<td>1,6-diisocyanatohexane</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>iPrOH</td>
<td>Isopropanol</td>
</tr>
<tr>
<td>(M_n)</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>LDI</td>
<td>L-lysine diisocyanate ethyl ester</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>MBCP</td>
<td>Multiblock Copolymer</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Magnesium Sulfate</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NR</td>
<td>Nile red</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(D,L-lactide-co-glycolid)</td>
</tr>
<tr>
<td>Pluronic®</td>
<td>poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)</td>
</tr>
<tr>
<td>PPO</td>
<td>poly(propylene oxide)</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
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CHAPTER 1

INTRODUCTION
INTRODUCTION

The aim of this chapter is to provide insight into the principles behind the experimental work reported in this thesis. First, an introduction to thermogels and their therapeutic applications are given, followed by an overview of thermogel with acid labile linker which triggers biodegradation of polymer in response to a physiological pH change. The chapter concludes by discussing the advantages the multiblock copolymers with pH/temperature dual sensitivity present in targeted drug delivery therapy.

Therapeutic interventions employing smart bio-receptive materials that are sensitive to biological signals or pathological idiosyncrasies are appealing therapeutic platforms\textsuperscript{1-4}. Bio-responsive drug delivery systems are able to release therapeutic molecules in response to either distress signals originating from diseased tissues or actuated by physiological environment such as change in pH, temperature, ionic strength, secretion of enzymes, activity of microbes, and generation of reactive oxygen species (ROS)\textsuperscript{2,5-9}. Among the many stimuli responsive materials, thermogels have gained attention as a potential medium of drug delivery due to their possibility as minimally invasive implantation\textsuperscript{10,11}.
TEMPERATURE SENSITIVE POLYMER

A thermogel is an aqueous solution of polymer which undergoes phase transition from solution to gel/solid as temperature changes\textsuperscript{12,13}. There has been considerable interest in utilizing the unique properties of thermogel for various biomedical applications, some of which include pseudo-bone thermogel engineered with Poly propylene fumarate, PEG/polyester for treatment of diabetes, glycol chitosan for biomedical material, PLGA-PGE-PLGA for glaucoma treatment and PEG-L-PA for stem cell delivery\textsuperscript{14-20}. While most of the active research is limited to lab research, OncoGel (Protherics) is the only thermogel based product that has found success for clinical application and is in the Phase II of the clinical trials\textsuperscript{21}. OncoGel is a biodegradable PLGA/PEG polymeric cancer-targeting drug.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{thermogel_phase_transition.png}
\caption{Phase transition of thermogel. (a) As temperature increases, polymer strains aggregate and form micelle like components. (b) At low temperature (4 °C) the polymer solution is in solution phase, and at high temperature (37 °C) the solution changes to gel/solid.}
\end{figure}
delivery formulation that provides sustained release of paclitaxel at the site of action\textsuperscript{22}. Naked DNA delivery system developed based on Pluronic\textsuperscript{®} was also reported\textsuperscript{23}. Pluronics\textsuperscript{®}, also known as Poloxamers, are polymers composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock which undergo temperature induced physical gelation at a certain concentration\textsuperscript{24}.

**PLURONIC\textsuperscript{®} P104**

Pluronics are triblock copolymers composed of one hydrophobic block (PPO) in the center with two hydrophilic strains (PEOs) that respond to temperature change\textsuperscript{24}. The most widely accepted gelation theory of Plurnoic\textsuperscript{®} is micelle packing (Figure 1. 1. (a))\textsuperscript{25}. At a particular concentration of polymer in the aqueous medium, the polymer strain aggregates and forms micelle like structure\textsuperscript{26}. As the temperature rises the reactivity of the polymeric strain changes and forms gel (Figure 1. 1. (b)). Pluronic\textsuperscript{®} solution forms thermal reversible gels\textsuperscript{25,27}. As temperature decreases, packed micelle-like-structures untangle into free strains and the gel becomes solution (Figure 1. 2.). Pluronic\textsuperscript{®} P104 was selected as a

![Figure 1. 2.](image)

**Figure 1. 2.** Pluronic\textsuperscript{®} P104 is composed of 61 PPO segments with 27 PEO segments on both ends. The thermal gelation of Pluronic\textsuperscript{®} is reversible. As temperature decreases, the gel-sol phase transition occurs.
component for MBCP synthesis because of its gelation property. The gelation property is closely related to the length of hydrophobic and hydrophilic components of the polymer. Pluronic® P104 has molecular weight of 5,900 Da and PPO/PEO ratio of 1.15. Letter ‘P’ of P104 represents its physical state at room temperature, paste, and the number 104 represents the proportion of hydrophobic strains and hydrophilic strains. The multiplication of first two digits by 300 represents the molecular weight of the hydrophobic portion (PPO) and the multiplication of the last one digit by ten represents percentage of hydrophilic segments (PEO) of Pluronics®. Pluronic® P104 has 3,000 Da PPO segments with 40% composition of PEO. Despite the many advantages of Pluronics, it is not suitable for long-term drug delivery application because of its short gel status (1<day) in physiological condition. The aim of this study is to design a durable and biodegradable thermogel with disease-responsive drug release attributes by introducing multiple sensitivities to the characteristics of a thermogel.

**PH OF PHYSIOLOGICAL ENVIRONMENT**

The normal physiological pH of human tissue is known to be neutral (7.4~7.6) but the value varies by region. Nasal cavity and lysosome are known to have acidic pH condition (average pH 6.0 ~ 6.5). Change in pH is a characteristic manifestation of a disease situation and it has been observed that conditions of inflammation and cancer are often accompanied with change in pH of the affected tissue. The tumor cells tend to have a wider range of pH from 5.0 to 7.6. The study done by Wike-Hooley et al. reported that the tumor tissues are more acidic than healthy tissues. In this direction,
biodegradable polymers that are sensitive to temperature and pH have gained particular attention as an effective biomaterial that are liquid below body temperatures and provide controlled drug release by forming in situ gels at body temperature.

**PH INDUCED HYDROLYSIS OF KETAL LINKER**

Acetal and ketal group both undergo acid catalyzed hydrolysis but the relative hydrolysis rate of ketal linkages is up to 1,000-fold faster than acetal linkages\textsuperscript{36-39}. Briefly, when an oxygen in ketal is protonated, the lone pair of electrons on the other oxygen attacks the protonated oxygen. Then water attacks the carbonyl carbon to give hemiketal and a hydroxyl group. The lone pair of hydroxyl group attacks the oxygen on the other side of hemiketal and result in acetone and hydroxyl group\textsuperscript{40}. Previously synthesized temperature/pH dual sensitive polymer with acetal linkage showed complete degradation in 4 days at pH 5.0 and retention of acetal linkage for 30 days at pH 7.4\textsuperscript{41}. To enhance the response speed to a changed environment, ketal linkage was chosen instead of acetal linker.

\textbf{Figure 1.3.} Acid-triggered hydrolysis of ketal linker. The ketal hydrolysis is up to 1,000 folds faster than acetal hydrolysis.
DESIGNING THE SIMPLE SYNTHESIS

The simple one-pot synthesis was achieved using diisocyanates. Isocyanates are electrophilic functional group composed of -N=C=O. The conjugation between amine and hydroxy requires catalytic agents such as NHS, CDI, EDC and 4-NPC/pyridine and followed by multiple washing steps afterwards\textsuperscript{40,42,43}. Diisocyanate has two isocyanate groups which can actively react with nucleophiles. Nucleophilic hydroxyl and amine groups rapidly react with electrophilic isocyanates without a presence of catalytic agent and reduce the purification steps\textsuperscript{42}. One end of diisocyanate reacts with amine to form urea and the other diisocyanate reacts with hydroxyl to form urethane. They ultimately create long chains of multiblock copolymer\textsuperscript{44} (Figure 1.4).

\begin{equation}
\begin{array}{c}
R_1\text{N}^\equiv\text{C}=O \\
\text{(a)} \\
\text{(b)} \\
R_1\text{N}=\text{C}=O \\
\text{N}^\equiv\text{C}=O \\
R_1\text{N}\text{H} \\
\text{O} \\
\text{O} \\
\text{R}_3 \\
\text{R}_3 \\
\end{array}
\end{equation}

Figure 1.4. Isocyanate reaction with (a) amine to form urea and (b) hydroxyl to form urethane.

Two non-toxic diisocyanates were chosen in this experiment\textsuperscript{45}: L-lysine diisocyanate ethyl ester (LDI) and 1,6-diisocyanatohexane (HDI) (Figure 1.5.). The reactivity of the isocyanate is influenced by the steric hindrance of adjacent complex. HDI is structurally symmetrical so that isocyanates at the both ends have equal strength of reactivity. LDI, on the other hand, has an ethyl ester group adjacent to an isocyanate group which differentiates the reactivity of two isocyanates. The isocyanate group adjacent to ethyl ester has lower reactivity over the other isocyanate group. This allows successful
conjugation of LDI-BAP-LDI complex. HDI, on the other hand, has a symmetrical structure which gives equal chance for both isocyanates to react with nucleophiles.

![LDI and HDI](image)

**Figure 1.5.** L-lysine ethyl ester diisocyanate (LDI) and 1,6-diisocyanatohexane (HDI)

**ORGANIZATION OF THE THESIS**

In this work, we synthesized and characterized acid labile temperature sensitive polymers by simple one-pot two step reaction to overcome previous methods which require catalytic agents, multiple addition steps and numerous purification steps. The synthesized pH/temperature dual sensitive multiblock copolymers were prepared to target acidic environments such as nasal cavities, tumor tissues and lysosomes. The designed polymer retains drugs in normal neutral pH condition, but ketal linkage breaks into acetone and Pluronic® unimer blocks and release drugs adsorbed in the hydrophobic cores in acidic environment.

**Chapter 2** proposes simple one-pot synthesis of pH/temperature dual sensitive multiblock copolymer and characterization. The general characteristics of the polymer such as molecular weight, polydispersity index, and critical micelle concentration are described in
this chapter. The methods to determine the temperature sensitivity and pH sensitivity of polymer are included.

Chapter 3 includes synthesis of multiblock copolymers with modification and characterization. The general characterization method including molecular weight, CMC, and sol-gel phase transition temperature are tested by the same method from Chapter 2. Acid-triggered drug release of multiblock copolymers was evaluated using nile red and irinotecan and their potential application in tumor targeted drug delivery system is included.
CHAPTER 2: SYNTHESIS AND CHARACTERIZATION OF PH/TEMPERATURE DUAL SENSITIVE POLYMER BY METHOD 1
EXPERIMENTAL

Materials

N-(2-hydroxyethyl)phthalimide, Triethylamine, Acetyl chloride, Benzene, 2-methoxypropene and L-lysine diisocyanate ethyl ester were obtained from Sigma-Aldrich (St. Louis, MO). 1,6-diphenyl-1,3,5-hexatriene and anhydrous dichloromethane were purchased from Acros Organics (New Jersey, NJ). Pluronic® P104 was kindly supplied by BASF (Ludwigshafen, Germany) and was dried by azeotropic distillation in benzene before use. All other chemicals were purchased from Thermo Fisher Scientific (Hampton, NH) and were used without further purification.

Preparation of Multiblock Copolymer

Synthesis of ketal linker, 2,2-bis(aminoethoxy)propane

BAP was synthesized by a two-step reaction involving 2-methoxypropene and N-(2-hydroxyethyl)phthalimide in the presence of p-toluene sulfonic acid as a catalyst as it was reported previously^{38,46}. Briefly, N-(2-hydroxyethyl)phthalimide (7.846 mmol, 1 eq.) was dried with 200 mL of dry benzene by azeotropic distillation. To the vigorously stirring solution was slowly added 2-methoxy propene (7.846 mmol, 1 eq.) at 0°C along with p-toluene sulfonic acid. After an hour of stirring at 0 °C, the solvent was evaporated over a period of 3 h to push the reaction forward by removing methanol. The reaction mixture was cooled to room temperature and TEA (12 mL) was added to quench the reaction. To
purify the reaction mixture acetyl chloride (3.4 mL) in dichloromethane was slowly added and stirred overnight. Then the mixture was precipitated in excess amount of hexane and recrystallized in ethyl acetate to collect the light-yellowish powder (yield: 45%). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.16 (s, 6H), $\delta$ 3.41 (t, 4H, $J = 6$), $\delta$ 3.62 (t, 4H, $J = 6$), $\delta$ 7.77 (m, 8H)

In the second step, phthalimide groups were deprotected by reflux in a 6 N sodium hydroxide solution overnight. The solution was extracted with CHCl$_3$/iPrOH (1/1) mixture three times and the collected organic layer was dried with MgSO$_4$. The organic layer was dried in vacuo and completely dried via azeotropic distillation with hexane to yield light yellow oil (yield: 40%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.35 (s, 6H), $\delta$ 1.55 (s, ~4H), $\delta$ 2.82 (t, 4H, $J=5.4$ Hz), $\delta$ 3.44 (t, 4H, 5.4 Hz)

Synthesis of MBCP-0

Polymerization was performed by a one-pot two-step reaction. In the first step, dried BAP (1 eq.) in anhydrous DCM was added dropwise to a solution of L-lysine ethyl ester diisocyanate (2 eq.) in DCM at -20 ºC. After 24 h stirring at room temperature, a solution of dried Pluronic® P104 (1 eq.) in benzene was added to the reaction mixture and stirred in the presence of dibutyltin dilaurate as a catalyst. At the end of the second step, the reactant was filtered and solvent was evaporated in reduced pressure, and finally precipitated in excess amount of petroleum ether. The precipitates were further purified with two cycles of heat-induced precipitation in slightly basic water. Collected polymer was lyophilized and stored at -20 ºC.
General Characterization

$^1$H-nuclear magnetic resonance (NMR) spectra of 2,2-bis(aminoethoxy)propane and MBCP-0 were recorded using Bruker Ultrashield 400 PLUS (Germany) by dissolving the samples in CDCl$_3$ at 400 MHz. Molecular weight of MBCP-0 was determined by Gel permeation chromatography (GPC) equipped with refractive index detector (Waters 2414), autosampler (Waters 717 Plus) and binary HPLC Pump (waters 1525), using Phenomenex Phenogel 10u 10E3A (300 X 7.8 mm) 10 micron column. THF was used as an eluent solvent at 1 mL min$^{-1}$ at 25 ºC and polystyrene standard samples of 1300 Da to 100 k Da were used to calibrate the results. Breeze 1.0 software was used to calculate the molecular weight and polydispersity index.

Determination of Critical Micelle Concentration

Critical micelle concentration (CMC) of MBCP was determined by a fluorescence dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH). From a 25% (w/v) polymer stock solution, 1 mL of sample solution with different concentration range from $10^{-5}$ to 5% (w/v) was prepared. Then, 10 µL of 0.4 mM DPH solution in MeOH was added to each solution and incubated for 24 h in a dark room. The absorbencies of the solutions were measured at wavelengths 377 and 391 nm with Genesys 6 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA)
Thermal gelation property

Test tube inverting method

From a 25% stock solution of MBCP-0, polymer solutions with concentrations of 10, 15 and 20% (w/v) were prepared and incubated overnight. The solutions were then placed in 1 ml glass vial 500 μL each. The sample vials were placed in water bath and temperature was adjusted gradually from 10 to 75 ºC. The temperature was raised 3 ºC at a time and sol-gel transition of solution was monitored by inverting the sample vial after the sample was settle at the temperature for 10 min. The temperature at where polymer solutions stopped flowing was recorded as a sol-gel transition temperature and where polymer gel precipitates (separated from aqueous medium) is recorded as gel-sol transition temperature.

Falling ball method

For the determination of dynamic viscosity, a solution of MBCP-0 at 20% (w/v) concentration was prepared and incubated overnight. Then 700 μL of solution was placed in an NMR tube with diameter of 4 mm and kept at 10 ºC for 15 min. The steel ball with diameter (D) of 2 mm and density of 7.97 g mL⁻¹ was dropped through the polymer solutions and the time (t) required to travel a specific distance (3 cm) was recorded with a temperature increment of 2 ºC. The polymer solution was incubated for 15 min per increment. The dynamic viscosity of polymer solutions (μ) was determined by following the Stokes equation⁴⁷,⁴⁸:

\[ \mu = \frac{(\gamma_s - \gamma_t)D^2}{18\nu} \]

The specific gravity of the ball (γ_s) was calculated by multiplying the density of the ball with the acceleration due to gravity (g =980 cm s⁻²). The specific gravity of the polymer
solution was determined by multiplying the density of the polymer solution ($\approx 1 \text{ g cm}^{-1}$) by the acceleration due to gravity ($g$). The velocity ($v$) of the ball was calculated by dividing the distance the ball travelled by time.

**Rheology**

Rheological property was studied using a HR-2 hybrid rheometer (TA Instruments, New Castle, DE) equipped with 25 mm diameter parallel plate geometry. A 20% solution (w/w) of MBCP-0 was prepared to determine the rheology of the polymer. Sample was heated from 20 °C to 50 °C while being subjected to a constant strain (0.5%) and frequency (0.5 Hz). The storage modulus ($G'$) and the loss modulus ($G''$) were recorded as a function of temperature to determine gelation temperature.

**pH induced polymer degradation**

In 1 mL centrifuge tubes, 100 μL of 25% MBCP-0 solution was prepared and incubated at 4 °C for 24 h. To this incubated samples, 500 μL of pH 5.0 buffer solution was added and incubated for predetermined time. The samples were prepared the same way for pH 7.4 using PBS buffer. The collected samples were lyophilized and dissolved in DCM. The tubes were then centrifuged for 10 min and the supernatant was collected and dried. The dried samples were than dissolved in THF and filtered with 0.45 μm nylon filter. The changes in molecular weight of samples was monitored using GPC.
RESULTS AND DISCUSSION

Previous polymerization methods required catalysts and multiple addition/purification steps. The present work provides a simple one-pot synthesis of MBCP-0 using L-lysine diisocyanate ethyl ester. LDI was used as a bridge to connect two BAP and Pluronic®. Briefly, diisocyanates react with primary amines at both ends of ketal linker 2,2-bis(amoethoxy)propane with a molar ratio of 2:1 (Figure 2.1.). The unreacted other end of diisocyanates react with dried Pluronic® P104 to form a long chain of pH/temperature dual sensitive polymeric strain. The reaction mixture was filtered after diluted in DCM and precipitated in petroleum ether after solvents were dried in reduced pressure. The final product was purified by heat induced precipitation in water and lyophilization. The molecular weight and polydispersity index of polymer were studied with GPC as the result is shown in Table 2.1. The synthesized polymer had a molecular weight of 42,500 Da and PDI of 1.23 with approximately 5~6 Pluronic® P104 repeating unit.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$</th>
<th>PDI</th>
<th>CMC</th>
<th>Number*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic® P104</td>
<td>7100 ± 110</td>
<td>1.02</td>
<td>0.300 ± 0.009</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(5900)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBCP-0</td>
<td>42500 ±1200</td>
<td>1.23</td>
<td>0.045 ± 0.009</td>
<td>5.98</td>
</tr>
</tbody>
</table>

Table 2.1. General characteristics of MBCP-0.
*: number of repeating Pluronic® P104 unimer
Figure 2. Synthetic scheme of MB-CP-0. BAP was reacted with LDI for 24 h. To the reaction mixture, dried Pluronic® was added with dibutyltin dilaurate as a catalyst and stirred for 24 h. The final product was collected by precipitation in petroleum ether.
The $^1$H NMR was used to confirm successful conjugation of three components. (Figure 2.2.) The peaks at 1.35 ppm and 4.25 ppm is related to ethyl group (-CH$_2$-CH$_3$) of LD1. The chemical shifts of 3.64 ppm, 3.54 ppm, 3.42 ppm, 1.13 ppm is attributed to the existence of Pluronic® P104. The methyl group of BAP was supposed to appear around 1.20 – 1.30 ppm but overlapped with a shift of methyl group on PPO (-CH$_3$) after synthesis.

The critical micelle concentration (CMC) is a concentration of surfactant above which polymer strains aggregate and form micelle-like structure to lower the surface tension$^{49}$. Pluronics® are triblock copolymers consisting of two hydrophilic side chains of poly(ethylene oxide) with central hydrophobic poly(propylene oxide) chain. At high temperature, the polymer strain aggregates and forms micelle-like structure with
hydrophilic outer layer with hydrophobic core in which a hydrophobic drug can be adsorbed. The CMC value for MBCP-0 was determined by DPH dye solubilization method (Figure 2.3)\textsuperscript{28}. Hydrophobic dye DPH was solubilized in the hydrophobic core of the polymer micelle giving a fluorescence. The UV absorbance differences between 377 nm and 391 nm were recorded to plot graphs and a crosspoint of two lines was determined as a CMC value. MBCP-0 had CMC of 0.045\% (w/v) respectively, which is lower than CMC value of Pluronic® P104. The CMC value of Pluronic® P104 known is 0.300\% (w/v)\textsuperscript{28}. Decrease in CMC value after synthesis is closely related to the molecular weight change of the polymer. The decrease in CMC value of MBCP-0 compared to Pluronic® is due to the increase in chain length after the conjugation\textsuperscript{50}.

![Figure 2.3](image)

**Figure 2.3.** Critical Micelle Concentration was determined using DPH. The cross point of two lines is CMC of MBCP-0.
Figure 2.4. Phase transition temperature of MBCP-0 determined by test tube inversion method for 10 – 25% polymer solution.

Figure 2.5. Dynamic viscosity determined by falling ball method. The phase transition temperature is 29.4°C for 20% MBCP-0. Solution
The temperature sensitivity of MBCP-0 was determined using three different methods: test tube inversion method, falling ball method, and rheometer. The sol-gel and gel-sol phase transition temperatures were determined by the test tube inversion method. (Figure 2.4) The polymer solutions concentration ranges from 10% to 25% were prepared in glass vials. The vials were placed in a water bath and the thermal gelation as temperature changes was recorded. The 10% polymer solution had narrow gel phase from 37 ºC to 39 ºC and the 15% solution had 35 ºC to 45.5 ºC. The 20% and 25% showed sol-gel/gel-sol phase transition at 31/63.5 ºC and 27.4/69.0 ºC respectively. The change in sol-gel/gel-sol transition temperature was closely related to the concentration of polymer.

Dynamic viscosity of MBCP-0 was measured by falling ball method. Figure 2.5 demonstrates the results of the experiment. The speed of the metal ball passing through the polymer solution at different temperatures was calculated based on the distance the ball travelled and time taken to travel that distance. Then the dynamic viscosity was calculated.

![Figure 2.5](image)

**Figure 2.5.** Storage modulus G’(■) and loss modulus G”(▲) of MBCP-0. The point where two lines intercept (28.5 ºC) is the phase transition temperature.
based on Stokes equation. The substance with viscosity above 100 Pa*s is considered as a solid state and below 50 Pa*s is considered as a liquid state. The sol-gel phase transition temperature for 20% polymer solution, the point where two slopes cross, was 29.4 °C. Additionally, the effect of temperature on polymeric solution was studied using rheometer (Figure 2.6). The performance temperature was subsequently increased from 20 °C to 50 °C while being subjected to a constant strain and frequency. The storage modulus G’ represents the elastic portion of energy and the loss modulus represents the viscous portion of energy. The sol gel-phase transition temperature of MBCP-0, where storage modulus

![Figure 2.7](image)

**Figure 2.7.** pH triggered degradation study using GPC for MBCP-0. The result does not show significant molecular weight change of MBCP-0 at both pHs 5.0 and 7.4
(G’) and loss modulus (G”) crosses, was 28.5 °C which is consistent with the conclusion of the test tube inversion method and falling ball method. The sol-gel transition temperature of 20% MBCP-0 solution measured by three different methods fall within the 28 °C to 31 °C range. A solution of MBCP-0 had desired temperature sensitivity which is liquid at low temperature and gel near body temperature (37 °C). The change in temperature sensitivity of polymer from pluronic® is due to the change in molecular weight\textsuperscript{50,51}.

The acid triggered degradation of the polymer was studied with gel permeation chromatography (GPC). The polymer solution was incubated at two different pH conditions of 5.0 and 7.4 respectively. At predetermined time points, samples were collected from the incubated polymer solution to monitor the molecular weight change. Figure 2.7 shows the results of GPC experiments. The experimental results indicate that the polymer did not show any sign of molecular weight change and retained its molecular

![Design](image1)

![Result](image2)

**Figure 2.8.** Schematic explanation of MBCP-0 Synthesis. The synthetic addition order for pH/temperature dual sensitive polymer was designed
weight at both acidic (pH 5.0) and neutral (pH 7.4) condition for 30 days. Ketal linkers are very sensitive to acidic condition, so unchanged molecular weight cannot be explained. The polymer had targeted thermal-gelation temperature and targeted molecular weight except pH sensitivity.

While the result deviates from our expectations, a possible explanation for this failure could be due to the experimental design (Figure 2.8). In the first step, we predicted that when 1 equivalent BAP was slowly added to 2 equivalent LDI at -20 °C the LDI-BAP-LDI complex will form for further react with Pluronic®, but what was synthesized instead was a long chain of polymers alternating BAP and LDI forms at 1:1 ratio due to the good reactivity between amines and isocyanates52. Upon an addition of pluronic® to the reaction mixture, the left over 1 equivalent LDI reacts with hydroxyl (-OH) of pluronic® and forms polymer consisting of pluronic® and isocyanate alternating. The pluronic® and isocyanate alternating multiblock copolymer (MBCP-0) had 5 to 6 repeating pluronic® P104 blocks and increase in molecular weight gave MBCP-0 desired sol-gel phase transition temperature. The synthesized polymer MBCP-0 does not have pH-sensitive ketal linkers, and results in thermogel with desired phase transition characteristic, however, without pH sensitivity. The modification in method was necessary to synthesize polymers with pH and temperature dual sensitivity.
CONCLUSIONS

Novel pH/temperature dual-sensitive multiblock copolymer which responds to change in pH and temperature was designed. The multiblock copolymer (MBCP) which maintains its solution form at lower temperature and transitions to gel form at elevated temperature was successfully synthesized. The final product had expected temperature sensitivity but did not show any sign of acid triggered degradation.
CHAPTER 3:
SYNTHESIS AND CHARACTERIZATION OF PH/TEMPERATURE DUAL SENSITIVE POLYMER BY METHOD 2
(MODIFICATION TO METHOD 1)
EXPERIMENTAL

Materials

N-(2-hydroxyethyl)phthalimide, Triethylamine, Acetyl chloride, Benzene, 2-methoxypropene, L-lysine diisocyanate ethyl ester and 1,6-hexamethylene diisocyanate were obtained from Sigma-Aldrich (St. Louis, MO). 1,6-diphenyl-1,3,5-hexatriene and anhydrous dichloromethane were purchased from Acros Organics (New Jersey, NJ). Pluronic® P104 was kindly supplied by BASF (Ludwigshafen, Germany) and was dried by azeotropic distillation in benzene before use. All other chemicals were purchased from Thermo Fisher Scientific (Hampton, NH) and were used without further purification. BAP was synthesized as the method describe in Chapter 2

Synthesis of MBCP1 and MBCP2

Polymerization was performed by a one-pot two-step reaction. In the first step, dried Pluronic® P104 (1 eq.) in dried benzene was added dropwise to a solution of diisocyanate (2 eq.) in dichloromethane at -20 ºC. After 24 h stirring at room temperature, a solution of BAP (1 eq.) in dichloromethane was added to the reaction mixture. At the end of the second step, solvent was evaporated in reduced pressure and precipitated in excess amount of petroleum ether. The precipitates were further purified with two cycles of heat-induced precipitation in slightly basic water. Collected polymer was lipolyzed and stored at -20 ºC.
General Characterization of MBCP1 and MBCP2

$^1$H-nuclear magnetic resonance (NMR) spectra of 2,2-bis(amo-noethoxy)propane and MBCP were recorded using Bruker Ultrashield 400 PLUS (Germany) by dissolving the samples in CDCl$_3$ at 400 MHz Molecular weights of MBCP1 and MBCP2 were determined by Gel permeation chromatography (GPC) equipped with refractive index detector (Waters 2414), autosampler (Waters 717 Plus) and binary HPLC Pump (waters 1525), using Phenomenex Phenogel 10u 10E3A (300 X 7.8 mm) 10 micron column. THF was used as an eluent solvent at 1 mL min$^{-1}$ at 25 °C and polystyrene standard samples of 1300 Da to 100 k Da were used to calibrate the results. Breeze 1.0 software was used to calculate the molecular weight and polydispersity index. Agilent Cary 660 FTIR spectrophotometer (Santa Clara, Ca, USA) was used to study Fourier transform infrared (FT-IR) spectroscopy of urea and urethane bond formation between BAP & isocyanates and isocyanates & Pluronic®.

Determination of Critical Micelle Concentration

Critical micelle concentrations (CMC) of MBCP1 and MBCP2 were determined by a fluorescence dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH). From a 25% (w/v) stock solutions of two polymers, 1 mL of sample solution with different concentration range from $10^{-5}$ to 5% (w/v) was prepared. Then, 10 μL of 0.4 mM DPH solution in MeOH was added to each solution and incubated for 24 h in a dark room. The absorbencies of the solutions were measured at wavelengths 377 and 391 nm with Genesys
6 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The point where the line crosses is determined as CMC.

**Thermal gelation property**

*Test tube inverting method*

From a 25% stock solution of MBCP1 and MBCP2, polymer solutions with concentrations of 10, 15 and 20% (w/v) were prepared and incubated overnight. 500 μL of each solution was then placed in 1 ml glass vial. The sample vials were placed in water bath and temperature was adjusted gradually from 10 to 75 °C. The temperature was raised by 3 °C at a time and sol-gel transition of solution was monitored by inverting the sample vial after allowing the sample to settle at that temperature for 10 min. The temperature at which polymer solutions stopped flowing was recorded as a sol-gel transition temperature and where polymer gel precipitates (separated from aqueous medium) is recorded as gel-sol transition temperature.

*Falling ball method*

For the determination of dynamic viscosity, solutions of MBCP1 and MBCP2 at 20% (w/v) were prepared and incubated overnight. Then 700 μL of each solution was placed in an NMR tube with diameter of 4 mm and kept at 10 °C for 15 min. The steel ball with diameter (D) of 2 mm and density of 7.97 g mL⁻¹ was dropped through the polymer solutions and the time required to travel a specific distance (3 cm) was recorded with a temperature increment of 2 °C. The polymer solution was incubated for 15 min per
increment. The dynamic viscosity of polymer solutions (μ) was determined by following the Stokes equation:

\[ \mu = (\gamma_s - \gamma_t)D^2/18v \]

The specific gravity of the ball (γs) was calculated by multiplying the density of the ball with the acceleration due to gravity (g = 980 cm s\(^{-2}\)). The specific gravity of the polymer solution was determined by multiplying the density of the polymer solution (≈ 1 g cm\(^{-1}\)) by the acceleration due to gravity (g). The velocity (v) of the ball was calculated by dividing the distance the ball travelled by time. (n = 3)

**pH induced polymer degradation**

The changes in molecular weight of polymers at different pH was monitored using gel permeation chromatography (GPC) equipped with refractive index detector (Waters 2414), autosampler (Waters 717 Plus) and binary HPLC Pump (waters 1525), using Phenomenex Phenogel 10u 10E3A (300 X 7.8 mm) 10 micron column. THF was used as an eluent solvent at 1 mL min\(^{-1}\) at 25 °C. The samples were prepared in 1 mL centrifuge tubes. 100 μL of 25% MBCP1 and MBCP2 solutions were placed in the tubes and incubated at 4 °C for 24 h. To this incubated samples, 500 μL of pH 5.0 buffer solution was added and again incubated for predetermined time. A similar procedure was followed to prepare samples for pH 7.4 using pH 7.4 PBS buffer. The collected samples were lyophilized and dissolved in DCM. The tubes were than centrifuged for 10 min and the supernatant was collected and dried. The dried samples were than dissolved in THF and filtered with 0.45 μm nylon filter.
**pH triggered drug release studied using NR**

For drug release study of polymers, nile red (NR) was chosen as a hydrophobic model drug. Solutions of MBCP1 and MBCP2 were prepared at a concentration of 0.25\% (w/v). Then 50 μL of 0.1 mg/mL NR solution in ethanol was added to 5 mL of each polymer solution. The polymer solutions were incubated for 24 h in dark place. To a 950 μL solution of MBCP solution, 50 μL of 2 M phosphate buffer solutions at pHs 5.0 and 7.4 were added each and fluorescence intensity change over 24 h were measured. A solution of Pluronic® P104 was prepared by the same method and treated with PBS buffers at pHs 5.0 and 7.4 to determine the baseline. The fluorescence intensity of NR in test solutions were recorded with Perkin-Elmer LS 50 at excitation 550 nm and wavelengths from 570 nm to 750 nm.

**In vitro drug release studies using Irinotecan**

Irinotecan was selected as a model drug the releaser properties of the synthesized polymer under pH and temperature triggered conditions. Solutions of MBCP1 and MBCP2 at 20\% (w/w) were prepared at 4 °C to load irinotecan with final concentration of 1 mg/mL. To 2 mL centrifuge tubes, 250 μL of polymer solutions were placed each and kept at 4 °C. After 1 h, tubes were placed in 37 °C bath for 15 min for gelation. One milliliter of each phosphate buffer solutions at pH 5.0 and 7.4 were added to each vial. The buffers were pre-heated to the same temperature (37 °C). The release medium was collected and replaced with new PBS solution after predetermined time while maintaining the incubation temperature at 37 °C. The collected samples were examined by UV spectroscopy at 256 nm.
RESULTS AND DISCUSSION

Acid-labile biodegradable temperature sensitive polymers were synthesized and characterized in this study. The polymers were synthesized by simple one-pot synthesis using diisocyanate (L-lysine diisocyanate ethyl ester or 1,6-diisocyanatohexane) to conjugate thermodgel Pluronic® P104 and ketal linker 2,2-bis(aminoethoxy)propane as it is described in Figure 3.1. The reaction was performed in anhydrous condition to avoid early termination of polymerization. The highly reactive diisocyanate was chosen as the bonder to connect Pluronic® and BAP without using a catalytic agent. In the presence of water,

\[
\begin{align*}
\text{Pluronic}® & \quad \text{OCN-R-NCO} \\
\text{Pluronic}® & \quad \text{R}_1: \quad \text{OCOCH}_2\text{CH}_3 \\
\text{Pluronic}® & \quad \text{R}_2: \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2
\end{align*}
\]

Figure 3.1. Synthetic scheme of MBCPs. Pluronic® was activated with diisocyanates and then BAP was added. The mixture was stirred for 24 h and collected by precipitation in petroleum ether
Figure 3.2. Synthesis and purification of MBCPs. The retention time was determined by GPC where (a) Pluronic® P104 (b) after precipitation in petroleum ether and (c) after heat-induced precipitation in water.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$</th>
<th>PDI</th>
<th>CMC</th>
<th>Number*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic® P104</td>
<td>7100 ± 110 (5900)</td>
<td>1.02</td>
<td>0.300 ± 0.009</td>
<td>-</td>
</tr>
<tr>
<td>MBCP1</td>
<td>41760 ±1000</td>
<td>1.30</td>
<td>0.049 ± 0.009</td>
<td>5.88</td>
</tr>
<tr>
<td>MBCP2</td>
<td>41230 ±1200</td>
<td>1.34</td>
<td>0.045 ± 0.009</td>
<td>5.80</td>
</tr>
</tbody>
</table>

Table 3.1. The general characterization of MBCP 1 and MBCP2

number*: number of repeating Pluronic® P104 unimer
isocyanate undergoes hydrolysis and results in carbon dioxide and amine derivative which terminates further conjugation with BAP. Briefly, dried Pluronic® P104 was reacted with diisocyanate at a molar ratio of 1:2 for 24 h. At the end of the first step, BAP was reacted at a molar ratio of 1:1 between BAP and Pluronic® P104. The reaction mixture was stirred for 24 h and upon completion of the synthesis, solvents were dried under reduced pressure. The product was collected by precipitation twice in excess amount of petroleum ether. Unreacted and/or early terminated Pluronic monomers were washed via temperature-induced precipitation in water to result in MBCP1 and MBCP2 with molecular weights ($M_n$) of 41,760 Da and 41,230 Da with 5~6 Pluronic® P104 block unimer units (Table 3.1). The molecular weights and polydispersity index (PDI) of polymers were characterized using gel permeation chromatography (GPC). The purification of the final product was monitored with GPC and the result is shown in Figure 3.2. The PDI values of the final products were 1.30 and 1.34. The pH of water used in purification step was adjusted to 7.4 with sodium bicarbonate to avoid the hydrolysis of ketal linker in naturally acidic di water.

The chemical configurations of MBCP1 and MBCP2 were confirmed by $^1$H NMR spectra. The $^1$H NMR spectrum of MBCP1 is shown in Figure 3.3. (a) and of MBCP2 is shown in Figure 3.3. (b). The major peaks were assigned and integration was taken to determine the molar ratio. Figure 3 (a) shows the proton peaks of ethyl group of LDI at 1.30 ppm and 4.30 ppm and methyl group of BAP at 1.35 ppm. The ratio of integration taken at 1.35 ppm (BAP) and 1.30 ppm & 4.30 ppm (LDI) was 3:5 which indicates a 1:2 conjugation ratio between BAP and LDI and confirms successful synthesis of MBCP1.
Figure 3.3. $^1$H NMR spectra of (a) MBCP1 and (b) MBCP2 in CDCl$_3$. 
In Figure 3 (b), peaks for six methyl groups of 1,6-diisocyanatohexane correspond to 1.48 ppm, 1.72 ppm, and 3.15 ppm. The methyl (-CH₃) groups of BAP peak was clearly shown at 1.33 ppm to confirm successful conjugation of BAP and Pluronic® P104.

Fourier transform infrared spectroscopy (FTIR) was performed to determine the successful conjugation of BAP(amine)-isocyanate and isocyanate-Pluronic®(hydroxy). Figure 3.4 (c) is the FTIR spectrum for Pluronic® P104 and used as reference for synthesized MBCP1 and MBCP2. From Figure 3.4 (a) and (b), we can infer that the absorption bands $\nu = 1714$ cm$^{-1}$ and $\nu = 1619$ cm$^{-1}$ correspond to the urea/urethane component formed from conjugation of isocyanate with amine and hydroxy.

![FT-IR spectra](image)

Figure 3. 4. FT-IR of (a) MBCP2 (b) MBCP1 and (c) Pluronic P104. New bands appeared at $\nu = 1600-1750$ cm$^{-1}$ which infer C=O of urea/urethane.
In aqueous medium, surfactant strains aggregate to form micelle like structures to minimize the surface tension. As the polymer strain aggregates, the hydrophobic dye DPH incorporates into the hydrophobic core of micelle and exhibits UV absorbance\textsuperscript{28}. The values of CMC for MBCP1 and MBCP2 was determined by DPH dye solubilization method (Figure 3.5). UV intensity differences between 377 nm and 391 nm were recorded to plot graphs and a crosspoint of two lines was determined as a CMC value. MBCP 1 and MBCP 2 had CMC of 0.049\% (w/v) and 0.045\% (w/v) respectively, which is lower than CMC value of Pluronic® P104. The CMC value of Pluronic® P104 known is 0.300\% (w/v)\textsuperscript{28}. The decrease in CMC values of MBCP1 and MBCP2 is due to increase in molecular weight of polymer\textsuperscript{50}. The decrease in CMC value may indicate a change in temperature stimulated sol-gel transition behavior.

**Figure 3.5.** Critical micelle concentration determined by DPH solubilization method for MBCP1 and MBCP2. The cross point where two slopes meet is the CMC value.
The temperature sensitivities of MBCP1 and MBCP2 were evaluated by test tube inversion method and falling ball method. The sol-gel and gel-sol transition temperatures of MBCP1 and MBCP2 polymer solution of different concentrations were determined by test tube inversion method (Figure 3.6.). The polymer solutions with concentrations of 10, 15, 20 and 25% (w/w) were prepared and placed in a water bath and the vials were inverted to check the flowability at different temperature. A solution of 10% MBCP1 did not have sol-gel phase transition. Instead, the solution formed a sluggish solution and precipitated out as the temperature increased. The MBCP2 solution at the same concentration formed turbid gel at 43.3 ºC and retained shape until 54.0 ºC. The 15% solutions of polymers had phase transition temperature near body temperature (gel-sol/sol-gel: 36.0/55.6 ºC and 38.7/59.0 ºC). Solutions of 20% MBCP1 and MBCP2 had sol-gel transition temperature of 30.7 ºC and 32.6 ºC and gel-sol transition temperature of 62.2 ºC and 63.5 ºC respectively.

Figure 3.6. Phase transition graph of MBCP1 and MBCP2 solutions range from 10 to 25%. Test tube inversion method was performed to determine sol-gel and gel-sol transition temperature.
Solutions of 25% MBCP1 and MBCP2 were at a gel phase from 26.5 °C to 69.3 °C and from 25.5 °C to 71.2 °C each respectively. The gelation temperature of MBCP2 was slightly higher than MBCP1 over all.

The dynamic viscosity of the polymers was studied by falling ball method as a function of temperature. The result of the experiment is shown in Figure 3.7. The time taken for a steel ball to vertically travel through the predetermined length of polymer solution was recorded with temperature increments of every 2 °C. Solutions of 20% MBCP1 and MBCP2 were placed in 4 mm NMR tube with 3 cm marks. The specific gravity of the ball, the specific gravity of the polymer solution, the velocity of the falling ball and the diameter of the ball were determined to calculate the dynamic viscosity (\(\mu\)). A sample with the viscosity value below 50 Pa*s\(^{-1}\) is considered to be in the "solution state" and above 100 Pa*s\(^{-1}\) is considered to be in "gel/solid state"\(^{53}\). For a low viscous solution,

\[\text{Figure 3.7. Dynamic viscosity of MBCPs was studied by falling ball method. The velocity of the ball traveling through a 20% polymer solution as temperature changes (range from 10 to 40 °C) was recorded and converted to dynamic viscosity (Pa-s).}\]
the ball travels through the solution rapidly and as the viscosity increases, the time taken for the ball to travel through the medium increases. The graph shows a sharp change in slope at the 28 °C to 32 °C range, indicating sol-gel transition of MBCP 1 and MBCP 2. The gelation temperature of MBCP 1 and MBCP 2 were determined based on falling ball method was 29 °C and 32 °C.

The sol-gel transition temperature of Pluronic® P104 solution (<20% w/v) is above 65 °C which is higher than body temperature. The gelation temperature of MBCP1 and MBCP2 were evaluated by two methods and the phase transition temperature of 20% polymer solutions were within 29 °C to 32 °C range which is lower than the body

![Figure 3. 8. Acid catalyzed degradation of MBCP1 studied with GPC. MBCP1 showed fast degradation as within 1 h at pH 5.0 and retained molecular weight for 48 h at pH 7.4.](image-url)
temperature. There was no significant difference in sol-gel transition found between MBCP1 and MBCP2 because the phase transition temperature of the polymer is largely associated with the hydrophobic chain length and the molecular weight of the polymer\textsuperscript{44,55}. Both polymers had 5 to 6 repeating Pluronic® P104 blocks which gave two polymers similar temperature sensitivity.

The molecular weight change of polymers at different pH was visualized with GPC. The results of the experiment are shown in Figure 3.8. The polymer samples were incubated at two different pH conditions of 5.0 and pH 7.4 respectively, for predetermined time and subsequently analyzed with GPC. At pH 5.0, the polymer showed almost complete degradation after first 1 h. The peak shift towards the left indicates reduction in molecular weight for GPC. After 1 h at pH 5.0, most peak has shifted towards the left, indicating a component which has similar molecular weight as pluronic has formed. The rate of degradation is much faster than the previously reported pH/temperature dual sensitive polymer with acetal linkage which endured complete degradation after 4 days\textsuperscript{41}. This is due to the faster hydrolysis rate of ketal over acetal which could be faster by 1,000 folds\textsuperscript{56}. The rapid response to the stimuli of MBCPs gives them potential as targeted drug delivery carriers.

Nile red (NR) fluorescence spectroscopy was used to monitor the drug release behavior of the polymeric micelle at different pHs. The hydrophobic dye NR is solubilized in the hydrophobic core of the polymer to show fluorescence but quenches light when it is released to the aqueous medium\textsuperscript{57,58}. The intensity of NR is used as an indicator to measure the amount of drug released from the polymeric micelle. In acidic medium, the ketal linkers
Figure 3.9. Nile red release study (a) change in fluorescence intensity of MBCP2 at pH 5.0 over 24 h. (b) NR release profile of MBCP1 and MBCP2 at pHs 5.0 and 7.4
connecting Pluronic® unimers cleave which increases the CMC and releases micelle-like structures of polymer strains. As NR is released into the aqueous medium, the fluorescent intensity decreases as is shown in Figure 3.9. (a). The concentration of polymer solution (0.25%) was selected below CMC of Pluronic® P104 (0.300%) to avoid the Pluronic® effect after complete degradation of MBCPs. Because the fluorescence intensity of NR may be affected by the dilution and buffer (strength, concentration) the same condition Pluronic® P104 solution with nile red was prepared in the same concentration and with the same buffers (pH 5.0 and 7.4). The intensity of prepared Pluronic® solution was measured and used to correct the baseline. As the result is shown in Figure 3.9. (b), a large amount of NR was released from solution of MBCP1 and MBCP2 solution at pH 5.0, while the solutions at pH 7.4 maintained fluorescence intensity with neglectable amount of reduction. Accordingly, it is clear that the hydrolysis of ketal linker that occurs at pH below 7.0 promotes the release of NR.

A correlated trend was observed for the drug release study from a hard gel. The release of irinotecan from polymeric gel of MBCP1 and MBCP2 was studied by UV visible spectrometry (Figure 3.10.). Irinotecan loaded 20% MBCP1 and MBCP2 solutions were placed in vials and incubated at 37 °C for gelation. To harden gels, the same temperature phosphate buffer solutions at pH 5.0 and 7.4 were placed without interrupting the gel. The release medium was collected after the predetermined time and tested using UV spectrometer. The amount of drug release was calculated from the absorbance values by preparing known standard solutions and dividing the absorbance with slope. The gels of MBCP1 and MBCP2 both showed complete release of drug in less than a day in an acidic
medium. At pH 7.4 the gels retained their structure and did not release the drug for 48 h and only after the pH of the release medium was changed to 5.0, the gels released the incorporated drug-load, thus exhibiting their pH dependent release behavior. The release rate of MBCP1 gel was slightly faster than MBCP2 in an acidic environment. The polyketal polymers tend to degrade by surface erosion as observed by the reduce in volume. The volume of the polymeric gel was reduced in an acidic medium but did not change at neutral pH.

**Figure 3.10.** pH-stimulated Irinotecan release profile of MBCP1 and MBCP2. Irinotecan was loaded to the gel state polymers and cultured at pH 5.0 and pH 7.4.
CONCLUSION

Novel pH/temperature dual-sensitive multiblock copolymers which respond to change in pH and temperature were designed and successfully synthesized via a simple one-pot reaction. Aqueous solutions of the polymers underwent gelation below body temperature (37°C) and degraded upon exposure to an acidic environment. MBCP1 and MBCP2 were able to release incorporated model drugs at pH 5.0 but retained the drugs at pH 7.4. The quantitative analysis confirmed temperature/pH dual sensitivity of the polymers. It can be inferred from the results that the synthesized MBCPs form an excellent bio-responsive drug delivery platform.
CHAPTER 4:

FUTURE RESEARCH PLAN
The temperature/pH dual sensitive polymers MBCP1 and MBCP2 were successfully synthesized and characterized for their release behaviour. However, upon degradation, ketal linker hydrolysis leads to the generation of acetone and pluronic unimer block. Thus, toxicity study for MBCPs is necessary towards establishing its efficacy and safety as an effective drug delivery system. Previous report on the toxic effect of MBCPs have reported it to be inert. The group developed microparticles with pH sensitive polymer consisting of BAP and electrophilic components. The particles, subjected to cell viability studies using macrophages, showed no significant toxicity up to concentration of 1 mg/mL. The particles were also tested for toxicity studies on the degradation by-products with no apparent evidence of toxic response\textsuperscript{38}. An inference can be established from the report of Fréchet et al. where the ketal linker BAP component is devoid of any significant toxic effect before/after the degradation below certain concentration. It can thus be hypothesized that MBCPs synthesised in this project will have better cell viability due to fewer number of BAP per polymer strain. Cytotoxicity of Pluronic\textregistered was reported by several research groups\textsuperscript{12,41}. According to these studies, the toxicity of pluronic\textregistered was concentration dependent so studies on cytotoxicity of synthesized multiblock copolymer and degraded by-product will expand the therapeutic application of MBCPs.


VITA

Hyung Kyung Lee was born in Seoul, South Korea on January 8, 1990. She is the daughter of Jun hyup Lee and In-ok Park. She graduated from University of Mississippi (Oxford, MS) where she obtained her Bachelor’s degree in chemistry in May of 2015. During her Bachelor’s program, she joined the research lab of Dr. Seongbong Jo at the Department of Pharmaceutics and Drug Delivery in the School of Pharmacy at The University of Mississippi as an undergraduate student researcher. August of 2015, she officially joined research group of Dr. Jo to pursue Master’s Degree in Pharmaceutical Sciences.

HONORS and FELLOWSHIPS

Summer Research Assistantship Awards, University of Mississippi 2016