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Pluronic-based dual-stimuli sensitive polymers capable of thermal gelation and pH-dependent degradation for *in situ* biomedical application

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Abstract

Thermo-sensitive hydrogels are considered ideal for applications in the biomedical fields for their biocompatibility, flexibility, tissue-like water content, and reversible gelation property. By adjusting sufficient hydrophilic-hydrophobic balance in block copolymer structure, thermogel's critical gelation temperature can be modified to be near the physiological temperature, which makes it an appealing candidate for *in situ* gel depot. In this study, we report successful syntheses of novel multiple block copolymer compounds, denoted as dual-stimuli sensitive polymers (DSSPs), by copolymerizing Pluronic® P104 (7,100 Da) and 2,2-bis(aminoethoxy)propane (BAP) using diisocyanate linkers, L-lysine ethyl ester diisocyanate (DSSP-1) and 1,6-hexamethylene diisocyanate (DSSP-2). Through effective elongation of polymer chain lengths (DSSP-1: 41,760 Da, DSSP-2: 41,230 Da), Pluronic® P104's reversible thermal gelation properties were enhanced, as demonstrated by lowered critical gelation temperatures (DSSP-1: 36°C, DSSP-2: 38.7°C; 15 wt. %) that is near the physiological temperature. Furthermore, integration of acid-labile BAP allowed rapid pH-dependent degradation of the polymer, which was displayed by gel permeation chromatography (GPC) and release profiles of Nile red and irinotecan from polymeric micelles and gels, respectively.

Graphical abstract

Integration of acid-labile ketal, BAP, into copolymerization of Pluronic® P104 yielded dual-stimuli sensitive polymer, DSSP, that is capable of reversible thermal gelation near physiological temperature and rapid pH-dependent degradation via acid-catalyzed hydrolysis.

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Introduction

In recent years, there has been a considerable interest in developing smart bio-responsive materials as therapeutic platforms in various biomedical fields, where drug delivery application has been highlighted for its capability to achieve selective delivery to target sites in response to physiological signals, such as changes in pH,^{1,2} temperature,^{3,4} redox capacity,^{5,6,7} and enzymatic activity.^{8,9,10} Among such platforms, polymeric hydrogels have particularly been recognized due to their advantageous characteristics such as biocompatibility, physical flexibility, and tissue-like high water content.¹¹ In light of this, various innovative approaches have been attempted to engineer effective hydrogel systems, where thermoresponsive hydrogel (thermogel) arose as a potent candidate to achieve minimally-invasive therapeutic interventions. As thermogels are capable of nearly instantaneous *in situ* solution-to-gel transition upon appropriate temperature change, they are considered ideal for direct injection to the desired administration site(s).

As critical gelation temperature (CGT) is the point where sol-gel phase transition occurs for thermogels, achieving CGT that is near the physiological temperature is considered essential in designing *in situ* gel systems. Unlike how permanent gel networks form by covalent intermolecular crosslinking, reversible thermogels form by crosslinking based on relatively weak physical interactions.^{12,13,14} To be specific, a dynamic equilibrium is present between hydrophilic and hydrophobic segments in reversible gels, where temporary gelation is driven by intermolecular hydrophobic interactions.^{12,13,14} As such, maintaining sufficient hydrophilic-hydrophobic balance in gel structure via effective block copolymer design became a prominent trend in the industry.

Accordingly, biomedical applications of thermogels have been exemplified by various block copolymer systems, some of which include: thermogels based on poly(propylene fumarate) (PPF) for bone tissue engineering,¹⁵ poly(ethylene glycol)-b-poly(L-alanine) (PEG-L-PA) for tissue engineering,¹⁶ PEG/polyester copolymers for controlled delivery of liraglutide,¹⁷ poly(lactic-co-glycolic acid)-PEG-poly(lactic-co-glycolic acid) (PLGA-PEG-PLGA) for

controlled delivery of activin receptor-like kinase 5 (ALK-5) inhibitors for glaucoma filtration surgery,¹⁸ PEG-1,4-bis(2-hydroxyethyl)piperazine (PEG-HEP) based polyether-polyurethane copolymers for on-demand intravaginal drug delivery,¹⁹ and Pluronic® block copolymers for therapeutic gene delivery.²⁰

In addition to temperature-sensitivity, more recently developed systems have evolved to furnish dual-stimuli sensitivity for amplified bio-responsiveness. For example, poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl) aminomethylstyrene]-b-poly-(ethylene glycol)-b-poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl) aminomethylstyrene] (PMNT-PEG-PMNT) has been synthesized as redox-active injectable gel (RIG), which demonstrated exceptional sustained release profile for anionic model drug.²¹ In another example, β -cyclodextrin-2-methacrylic acid-N,N'-methylene diacrylamide copolymer was synthesized for both pH- and temperature-responsive drug release, which was demonstrated using the model drug, atorvastatin.²² Furthermore, PEG-PPG-spiropyran block copolymer system, denoted as EPS, has also been reported to exhibit light- and temperature-sensitive reversible gelation.²³ As extensively demonstrated in aforementioned cases, providing dual-stimuli sensitivity to hydrogel systems has significantly broadened the scope of potential applications.

Among various biomedical platforms that aimed to furnish pH-sensitivity, acetal or ketal linkers that are prone to acid-catalyzed hydrolysis have widely been incorporated previously. One of the best known of such linkers is 2,2-bis(aminoethoxy)propane (BAP) linker, which is a bifunctional amino ketal linker that was first synthesized by Fréchet and co-workers to develop acid-degradable polyacetal microparticles for drug delivery.^{24,25,26} In further applications, BAP was used as a core crosslinking agent in block copolymer of methoxy triethylene glycol methacrylate (mTEGMA) and pentafluorophenyl methacrylate (PFPMMA), as well as block copolymer of acetylated glycosylated block and pentafluorophenyl (PFP)-activated ester block to fabricate pH-degradable nanogels for immunological applications.^{27,28}

In this study, BAP was copolymerized with Pluronic® P104, a thermosensitive triblock copolymer of PEG-PPG-PEG, via diisocyanate linkers to yield novel dual-stimuli sensitive polymers (DSSP) capable of thermoresponsive gelation as well as pH-dependent degradation. By purposefully elongating the chain length of Pluronic® through copolymerization, we expected to achieve lower gelation temperature due to enhanced intermolecular hydrophobic interactions. Furthermore, integration of BAP linker between alternating Pluronic® units provided rapid degradation pathway for the corresponding thermogels via acid-catalyzed hydrolysis. In order to effectively demonstrate potential applicability of DSSPs as injectable *in situ* drug delivery platform, dynamic viscosity of polymeric gel was investigated as a function of temperature and pH-dependent release of Nile red and irinotecan from polymeric micelles and gels, respectively, was simulated.

Experimental

Materials

N-(2-hydroxyethyl)phthalimide, triethylamine, benzene, 2,2-bis(aminoethoxy) propane, L-lysine ethyl ester diisocyanate (LEEDI) and 1,6-hexamethylene diisocyanate (HMDI) were

purchased from Sigma-Aldrich (St. Louis, MO). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and anhydrous dichloromethane (DCM) were purchased from Acros Organics (New Jersey, NJ). Pluronic® P104 was kindly supplied by BASF (Ludwigshafen, Germany) and was dried via azeotropic distillation in benzene before use. All other chemicals were purchased from Thermo Fisher Scientific (Hampton, NH) and were used without further purification.

Synthesis of DSSP-1 and DSSP-2

Two-step synthesis was carried out in one pot. After removing moisture from Pluronic® P104 (10 g; 1.69 mmol) via azeotropic distillation using benzene (majority distilled off), the solution was added drop-wise to diisocyanate (LEEDI or HMDI) (3 mmol) in 10 mL of DCM at -20 °C. The temperature of the mixture was then slowly brought up to room-temperature and it was left to stir for additional 24 hours. Next, BAP (3 mmol) dissolved in 5 mL of DCM was added to the same reaction mixture, which was left to stir at room-temperature overnight. The mixture was then concentrated under reduced pressure and the crude product was obtained via excess petroleum ether precipitation. Further purification was carried out via two cycles of heat-induced precipitation in 10 mM sodium bicarbonate solution and finished with one cycle in water to neutralize the pH. The resulting polymer product was lyophilized and stored at -20 °C in order to prevent hydrolysis. (Yield% = 84% for DSSP-1 and 89% for DSSP-2)

¹H NMR and GPC Characterizations of DSSP-1 and DSSP-2

The structures of DSSP-1 and DSSP-2 were characterized and confirmed via ¹H NMR (CDCl₃) using Bruker Avance 400 MHz spectrophotometer. Molecular weights (M_n) of DSSPs were determined by gel permeation chromatography (GPC) system (Waters, Milford, MA) equipped Waters 1525 binary pump, Waters 717plus auto sampler, Waters 2414 refractive index detector, and Phenogel 10μ 10E3A column (300×7.8 mm, 10 micron). The mobile phase was composed of 100% HPLC-grade THF, eluting at a flow rate of 1.0 mL/min. at 25 °C. Using Breeze 3.3 software, a calibration curve (R² = 0.999) was constructed using polystyrene standards.

Determination of Critical Micelle Concentration (CMC)

CMCs of DSSP-1 and DSSP-2 were determined by dye solubilization method using DPH. 1 mL polymer solutions ranging from 1.0×10^{-5} to 5.0% (w/v) were prepared via serial dilutions of the stock solution. 10 μL of 0.4 mM DPH solution (methanol) was added to each solution and incubated in the dark for 24 hours. The DPH absorbance was then measured at 377 nm and 391 nm using Genesys 6 UV-Vis spectrophotometer (Fisher Scientific, Waltham, MA). The absorbance values were plotted against log of concentration (% w/v) for CMC determination.

Thermal gelation property

Test tube inversion method—From 25% stock solutions of DSSP-1 and DSSP-2, polymer solutions 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/min 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) was recorded as polymer-rich/solvent-rich phase transition temperature.

Falling ball method—For the monitoring of dynamic viscosity, solutions of 20% (w/v) DSSP-1 and DSSP-2 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. A steel ball with diameter (D) of 2 mm and density of 7.97 g/mL was dropped into the polymer solutions and the time required to travel pre-determined distance (3 cm) was recorded at different temperatures (consistent increments of 2 °C). The polymer solutions were incubated for 15 minutes per increment. The dynamic viscosity of polymer solutions (μ) was determined by using the Stokes equation:

$$\mu = (\gamma_s - \gamma_l)D^2/18v \quad (1)$$

The specific gravity of the ball (γ_s) was calculated by multiplying the density of the ball with the acceleration due to gravity ($g = 980 \text{ cm}\cdot\text{s}^{-2}$). Briefly, the specific gravity of the polymer solution was determined by multiplying the density of the polymer solution ($\approx 1 \text{ g}\cdot\text{cm}^{-3}$) by the acceleration due to gravity (g). The velocity (v) of the ball was calculated by dividing the distance the ball traveled by time.

pH-Induced polymer degradation

The changes in molecular weight of polymers at different pH were monitored by GPC as described previous for polymer characterization. 100 µL of 25% DSSP solutions were placed in the tubes and incubated at 4 °C for 24 h. To the incubated samples, 500 µL of pH 5.0 buffer solution was added and further subjected to incubation for a predetermined time. A similar procedure was followed to prepare samples using pH 7.4 PBS buffer. The collected samples were lyophilized and dissolved in DCM. The tubes were then centrifuged for 10 minutes and the supernatant was collected and dried. The dried samples were then dissolved in THF and filtered using 0.45 µm nylon filter.

pH-Triggered Nile red (NR) release

For the evaluation of pH-sensitivity of synthesized polymers, a fluorescent dye, NR, was chosen as a hydrophobic model compound. DSSP solutions were prepared at a concentration of 0.25% (w/v). Then, 50 µL of $0.1 \text{ mg}\cdot\text{mL}^{-1}$ NR solution in ethanol was added to 5 mL of each polymer solution. The polymer solutions were incubated for 24 hours in the dark. To each 950 µL of polymer solution, 50 µL of 2 M phosphate buffer solutions at pH's 5.0 and 7.4 were added and resulting changes in fluorescence intensity were measured for 24 hours. A solution of Pluronic® P104 was prepared by the same method and treated with PBS buffers at pH's 5.0 and 7.4 as control. The fluorescence intensity at 620 nm of NR (excitation

at 550 nm) in test solutions were recorded with Perkin-Elmer LS 50B fluorescence spectrophotometer (Perkin Elmer, Waltham, MA).

***In vitro* drug release studies using irinotecan**

Irinotecan was selected as a model drug to monitor the release profiles of the synthesized polymeric gels under pH and temperature triggered conditions. DSSP solutions of 20% (w/w) were prepared at 4 °C to load irinotecan with final concentration of 1 mg*mL⁻¹. To 2-mL centrifuge tubes, 250 µL of drug-loaded polymer solution was placed and kept at 4 °C. After 1 hour, tubes were placed in 37 °C bath for 15 minutes for gelation. 1 mL of each phosphate buffer solutions at pH 5.0 and 7.4 was 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 at predetermined time points while maintaining the incubation temperature at 37 °C. The drug concentrations in the collected samples were determined by UV-Vis spectroscopy at 256 nm.

Statistical Analysis

The experimental data in this research paper is presented as the mean of 3 test samples and the error bars indicate the standard deviation of test group (mean ± SD). Statistical significance of the data was determined using a t-test and P value less than 0.05 was considered to be statistically significant.

Results and Discussion

In this study, novel biodegradable polymers capable of pH-sensitive degradation and thermal gelation were successfully synthesized and characterized. The syntheses of these dual-stimuli sensitive polymers (DSSP-1 and DSSP-2) were carried out using either LEEDI or HMDI as the linker to conjugate Pluronic® P104 and an acid-labile linker, BAP, to ultimately yield multiple block copolymers as depicted in Scheme 1. After necessary purification steps to remove unreacted monomer units, resulting polymers were characterized and confirmed via GPC (Figure 1) and ¹H NMR ²⁹ (Figure 2). As listed in Table 1, the molecular weights (M_n) of DSSP-1 and DSSP-2 were determined to be 41,760 Da (PDI = 1.30) and 41,230 Da (PDI = 1.34), respectively. These results indicated that both polymers consisted of around five Pluronic® P104 units, which was comparable to previously reported Pluronic®-based block co-polymers. As suggested in previous studies, most probable reasons for relatively low degrees of polymerization may be due to insufficient removal of adsorbed water via azeotropic distillation and/or hindered accessibility of small linking molecules toward terminal hydroxyl groups of bulky Pluronic® units.⁴

One of the most important characteristics of Pluronic® polymer molecule is its amphiphilicity that allows self-assembly into micelle-like structures in aqueous media. It is known that critical micelle concentration (CMC) – characteristic polymer concentration at which self-assembly occurs – is affected by both the composition and molecular weight of the given compound. As the chain length increases via polymerization, the compound's CMC is expected to decrease as elongated hydrophobic segments gain enhanced

intermolecular aggregation property.¹³ Such aggregation into micelles is driven by dehydration of PPG blocks at concentrations above the CMC. Thus, we were able to assess the success of polymerization by comparing the CMCs of the synthesized polymers to that of the corresponding Pluronic® P104 unit.

Here, hydrophobic dye (DPH) solubilization method was utilized to determine the CMCs of DSSPs. With micellization of the polymers, DPH's characteristic absorbance at 377 nm and 391 nm ($A_{377\text{nm}} - A_{391\text{nm}}$) was expected to increase collectively with its solubility in aqueous media as hydrophobic DPH becomes incorporated within the hydrophobic cores of polymeric micelles.³⁰ As displayed in Figure 3, the CMCs of DSSP-1 and DSSP-2 were determined to be 0.049% (w/v) and 0.045% (w/v), respectively. In accordance to our hypothesis, these results indicated that the synthesized polymers exhibited substantially lower CMC values than the reported value of Pluronic® P104 – 0.300% (w/v). Here, it is worth mentioning that differences in monomer unit composition could also lead to variations in CMCs. According to previous reports on Pluronic®-incorporated multi-block copolymers, P104-based polymer displayed significantly lower CMC value than its P85 counterpart, as P104 is composed of higher PPG-to-PEG ratio than P85.⁴ Such trait indicated that micellization property of synthesized polymers can be precisely controlled and altered based on need, which can be extremely advantageous for biomedical applications.

Another important aspect of Pluronic®, and an extension from micellization, is regarded as its gelation property. Unlike covalently-crosslinked polymers' irreversible gelation properties, Pluronic®-incorporated block co-polymers exhibit reversible solution-to-gel-to-solution (sol-gel-sol) transitions at characteristic concentrations and temperatures. Although various theories have been proposed to describe such phenomenon, hydrophilic-hydrophobic equilibrium approach is accepted to be one of the more rationale mechanisms. This theory asserts that equilibrium exists between the hydrophobic and hydrophilic segments in amphiphilic polymer solutions, where gelation is mediated by hydrophobic inter-micellar interactions, in this case, micellar packing. Thus, raising the solution temperature expedites dehydration of hydrophobic segments (PPG) and gelation can consequently occur in a temperature-dependent manner.

As shown in Figure 4, polymer solutions of varying concentrations (10 to 25 wt.%) were tested for phase transition temperatures via test tube inversion method, where higher polymer concentration indeed resulted in lower sol-gel transition temperatures and extended gel phase due to presumed enhancement in aggregation activity. At temperatures below critical gelation temperatures (CGT), the respective polymer solutions remained in free-flowing solution form. Once the temperature reached CGT, however, the gelation took place immediately, which was observed by evident decreases in solution transparency as well as increases in relative viscosity. Such effective and immediate sol-gel transition upon stimulus demonstrated DSSPs' competence to be utilized as local drug delivery platform via needle injection.

Moreover, these results further validated successful polymerization, as their overall ranges of CGT and critical gelation concentrations (CGC) were notably lower than the corresponding ranges reported for unmodified Pluronic® units. As analogous to micellization, lengthened

hydrophobic chains of DSSPs could significantly enhance inter-micellar chain entanglements and consequently facilitate gelation in relatively mild conditions as compared to the more extreme conditions required for gelation of monomer units. Furthermore, it is noteworthy that 15 wt.% solutions for both DSSPs exhibited gelation near body temperature (DSSP-1: 36.0 °C; DSSP-2: 38.7 °C), which suggested their suitability for various biomedical applications, for example, as *in situ* depot for controlled drug release.

The respective gelation property was further confirmed by studying dynamic viscosities of the synthesized polymers (20 wt.%) as a function of temperature. Here, specific gravities of the ball and polymer solution, falling velocity, and ball diameter were used to determine viscosity (μ). As shown in FIGURE 5, sharp increase in viscosity was observed for both polymers between 29°C and 32°C, which indicated sol-gel transition of the polymers in the respective temperature range. Considering sol-gel transition temperature of 20 wt.% Pluronic® P104 solution is reported to be above 65 °C, it was, again, clear that DSSPs exhibited enhanced gelation property as a result of polymerization. It should be noted that minor differences in results existed between test tube inversion and falling ball methods, presumably due to slower equilibration of polymer solutions at given temperatures. Although the results from falling ball experiment may indicate more reproducible and accurate transition temperatures, the test tube inversion experiment demonstrates gelation kinetics in a more practical manner.

After confirming temperature-dependent gelation property of DSSPs, we assessed pH-dependent polymer degradation via GPC to further validate our hypotheses. The BAP linker incorporated between repeating Pluronic® units is a bifunctional amino ketal that can undergo acid-catalyzed hydrolysis. The main reason for choosing this particular acid-labile linker was to provide more rapid degradation mechanism than previously reported polymers with acetal linkers, which took up to 4 days to reach complete degradation. In addition, hydrolysis of BAP releases acetone as a relatively non-toxic degradation product.²⁵ As hydrolysis of ketals is accepted to be much faster (up to 1,000-fold) than that of acetal, BAP incorporation was a rational choice to achieve enhanced response towards pH changes.

As displayed through the overlays of GPC chromatograms in Figure 6, the peak retention time of DSSP-1 shifted from 6.2 minutes to around 7.8 minutes and 8.9 minutes by the first hour of incubation in acidic medium of pH 5.0. Such behavior suggested successful degradation of the polymer into multimer and monomer units, as longer retention time in GPC correlates to lower molecular weight (MW). Moreover, we were able to conclude that degradation was already at completion within the first hour as the peak retention times of 24-hour sample were identical to those of 1-hour sample. In the resulting chromatograms, the peak at around 7.8 minutes represented multimer units that could not undergo complete degradation to monomer units, which were represented by the 8.9-minute peak. The most probable reason for incomplete degradation may be due to the portion of multiblock copolymer synthesized by alternating insertion of Pluronic® and LEEDI (or HMDI) in DSSPs, where some Pluronic® units may have ended up next to one another during polymerization. Consequently, such segments without BAP could not undergo acid-catalyzed hydrolysis. The fact that DSSP-1's original retention time remained relatively intact in neutral pH of 7.4 for 48 hours confirmed that the changes observed in pH 5.0

incubation were attributed to BAP hydrolysis in an acidic environment. It should be noted that due to the presence of ether functional group, Pluronic[®] units are subject to elimination via excretion rather than biodegradation. As such, it is critical for Pluronic[®]-based copolymer systems for biomedical applications, to have a definite disassembly mechanism into monomeric fragments in order to facilitate bio-elimination.

In order to further validate pH-dependent degradation of DSSPs, Nile red (NR) release from polymeric micelles was monitored at pH 5.0 and 7.4 via fluorescence spectroscopy. As analogous to micellar incorporation of DPH for CMC determination, NR could also be incorporated into micellar core through hydrophobic interactions. It is widely known that NR is a phenoxazine dye that becomes fluorescent only in hydrophobic environments. Since its fluorescence consequently becomes auto-quenched in insoluble aqueous media, we were able to study the release rate of NR from polymeric micelles (dispersed in aqueous medium) as an inverse function of fluorescence intensity. In agreement to our hypothesis, Figure 7(a) shows that NR's fluorescence at 620 nm decreases substantially over time under acidic condition (pH 5.0) and reaches near the baseline by the 24-hour mark, indicating complete release of the dye into aqueous media. In Figure 7(b), such trend is shown in release percentages, where virtually no dye release occurred for 24 hours at pH 7.4, while significant release was observed within the same timeframe at pH 5.0 for both DSSP-1 and DSSP-2 micelles. Taken together, it was clear that NR release was induced by acid-catalyzed disassembling of polymeric micelles.

Furthermore, drug delivery application of DSSPs was simulated by monitoring drug release trend from DSSP gels via UV-Vis spectroscopy. The model drug chosen for this study was irinotecan, which is a commercialized chemotherapeutic agent that acts as a topoisomerase-1 inhibitor. As irinotecan's potency is known to substantially decrease upon lactone hydrolysis at basic pH, gel formulation using acid-labile polymers may be particularly favorable for retaining stability of the drug. Furthermore, as chemotherapeutic agents are prominently characterized by their non-specific toxicity, stimuli-sensitive delivery method is an appealing option to increase their specificity towards malignant tumor cells over normal cells. In Figure 8, pH-sensitive drug release trends over 54 hours are displayed, where nearly identical trends were observed for both DSSP gels, which indicated that our polymerization method was consistent and effective. For both DSSPs, incubation at pH 5.0 led to drug release of up to 99% within 20 hours, while incubation at pH 7.4 showed limited drug release at around 40% by the 48-hour mark. However, in comparison to previously-synthesized Pluronic[®] copolymers with acetal linkers, denoted as MBCP-1 and MBCP-2, DSSPs' drug release in neutral pH was relatively high as MBCPs managed to limit *in vitro* release of FITC-dextran at around 30% by the 10th day of incubation in similar conditions.⁴ Respective differences in release profiles suggested that gelation of DSSPs is relatively less rigid and more prone to concentration-gradient diffusion of incorporated compounds. Nevertheless, it was also clear that DSSPs' pH-dependent release profiles were far superior, as MBCPs' FITC-dextran release in acidic environments, pH 5.0 and 6.5, took from 2 to 9 days for completion.⁴ The rapid drug release observed from DSSP gels can be attributed to ketal hydrolysis, which is up to 1,000-fold faster than acetal hydrolysis as previously mentioned.^{31, 32, 33} Moreover, pH-sensitive stepwise release trend was also shown by lowering the pH of the control group (pH 7.4) at 48-hour mark, which led to rapid increase

in release rates and complete drug release by the 54-hour mark. Ultimately, such evident pH-sensitive degradation of DSSPs, demonstrated by NR and irinotecan release, suggested their potential for target-specific drug delivery which can prevent systemic toxicity and dose-limiting side effects.

Conclusions

Dual-stimuli sensitive polymers, denoted as DSSP, based on novel block copolymer design have been synthesized and characterized in this study. The integration of acid-labile ketal linker with thermo-sensitive Pluronic[®] units yielded reversibly thermogelling multiple block copolymers capable of acid-sensitive degradation within effective timeframe. Furthermore, respective thermogelation was demonstrated to occur near the physiological temperature, which suggested DSSP's potential to be utilized for biomedical applications. As such, pH-sensitive drug release patterns from DSSP micelles and gels were also simulated using Nile red and irinotecan as model drug compounds.

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Figure 1. (A) GPC chromatograms of Pluronic® P104, (B) crude and unpurified DSSP-1, (C) purified DSSP-1. After purification, unreacted Pluronic® P104 units are removed.

Figure 2.
 ^1H -NMR (400 MHz, CDCl_3) spectra of DSSP-1 and DSSP-2.

Figure 3. DPH absorbance differences between 377 nm and 391 nm plotted as a function of log of (DSSP w/v %). CMC values of DSSP-1 and DSSP-2 are defined at the cross-section points of the corresponding lines.

Figure 4. Phase diagrams of aqueous DSSP solutions at different concentrations (w/v %). The phase transition temperatures were determined via test tube inversion method.

Figure 5. Dynamic viscosity of 20 wt.% DSSP solutions studied via falling ball method. As indicated with dotted line on the graph, 100 Pa-s was determined as the sol-gel transition point.

Figure 6. GPC study of pH-dependent degradation of DSSP-2. As shown in two sets of overlays, pH 5.0 incubation induced rapid degradation of DSSP-2 into multimer and monomer (Pluronic® P104) units within 1 hour, while pH 7.0 incubation induced limited degradation for up to 48 hours.

Figure 7. pH-dependent NR release profiles from DSSP micelles. (Left) Fluorescence of NR at 620 nm becomes auto-quenched as it releases into insoluble aqueous medium (pH 5.0). (Right) NR release from DSSP micelles in pH 5.0 media is rapid and completes within 24 hours, while release in pH 7.4 is very minimal.

Figure 8.

pH-dependent irinotecan release profiles from pre-formulated DSSP gels. Again pH 5.0 media induced significant drug release, while pH 7.4 limited release by 40% for up to 48 hrs. By lowering the control groups' pH at 48-hour mark, step-wise release trends could be observed (Error bars represent standard deviation from triplicated results, $n=3$).

Scheme 1.
Synthetic scheme for DSSP-1 and DSSP-2.

Table 1

Molecular weights (M_n) and CMC values for synthesized DSSPs in comparison to Pluronic[®] P104.

Polymer	M_n	PDI	CMC	# Units
Pluronic [®] P104	7100 ± 110 (5900) [*]	1.02	0.300 ± 0.009	—
DSSP-1	41760 ± 1000	1.30	0.049 ± 0.009	5.88
DSSP-2	41230 ± 1200	1.34	0.045 ± 0.009	5.80

^{*} BASF-provided M_n value.