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Tuning Properties of Poly(ethylene glycol)-*block*-poly(simvastatin) Copolymers Synthesized via Triazabicyclodecene

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Abstract

Simvastatin was polymerized into copolymers to better control drug loading and release for therapeutic delivery. When using the conventional stannous octoate catalyst in ring-opening polymerization (ROP), reaction temperatures 200 °C were required, which promoted uncontrollable and undesirable side reactions. Triazabicyclodecene (TBD), a highly reactive guanidine base organocatalyst, was used as an alternative to polymerize simvastatin. Polymerization was achieved at 150 °C using 5 kDa methyl-terminated poly(ethylene glycol) (mPEG) as the initiator. ROP reactions with 2 kDa or 550 Da mPEG initiators were also successful using TBD at 150 °C instead of stannous octoate, which required a higher reaction temperature. Biodegradability of the poly(simvastatin) copolymer in phosphate-buffered saline was also improved, losing twice as much mass than the copolymer synthesized via stannous octoate. The three copolymers exhibited modified rates of simvastatin release, demonstrating tunability for drug delivery applications.

Keywords

poly(simvastatin); simvastatin; diblock copolymer; triazabicyclodecene; drug delivery

1. INTRODUCTION

A wide range of catalysts with different mechanisms of action have been used to synthesize degradable polyesters for biomedical applications. Common catalysts that mediate ring-

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CONFLICT DISCLOSURE

DAP has an equity interest in Regenera Materials, LLC, a start-up company for commercializing novel biomaterials for tissue regeneration.

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opening polymerization (ROP) of lactone-incorporated monomers include tin (II) ethylhexanoate (stannous octoate) and other organotin compounds [1]. Aluminum-, lanthanum-, and zinc-based alkoxides have also been used in the synthesis of high molecular weight (MW) poly(lactic acid) (PLA), poly(lactic-co-glycolic acid), and poly(ϵ -caprolactone) [1–3].

Stannous octoate and other metal and alkaline earth catalysts are known to be efficient [4], while enzymatic, acidic, and organic catalysts have reportedly shown lower reactivity, producing low MW polymers [1, 5]. Catalyst reactivity, however, can be altered by modifying reaction conditions, the type or size of lactone monomer incorporated into the feed, or functional groups of these catalysts. For example, changing the diamine bridge of aluminum salen complexes from ethylene to dimethylpropylene led to significantly increased polymerization rates of small L-lactide, ϵ -caprolactone, ϵ -decalactone, and β -butyrolactone monomers, while low reactivities with ω -pentadecalactone and other macrolactones were not significantly affected [6]. Also, ROP reactions with diazabicycloundecene and N-methylated triazabicyclodecene (TBD) organocatalysts generated polylactide MWs of 18 and 21 kDa, respectively, in the presence of pyrenebutanol in chloroform under optimized conditions [7]. The type of catalyst used under specific reaction conditions can affect the degree of polymerization and the resulting quality of the polymer synthesized.

In our previous studies, stannous octoate-mediated coordination-insertion ROP was used to synthesize a newly developed poly(simvastatin)-poly(ethylene glycol) diblock copolymer with potential anti-inflammatory, angiogenic, and osteogenic properties following degradation. While the catalyst was successful in mediating poly(simvastatin) propagation with a methyl-terminated poly(ethylene glycol) (mPEG) initiator, a narrow and high reaction temperature window served as a limitation that also promoted undesirable transesterification reactions. After preliminary attempts with other metal and organocatalysts, TBD was ultimately selected because of its efficient performance at ambient temperatures [7], ability to work without a co-catalyst, metal-free process, and accessibility. TBD was also reported to rapidly catalyze synthesis of 26 kDa PLA, of which the MW could be modified by changing the molar ratio of initiator to monomer in the feed [8].

In the present study, the TBD-mediated poly(simvastatin) reaction was compared with the stannous octoate-mediated reaction under similar conditions. Polymerization via TBD was also evaluated with different MW mPEGs, catalyst percentages, and molar ratios of simvastatin to mPEG. Hydrolytic degradation of the resulting poly(ethylene glycol)-*block*-poly(simvastatin) (PSIM-mPEG) copolymers was also analyzed by measuring mass loss and drug release.

2. EXPERIMENTAL

2.1 Materials

Simvastatin was purchased from Haorui Pharma-Chem (Edison, NJ). Triazabicyclodecene, monomethyl ether poly(ethylene glycol) (mPEG), anhydrous toluene, anhydrous diethyl ether, dichloromethane (DCM), and deuterated chloroform (CDCl_3) were obtained from

Sigma-Aldrich (St. Louis, MO). Tetrahydrofuran (THF) stabilized with 3,5-di-tert-butyl-4-hydroxytoluene (BHT) was procured from Fisher Scientific (Pittsburgh, PA).

2.2 Synthesis of poly(ethylene glycol)-*block*-poly(simvastatin)

Microscale reactions of PSIM-mPEG using stannous octoate have been previously described [9]. Macroscale reactions (2 g) were conducted using simvastatin as the monomer and mPEG (550, 2000, or 5000 Da) as the initiator. Molar ratios of 100:2 for simvastatin to mPEG 550, and 100:1 for simvastatin to mPEG 2000 or 5000 Da were used in the feed to synthesize PSIM-mPEG(550), PSIM-mPEG(2k), and PSIM-mPEG(5k), respectively. Simvastatin and mPEG were dried in a round bottom flask embedded in a silica sand bath at 120 °C for 1 hr under a continuous flow of nitrogen gas. The internal bulk temperature was increased to 150 °C for an additional hour before adding 1 wt% of TBD to the homogeneous melt. Each reaction ran for 24 hr.

Microscale reactions (0.4 g) were also conducted for PSIM-mPEG(550) copolymers with a TBD catalyst percentage of 0.1 or 1 wt%, a 100:1, 100:2 or 100:10 simvastatin to mPEG molar ratio, and crude samples taken at 0, 4, 12, 18, or 24 hr reaction times.

Polymer dissolved in DCM was slowly added to cold diethyl ether at a 1:7 v/v ratio of DCM to ether and vacuum filtered to purify the crude PSIM-mPEG(5k) product. The purification process for PSIM copolymers with lower MW mPEG blocks involved slowly adding cold diethyl ether to the polymer in DCM solution at a 1:20 v/v ratio, followed by centrifugation and decantation of the supernatant.

2.3 Physico-chemical characterization

2.3.1 Gel Permeation Chromatography (GPC)—A Shimadzu Prominence LC-20 AB HPLC system connected to a Waters 2410 refractive index detector was used to measure the weight-average molecular weights of simvastatin, mPEG (550, 2000, and 5000 Da), and the crude PSIM copolymers. Two Resipore columns in series (300 × 7.5 mm, 3 μm particle size; Agilent Technologies) were used for separation. Samples were dissolved in THF at 5 to 10 mg/ml. THF was also used as the mobile phase at a 1.0 ml/min flow rate. Polystyrene standards were used to calculate MW ranged from 160 Da to 430 kDa.

2.3.2 Nuclear Magnetic Resonance (NMR) Spectroscopy—¹H-NMR spectra were obtained to characterize the PSIM-mPEG (5k) copolymer and a melted mixture of simvastatin and mPEG at a 100:1 molar ratio using a 400 MHz Varian Gemini NMR instrument connected to a VnmrJ software interface. Samples weighing 5 to 7 mg each were dissolved in 1 ml of CDCl₃, transferred into NMR tubes, and analyzed for additional structural characterization. The number of simvastatin monomers present in the diblock copolymer was calculated by integrating the area under the peaks representing simvastatin relative to those associated with 5 kDa mPEG, of which the number of H atoms in its structure was known. With this ratio, the number of protons in the poly(simvastatin) block of the copolymer was calculated and divided by the known number of protons in simvastatin to get the number of simvastatin monomers in the diblock copolymer.

2.3.3 Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS)—Degradation products of PSIM-mPEG(5k) were analyzed using a positive ion mode Bruker Ultraflex extreme MALDI-TOFMS. The procedure used for sample analysis was previously described [9].

2.3.4 *In Vitro* Degradation—Films of each copolymer (10–15 mg) were made by adding a small amount of DCM to polymer to create a viscous solution (70% w/v) that was pipetted onto a Teflon sheet to dry overnight. Each film was gently shaken in 1.5 ml of phosphate-buffered saline (PBS), pH 7.4, at 37 °C. Supernatant was collected and the medium completely replaced every 12 hr the first day, every other day the first week, and at 2 to 5 d for the remainder of the 44 d degradation period. The remaining samples were dried and weighed after 6 weeks to measure total mass loss.

2.3.5 High Performance Liquid Chromatography (HPLC)—A Shimadzu Prominence LC-20 AB HPLC system was used to analyze supernatants collected from the mass loss study. One Luna C18 column (150 × 4.60 mm, 5 μm particle size) was used with an isocratic mobile phase of acetonitrile and water with 0.1% trifluoroacetic acid (70:30 v/v). Absorbance was measured at 240 nm.

2.3.6 Statistical Analysis—Two-way ANOVA with a Bonferroni post-test was performed on the kinetic data to test effects of reaction time, catalyst percentage, and molar ratio on MW, yield, and percent composition of the copolymer. The same analysis was applied to the simvastatin amounts released during copolymer degradation. Values of $p < 0.05$ were deemed statistically significant. Data are plotted as mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1 Polymerization Mechanism

The ROP mechanism governed by TBD is anionic. From the literature, one theorized mechanism suggests that the amidine imine nitrogen of the nucleophilic catalyst attacks the carbonyl group on the lactone ring of simvastatin to form a temporary intermediate as the acyl bond is broken. The secondary amine in the guanidine-based organocatalyst attracts the alcohols within the reaction mixture (i.e., both on mPEG and the propagating poly(simvastatin) block) via hydrogen bonding. This action propagates the PSIM block of the PSIM-mPEG diblock copolymer [8]. However, computational analysis comparing the transitional state energies of proposed TBD-mediated ROP reactions with L-lactide and methanol showed that the intermediate steps carried out via hydrogen bonding had lower energy transitional states compared to nucleophilic attraction throughout the reaction. The lower energy states due to hydrogen bonding indicated a relatively more stable mechanism [10].

In the proposed mechanism shown in Scheme 1, the amidine imine nitrogen of TBD attracts the hydrogen on the alcohol, in this case mPEG, to activate it. The activated alcohol then attacks the carbon of the carbonyl group of the lactone ring of simvastatin. The catalyst then changes orientation, subsequently hydrogen bonding to the oxygen in the C–O bond in the lactone ring, while the secondary amine remains hydrogen bonded to the oxygen in the

carbonyl group. This transitional state initiates opening of the lactone ring. TBD is reformed after the hydrogen migrates away from the amidine imine to form the hydroxyl end-group of the propagating polymer [10].

3.2 Stannous Octoate vs. TBD Catalyst Mediated Reactions

The mole percentages of simvastatin, intermediates, and copolymer throughout the reaction using stannous octoate or TBD as a catalyst are displayed in Figure 1. Within 24 hr, a decrease in simvastatin monomer was observed at a conversion rate of 0.052 hr^{-1} with 27.2% of simvastatin remaining when using TBD as a catalyst at $150 \text{ }^\circ\text{C}$. In contrast, stannous octoate did not lead to monomer conversion at this temperature. At 200 and $230 \text{ }^\circ\text{C}$, however, stannous octoate catalyzed rapid conversion, at 0.179 and 0.351 hr^{-1} , respectively, 3 and 7 times the rate for TBD. Total monomer consumption was reached at 12 and 8 hr for the stannous octoate mediated reactions at 200 and $230 \text{ }^\circ\text{C}$, respectively.

The degree of polymerization achieved by an ROP reaction is greatly influenced by the nature of the catalyst, cyclic monomer, or alcohol incorporated in the feed. Stannous octoate has the necessary electrophilic qualities possessed by its metal cation center, and it gains nucleophilic properties once formed into an alkoxide, becoming a stereoselective catalyst in the mediation of ROP. The newly formed tin alkoxide is subsequently attracted to the carbon of the lactone carbonyl group and cleaves the lactone ring of the monomer at the acyl bond, propagating the polymer chain via a pseudo-anionic coordination-insertion ROP mechanism. However, stannous octoate is a much larger molecule than TBD with a MW of 405 Da, three times that of TBD at 139 Da (Figure 2). After forming a complex with the alcohol, or 5 kDa mPEG, the resulting metal alkoxide complex becomes even larger.

TBD is highly basic (pK_a 26) and requires no cocatalyst, such as thiourea, in the ring-opening of cyclic esters, compared to some of its monocyclic phosphazene, amidine, and guanidine counterparts. The bicyclic structure of TBD also has two active nitrogen centers that allow for electrophilic and nucleophilic bifunctionality, activating simvastatin's lactone ring and the hydroxyl group of the mPEG initiator, respectively, via hydrogen bonding [11, 12]. This bifunctionality removes a possible limiting step otherwise necessary for stannous octoate to form an alkoxide in order to initiate polymerization. These advantageous characteristics associated with the smaller and less sterically hindered structure may give TBD relatively heightened sensitivity, selectivity, and ease in mediating ROP with mPEG and simvastatin at a lower temperature. These characteristics may also explain why TBD was able to catalyze a ROP reaction of PSIM at $150 \text{ }^\circ\text{C}$, where stannous octoate was unsuccessful. TBD-mediated reactions were also carried out at internal temperatures lower than $150 \text{ }^\circ\text{C}$ (results not shown). Those reactions, however, did not produce significant polymerization, showing that the window for polymerization is still small, but still with a lower temperature threshold than would be necessary for stannous octoate to polymerize simvastatin.

Monomer conversion increased with reaction temperature when catalyzed by stannous octoate. Even after total monomer consumption, however, the polymer composition in the crude product continued to increase as the relative percentage of intermediates declined, suggesting an addition of intermediate products to the copolymer structure after monomer

consumption. This may be due to the secondary hydroxyl group on the simvastatin lactone ring acting as another reactive site, potentially forming intermediates of dimers and small oligomers that are subsequently incorporated into the copolymer.

3.3 NMR and Mass Spectrometry of PSIM-mPEG (5k) via TBD

H-NMR was performed to further support the MW of PSIM-mPEG(5k) measured via GPC. As seen in the control of Figure 3a, the chemical shifts represented by r, s, and t (4.21, 3.71, and 3.48 ppm) represent mPEG within the mixture. Simvastatin was identified by the remaining chemical shifts within the spectrum. Seventeen simvastatin monomers were calculated to be incorporated into the PSIM-mPEG(5k) copolymer sample tested, leading to a MW of 12 kDa.

In the NMR control spectrum, the repeating ethylene oxide unit of mPEG is represented by the chemical shift at 3.71 ppm, and the hydroxyl and methyl oxide groups of the polymer block are represented by the 4.21 and 3.48 ppm chemical shifts, respectively. The disappearance of the 4.21 ppm chemical shift in the copolymer spectrum reflects incorporation of the mPEG hydroxyl moiety into the copolymer backbone after initiation. The absence or shift change of e and f, which represent the lactone ring (Figure 3a) in the copolymer spectrum reflect a change in the lactone structure or molecular environment as a result of ROP. The broadened peaks in Figure 3b indicate synthesis of a polymerized form of simvastatin. Broadening of the base of the peaks was observed between 5.38 to 6.05 ppm, with letters a through d representing more aromatic portions of the simvastatin monomers.

Figure 4 displays the mass-to-charge ratio (m/z) spectra of PSIM-mPEG(5k) degradation products. The inset in Figure 4a shows simvastatin represented by the parent ion of 441 m/z . The low MW degradation product spectrum (Figure 4a) contained multiple peaks close to that of simvastatin at 404, 422, 439, 457, and 480 m/z , but the intensities were low relative to the peak of highest abundance at 522 m/z . To the right of the most abundant species, a multitude of smaller peaks was seen, ranging from 540 to 957 m/z .

In the mass spectra of low MW degradation products, the 404 m/z peak represents fragmented simvastatin. The 422 to 480 m/z peaks, alongside the peak of highest relative abundance at 522 m/z , reflect open or closed-ring simvastatin in the presence of a combination of salt adducts remaining from the PBS (i.e., Na^+ , K^+ , and H^+) used to carry out polymer degradation. The alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix (189 m/z) used in the analysis may also contribute to the m/z values. Peaks ranging from 540 to 957 m/z indicate the presence of simvastatin dimers and combined salt adducts or matrix in the degradation products.

In the spectrum of the higher MW degradation products (Figure 4b), a wide distribution was observed, with the highest relative abundance at 5561 m/z . The most prevalent peak was slightly higher than the theoretical average MW of the 5 kDa mPEG polymer used as an initiator in the synthesis of the PSIM-mPEG copolymer. The base of peaks representing the higher MW degradation products was broadened compared to the sharp and distinct peaks seen in the mPEG control (Figure 4b inset), indicating the varied distribution of complex

ions present in this m/z range, possibly representing mPEG and mPEG with attached simvastatin monomer degradation products.

3.4 Poly(simvastatin) Copolymer Synthesis using Different mPEGs

Chromatograms of purified PSIM-mPEG copolymers initiated with 5k, 2k, or 550 Da mPEG and catalyzed via TBD are shown along with their respective reactants in Figure 5. The resulting weight-average MW of PSIM-mPEG(5k), PSIM-mPEG(2k), and PSIM-mPEG(550) copolymers synthesized were 15, 13, and 20 kDa, respectively, with polydispersity indexes (PDIs) of 2.1, 2.2 and 2.5, respectively. The mPEG initiators with theoretical MWs of 5 and 2 kDa were measured to have slightly higher MW values of 7.4 and 2.5 kDa via GPC, while the 550 Da mPEG and simvastatin registered lower MW values of 0.4 and 0.2 kDa, respectively. ROP reactions with 5k mPEG led to successful simvastatin polymerization when mediated by stannous octoate. With 2k and 550 Da mPEG, however, polymerization reactions were not successful when mediated by stannous octoate or other selected catalysts, such as lanthanum isopropoxide, aluminum isopropoxide, tin triflate, lipase B from *Candida antarctica*, HCl-EtO₂, potassium methoxide (KOMe), 1,3-dimesitylimidazol-2-ylidene (IMes), and diazabicycloundecene (DBU) under varied reaction conditions (results not shown).

TBD successfully catalyzed the synthesis of PSIM-mPEG copolymers using 5k, 2k, and 550 Da mPEG as initiators at 150 °C in melt conditions, possibly as a result of high reactivity combined with less steric hindrance due to its small molecular size. The poor performance of stannous octoate in the same conditions possibly indicated steric hindrance between the catalyst and the simvastatin structure and hindered catalyst stereoselectivity.

3.5 Kinetics

Based on the copolymer and mPEG MWs measured via GPC, the PSIM copolymer initiated with the lowest MW mPEG had the highest percentage of simvastatin incorporated in the copolymer at 98%. PSIM-mPEG(2k) and PSIM-mPEG(5k) had 80 and 51% of drug incorporated, respectively. Percent yields of the three copolymers exhibited an opposite trend, however, with the 550 Da mPEG-initiated copolymer having the lowest yield, which increased as the MW of the mPEG block increased (Table 1). This trend may be due in part to the decreased solubility of mPEG in ether as its MW increases.

The initial MWs measured for the three PSIM copolymers were significantly different ($p < 0.0001$) due to the MW differences of the mPEG initiators used for each reaction (Figure 6a). The MW of the PSIM-mPEG(5k) copolymer remained significantly greater than for the other two copolymers throughout the reaction ($p < 0.0001$). PSIM-mPEG(2k) also had a significantly greater MW than PSIM-mPEG(550) did after 12 and 18 hr ($p < 0.001$) and 24 hr ($p < 0.0001$).

The difference between the initial and highest weight-average MW reached throughout the reaction, which correlates with the number of simvastatin monomers attached, was greatest for the 550 Da mPEG initiator. This difference decreased as MW of the mPEG initiator increased. The trend was further reflected by the rates of polymerization throughout the 24 hr reaction period. Within the first 4 hr, the PSIM-mPEG(550) reaction had the greatest

change in MW, increasing by 5 kDa and plateauing at approximately 6.1 kDa for the remainder of the reaction period. PSIM-mPEG(5k) had the smallest change in MW growth in the first 4 hr, and the slowest first-order polymerization rate of 0.021 h^{-1} for 18 hr before undergoing a MW decrease from 13.4 to 12.6 kDa. The PSIM-mPEG(2k) reaction showed a smaller 4 hr MW change and had a lower polymerization rate than PSIM-mPEG(5k) did, but its rate was higher than for the PSIM-mPEG(550) reaction. The total MW increase achieved was 6 kDa at a rate of 0.037 h^{-1} . From initial MWs of 0.67, 3, and 9 kDa, copolymer MWs of 5.8, 9, and 13 kDa were achieved for PSIM-mPEG(550), PSIM-mPEG(2k), and PSIM-mPEG(5k), respectively.

Although the differences in MW growth within the first 4 hr were not significant in Figure 6b, there was a noticeably greater change observed for the reaction initiated with a 100:10 ratio of simvastatin to 550 Da mPEG. Unexpectedly, reactions with the smaller amounts of 550 Da mPEG (100:1 and 100:2 molar ratios of simvastatin to mPEG) achieved lower MWs after 4 hr ($p < 0.0001$ and $p < 0.001$, respectively) compared to the feed containing the most mPEG (100:10 sim to mPEG). The MW obtained from the 100:1 molar ratio feed remained significantly lower than the others after 12 ($p < 0.001$), 18, and 24 hr ($p < 0.0001$). The 100:2 molar ratio feed generated a significantly lower MW than only the 100:10 feed after 4 hr ($p < 0.001$).

The 100:1 and 100:10 sim to 550 Da mPEG molar ratios with the smallest amount of catalyst resulted in the greatest MW growth (Figure 6c) when comparing the effects of different molar ratios and weight percentages of TBD. MW growth was statistically insignificant in the 100:10 melt with 0.2 wt% TBD during the first 4 hr. Despite the initial lag, the polymerization rate during the first 12 hr was 0.221 hr^{-1} , leading to an 8.3 kDa crude copolymer. In the last 12 hr, the rate decreased to 0.008 hr^{-1} , resulting in a 9.1 kDa crude copolymer product formed. In the 100:1 melt with 0.2 wt% TBD, a 5.1 kDa MW was formed during the first 4 hr, followed by a 0.026 h^{-1} rate for the remainder of the reaction to yield a 9.9 kDa polymer. MW differences between the two ratios at 0.2 wt% were insignificant after 4 hr. Reactions with 1 wt% TBD were completed within 4 hr. Upon completion, however, MWs plateaued at lower values than their counterparts did with 0.2 wt % TBD, which were 8.4 and 6.1 kDa for the 100:10 and 100:1 molar ratio feeds, respectively. The final MWs were 8.5 and 5.8 kDa for the 100:10 and 100:1 molar ratio feeds, respectively. The 100:2 melt with 1 wt% TBD reached a MW of 6.9 kDa within the first 4 hr and had a first-order rate of 0.011 h^{-1} for the rest of the reaction period, resulting in an 8.5 kDa crude product.

Due to the low yield of PSIM-mPEG(550), the amount of catalyst and molar ratios of monomer to initiator were modified to improve yield and the degree of polymerization. The kinetics of the PSIM-mPEG(2k) and PSIM-mPEG(5k) copolymers were also monitored over a 24 hr period. The slower rate of polymerization as the MW of the mPEG initiator increased suggests that the larger mPEG molecules provided greater steric hindrance. The computational analysis of Chuma *et al.* showed that alcohols with bulky, more sterically hindering side groups increase the potential for destabilizing the transition state needed to initiate and continue polymerization [10]. Although mPEG does not have bulky side groups,

this reasoning could apply to the mPEG size and resulting conformations it assumes in the melt.

The majority of MW growth of PSIM-mPEG(550) synthesized with 1 wt% TBD was complete within 4 hr, which plateaued for the remainder of the 24 hr reaction period. This may be explained by the rapidly reactive nature of TBD. At 0.1 mol%, TBD was shown to fully polymerize lactic acid into PLA in 1 min [7]. Also, a 6 d polymerization reaction with cyclic trimethylene carbonate, carried out by MTBD, a guanidine equivalent of TBD, was shown to be complete in a significantly shorter time of 15 min when mediated by TBD [12, 13]. TBD is known to be the most basic of its “superbase” counterparts, resulting in polymerization rates that almost equal those catalyzed by N-heterocyclic carbenes, which can take seconds to complete a reaction. Future potential avenues could investigate the kinetics of TBD-mediated PSIM copolymer reactions in a shorter time period for more precise polymerization rates within the 4 hr period.

Opposing relationships between catalyst concentration and molecular weight of various polymers have been presented in literature [5, 14, 15]. One of these investigations includes the analysis of trioxane monomer to p-chlorophenyldiazonium hexafluorophosphate catalyst molar ratios ranging from 5,000 to 20,000. At ratios up to 8,000, the resulting polymer molecular weight was inversely proportional to catalyst concentration, reaching maximum MW values. As the catalyst concentration continued to decrease with monomer to catalyst ratios between 8,000 and 20,000, the relationship to polymer molecular weight became directly proportional [14]. A range of different initiator concentrations tested with trioxane had no effect on the resulting poly(trioxane) MW in melt conditions, but the same range explored in solution caused a decrease in MW as the initiator concentration increased [15]. The type of catalyst used can also influence the relationship between catalyst concentration and MW. Increasing concentrations of catalysts such as sulfuric acid and titanium (IV) butylate were shown to mediate synthesis of lower molecular weight poly(lactic acid) during polycondensation of L-lactide [5]. A similar inverse relationship was seen in the present studies where polymerization rates and resulting MWs of PSIM-mPEG(550) reactions increased as the TBD catalyst weight percentage decreased. This relationship may result from the chosen catalyst to reactant ratios leading to reactions above or below a specific energy threshold, where the relationship between simvastatin monomer and TBD catalyst is inversely proportional. Mechanistically, increasing the catalyst content between 0.2 and 1 wt % may contribute to an increase of chain transfer reactions mediated by the catalyst, where the hydroxyl terminal end group of a propagating chain, activated by the TBD catalyst, may attack the ester group within the backbone of another neighboring or growing chain [16]. This action can lead to a decrease in MW of the polymer chain and, ultimately, a reduced weight-average MW.

An increased monomer to initiator ratio typically results in greater MW because fewer initiation sites are present, which leads to longer polymer chains at high monomer conversion. An example of this occurrence is seen with the ROP of ϵ -caprolactone using a calcium methoxide catalyst, which increased the resulting poly(ϵ -caprolactone) MW from 5 to 11.4 kDa at 100% conversion as monomer/initiator ratio increased from 20 to 100 [17]. While this trend was maintained in PSIM-mPEG(550) crude products with 0.2 wt% TBD,

the resulting MW decreased as the initial simvastatin to 550 Da mPEG initiator ratio increased using 1 wt% TBD. This unexpected latter observation may be influenced by low monomer conversion in the crude product for the most hydrophobic copolymer, which partially explains its low yield. Increasing the initiator present in the melt (i.e., lowering the monomer to initiator ratio) provides more initiator sites for potential polymer propagation. Even though this would lead to shorter chains or a lower MW polymer at nearly 100% conversion, the crude products show monomer conversion is still far from complete. At this stage, a larger number of chains present compared to the remaining monomer in the crude may exert a greater influence on the overall average MW increase seen in the crude products as the 550 Da mPEG initiator amount increased in the feed.

Transesterification reactions and depolymerization with extended time may explain the slight decreases in MW nearing the end of reaction for PSIM-mPEG(5k) and PSIM-mPEG(550) with 1wt% TBD. TBD and similar organocatalysts show significantly greater binding affinity to cyclic lactones than acyclic esters, which contributes to transesterification reactions and may contribute to the low PDIs measured in the crude products after 24 hr [12].

3.6 Crude vs Purified Polymers

Figure 7 shows chromatograms of the PSIM-mPEG(550) copolymer before and after purification via DCM/cold ether. The crude copolymer in the kinetic reactions reached a maximum of MW of 6–7 kDa in contrast to the purified 19–20 kDa copolymer noted earlier. After precipitation, the purified product showed a peak that noticeably shifted left to an elution time of 15.1 min compared to the crude peak, which remained at 16.7 min. The measured weight-average MW of the polymer peak in the crude product was 9 kDa, while the purified form was 21 kDa.

The significant difference in MW distribution between the crude PSIM-mPEG(550) (approximately 6 kDa) and the purified copolymer (20 kDa) was due to the large amount of low MW products remaining in the crude samples decreasing the weight average MW. Also, the high MW purified product represented only approximately 2% of the crude product. The PSIM copolymer yield increased as the MW of the mPEG incorporated into the feed increased. Analysis of purified samples may have resulted in a different MW trend between the copolymers, but the small amounts of crude product collected from the microscale reactions (5 to 10 mg) made purification impractical.

For macroscale reactions, investigating new solvent/anti-solvent phase precipitation systems may help to improve the yield of the PSIM-mPEG(550) copolymer. The increased hydrophobicity of the copolymer may be influencing its solubility in the DCM/diethyl ether combination used for purifying PSIM-mPEG(5k) and PSIM-mPEG(2k), thereby affecting its yield.

3.7 Degradation

A degradation study was conducted to compare mass loss and cumulative amounts of simvastatin released from the PSIM copolymers (Figure 8). After 6 wk, an average total mass loss of 28, 29, and 7% was observed, from which cumulative amounts of 12, 5.2, and 0

μg of simvastatin were released from PSIM-mPEG(5k), PSIM-mPEG(2k), and PSIM-mPEG(550), respectively. While mass loss for the 5 kDa and 2 kDa mPEG-initiated PSIM copolymers was similar, both exhibited significantly greater mass loss compared to PSIM-mPEG(550), the most hydrophobic copolymer ($p < 0.05$). PSIM-mPEG(5k) and PSIM-mPEG(2k) each exhibited differing rates of simvastatin release in later stages of degradation compared to the rates seen initially. From 12 hr up to 10 days, 0.578 and 0.230 $\mu\text{g}/\text{d}$ of simvastatin was released from PSIM-mPEG(5k) and PSIM-mPEG(2k), respectively, which changed to slower rates of 0.124 and 0.052 $\mu\text{g}/\text{d}$, respectively, for the remainder of the 44 d study. In the first 12 hr, a burst release of 1.6 and 1.1 μg was measured from the two copolymers, respectively. PSIM-mPEG(5k) released a significantly greater amount of simvastatin than PSIM-mPEG(550) did during the 44 d period ($p < 0.0001$) and PSIM-mPEG(2k) on day 3 ($p < 0.001$) and the remainder of the study ($p < 0.0001$). Simvastatin release from the PSIM-mPEG(550) copolymer was negligible over the 44 d period. By visual observation, PSIM-mPEG(5k) experienced the most erosion, which began the earliest. PSIM-mPEG(550) remained the most intact with breakage observed much later during the degradation period.

Degradation of poly(simvastatin) is caused by cleavage of hydrolytically labile ester bonds throughout the polymer chain, ultimately releasing molecules of simvastatin. The degradation rate of poly(simvastatin) can potentially be tuned by modifying the composition of the diblock copolymer. While simvastatin, and subsequently its respective polymerized form, is hydrophobic, mPEG is hydrophilic, giving the resulting PSIM-mPEG copolymer an amphiphilic nature. The higher the MW of mPEG incorporated into the diblock copolymer, the more hydrophilic the resulting copolymer becomes. Generally, the more hydrophilic the copolymer, the faster it should degrade in a physiological environment. While PSIM-mPEG(2k) had a slightly higher average mass loss than PSIM-mPEG(5k), this difference was insignificant.

The order of increasing mass loss during degradation of the PSIM copolymers correlated with cumulative release of simvastatin. An increasing degradation rate with increasing hydrophilicity was more evident when comparing the amount of simvastatin released. PSIM-mPEG(5k) released 2.3 times more simvastatin than did the less hydrophilic PSIM-mPEG(2k) copolymer, with both releasing significantly more than PSIM-mPEG(550), which experienced insignificant degradation. Concentrations of 0.3–2.4 $\mu\text{g}/\text{ml}$, 0.07–0.8 $\mu\text{g}/\text{ml}$, and 0–0.01 $\mu\text{g}/\text{ml}$ were reached for PSIM-mPEG(5k), PSIM-mPEG(2k), and PSIM-mPEG(550), respectively. Concentrations from PSIM-mPEG(5k) were within and above the range reported for simvastatin to have therapeutic effects *in vitro*, while the range for PSIM-mPEG(2k) was at the bottom and slightly below this window [18]. Note, however, the small size (10–15 mg) of the samples; the total amount of simvastatin release can be adjusted via the mass of copolymer used. The negligible amount of simvastatin released from PSIM-mPEG(550), the most hydrophobic of the PSIM copolymers, during the 8 wk time period may be due to hydrophobic interactions between the PSIM block within the copolymer matrix and potentially free simvastatin trapped within the matrix. Both are extremely hydrophobic, which would lead to a considerably slow rate of simvastatin release from the matrix into the aqueous medium, as seen by similar drugs incorporated in hydrophobic block copolymer delivery systems [19]. A loss of other degradation products, such as mPEG,

simvastatin oligomers, or mPEG with limited simvastatin monomers attached, most likely contributed to the significantly greater total mass loss than simvastatin released. Within a 44 d degradation period, the PSIM-mPEG(5k) copolymer synthesized via TBD lost twice as much mass than did the same copolymer synthesized via stannous octoate in a previous study [9]. The comparison may indicate a greater amount of side reactions leading to less degradable byproducts formed in the stannous octoate mediated reaction as a result of being conducted at a higher temperature of 230 °C.

4. CONCLUSION

Triazabicyclodecene, a highly reactive organocatalyst, was able to mediate the ROP of poly(simvastatin) diblock copolymer using different MW mPEGs at a lower reaction temperature than the previously used stannous octoate catalyst. This provides the advantage of modifying hydrophilicity or hydrophobicity of the biomaterial that, in turn, alters degradation rate for therapeutic treatments of different timescales. The potential to polymerize simvastatin at a lower temperature also decreases the incidence of side reactions that generate undesirable byproducts that interfere with degradation of the polymeric biomaterial. Lower reaction or processing temperatures would also be more energy- and cost-efficient at an industrial manufacturing scale. The resulting hydrophilic copolymer synthesized via TBD was capable of losing significantly more mass within the same time period than the same copolymer synthesized via stannous octoate. Simvastatin release was modified as a result of using different MW mPEG initiators for synthesis. Synthesizing and developing a poly(simvastatin)-mPEG diblock copolymer with tunable degradation properties is desirable for tissue therapeutic applications.

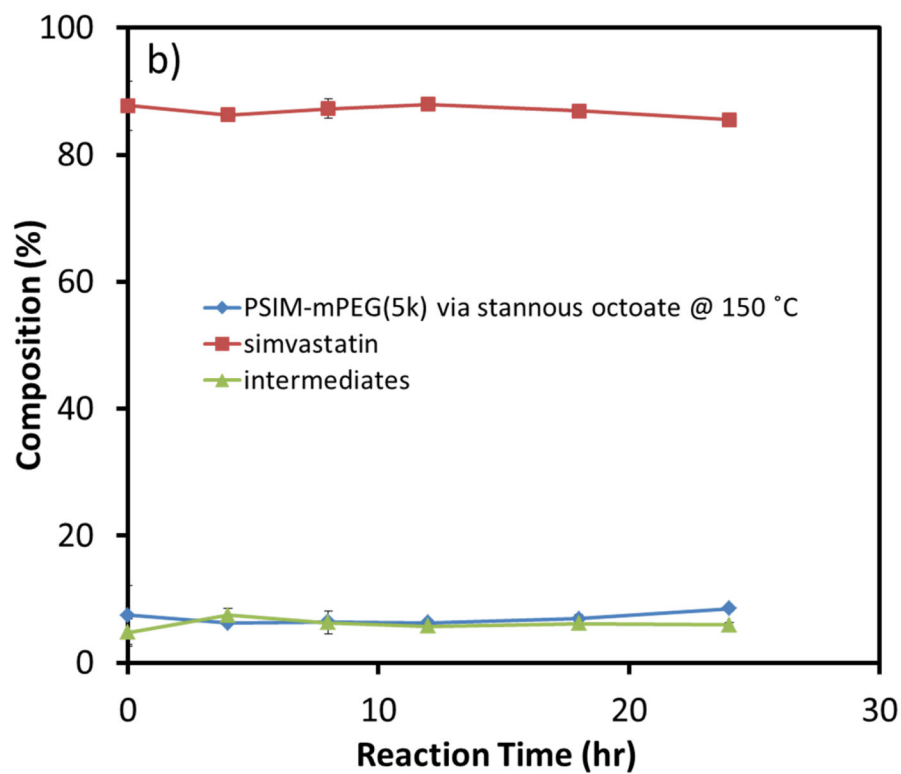
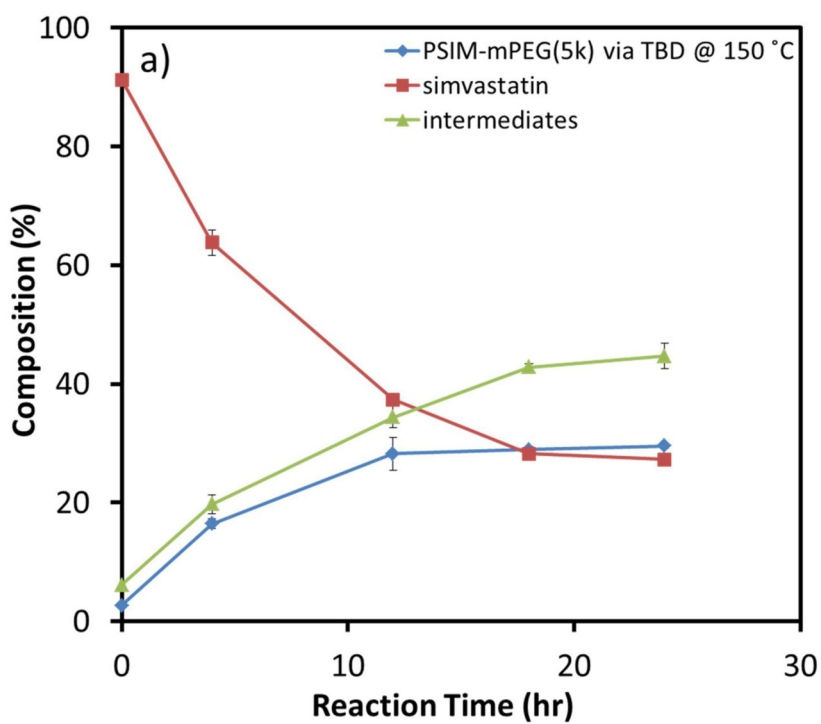
Acknowledgments

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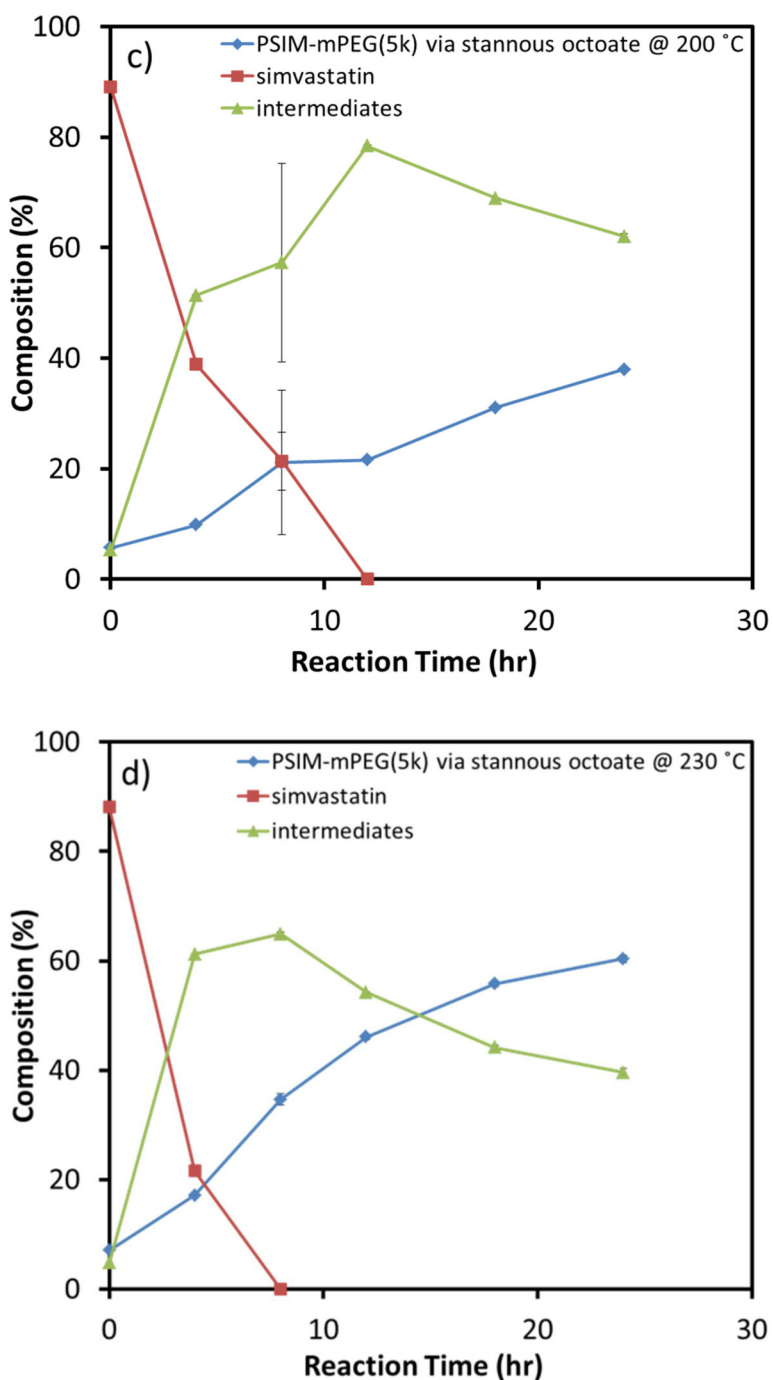


Figure 1. GPC measurements of the percentages of copolymer, intermediates, and simvastatin monomer throughout the reactions in the crude products of a) PSIM-mPEG(5k) synthesized via TBD at 150 °C, b) PSIM-mPEG(5k) synthesized via stannous at 150 °C, c) PSIM-mPEG(5k) synthesized via stannous at 200 °C, and d) PSIM-mPEG(5k) synthesized via stannous at 230 °C.

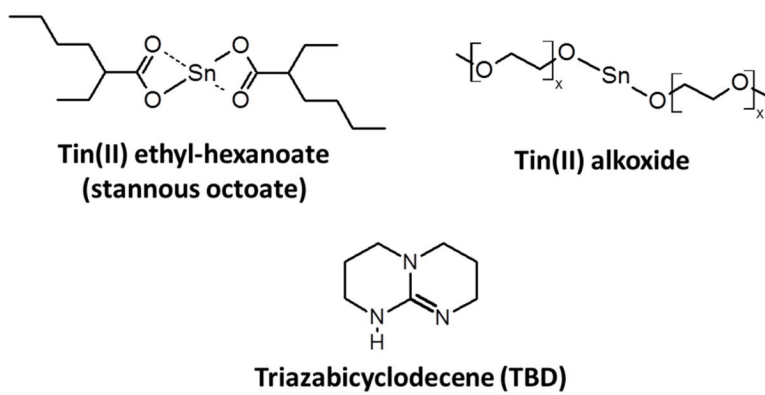


Figure 2.
Structures of stannous octoate and TBD catalysts used in the ROP of simvastatin.

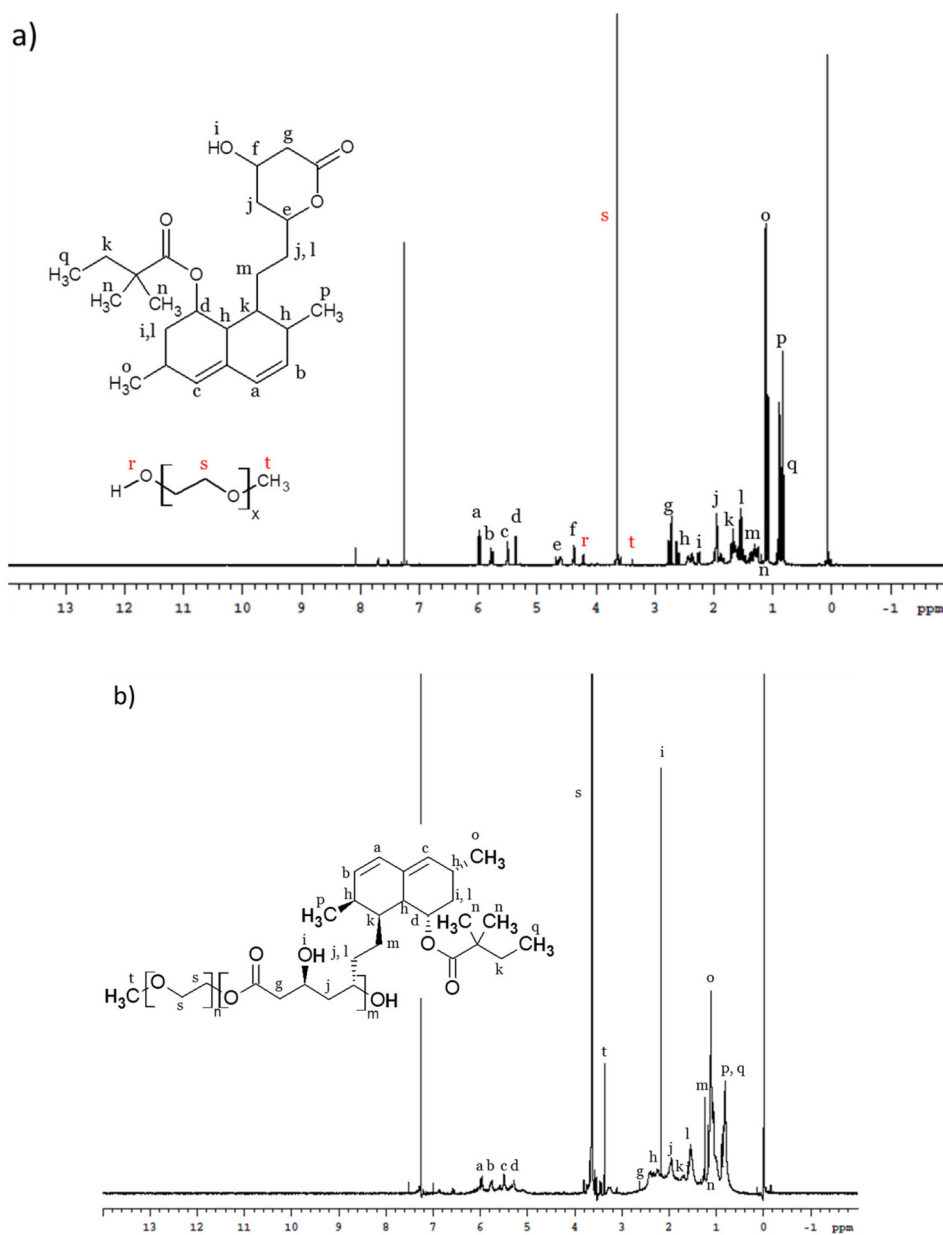


Figure 3. H-NMR spectra of a) a 100:1 mixed control of simvastatin and mPEG and b) the PSIM-mPEG(5k) copolymer synthesized via TBD. Figure 3a was reproduced from Ref. 9 with permission from the Royal Society of Chemistry.

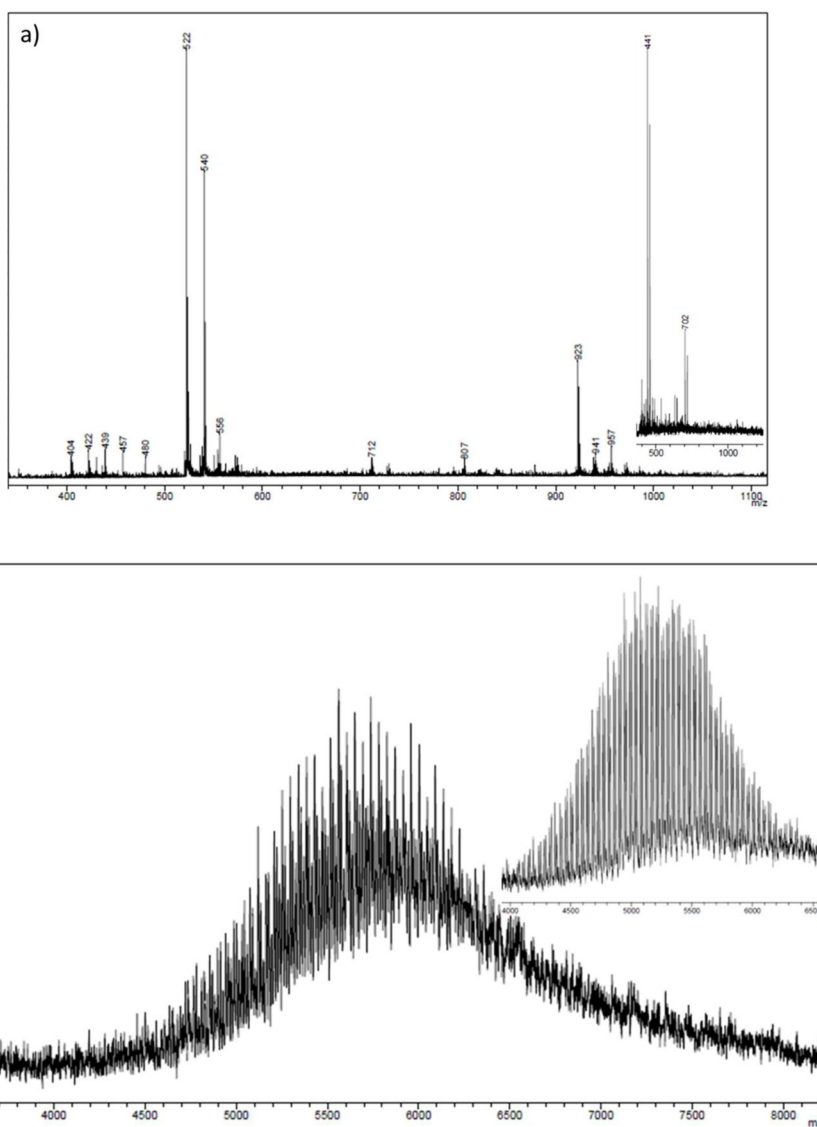
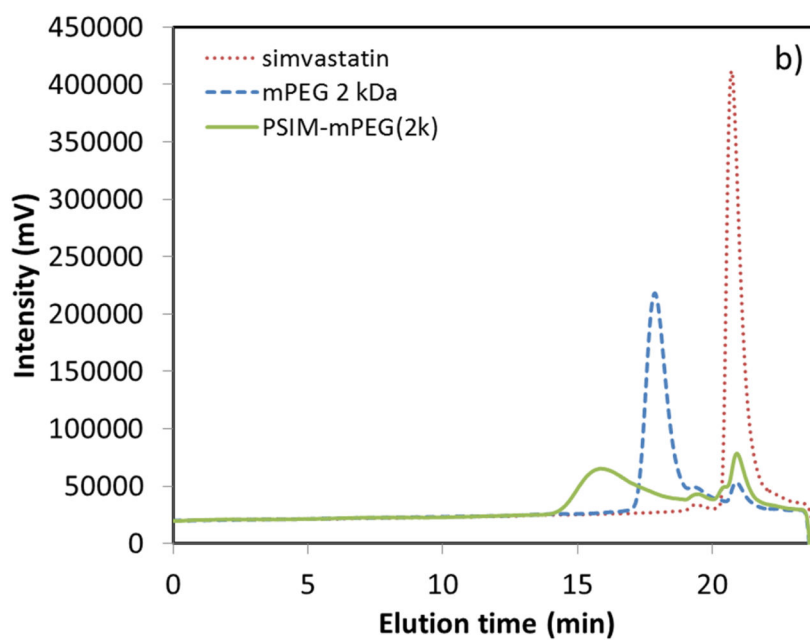
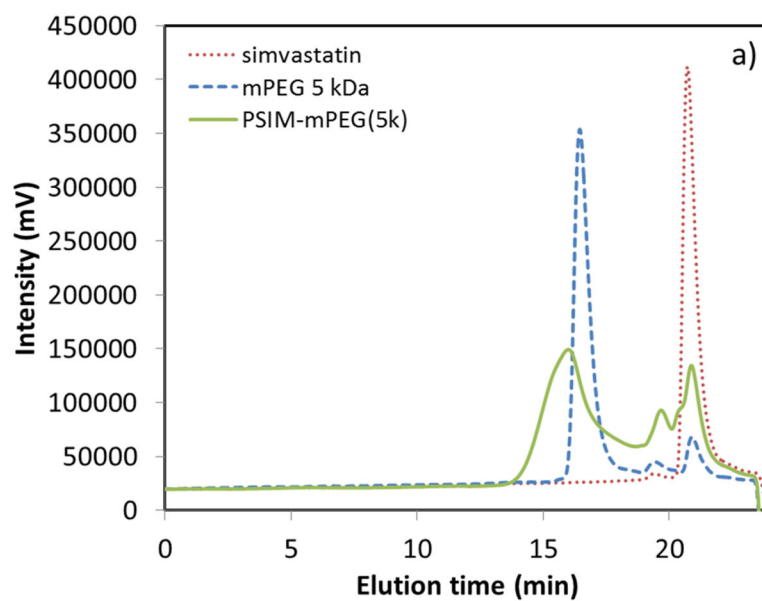


Figure 4. Mass spectra of a) low MW PSIM-mPEG(5k) degradation products compared to simvastatin control and b) high MW PSIM-mPEG(5k) degradation products compared to mPEG control. Insets of controls in Figure 2a and 2b were reproduced from Ref. 9 with permission from the Royal Society of Chemistry.



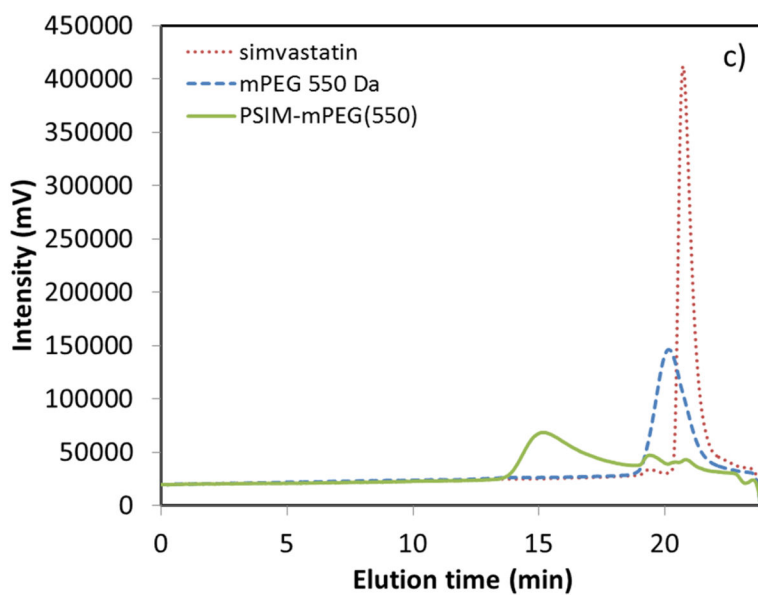
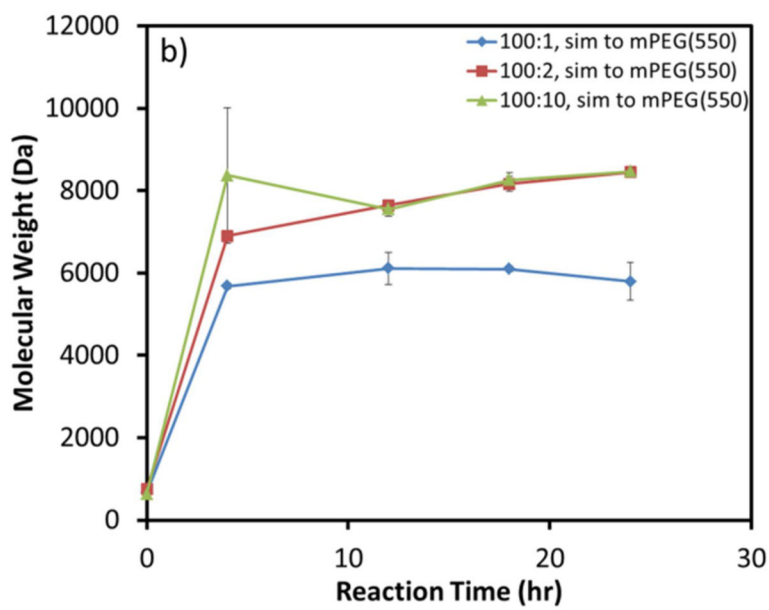
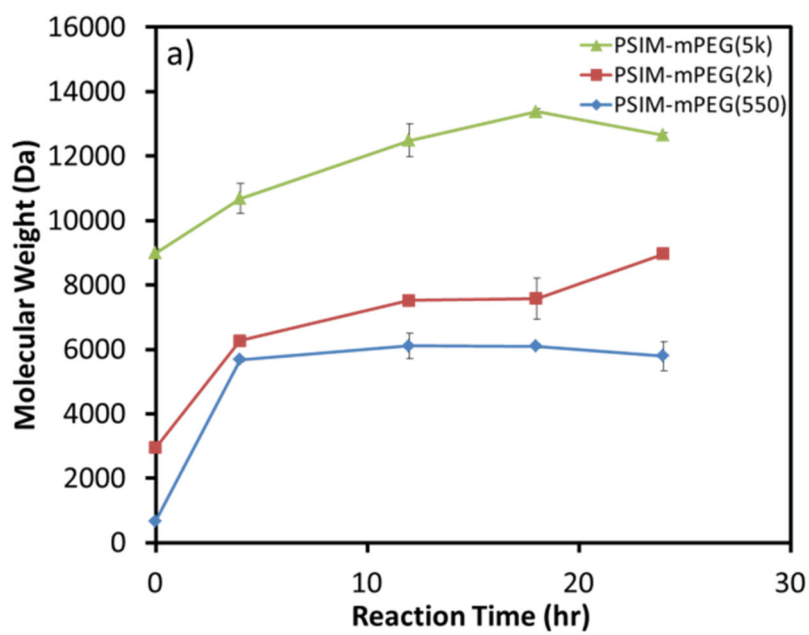


Figure 5. GPC chromatograms of poly(simvastatin) synthesized via TBD using a) 5kDa, b) 2kDa, and c) 550 Da mPEG initiators compared with reactants.



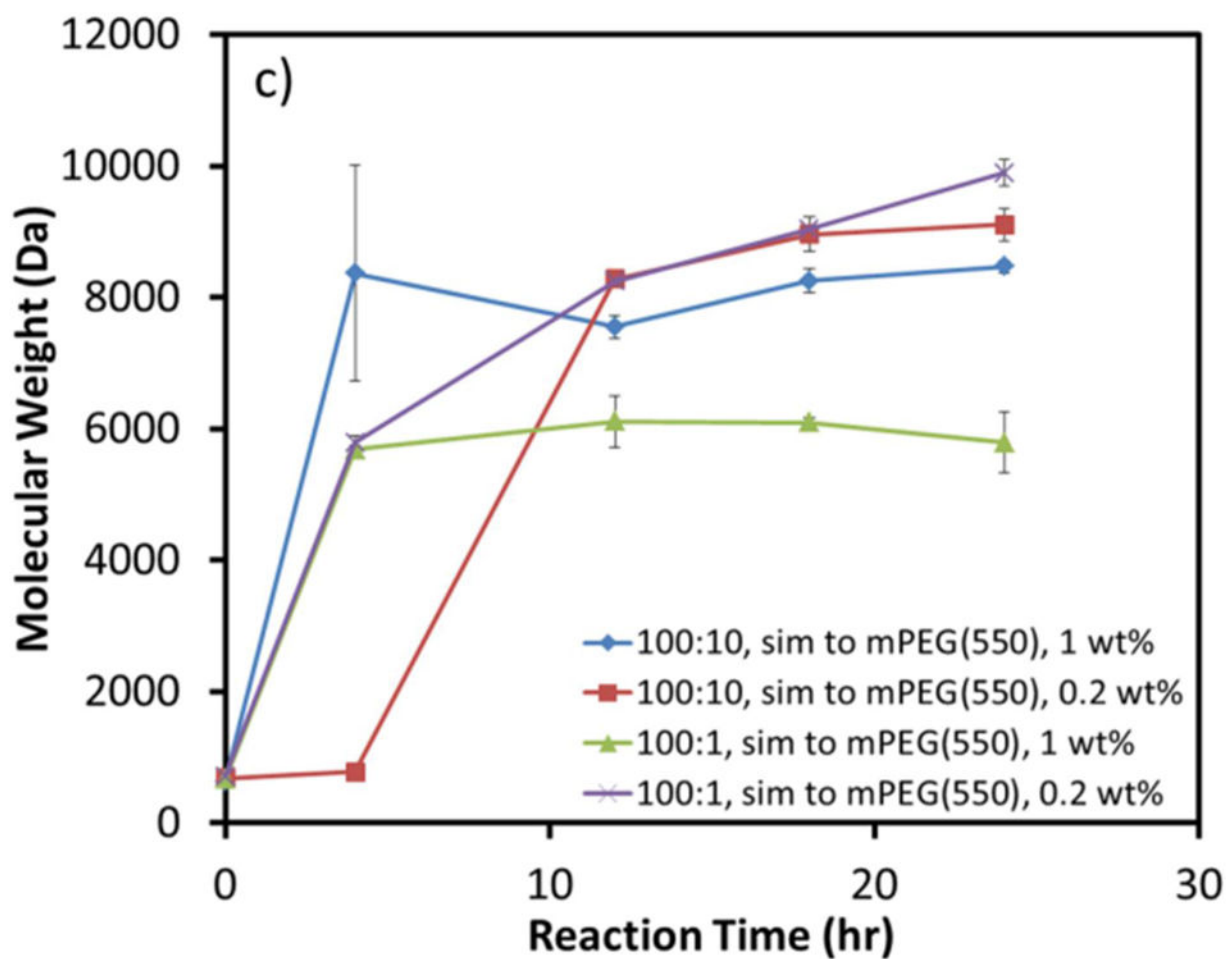


Figure 6. MW growth kinetics for PSIM-mPEG copolymers synthesized with: a) different mPEG MWs with 1 wt% TBD, b) different molar ratios of simvastatin to mPEG (550 Da), and c) two different amounts of catalyst at two different molar ratios.

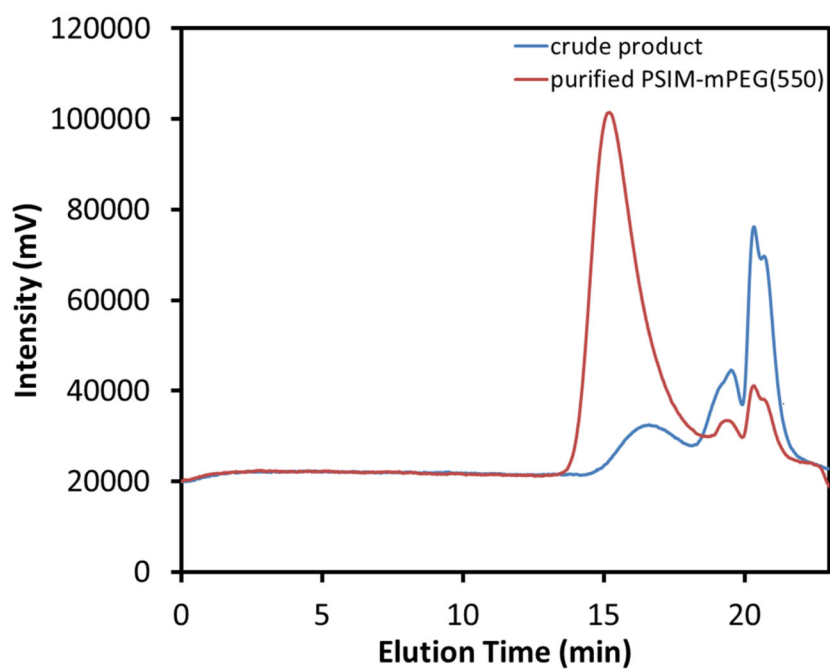


Figure 7.
Chromatograms of PSIM-mPEG(550) before and after purification.

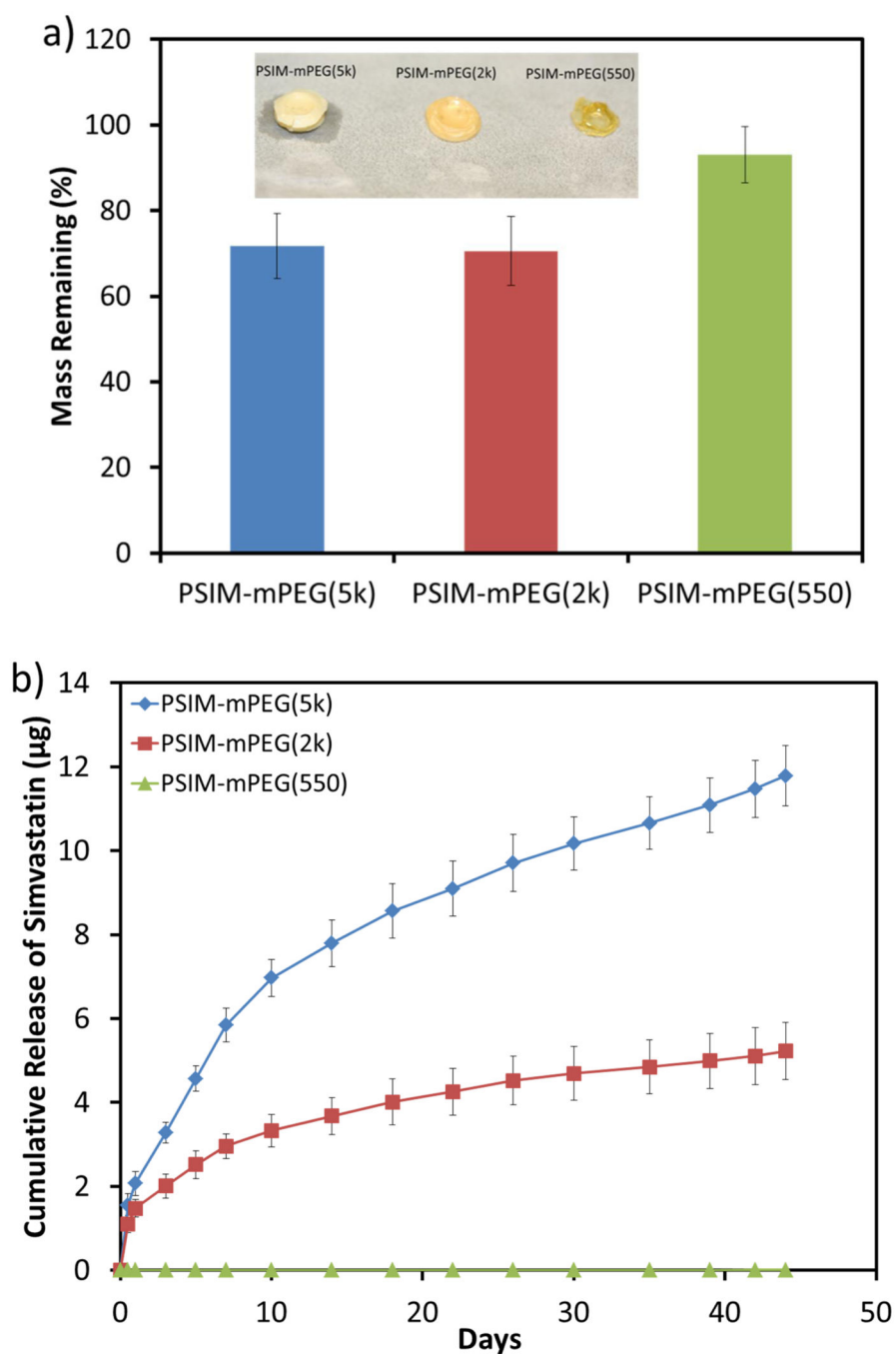
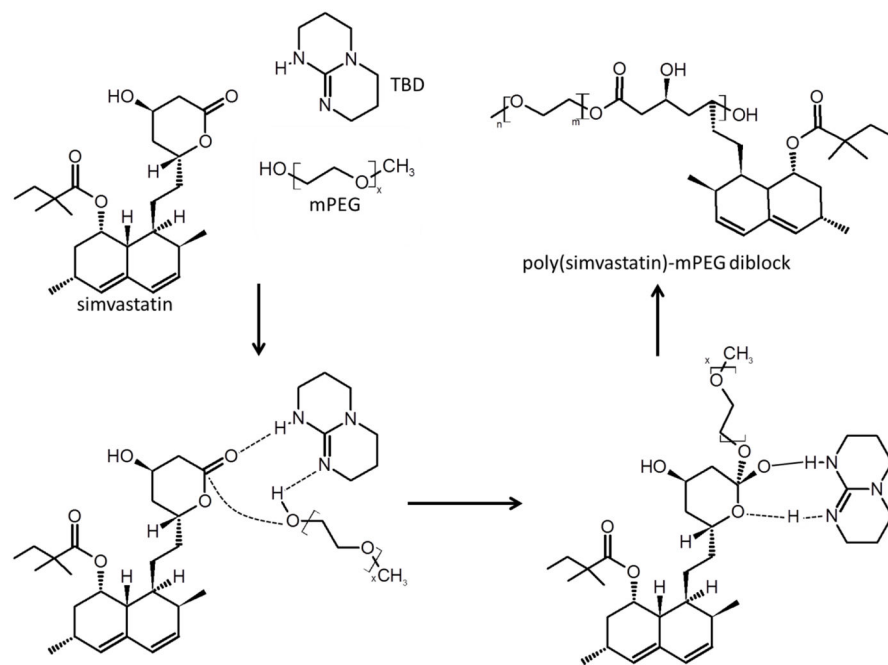


Figure 8. Degradation of PSIM-mPEG(5k, 2k, and 550) diblock copolymers showing a) final mass remaining and b) resulting simvastatin release.



Scheme 1.
Proposed ROP mechanism using TBD catalyst via hydrogen bonding.

Table 1

GPC measurement of simvastatin and mPEG composition in copolymers.

Copolymer	Molar ratio	Sim in copolymer	Sim monomers per mPEG initiator (#)	Yield
PSIM-mPEG 5k	100:1	51%	39	17–30%
PSIM-mPEG 2k	100:1	80%	52	~13%
PSIM-mPEG 550	100:2	98%	96	~2%

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