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Synthesis and Characterization of Degradable Polymers

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SYNTHESIS AND CHARACTERIZATION OF DEGRADABLE POLYMERS

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By

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LIST OF ABBREVIATIONS USED IN THIS WORK

ATRP   atom transfer radical polymerization

DFI   difunctional initiator

DMF   N,N-dimethylformamide

EBiB   ethyl α-bromoisobutyrate

MMA   methyl methacrylate

NMR   nuclear magnetic resonance

PDI   polydispersity index

PMDETA   N,N,N',N'',N''-pentamethyldiethylenetriamine

SEC   size-exclusion chromatography

THF   tetrahydrofuran

ABSTRACT

A degradable polymer with proposed applications in drug delivery was synthesized and characterized. This polymer was grown from a difunctional initiator, a "core" that is able to grow two polymer chains and able to degrade in the presence of oxidizing agents such as peroxides. During the first part of this project, poly(methyl methacrylate) was synthesized via atom transfer radical polymerization using commercially-available reagents in order to develop an optimal synthesis technique. This technique was then used to synthesize the degradable polymer. After introducing hydrogen peroxide, the molecular weight of the degradable polymer decreased in two different experiments, confirming its hypothesized behavior.

VITA

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Research

INTRODUCTION

This project focuses on the synthesis and characterization of a degradable polymer with a proposed use in drug delivery. The crux of this project is the difunctional initiator (DFI) which is able to grow two chains, somewhat like a star polymer. It is also able to degrade, separating the chains, when exposed to oxidizing agents. Degradation in the presence of oxidizing agents, rather than reducing agents like existing degradable polymers, is important for a potential use of the polymer in drug delivery. Hydrogen peroxide is present in the human body in low concentrations, but this concentration is higher in cancer cells.[1] By using this degradable polymer as packaging for a chemotherapy drug, the drug can be contained until reaching cancer cells, at which point the packaging breaks down due to the elevated peroxide concentration and the payload is released.

Synthetic polymers are important industrial products and constitute a major area of research and development in materials science. The simplest polymers are single chains, but much research is focused on more complex structures. Star polymers, for example, consist of many chains bonded to a central initiator, a molecule from which polymer chains begin growth.[2] Polymers may also contain units that allow novel functions, such as degradation when exposed to reducing agents.[3] Complex polymers are often synthesized using atom transfer radical polymerization (ATRP). ATRP is a polymer synthesis technique and a kind of so-called "living" polymerization, a family of reactions first discovered in 1982. Living polymerizations do not self-terminate but instead enter a dormant state that can be reactivated at a later time and allow the polymer to continue growing. ATRP allows a high degree of control over polymer characteristics, with tight

distributions of weights; i.e., most polymer chains are close to identical lengths with little variation. This is quantified by the polydispersity index (PDI).[2]

The structure of the DFI is shown in Figure 1. Two tertiary bromides are present. A polymer chain can grow at each of these during ATRP synthesis. The tertiary bromides exist in an environment similar to that of the tertiary bromide in ethyl α-bromoisobutyrate (EBiB), a commercially-available initiator shown in Figure 2. Due to this similarity, synthesis techniques developed during this project for EBiB should be transferable to the DFI.
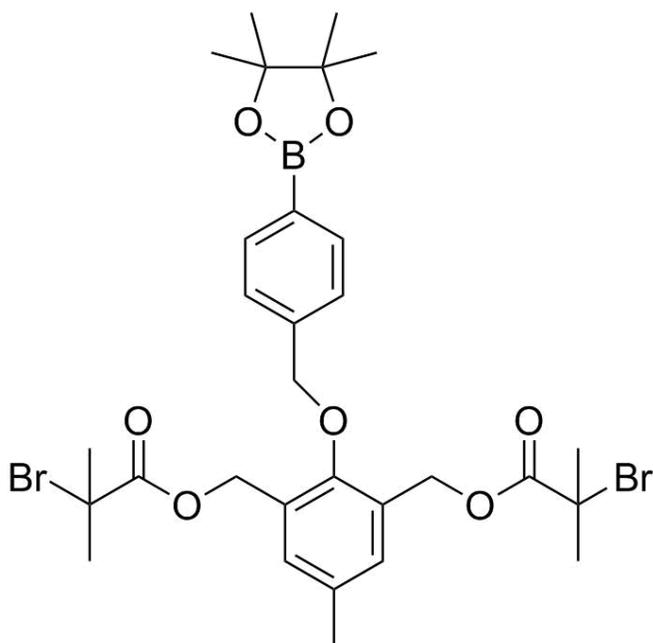

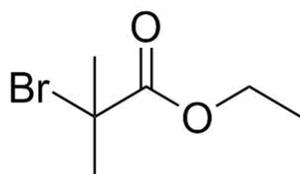*Figure 1. The degradable difunctional initiator.*

Figure 2. The structure of EBiB, a commercially-available ATRP initiator.

The boronic ester allows the DFI to degrade in the presence of oxidizing agents at biologically relevant concentrations.[4] The degradation mechanism is shown in Figure 3. This mechanism results in the separation of the two chain growth sites.
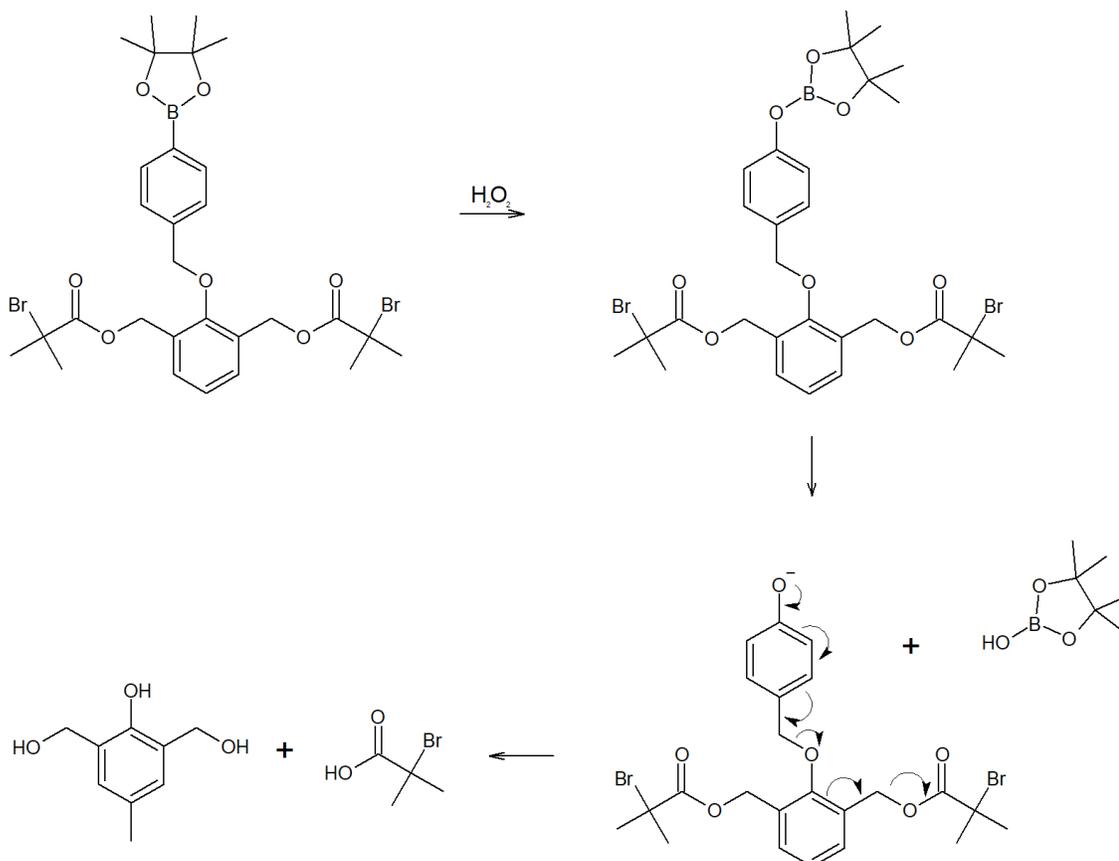

Figure 3. The mechanism of the DFI's degradation after exposure to peroxide.[4]

This project can be divided into two stages. First, an optimal ATRP synthesis focusing on minimizing molecular weight distributions is developed for EBiB. Second, this synthesis is adapted for use with the DFI in order to produce and characterize degradable polymers.

The first monomer addition step of ATRP synthesis using EBiB occurs as shown in Figure 4. The synthesis used in this research is mediated by a copper-containing metal complex.
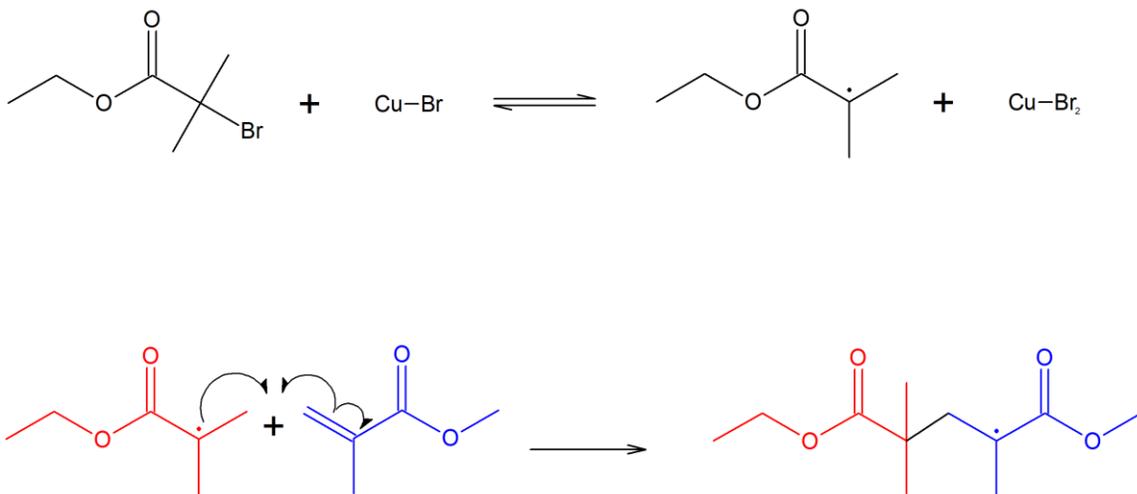

Figure 4. The mechanism of metal complex-mediated ATRP including activation (top) and radical addition (bottom).

The synthesis requires several components: the initiator, monomer, and catalyst. Initiators are organic compounds that contain a halogen, often bromine, bonded to a carbon atom that can form a stable radical intermediate. EBiB and the DFI, for example, form a radical tertiary carbon. This intermediate is formed when the halogen leaves the initiator and oxidizes the catalyst, which is a salt bound to an organic-soluble ligand.[5,6] When using copper(I) catalysts specifically, there are shortcomings. First, copper is toxic and thus polymers produced from copper catalysts may not be safe in medical applications.[2] Second, copper(I) is readily oxidized in air to form copper(II). Copper(I) must either be prepared in advance of the reaction or produced *in situ*.[7]

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Changing any part of the catalyst can significantly alter both reaction rates and polymer characteristics. For example, a set of catalysts using the same transition metal ion and the same ligand with varied counterions produces varying rates of activation in otherwise-unchanged reactions.[8,9] The first part of this research investigated the effects of catalyst concentration and counterion species in an ATRP reaction on the characteristics of the resulting polymers.

PROCEDURE

Several reagents were prepared before synthesis. Stock solutions of the ligand N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA) and the initiator ethyl α-bromoisobutyrate (EBiB) in anisole at concentrations of 0.375 M were prepared and stored in vials. Copper(II) bromide and copper(II) chloride were reduced to their respective copper(I) halide salt by stirring overnight in glacial acetic acid and washing in ethanol the following day. After drying in a vacuum oven, the copper(I) halide salts were stored under air. The monomer methyl methacrylate (MMA) used in these experiments is shipped with an inhibitor; the inhibitor was removed by filtering the MMA through a basic alumina column immediately before synthesis.

An air-free environment needed to be created. The chosen copper halide and a magnetic stirbar were added to a Schlenk flask. The flask was sealed with a rubber septum and attached to a Schlenk line. Several vac-refill cycles were performed to replace oxygen with argon, an inert gas. Pure anisole and the stock solutions of PMDETA and EBiB were sparged with argon for approximately 20 minutes. Once thoroughly degassed, these liquid reagents were injected into the Schlenk flask through the septum. Upon the addition of the

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PMDETA stock solution, a green solution formed in the flask, indicating the presence of dissolved copper ion. EBiB was always added last; as the initiator, its addition triggers the ATRP reaction. The flask was submerged in an oil bath at roughly 60°C. When using EBiB, the solution turned yellow, and dark solid particles were observed.

This procedure was modified when using the DFI. Because the DFI is solid at room temperature, it was added to the flask alongside the copper halide and stirbar before the flask was sealed. The reaction was triggered by the injection of MMA through the septum. The solution remained green when using the DFI, and no solid particles were observed.

Samples were removed periodically in order to track the progress of the reaction using nuclear magnetic resonance spectroscopy (NMR) and size-exclusion chromatography (SEC). Because ATRP quickly dies in air, each sample provides a "snapshot" of the reaction at a given time once removed and exposed to oxygen. Each sample was filtered through a pipette column packed with basic alumina in order to remove copper, which can damage the SEC and interfere with NMR spectra. Alumina near the tops of the columns turned blue, and the filtered samples were colorless or white. Portions of the samples were rotovapped in order to remove anisole. Other portions were dissolved in deuterated chloroform for use in the NMR.

In some experiments, poly(methyl methacrylate) was purified once the reaction was completed. A fritted funnel was filled with basic alumina, inserted into a vacuum flask, and wetted with dichloromethane. The contents of the Schlenk flask were poured through the funnel, and the filtered liquid was collected in the vacuum flask. The contents of the vacuum flask were transferred to a round-bottom flask and removed via a rotovap, leaving a substance that visually resembled hot glue stuck to the glass. This was dissolved in a

small amount of dichloromethane and added dropwise to a beaker filled with methanol. Unreacted monomer and oligomers, short polymer chains, are soluble in methanol, but long polymer chains are not. The purified poly(methyl methacrylate) appeared as white strands in the beaker. The contents of the beaker were poured through filter paper, and the solid was collected in a vial and dried in a vacuum oven overnight.

Purified polymer samples were used in degradation experiments. Approximately 10 mg of polymer was weighed out and placed in a vial. For each milligram of polymer, 1 mL of N,N-dimethylformamide (DMF) was added and 10 μL of 30% hydrogen peroxide was added, giving the peroxide a concentration of about 100 mM.[4] In order to allow the polymer to fully degrade, the solution was stirred for four days. DMF and leftover hydrogen peroxide were removed via a rotovap and, due to their high boiling points, heated using a heat gun rather than a water bath.

RESULTS AND DISCUSSION: OPTIMIZATION OF POLY(METHYL METHACRYLATE) SYNTHESIS

Three experiments were devised using EBiB, the commercially-available initiator, in order to determine the best conditions to use with the DFI, which is available only in small quantities and time-consuming to produce. In the first experiment, baseline conditions for ATRP synthesis were established. These included a 200:1 molar ratio of monomer to initiator and a 1:1 ratio of catalyst and ligand to initiator. Additionally, a 3 M concentration of MMA was chosen. In the next two experiments, the conditions were varied. The second experiment replaced copper bromide with copper chloride. The third experiment used copper bromide again, but at half the concentration of the first experiment.

The quantities of reagents are given in Table 1. The reactions were conducted at approximately 65°C in anisole.

Species	Equivalents (Exp. 1)	Equivalents (Exp. 2)	Equivalents (Exp. 3)
MMA	200	200	200
EBiB	1	1	1
PMDETA	1	1	0.5
CuBr	0.92	---	0.5
$CuBr_2$	0.064	---	0.1
CuCl	---	1.25	---
Total Cu ion	0.984	1.25	0.6

Table 1. Equivalents of reagents used in the first three ATRP synthesis.

When using copper bromide, the reaction vessel was intentionally spiked with a small amount of oxidized copper(II). This was predicted to reduce PDI by reducing the rate of reversible oxidation of copper(I), resulting in a reduced rate of radical formation and increased abundance of dormant initiators and polymers.[10] During the second and third experiments, excess copper salt was added compared to the desired molar ratio between the catalyst and initiator. Excess salt, however, does not appear to affect reaction rate. Any

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amount of copper ion which goes beyond the amount of ligand present does not impact the reaction rate because the ligand limits the amount of catalyst which may form *in situ*.[11]

During the first experiment using the baseline conditions, several samples were extracted while the reaction ran. Results from the SEC overlaying data from five samples is shown in Figure 5. Plots relating molecular weight and PDI as functions of monomer conversion are shown in Figure 6.
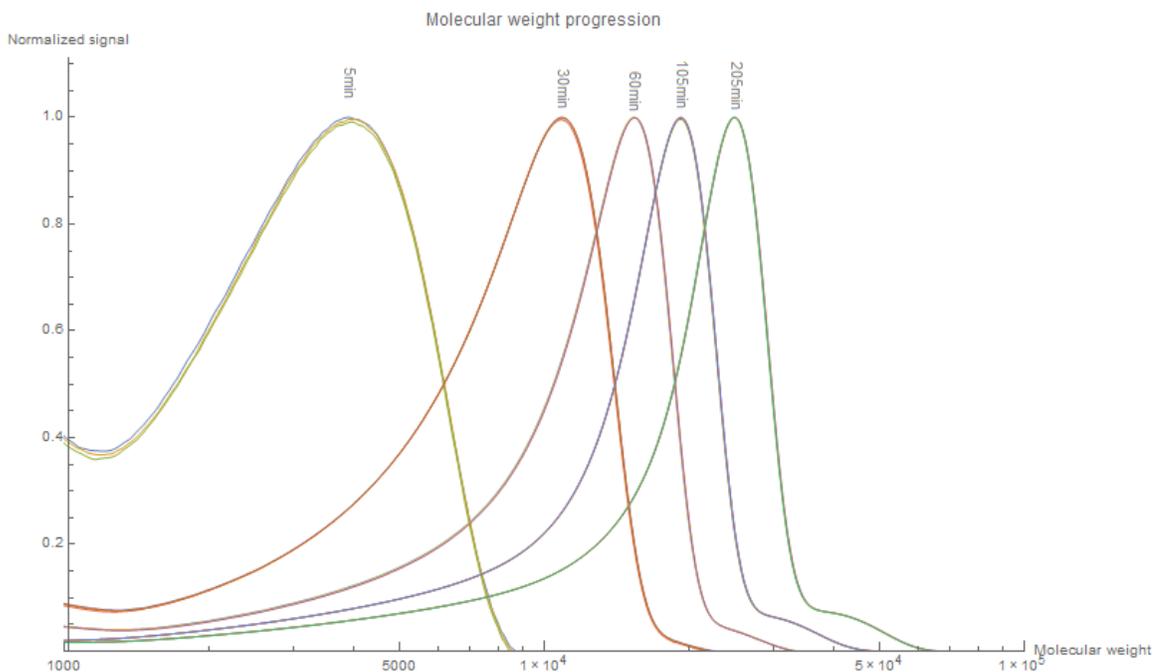

Figure 5. The progression of molecular weight over time in an ATRP synthesis using the baseline conditions.
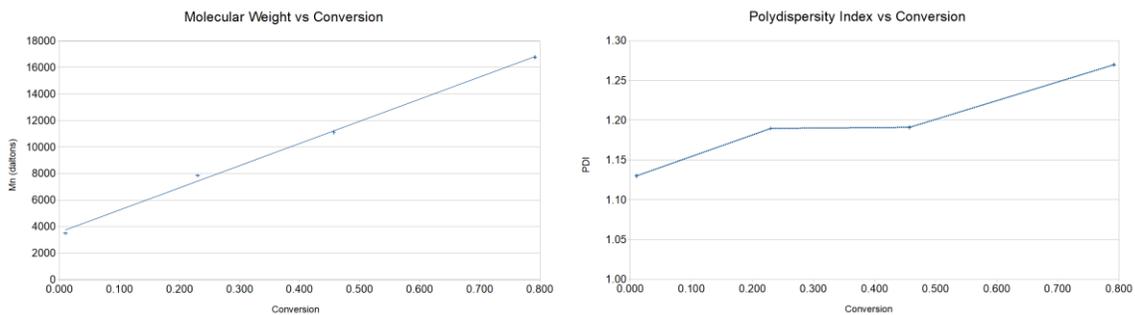
Figure 6. Polymer characteristics as functions of monomer conversion using the baseline ATRP conditions.

PDI was approximately 1.19 after 30 minutes (23% conversion) and 60 minutes (46% conversion). This PDI was higher than desired for a basic ATRP reaction using methyl methacrylate. The number-average molecular weight reached 11000 daltons after 60 minutes and 17000 daltons after 205 minutes.

These results were not satisfactory. PDI was considered too high for such common reagents in a basic ATRP synthesis. This prompted the development of new conditions in an attempt to reduce PDI. Several changes were proposed in order to decrease PDI, and a target of 1.15 was set.

During the second experiment, the conditions were altered and copper bromide was replaced with equal equivalents of copper chloride. Using chloride as a counterion yields a slower reaction than bromide[9] and was predicted to produce a tighter distribution of polymer weights. The reaction was started at 85°C but quickly decreased to 70°C within 15 minutes.

An important caveat to be considered when using CuCl alongside a brominated initiator is that the reaction vessel does not contain only chloride but rather a mix of both chloride and bromide. Because EBiB ejects a bromine atom when reacting with MMA, some amount of CuBr forms and catalyzes some propagation steps. Regardless, the overall reaction rate in this mixed halide reaction is lower than when using exclusively bromide.

Like the first experiment, samples were extracted while the reaction ran. SEC data for several samples is overlaid in Figure 7. Polymer characteristics are related to time in Figure 8.
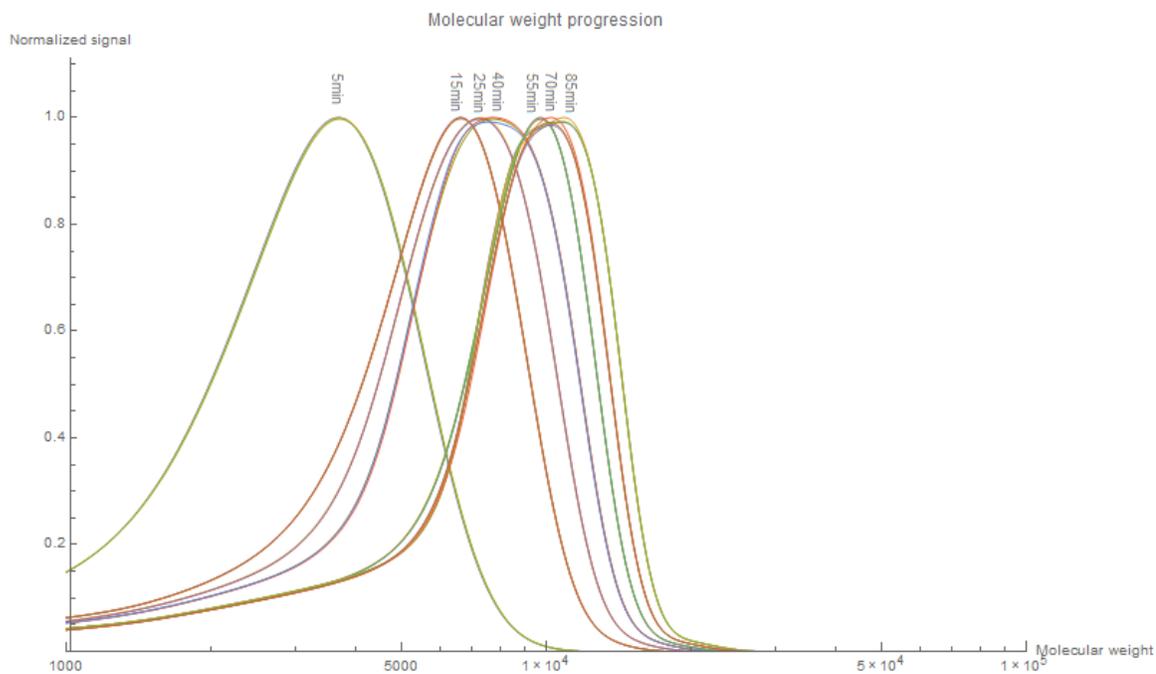

Figure 7. The progression of molecular weight over time in an ATRP synthesis using CuCl rather than CuBr.
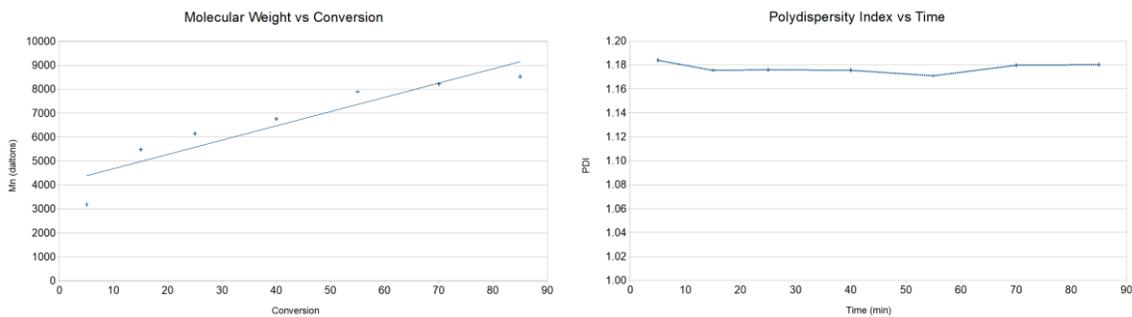

Figure 8. Polymer characteristics as functions of time.

PDI was noticeably lower in this experiment than in the first, dropping to a minimum of 1.17 at 55 minutes and reaching 1.18 after 85 minutes. The number-average molecular weight reached 7900 daltons after 55 minutes and 8500 daltons after 85 minutes when the reaction was terminated.

NMR spectra are not available for this experiment due to errors in sample handling. Anisole, the solvent, was removed from extracted samples in the rotovap for use in the SEC. This procedure also removed unreacted MMA, and no portion had been preserved for NMR use only. Because of the variable amount of anisole remaining and total removal of MMA, it is not possible to quantify conversion. Polymer characteristics are instead related to reaction time.

Like the second experiment, the third experiment was designed to slow the rate of propagation. However, instead of using slow copper chloride, a reduced amount of fast copper bromide was used instead. During the third experiment, half the equivalents of both copper bromide and the ligand were used in comparison to the first experiment. The reaction was run at approximately 55°C. SEC data is overlaid in Figure 9, and this data is related to NMR spectra in Figure 10.
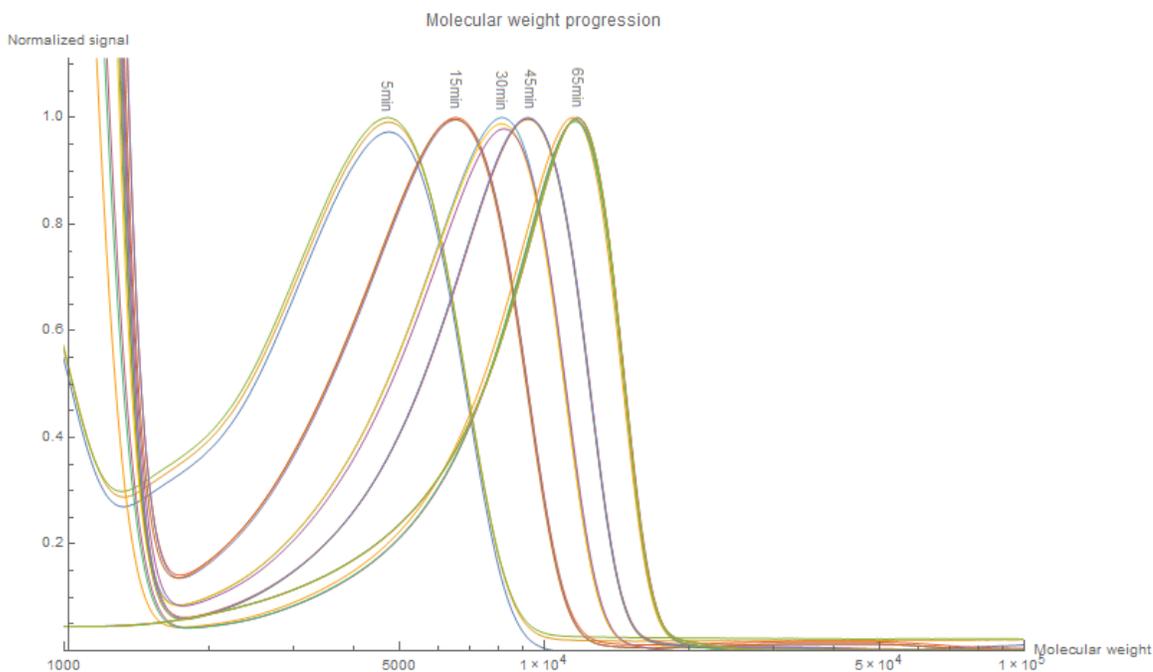
Figure 9. The progression of molecular weight over time in an ATRP synthesis which used the original initiator and counterion but with half the amount of ligand and catalyst.
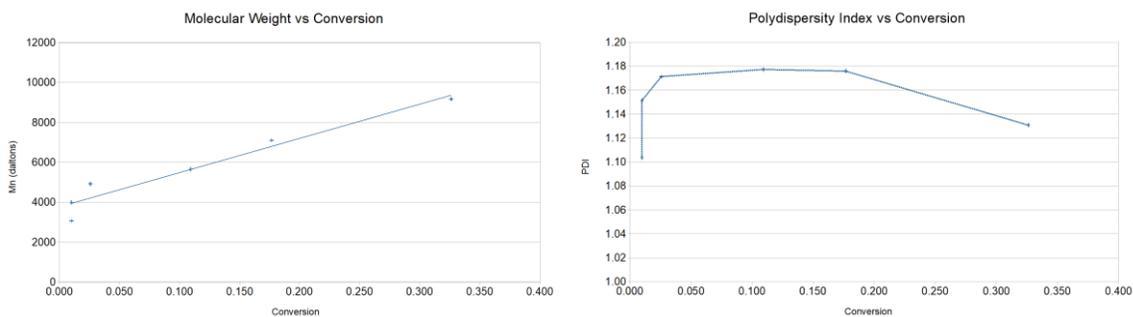

Figure 10. The characteristics of a polymer versus monomer conversion when using excess initiator relative to catalyst.

PDI was quite good and remained below 1.18 for the entirety of the reaction. At 65 minutes, it was about 1.17. When the reaction was terminated at 100 minutes, it dropped to 1.13. This is the lowest PDI observed yet.

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The number-average molecular weight did not increase quickly, as expected. At 65 minutes, it was only 7100 daltons, the lowest weight of any of the three reactions. This may not be significant, however. The temperature in this reaction is also lower than in the others and likely contributed to the observed decreased rate. When the reaction was terminated at 100 minutes, the number-average molecular weight had only increased to 9200 daltons.

Figure 9 does include the peak produced by the sample taken at 100 minutes, but it perfectly overlaps the peak produced by the sample taken at 65 minutes. It is unclear why the number-average molecular weight was so much higher and PDI was so much lower, however, since the chromatographic data appears to overlap. Figure 10 indicates that PDI may not have reached a minimum and may have continued to decrease if the reaction were allowed to run longer.

RESULTS AND DISCUSSION: SYNTHESIS, CHARACTERIZATION, AND DEGRADATION OF A DEGRADABLE POLYMER

The second set of experiments involved polymers synthesized using the novel boronic ester-based DFI rather than the readily-available EBiB. The conditions chosen were based on the second experiment in the previous section, which used copper(I) chloride as a catalyst in a 1:1 molar ratio with EBiB. The stoichiometry was modified. Because each DFI molecule is able to grow two chains, its concentration was halved compared to the amount of EBiB used in the second experiment. Table 2 lists the reagents and quantities compared to the second experiment.

Species	Equivalents (Exp. 2)	Equivalents (DFI synthesis)
MMA	200	200
EBiB	1	---
DFI	---	0.5
PMDETA	1	1
CuCl	1.25	1.13

Table 2. Equivalents of reagents used in the first three ATRP synthesis.

Like the second experiment, an excess amount of copper(I) chloride was used with respect to the ligand, but this was not expected to affect kinetics. The reaction was run at about 55°C and terminated after 200 minutes. Figure 11 shows an incomplete set of SEC data truncated for visual clarity, and Figure 12 shows the reaction progress as a function of monomer conversion and molecular weight.
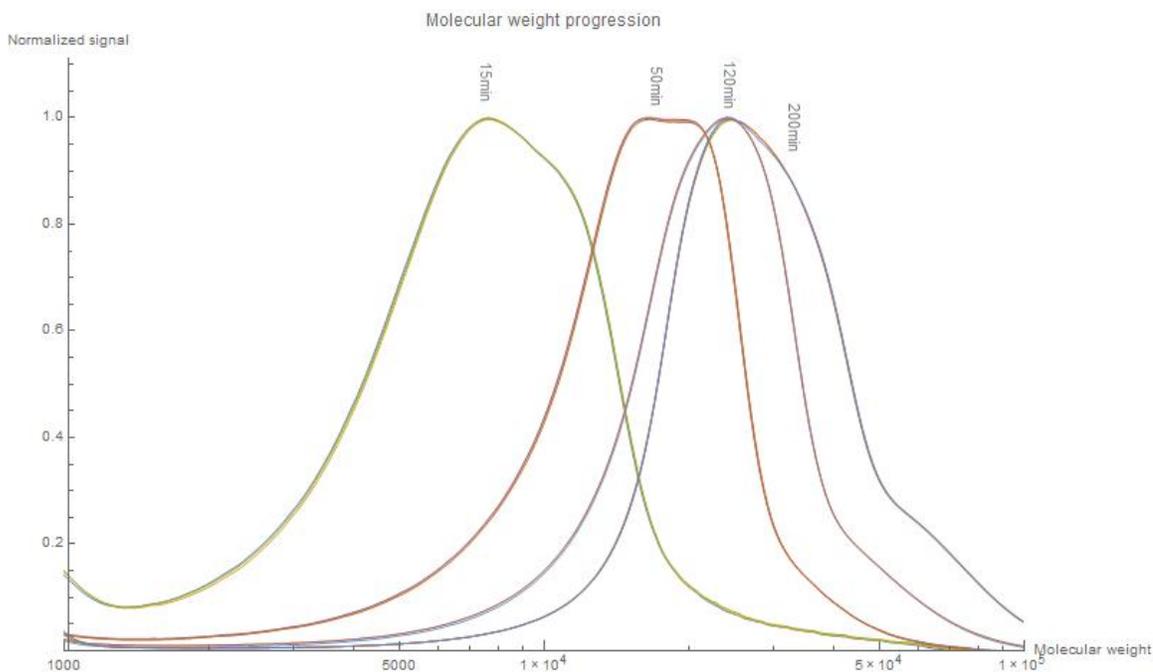
Figure 11. The molecular weights of the second, fourth, sixth, and eighth samples.
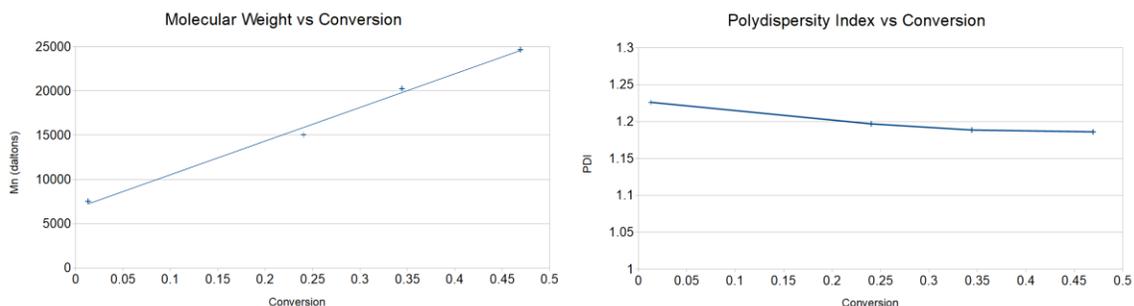

Figure 12. Molecular weight and polydispersity index as functions of monomer conversion.

The peaks shown in Figure 11 are much broader than usual, and the peak produced by the sample taken at 50 minutes appears almost bimodal. At 50 minutes, the number-average molecular weight was 15000 daltons with a PDI of 1.20. When the reaction was terminated after 200 minutes, the number-average molecular weight had reached 24700 daltons and PDI had decreased slightly to 1.18.

One data point has been dropped from the plots in Figure 12. The NMR spectrum of the sample taken at 5 minutes suggested that its monomer conversion was significantly higher than the conversion of the sample taken at 15 minutes. The best-fit line relating molecular weight to conversion has $R^2 > 0.99$ with or without this point. However, the PDI of this sample was extremely high at 1.52. The high PDI can probably be explained by instrumental errors. The SEC had not been operational for four weeks, and the molecular weight distribution of this sample is not consistent between consecutive injections from the same vial, nor does it visually resemble the distribution of any other sample.

The molecular weights are consistent with the second experiment that used EBiB and copper(I) chloride. Recall that the EBiB-based polymers in the second experiment had reached a number-average molecular weight of 7800 daltons after 55 minutes. This is about half the weight reached by the DFI-based polymers after 50 minutes, suggesting that the rate for an individual radical chain reaction is similar in both initiators. The DFI is able to undergo two such chain reactions simultaneously and thus grows overall at twice the rate.

Polymer from a different synthesis was chosen for use in a degradation study. This experiment was designed to compare the behavior of poly(methyl methacrylate) using different initiators, EBiB and the DFI, when exposed to hydrogen peroxide. An old batch of EBiB-based polymer was pulled from storage. Four vials were prepared, shown in Table 3.

	Initiator	Polymer mass (mg)	DMF (mL)	H_2O_2 (μL)
Vial 1	EBiB	6.9	7	0

Vial 2	EBiB	11	11	110
Vial 3	DFI	9.8	10	0
Vial 4	DFI	8.5	8.5	85

Table 3. Reagents used in a second degradation experiment.

Vials 1 and 3 were stirred for 93 hours, and Vials 2 and 4 were stirred for 96 hours. After completely degrading, DMF was removed via a rotovap, and solid residue in the vials was dissolved in THF and transferred to the SEC's autosampler. Figures 13 and 14 show molecular weight distributions with and without peroxide treatment overlaid.
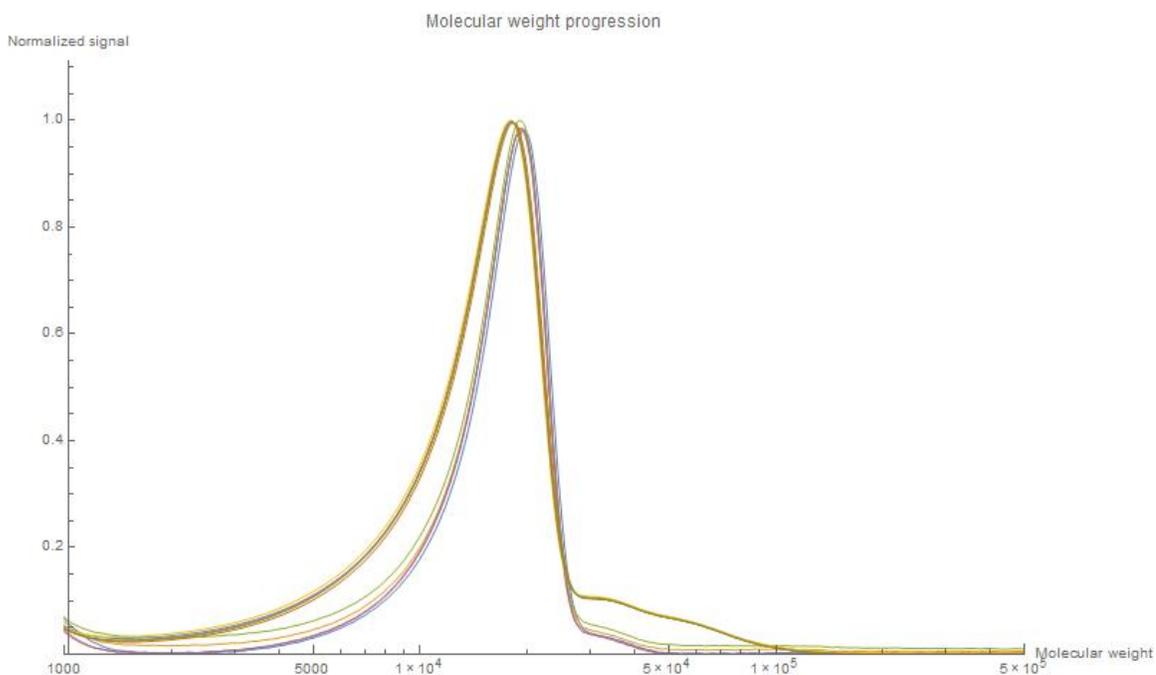

Figure 13. Overlaid plots of an EBiB-based poly(methyl methacrylate) polymer with and without hydrogen peroxide treatment.
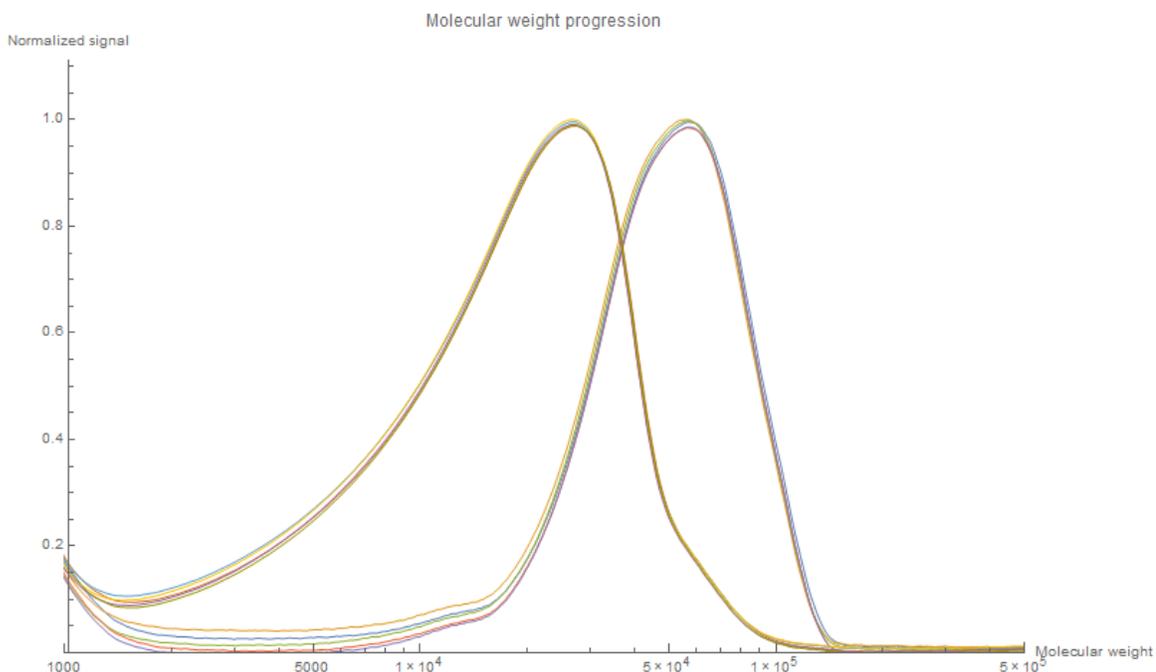
Figure 14. Overlaid plots of a poly(methyl methacrylate) polymer using our novel boronic ester-containing difunctional initiator with and without hydrogen peroxide treatment.

The number-average molecular weight of the EBiB-based poly(methyl methacrylate) is about 10% lower in the peroxide-treated sample, but its weight-average molecular weight is somewhat higher. The polymer used in Vials 1 and 2 may not have been homogenously distributed, and it is unlikely that the molecular weights are meaningfully different.

Figure 14 shows the DFI-based poly(methyl methacrylate) with and without peroxide treatment. There are clear signs of degradation here. The peroxide-treated sample has a PDI of 1.45 rather than 1.29, and its number-average molecular weight is 58% lower at 15700 daltons rather than 37700 daltons. This is a greater decrease in molecular weight than anticipated but reasonably close to the expected 50% decrease. The increase in PDI

suggests that there is a large distribution in chain lengths, which is consistent with the left curve.

CONCLUSION

Atom transfer radical polymerization is an effective technique for synthesizing well-characterized polymers with novel structures. A diverse set of initiators, monomers, catalysts, and ligands are available, and changing just one component alters characteristics of both the reaction and the synthesized polymers.

Boronic esters are useful functional groups which react with oxidizers and, when integrated into larger organic molecules, can add new functionality. The novel difunctional initiator is able to initiate two polymer chains' growth in ATRP. Because these chains are attached through boronic esters, oxidizers are able to break the difunctional initiator into two pieces and thus polymer chains. The boronic ester groups do behave as expected when introduced to a strong oxidizer.

Future work will include the synthesis of polymers containing multiple difunctional initiators which are able to degrade at multiple points within the chain. Experiments examining the characteristics of the degradable polymers as they are oxidized will be designed. In addition, we are working toward synthesizing a methyl methacrylate-based monomer with an ascorbic acid pendant group attached. This monomer is predicted to have antioxidant properties and reduce the rate at which the difunctional initiator degrades. However, this monomer has not yet been synthesized in the quantity needed, and a successful experiment focusing on degradation kinetics has yet to be developed.

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