# REVERSIBLY PHOTO-CROSSLINKABLE POLYCARBONATE-BASED POLYMERSOMES FOR DRUG ENCAPSULATION AND DELIVERY

by

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### Abstract

This thesis describes the preparation of polymersomes from poly(ethylene glycol)-*block*-polycarbonate (PEG-PC) copolymers functionalized with pendant coumarin groups. Coumarin groups undergo photo-reversible dimerization when irradiated with specific ultraviolet wavelengths, so they can be used to prepare polymers with photo-responsive properties. In this case, the pendant coumarin groups enable stabilization of the polymersome membrane through photo-crosslinking of the hydrophobic block.

Initially, several novel cinnamoyl and coumarin functionalized cyclic carbonate monomers were synthesized using ester, ether, or amide linkages. While the homopolymerization of these functionalized monomers proved challenging due to their high melting points, both cinnamoyl and coumarin functionalized monomers were successfully copolymerized with trimethylene carbonate (TMC) at 100  $^{\circ}$ C using a catalyst-free melt polymerization process where the TMC doubled as a solvent for the higher melting point monomer. Using this system, polycarbonate copolymers with up to 33% incorporation of the functionalized monomers were prepared. In addition, an investigation of some anomalous polymerization results identified previously unreported triethylamine-based catalysts for the melt polymerization of carbonate monomers. These studies also demonstrated that the catalyst-free polymerization of TMC occurs faster and at lower temperatures than previously reported.

Subsequently, the photo-crosslinking of cinnamoyl and coumarin functionalized polycarbonates was compared and coumarin was identified as the more effective crosslinking agent when using 300-400 nm UV. An investigation of the photo-reversibility of the coumarin dimerization revealed no discernible change in the properties of crosslinked networks, but rapid photo-reversion in dilute solutions. The photo-crosslinking and photo-reversion kinetics of the coumarin functionalized polycarbonates were determined to be second-order in both cases.

Finally, the self-assembly of PEG-PC diblock copolymers functionalized with coumarin was examined and both reverse solvent evaporation and solvent displacement were found to induce self-assembly, with hydrophilic mass fractions (*f*-factors) of 12-28% resulting in the formation of solid microparticles and nanoparticles and *f*-factors of 33-43% resulting in the formation of polymersomes. The stabilization of these polymersome membranes through photo-initiator-free photo-crosslinking was demonstrated with the crosslinking allowing polymersomes to withstand centrifugation at 12,000 x g. In addition, the encapsulation of calcein, as a model small molecule drug, in the stabilized polymersomes was successfully demonstrated using confocal microscopy.

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## **List of Abbreviations**

- AC, acrylated carbonate monomer
- AMC, 7-amino-4-methylcoumarin
- AMD, age-related macular degeneration
- ANOVA, analysis of variance
- BTMC, benzyloxytrimethylene carbonate
- CAM, 5-(cinnamoylamino)-5-methyl-1,3-dioxan-2-one
- CDI, 1,1'-carbonyldiimidazole
- CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine
- CDCl<sub>3</sub>, deuterated-chloroform
- CMC, critical micelle concentration
- CMVR, cytomegalovirus retinitis
- COM, 5-cinnamoyloxymethyl-5-methyl-1,3-dioxan-2-one
- DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene
- DCM, dichloromethane
- DLS, dynamic light scattering
- DMF, dimethylformamide
- DMSO, dimethylsulfoxide
- DMT-MM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
- D<sub>p</sub>, degree of polymerization
- DNA, deoxyribonucleic acid
- DSC, dynamic scanning calorimetry
- EVA, ethylene-vinyl acetate
- EGFP, enhanced green fluorescent protein
- FDA, United States Food and Drug Administration

GFP, green fluorescent protein GPC, gel permeation chromatography HBr, hydrobromic acid HCl, hydrochloric acid HPLC, high performance liquid chromatography LC, liquid chromatography MAC, 5-(4-methylcoumarin-7-carbamoyl)-5-methyl-1,3-dioxan-2-one mPEG, poly(ethylene glycol) monomethylether M<sub>n</sub>, number average molecular weight M<sub>p</sub>, weight average molecular weight MU, 4-methylumbelliferone MUC, 5-(4-methylumbelliferyloxycarbonyl)-5-methyl-1,3-dioxan-2-one MUM, 5-(4-methylumbelliferyloxymethyl)-5-methyl-1,3-dioxan-2-one NMR, nuclear magnetic resonance NPC, neopentylene carbonate ODTx, On Demand Therapeutics PBC, poly(benzyl carbamate) PBS, phosphate-buffered saline PEG, poly(ethylene glycol) PEG-DME, poly(ethylene glycol) dimethylether PEG-PC, poly(ethylene glycol)-polycarbonate PLGA, poly(lactide-co-glycolide) PMA, poly(methacrylate) PVA, poly(vinyl alcohol) RNA, ribonucleic acid

SEM, standard error of the mean SnOct<sub>2</sub>, stannous octoate TBD, triazabicyclodecene TEA, triethylamine TEA·HCl, triethylamine hydrochloride THF, tetrahydrofuran TMC, trimethylene carbonate UV, ultraviolet UV-Vis, ultraviolet-visible spectrophotometry VEGF, vascular endothelial growth factor

# Chapter 1

# Introduction

## **1.1 Traditional Intraocular Drug Delivery**

According to the CNIB, treating vision loss in Canada cost \$15.8 billion in 2007<sup>1</sup> and retinal diseases account for approximately 25% of vision loss cases.<sup>2</sup> For example, age-related macular degeneration (AMD) is the leading cause of blindness in the developed world and affects 25-30 million people worldwide.<sup>3</sup> Many pathways have been explored for retinal drug delivery, but there are issues associated with each one. First, eye drops, which have been successfully used to treat inflammation and irritation of the eye surface, are ineffective for treating retinal diseases as only 1–5% of the drug is absorbed into the eye and only a fraction of that reaches the retina.<sup>4</sup> Second, a few retinal drugs, such as antibiotics, can be delivered by intravenous injection. However, this delivery method results in systemic drug circulation, which significantly increases the risk of toxic side-effects.<sup>5</sup> Thus, most retinal drugs are delivered by direct injection into the eye.<sup>6,7</sup> While the treatment of retinal diseases such as age-related macular degeneration (AMD), diabetic retinopathy, and macular edema lasts for years, the half-life of most retinal drugs within the eye is quite short, typically 2-5 hours,<sup>8</sup> so regular injections are required, normally once every 4-6 weeks.<sup>6,7</sup> These injections are often painful and can lead to complications, such as vitreous haemorrhage, infection, and retinal tearing.<sup>9</sup> Therefore, a new approach is needed that can either reduce or eliminate the need for injections.

# 1.2 Advances in Intraocular Drug Delivery

#### **1.2.1 Implantable Devices**

One approach that has been explored to reduce the need for injections is to deliver the retinal drugs using reservoir-based delivery systems. The first drug delivery implant approved by the United States Federal

Drug Administration (FDA) for localized, sustained drug release in the eye was Vitrasert<sup>®, 6</sup> This product is an intravitreal implant for the controlled delivery of ganciclovir to treat cytomegalovirus retinitis (CMVR) in immunocompromised patients and was introduced in 1996.<sup>10,11</sup> In this device, a 2.5 mm diameter, 1 mm thick tablet-shaped core containing 4.5 mg of ganciclovir is completely coated with poly(vinyl alcohol) (PVA) and discontinuously coated with ethylene-vinyl acetate (EVA).<sup>9</sup> The gaps in the EVA coating provide release windows and the rate of the ganciclovir diffusion through the exposed PVA coating controls the drug release rate. Vitrasert<sup>®</sup> is designed to release the drug over a period of 5 to 8 months at a rate of 1 to 2  $\mu$ g/h.<sup>6</sup> This release regimen has been shown to be highly efficacious at treating CMVR with an increase in the median disease progression time from 15 days in the deferred treatment group to 226 days in the implant group.<sup>12</sup> Another study found Vitrasert® to be more effective than intravenous delivery of ganciclovir and to eliminate the risk of systemic toxicity.<sup>13</sup> However, the Vitrasert® delivery vehicle is not biodegradable, so it requires surgical removal after the treatment is complete. Whether the need for this surgical removal is offset by the more reproducible drug release rates obtained by not using a biodegradable device is subject to debate.<sup>9</sup>

A similar approach was used to design Retisert<sup>®</sup>, which is used for delivering fluocinolone acetonide to treat recurrent posterior uveitis.<sup>14</sup> This drug delivery vehicle consists of a pure drug tablet contained in a silicone elastomer cup with a release orifice and a PVA membrane between the tablet and the release orifice.<sup>15</sup> The silicone elastomer cup assembly is attached to a PVA suture tab with silicone adhesive and the entire device is approximately 3 mm x 2 mm x 5 mm.<sup>16</sup> Similar to Vitrasert<sup>®</sup>, this device is surgically implanted into the vitreous humour through a small incision and the drug release rate is controlled by diffusion through the PVA. This delivery method is designed to deliver the drug at a rate of approximately 2  $\mu$ g/day for a minimum of 2.5 years.<sup>16</sup> The prolonged drug delivery period allowed by this device is attractive for treating an assortment of retinal diseases. However, once again this device is non-degradable and requires surgical removal at the end of the treatment period.

In addition, several other reservoir devices are in development or preclinical testing. For example, Varner *et al.* patented a device for transconjunctival implantation with a helical shape to provide the maximum surface area for diffusion for the minimum implantation size.<sup>17</sup> Preferred materials for the construction of these devices include known shape memory polymers, such as AB-polymer networks based on oligo( $\epsilon$ -caprolactone) dimethacrylate and n-butyl acrylate, but any polymer that is biocompatible and insoluble in the fluids present in the eye could be used.<sup>17</sup> In addition, a flexible material reduces the translation of slight device movements to the retina, which minimizes the risk of retinal tearing.<sup>17</sup> SurModics Pharmaceuticals has incorporated this drug delivery vehicle into the iVation device, which is designed to deliver triamcinolone acetonide for the treatment of diabetic macular edema and is currently in phase II testing.<sup>15</sup>

### **1.2.2 Injectable Devices**

Vitrasert<sup>®</sup>, Retisert<sup>®</sup>, and related methods require surgical implantation, which can potentially lead to complications. However, the potential benefits of sustained ocular drug delivery at a consistent release rate has led to a new class of injectable delivery devices. Iluvien<sup>®</sup> is a third-generation device designed to be inserted using a proprietary 25-gauge needle instead of surgical implantation that was approved by the FDA in September 2014 for the delivery of fluocinolone acetonide in the treatment of diabetic macular edema.<sup>15,18</sup> There are two dosage forms with the high dosage form capable of delivering 0.5 µg/day for 18-24 months and the low dosage form delivering 0.2 µg/day for 24-30 months.<sup>19</sup> Controlled drug release for both forms is achieved by diffusion through the end caps of a small (3.5 mm in length and 0.37 mm in diameter) cylindrical device.<sup>20</sup> These end caps are made of the same PVA polymer matrix as Retisert<sup>®</sup> in the higher dosage form and of a silicone bioadhesive in the lower dosage form.<sup>19</sup>

Preparation of injectable intravitreal implants from biodegradable polymers has also been explored. For example, Allergan received FDA approval in 2009 to market a biodegradable, injectable implant known as Ozurdex<sup>®</sup>.<sup>21</sup> This product delivers dexamethasone to treat several ocular diseases including uveitis, central retinal vein occlusion, and macular edema after branch retinal vein occlusion. Ozurdex<sup>®</sup> is a 6.5 mm by

0.45 mm rod of poly(lactide-*co*-glycolide) (PLGA) containing 0.7 mg of dexamethasone that can be injected with a 22-gauge needle.<sup>22</sup> Peak dose delivery lasts for 2 months and lower sustained release continues for another 4 months.<sup>22</sup>

### **1.2.3 Other Injectable Systems**

Another delivery system called Verisome has been used in phase 2 clinical trials for the delivery of triamcinolone acetonide in conjunction with injections of ranibizumab.<sup>23</sup> This injectable system forms a liquid drug depot in the vitreous cavity and consists of a variety of excipients, such as carbonates, tocopherols and citrate ester.<sup>23</sup> It can provide measurable drug release for 180 days with a 6.9 mg initial loading or 360 days with a 13.8 mg loading.<sup>23</sup>

In addition, a variety of particulate drug delivery systems are being explored. These typically consist of small biodegradable colloidal systems, including liposomes, microparticles, and nanoparticles. Liposomes, which are colloidal spheres composed of phospholipids, such as lecithin and phosphatidylcholine, can encapsulate hydrophilic within their aqueous core and/or hydrophobic drugs within the lipid bilayer.<sup>24</sup> Verteporfin (Visudyne, QLT Inc., Vancouver, BC) was the first drug using a liposomal delivery vehicle to be approved for ophthalmic applications, specifically for the treatment of neovascularization due to AMD, pathologic myopia, or presumed ocular histoplasmosis.<sup>22</sup> Subsequently, there has been considerable interest in liposomal delivery systems as they can protect oligonucleotides from degradation by nucleases<sup>25</sup> and increase the retention time of many drugs in the vitreous, including bevacizumab,<sup>26</sup> ganciclovir,<sup>27</sup> and ciprofloxacin.<sup>28</sup>

Microparticles and nanoparticles typically provide greater stability than liposomes and while drug delivery systems based on them have yet to be approved for market, there are many systems under development.<sup>22</sup> For example microspheres composed of PLGA and 1 mg triamcinolone acetonide were well tolerated and outperformed a 4mg injection of triamcinolone acetonide over a 6-12 month period<sup>29</sup>. Similarly, PLGA nanospheres loaded with bevacizumab provided 3 months of sustained release.<sup>30</sup>

#### **1.2.4 Current State**

Overall, reservoir-based drug delivery devices have been demonstrated to be more effective at drug delivery to the posterior of the eye than traditional delivery methods. Their advantages include sustained drug release, improved drug stability, and good control over the drug release kinetics. However, these devices require surgical implantation or a proprietary injection system and their structural complexity can require several manufacturing steps, which leads to additional expense. In addition, failure of a reservoir device results in dose-dumping, which can be particularly problematic in the eye given its small volume. Finally, these delivery vehicles are limited to small hydrophobic drugs that have sufficient diffusivity in the typical polymer coatings to be released.

Newer colloidal systems, such as polymersomes and liposomes, can provide many of the same advantages in terms of drug delivery while simplifying the formulation process and expanding the range of drugs that can be delivered. Since they use diffusion or *in situ* degradation to control the drug release,<sup>9</sup> there is no way to externally modify the release rate. A drug delivery device in which drug release could be externally-triggered would be highly desirable as it would permit fine control over drug release and could help optimize therapeutic outcomes for individual patients.<sup>31,32</sup> However, depending on the external stimulus used, it might be necessary to use an *in situ* gelling hydrogel to keep the polymersomes trapped in a certain location (Figure 1.1).



Figure 1.1: Conceptual diagram of how an externally-triggerable polymersome-based drug delivery device might be implemented. Polymersomes loaded with a green dye are shown as spheres. Hydrogel polymer chains are shown as black wavy lines.

# **1.3 Overall Objective: Preparation of a Photo-Triggerable Drug Delivery** Vehicle

The long-term motivation for this project was to develop a biodegradable intraocular drug delivery system that would eliminate the need for multiple injections by providing an external means of triggering or tuning the drug release rate. In support of this goal, the focus of my doctoral research was to develop photo-triggerable biomaterials that could potentially be used for drug delivery. Specifically, my approach involved synthesizing a series of novel reversibly photo-crosslinkable polycarbonates and examining their suitability for the preparation of drug delivery vehicles.

### 1.3.1 Specific Aim 1: Synthesis of Cyclic Carbonate Monomers with Photo-

### **Reversible Functionality**

In order to introduce photo-reversible functionality into a variety of polymers, a series of cyclic carbonate monomers with pendant photoactive moieties were synthesized (Figure 1.2). Specifically, cinnamoyl and coumarin groups, which are capable of [2+2]-cycloaddition, were attached via ester, amide, or ether linkages. A range of chemical linkages was employed as they can affect the ease of monomer preparation, the stability of the monomers and resulting polymers, the polymerizability of the monomers, and the photochemistry of the photoactive groups.



Figure 1.2: Cyclic carbonate monomers with pendant cinnamoyl (COM and CAM) or coumarin (MUM, MUC, and MAC) groups linked by various functional groups.

# **1.3.2 Specific Aim 2: Synthesis of Polycarbonate Homopolymers and Copolymers** with Photo-Reversible Functionality using Various Initiators

The polymerizability of each of the various cinnamoyl and coumarin functionalized monomers was examined. By examining the stability of the monomers under various polymerization conditions, their suitability for use in subsequent studies was assessed. In addition, their polymerization kinetics relative to the commercially available monomer trimethylene carbonate (TMC) were examined under a variety of polymerization conditions (temperatures, catalysts, and initiators) to determine optimum copolymerization conditions. Monomer conversion rates, % functionalized monomer incorporation, and end group fidelity were used to assess the dynamics during copolymerization and their suitability for use in various applications. End group fidelity is a parameter that quantifies the proportion of the polymer chains that contain the expected end-groups, e.g. the added alcohol initiator and the terminal hydroxyl group in the case of a ring-opening polymerization. It serves as a useful measure of polymerization control as it will be reduced by side-reactions and auto-initiation and is especially important when the initiator is being used to add a desired functionality to the polymer, which is a common method of preparing mono-functionalized polymers.



Figure 1.3: Ring-opening polymerization schematic for the synthesis of poly(carbonate) copolymers from TMC and a cinnamoyl or coumarin functionalized cyclic carbonate using benzyl alcohol as an initiator.

# **1.3.3 Specific Aim 3: Characterization of Extent and Reversibility of Polycarbonate Photo-Crosslinking**

The photo-crosslinking of the most promising polycarbonates was assessed in both bulk samples and in solution to determine the suitability of the polycarbonate copolymers for various applications. In addition, the efficiency of the photo-crosslinking with the cinnamoyl and coumarin functional groups was compared. Finally, the reversibility of the photo-crosslinking was examined.



*Figure 1.4: Schematic of the reversible [2+2] photo-cycloaddition reaction for an example polycarbonate with a pendant cinnamoyl group* 

## 1.3.4 Specific Aim 4: Preparation of Reversibly Photo-Crosslinkable Polymersomes

The self-assembly of amphiphilic diblock copolymers containing reversibly photo-crosslinkable polycarbonates was examined and the appropriate molecular weight and polymer composition ranges for self-assembly into polymersomes were identified. Finally, the encapsulation and release of fluorescent dyes was examined to determine the suitability of the polymersomes as photo-triggerable drug delivery vehicles.



Figure 1.5: Conceptual illustration of photo-reversible encapsulation and release of a drug (green circles) in polymersomes

# **Chapter 2**

# **Literature Review**

# **2.1 Functionalized Polycarbonates**

#### 2.1.1 Monomer Synthesis

Aliphatic polycarbonates are promising biomaterials as they can degrade in vivo, but unlike commonly used polyesters (e.g. poly(lactide-co-glycolide), they do not generate acidic degradation products.<sup>33</sup> In addition, a wide variety of carbonate monomers have been synthesized, including commercially available monomers such as trimethylene carbonate (TMC) and neopentylene carbonate (NPC).<sup>34</sup> By combining specific monomers, the hydrophobicity, degradability, viscoelasticity, and other properties of polycarbonates can be easily tailored.<sup>34</sup> One of the primary reasons for the wide assortment of synthesized carbonate monomers is the comparative ease with which functionalized cyclic carbonates can be prepared. Typically, cyclic carbonates are generated by converting a functionalized 1,3-propanediol into the corresponding 6membered cyclic carbonate using ethyl chloroformate.<sup>35</sup> This relatively simple synthesis process means that custom carbonate monomers are attractive candidates for introducing specific functionality into polyesters and polycarbonates. For example, carbonate monomers with pendant functional groups, such as protected hydroxyls,<sup>36</sup> azides,<sup>37</sup> and halides,<sup>38</sup> have been synthesized. Functionalized monomers have also been successfully copolymerized with commercially available non-functionalized monomers such as TMC and lactide, which have been used extensively in biomaterials applications.<sup>39–46</sup> Consequently, the preparation of cyclic carbonate monomers functionalized with photoactive functional groups could prove to be an excellent avenue for preparing new biomaterials for use in photo-responsive applications such as drug delivery.

#### 2.1.2 Polymerization Catalysis

Polycarbonates are typically synthesized by ring-opening polymerization of cyclic carbonate monomers.<sup>34,41,47</sup> Early studies of the ring-opening polymerization of carbonates used catalysts that were already used for the polymerization of cyclic esters, including stannous octoate (SnOct<sub>2</sub>).<sup>48</sup> Although SnOct<sub>2</sub> remains widely used, tin complexes have become increasingly controversial due to the potential toxicity of tin residues, especially in food or biomedical applications where extensive purification of the resulting polymers is required before they can be used.<sup>49,50</sup>

As a result, researchers have explored less toxic metal complexes such as zinc, calcium, and magnesium<sup>50,51</sup> and a variety of organic compounds,<sup>49,52</sup> such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),<sup>53</sup> triazabicyclodecene (TBD),<sup>53</sup> and trifluoroacetic acid<sup>54</sup> as catalysts for the preparation of both polyesters and polycarbonates. Both DBU and TBD have been reported to catalyze the polymerization of TMC at 25 °C in solution with moderate to excellent control over molecular weight and molar mass dispersity.<sup>42,53,55</sup> Of the two, TBD has been reported to have higher catalytic activity than DBU for TMC, with rapid monomer conversion, but poorer control over the polymerization resulting in an increased molar mass dispersity, which may be due to its ability to mediate acyl transfer reactions resulting in chain reshuffling.<sup>42,53</sup> In addition, thermal catalysis at high temperatures<sup>54,56-58</sup> or using microwave irradiation has been examined.<sup>58-60</sup> These studies have highlighted the fact that catalytic activity varies significantly between catalysts.<sup>53,61</sup> In addition, the choice of catalyst dictates the polymerization rate, polymerization conditions, purification process, polymer dispersity, and end group fidelity and thus warrants careful consideration based on the intended application for the polymer.

### **2.2 Protein Therapeutics**

Protein therapeutics were once rare, but they have grown considerably in number and frequency of use over the last few decades.<sup>62</sup> Since the approval of human insulin in 1982, which was the first recombinant protein therapeutic, the FDA has approved more than 130 protein therapeutics and many more are being

developed.<sup>62,63</sup> Protein therapeutics have a number of advantages relative to small molecules, including the ability to carry out complex biological functions, such as catalyzing chemical reactions,<sup>62</sup> and the ability to bind proteins or cell receptors with high affinity and specificity.<sup>63</sup> In the case of ocular diseases, several protein therapeutics, including ranibizumab (Lucentis) and aflibercept (Eylea) have been approved by the FDA to treat diseases such as AMD, retinal vein occlusion, and diabetic retinopathy by inhibiting the activity of vascular endothelial growth factor (VEGF).<sup>18</sup> These protein therapeutics are capable of binding VEGF with high specificity and thereby inhibiting its undesirable activity as a growth factor in the disease states.<sup>64</sup>

Unfortunately, proteins tend to suffer from poor stability due to chemical degradation,<sup>65</sup> unfolding,<sup>66</sup> denaturation,<sup>65,66</sup> and aggregation.<sup>65</sup> These changes can result in a loss of activity, by altering the threedimensional structure of the protein,<sup>62,66</sup> or trigger an immune response.<sup>67</sup> As a result, protein drugs are nearly always delivered by injection to avoid exposure to harsh environments, such as the gastrointestinal tract.<sup>62,66</sup> In addition, the encapsulation of the proteins into drug delivery vehicles has been of considerable interest as a means to stabilize them and extend their half-life.<sup>62,68–71</sup> However, specific care must be taken during the formulation of protein delivery devices to avoid exposure to conditions that could destabilize the proteins including organic solvents, shear forces, homogenization, and temperature changes.<sup>62,69,71,72</sup>

### 2.3 Triggerable Drug Release

Triggerable drug delivery is a growing research area as it allows drug release to be customized depending on the stimulus used.<sup>73–76</sup> For example, it can be used to provide a degree of targeting to drug delivery in the case of pH or enzyme sensitive systems. This premise has been commonly used in the design of drug delivery vehicles for cancer treatments as tumours have an acidic environment.<sup>73</sup> In the case of light or magnetic field triggerable drug delivery systems, they can be externally stimulated, which provides a noninvasive method to control the drug release.<sup>73,74</sup> Thus, a physician could easily alter the release rate of an implant in response to clinical symptoms or multiple drug doses could be delivered in a single device, thereby eliminating the need for regular injections. In particular, the use of light as a stimulus has been widely examined due to its convenience and the variety of ways that photo-responsiveness can be incorporated into drug delivery systems.<sup>75–77</sup> Photo-responsive drug delivery could be ideally suited for ocular applications due to the eye's inherent transparency, as shown by the clinical use of photodynamic therapies to treat neovascular diseases in the eye.<sup>64,78</sup> Triggerable drug delivery of ocular drugs could reduce the need for intravitreal injections, which would improve treatment safety while reducing patient discomfort.<sup>9</sup>

### **2.3.1 Photo-Triggerable Chemistry**

There are a diverse range of photo-responsive chemistries (Figure 2.1) that could be used to prepare a drug delivery vehicle. Many of these systems employ compounds that can either undergo a reversible isomerization<sup>79,80</sup> or form or cleave bonds<sup>81–85</sup> when photo-irradiated. The reversibility of these bonds is dependent on the compound used, with acrylates<sup>45,86</sup> and *o*-nitrobenzyl<sup>81,82,87–91</sup> resulting in irreversible bond formation or cleavage respectively, while anthracene,<sup>83,92</sup> cinnamoyl,<sup>84,93,94</sup> and coumarin,<sup>85,95–97</sup> are capable of photo-reversible bonding. These compounds have been incorporated into polymer backbones,<sup>88,89,91,93,96</sup> conjugated to the end of polymer chains to form crosslinked hydrogels,<sup>81,83,92</sup> and incorporated as pendant crosslinking nodes along the polymer backbone.<sup>94,95</sup> By exposing these materials to the appropriate wavelength of light, the disintegration of the polymer backbone, crosslinked hydrogel, or polymer network can be induced. Therefore, potential applications include tuning the refractivity of intraocular lenses<sup>98</sup> and photo-triggerable drug release.<sup>85</sup>



Figure 2.1: Chemical structures of several photo-responsive molecules

Many of these applications involve the reversible formation of photo-crosslinks between the polymer chains, which is commonly achieved through [2 + 2] photo-cycloaddition (Figure 2.2). This reaction consists of the reversible formation of a cyclobutane ring from two alkene groups when irradiated with photons of certain wavelengths of UV light and both cinnamoyl and coumarin groups are known to undergo this process.<sup>99</sup>



Figure 2.2: Schematic of the reversible [2+2] photo-cycloaddition reaction for an example polycarbonate with a pendant cinnamoyl group

The chemistry of cycloadditions has been extensively examined and they are widely used for the preparation of complex molecules with excellent control over the resulting stereochemistry. However, depending on

the number of electrons involved, the cycloadditions are typically either photochemically or thermally driven.<sup>100</sup> In 1965, Hoffmann and Woodward proposed a theoretical explanation based on molecular orbital symmetry for this variation in driving force.<sup>100</sup> They proposed that systems with 4n electrons, such as [2+2]cycloadditions proceed photochemically, while systems with 4n + 2 electrons, such as the [4+2] Diels-Alder reaction proceed thermally as this allows for the symmetry of the molecular orbitals to be maintained. The basis for this distinction is whether the molecular orbitals of the reactants correspond to the molecular orbitals of the product in their ground state or excited state (Figure 2.3). In the case of a thermal [2+2] cycloaddition where all the reactant electrons are in their ground state, the need to maintain orbital symmetry means that the  $\pi_{AS}$  bonding orbital correlates to the  $\sigma^*_{AS}$  antibonding orbital, which suggests that there would be a high energy barrier to overcome for this reaction to proceed (Figure 2.3A). Conversely, in the case of a photo [2+2] cycloaddition, where one reactant electron has been excited to the  $\pi^*_{SA}$  orbital, the reactant and product orbitals are closely correlated, which suggests that the activation barrier is minimal when the reactants are photochemically excited (Figure 2.3B). Thus, [2+2] cycloadditions are primarily expected to proceed photochemically and there are many examples of this experimentally.<sup>101</sup> Although thermal [2+2] cycloadditions are symmetry forbidden under these rules, they are possible under certain conditions. For example, the thermal [2+2] cycloaddition of coumarin has been reported at 85 °C under high vacuum<sup>102</sup> and the thermal [2+2] cycloreversions of several cyclobutane rings have been reported to proceed through a biradical mechanism.<sup>103</sup> However, the primary advantage of these [2+2] reactions is that they typically proceed exclusively photochemically and can be used to modify or crosslink polymers under relatively mild conditions with spatial and temporal control.<sup>101</sup>



Figure 2.3: Molecular orbital diagrams for [2+2] cycloaddition showing thermal (A) and photochemical (B) reactions

In addition, coumarin dimers have been reported to be reversible through a two-photon induced cleavage when irradiated with high intensity visible light.<sup>99</sup> Two-photon processes require the quasi-simultaneous absorption of two photons via a virtual state and were originally predicted by Maria Göppert-Mayer.<sup>104</sup> The development of high power lasers has allowed an assortment of two-photon processes to be investigated, including two-photon organic photoreactions.<sup>105,106</sup> The two-photon cleavage of coumarin dimers occurs when they absorb two photons of 532 nm visible light simultaneously.<sup>105</sup> Simultaneous activation by two photons requires a high photon density, which is only possible with a laser, so these dimers will not be cleaved by exposure to ordinary light intensities.<sup>107</sup> Finally, 532 nm lasers are already commonly used by ophthalmic surgeons for pan-retinal photocoagulation to seal ruptured blood vessels in patients with diabetic retinopathy, which could facilitate adoption of photo-triggerable delivery systems based on this photoactive group.<sup>85</sup>

#### 2.3.2 Photo-Triggerable Release by Directly Binding the Drug

One approach to triggerable drug delivery is to chemically link the drug to a polymer via a photo-reversible [2+2] cycloaddition. Drug delivery using this method has been demonstrated for small molecule drugs with an alkene bond,<sup>85</sup> and a patent was granted for the use of this approach as part of cataract surgeries.<sup>108</sup> However, the drug delivery vehicle used in this particular application requires implantation during the surgery, which limits its range of applications. A similar approach was utilized to prepare micelles capable of photo-triggerable delivery of 5-fluorouracil with the drug attached to pendant coumarin groups on the hydrophobic block of the diblock copolymers.<sup>109</sup> The incorporation of this technique into micelles makes the formulation injectable and thereby increases the range of applications. However, directly binding a desired drug to the polymer has the potential to alter the activity of the drug and is not suitable for large drug molecules such as proteins. The preparation of stabilized prodrugs with photo-cleavable functional groups such as *o*-nitrobenzyl and coumarin has also been explored.<sup>110</sup>

### 2.3.3 Photo-Triggerable Multi-Compartment Reservoirs

Another method of triggerable drug release that has been explored is to encapsulate the drug in capped multi-compartment reservoirs where the reservoir cap is composed of a photo-crosslinked polymer that will degrade when irradiated with the correct wavelength. The commercial applications of this method are being explored by MicroCHIPS and their subsidiary, On Demand Therapeutics (ODTx). MicroCHIPS was granted a method patent for triggerable drug delivery from capped reservoirs in February, 2011,<sup>111</sup> which describes a drug delivery vehicle that would require implantation. However, ODTx claims to have developed an injectable form,<sup>112</sup> which has been referenced in several recent patents and patent applications.<sup>113-115</sup> The patent descriptions suggest that both systems are composite devices with the reservoirs composed of an impermeable material such as silicone polymer or titanium with the exception of a small orifice that is capped with a photosensitive material. Thus, these devices would require a fairly complex manufacturing process. Since these devices are still in pre-clinical testing, their efficacy in treating

ocular diseases *in vivo* and the risk of long-term complications due to their non-biodegradable design have not been fully evaluated, but the multi-compartment design would provide the ability to deliver multiple drugs with the same device.<sup>78</sup> Consequently, multi reservoir-based approaches in which the entire drug delivery vehicle is photo-triggerable could be of interest as they would be simpler to manufacture.

### 2.3.4 Photo-Triggerable Polymer Hydrogels

Single reservoir drug delivery vehicles wherein the entire device is composed of a photo-responsive polymer have been extensively explored and often take the form of polymeric hydrogels.<sup>76</sup> This approach is best suited to macromolecular drugs, such as proteins, as the diffusion coefficient of proteins in a hydrogel network has an inverse relationship to their size.<sup>116</sup> Controlled drug release from these hydrogels is typically achieved through photo-responsive control of the mesh size of the hydrogel network.<sup>76</sup> For example, varying the mesh size of a poly(ethylene glycol) (PEG) based hydrogel by reversibly altering the dimerization of cinnamoyl groups resulted in a corresponding change in protein diffusion.<sup>116</sup> A similar result was observed for anthracene-functionalized PEG hydrogels with photo-responsive control over the release rate of lysozyme and bovine serum albumin.<sup>83</sup> Photo-controlled release of green fluorescent protein (GFP) from a dextran hydrogel has been demonstrated using a dual cyclodextrin and azobenzene system to provide photo-responsive control over the hydrogel crosslinks provided photo-triggerable release of GFP as the *o*-nitrobenzyl as part of the hydrogel crosslinks provided photo-triggerable release of vitamin B12, concanavalin A, and 250 nm nanobeads from a glycolipid-based photo-responsive hydrogel has been demonstrated.<sup>119</sup>

# 2.3.5 Photo-Triggerable Micelles and Polymersomes

Another class of drug delivery vehicles that can be synthesized using photo-responsive polymers are micelles and polymersomes. Formed by the self-assembly of diblock copolymers, these polymer constructs

provide a convenient way to encapsulate a variety of drugs. In particular, photo-responsive micelles have been widely examined using an assortment of photo-responsive methods.

The most common approach has been to encapsulate the drug into a photo-responsive hydrophobic micellar core.<sup>75</sup> In these systems, photo-irradiation typically causes a change in the hydrophobic block that results in an increase in hydrophilicity or polarity, which then either disrupts the micelle or induces sufficient swelling of the micellar core to permit drug release.<sup>75</sup> The first reported examples of this approach involved PEG-poly(methacrylate) (PMA) diblock copolymers in which the PMA block was functionalized with pendant photo-labile groups, such as pyrenylmethyl esters,<sup>120</sup> *o*-nitrobenzyl esters,<sup>121</sup> and (diethylamino)methylcoumarinyl esters (Figure 2.4).<sup>122</sup> In all three of these systems, photo-irradiation cleaves the functional group to generate free carboxylic acids, thereby converting the hydrophobic block into a hydrophilic block and disrupting the micelles. Conversely, micelles formed from PEG-poly(S-(-*o*-nitrobenzyl)-L-cysteine) diblock copolymers undergo swelling rather than disruption upon photo-cleavage of the *o*-nitrobenzyl group, which allowed them to release doxorubicin.<sup>123</sup>



Figure 2.4: Chemical structures of several photo-labile molecules with a wavy line showing the cleavage site

Another approach is to incorporate the photo-cleavable moieties into the backbone of the diblock copolymer, which would lead to rapid disintegration of the micelle core upon irradiation. Examples of this

method include PEG-polyurethane-PEG triblock copolymers with *o*-nitrobenzyl as a repeating unit in the hydrophobic block,<sup>124</sup> PEG-poly(ester amide) with *o*-nitrobenzyl as a repeating unit in the hydrophobic block,<sup>88</sup> poly(acrylic acid)-*b*-poly( $\gamma$ -methyl- $\epsilon$ -caprolactone) with *o*-nitrobenzyl linking the blocks,<sup>125</sup> and PEG-*b*-poly(butyl acrylate) with a cinnamoyl dimer linking the blocks.<sup>93</sup>

Finally, photo-reversible stabilization of the micellar core or corona has been explored through the incorporation of photo-crosslinkable pendant groups. Cinnamoyl groups have been used to stabilize the corona of poly(styrene)-*b*-poly(2-cinnamoylethyl methacrylate) micelles.<sup>126</sup> Meanwhile coumarin groups have been used to stabilize either the core of PEG-*b*-poly(coumarin methacrylate) micelles<sup>95</sup> or the shell of poly(dimethylaminoethyl methacrylate)-*b*-poly(methyl methacrylate-*random*-coumarin methacrylate) micelles.<sup>127</sup>

By comparison, photo-responsive polymersomes are a relatively new research area with only a few reported systems. In theory, all of the photo-responsive mechanisms used in micelles should be translatable to polymersomes as they are also formed by the self-assembly of amphiphilic copolymers. One group explored the incorporation of azobenzenes to temporarily disrupt polymersome membranes (Figure 2.5).<sup>128</sup> However, the incorporation of a photo-cleavable linkage between the hydrophobic and hydrophilic blocks has been the preferred approach as it provides a rapid and facile means to disrupt the polymersome membrane. For example, polymersomes have been prepared using a PEG-*b*-poly( $\varepsilon$ -caprolactone) with 2-nitrophenylalanine linking the blocks (Figure 2.5), which is another photo-cleavable group.<sup>129</sup> Thus, irradiation resulted in a disruption of the polymersome membrane and the release of encapsulated biocytin. Similarly, polymersomes composed of poly(acrylic acid)-*b*-poly( $\gamma$ -methyl- $\varepsilon$ -caprolactone) with *o*-nitrobenzyl linking the blocks converted to a micellar morphology following photo-irradiation, thereby releasing encapsulated enhanced GFP.<sup>130</sup> In a variation on this approach, polymersomes were prepared from a poly(N,N-dimethylacrylamide)-*b*-poly(benzyl carbamate) (PBC) copolymer where the PBC block was self-immolative and linked to the hydrophilic block by various photo-cleavable groups.<sup>131</sup> In this case,
irradiation with visible light (perylene-3-yl linker) (Figure 2.5) or UV (*o*-nitrobenzyl linker) triggered the disintegration of the entire hydrophobic block, which disrupted the membrane and released proteins such as alkaline phosphatase and lipase.<sup>131</sup> The formation of polymersomes from a triblock with *o*-nitrobenzyl incorporated into the repeating unit of the hydrophobic block has also been demonstrated.<sup>132</sup> Finally, dendrimersomes, which are similar to polymersomes, composed of dendrimers that incorporate *o*-nitrobenzyl throughout their backbone have been prepared and shown to release Nile Red and fluorescein.<sup>133</sup>



Figure 2.5: Chemical structures of additional photo-responsive molecules

While these approaches can provide a rapid and pronounced disruption of the membrane in response to photo-irradiation, there are potential advantages to exploring other photo-responsive approaches in polymersomes. In one case, polymersomes with an acrylate crosslinkable hydrophilic block and a *o*-nitrobenzyl cleavable hydrophobic block were prepared. This approach allowed the stabilization of the polymersome morphology by irreversibly crosslinking the hydrophilic layers, while having a photo-cleavable method to weaken the hydrophobic core of the membrane and enhance the diffusion of drugs across the membrane.<sup>134</sup> The stabilization of polymersome membranes through crosslinking has been shown to allow polymersomes to withstand de-hydration and re-hydration, high osmotic gradients, and immersion in organic solvents,<sup>135,136</sup> so the incorporation of a photo-reversible membrane stabilization approach similar to the core or corona stabilization of micelles is of interest.

## 2.4 Polymersomes

Polymersomes are composed of synthetic amphiphilic block copolymers that typically have a similar amphiphilicity to lipids, but much higher molecular weights.<sup>137</sup> The resulting polymer vesicles have significantly thicker membranes (8-21 nm), lower permeability, and higher stability than liposome membranes.<sup>138</sup> The preparation of polymersomes was first reported by Discher *et al.* in 1999<sup>139</sup> and they have subsequently become a significant research topic. Since polymersomes have a hydrophilic core in addition to the hydrophilic corona, their properties are actually similar to viral capsids composed of large poly(peptide) chains.<sup>137</sup> Consequently, there is considerable interest in developing polymersomes into drug delivery vehicles for RNA, DNA, and proteins, such as enzymes and antibodies. Polymersomes can be prepared from a wide range of copolymers, so it is relatively easy to tailor both their stability and permeability for a given drug delivery application. Research on methods of targeting the polymersomes to certain regions of the body and programming their disassembly is also being conducted.<sup>137</sup>

## 2.4.1 Factors Affecting Polymer Self-Assembly

The self-assembly of amphiphiles is a thermodynamically driven process that can be influenced by a variety of factors. First, polymer constructs, such as polymersomes, only form when surfactant concentrations are above the critical micelle concentration (CMC), which in aqueous solutions ranges from micromolar to picomolar values.<sup>137</sup> In other words, polymer vesicles are stable even when highly dilute. Further, the exchange rate of amphiphiles between constructs is generally proportional to the CMC and is typically on the order of a few hours for phospholipids.<sup>137</sup> Since the CMC decreases exponentially with an increase in size of the hydrophobic block(s), amphiphilic polymers with large hydrophobic regions generally lead to highly stable constructs.<sup>137</sup> Second, the morphology and stability of a polymer construct is heavily influenced by the selection of the copolymer, with factors such as the hydrophilic mass fraction, molecular weight, and chemical composition of the block copolymer all influencing polymer self-assembly.<sup>137</sup>

## 2.4.1.1 Hydrophilic Mass Fraction

The average molecular shape of individual polymer chains over time can be used to explain whether a given copolymer will lead to a membrane, cylindrical, or spherical morphology.<sup>140</sup> This shape is typically a reflection of the hydrophilic mass fraction (*f*-factor) of a block copolymer. A general rule-of-thumb for the formation of polymersomes is to use block copolymers with a phospholipid-like ratio of the hydrophilic blocks to the total mass ( $f = 35\% \pm 10\%$ ).<sup>137</sup> Copolymers that fall within this guideline tend to have cylindrically shaped polymer chains, which presumably facilitates their self-assembly into larger vesicle membranes. One possible explanation for why these chains have a cylindrical shape despite having a significantly smaller hydrophilic mass fraction is that hydration may balance the larger hydrophobic fraction.<sup>137</sup> When *f* is greater than 45%, micelles tend to result and when *f* is less than 25%, inverted microstructures are formed.<sup>137</sup> While the effects of chain chemistry and molecular weight on these rules have not been fully explored, they seem to generally hold true. Consequently, copolymers with an *f* value around 35% and an average molecular weight between 2000 and 20000 g/mol have been shown to form polymersomes.<sup>137</sup>

### 2.4.1.2 Molecular Weight

Cryogenic transmission electron microscopy of 100 to 200 nm polymersomes showed that their membrane diameter increased from 8 to 21 nm with increasing molecular weight.<sup>138</sup> Conversely, lipid membranes have a very narrow core diameter of approximately 3 nm, which reflects their significantly lower molecular weights.<sup>141</sup> Molecular dynamic simulations suggest that the vesicle membrane transitions from a bilayer with a low density midplane to a single shell of uniform density as the copolymer molecular weight increases.<sup>137</sup> In addition, measurements of the apparent membrane viscosity indicate that membrane fluidity typically decreases with increasing molecular weight.<sup>142</sup> These results are supported by an observed decrease in the lateral diffusivity of the polymers within the membrane with increasing molecular weight.<sup>143</sup> Overall, the fluidity of the membranes appears to decrease most significantly when the chains are long enough to entangle.<sup>142</sup> Finally, membrane permeability is significantly lower in polymersomes than in

phospholipid membranes, which is consistent with the changes in membrane thickness, density, and fluidity resulting from the change in amphiphile molecular weight.<sup>137,144</sup>

#### 2.4.1.3 Chemical Composition

Polymersomes are typically formed from linear diblock or triblock copolymers, but diblocks result in a membrane morphology that resembles that of a liposome or cell membrane while triblocks generate a morphology that is a cross between a monolayer and a bilayer with many chains traversing the membrane with their hydrophobic block.<sup>145,146</sup> In addition, the relative hydrophilicity/hydrophobicity of the copolymer blocks can have a significant impact on the properties of the polymersome membrane. For example, PEG-poly(butadiene) and PEG-poly(propylene oxide) both have PEG as the hydrophilic block, but the presence of the strongly hydrophobic poly(butadiene) results in dense, water impermeable membranes with greater chain entanglement in the hydrophobic region, while the much less hydrophobic poly(propylene oxide) results in loose, water permeable membranes.<sup>146</sup> This result suggests that the properties of a polymersome membrane can be tuned by selecting blocks with appropriate degrees of hydrophilicity/hydrophobicity provided that the difference between the hydrophilic and hydrophobic blocks is still sufficient to drive self-assembly.

## 2.4.2 Methods of Inducing Polymersome Self-Assembly

The first examples of polymers assembling into polymersomes involved fully synthetic diblock copolymers, such as PEG-poly(butadiene) and PEG-poly(ethylethylene) in a variety of aqueous conditions.<sup>139</sup> These constructs consisted of unilamellar polymer vesicles of varying diameters depending on the method used to induce their formation. When water was added to a several micron thick lamellar film of PEG-poly(butadiene), 1 µm diameter polymersomes were formed.<sup>139</sup> If the polymersomes were then sonicated, film-squeezed, or extruded, the constructs rearranged to generate 100 nm diameter polymersomes.<sup>139</sup> In general, the same methods that are used to induce the self-assembly of liposomes can

be used to generate polymersomes.<sup>147</sup> These include direct injection,<sup>148</sup> organic co-solvent approaches,<sup>149,150</sup> and other protein safe approaches.<sup>151–153</sup>

### 2.4.2.1 Direct Injection

One of the simplest methods for inducing polymersome formation, which was originally used to generate micelles, is to directly inject the amphiphilic polymer dissolved in an organic solvent into an excess of stirring water.<sup>148,154</sup> For this method, the block copolymer is typically dissolved in a water miscible solvent,<sup>152</sup> but the use of immiscible solvents, such as chloroform have also been reported.<sup>148</sup> However, this approach is a poor choice for protein encapsulation as the presence of an organic solvent could denature the proteins.<sup>152</sup> In addition, this method also tends to have very low encapsulation efficiencies, due to the excess water phase.<sup>155</sup>

## 2.4.2.2 Organic Co-Solvents

The rapid dilution of the organic solvent that occurs during direct injection can interfere with the selfassembly of some copolymers.<sup>150</sup> Thus, other methods that use a co-solvent to facilitate the suspension of diblock copolymers via a more gradual transition have been explored. For example, the addition of a similar volume of water to an organic solution of the polymer followed by removal of the organic solvent *in vacuo* provides a slower increase in the water content than direct injection.<sup>149</sup> This approach is similar to the reverse-phase evaporation technique used for the preparation of liposomes.<sup>156,157</sup> An extension of this approach is solvent displacement, where a polymer solution in a water-miscible solvent is gradually diluted by the addition of water.<sup>150,158</sup> The slow addition of water allows the polymer chains to gradually selfassemble and change their morphology in conjunction with the changing water to solvent balance, which resulted in an increase in the average particle size and a decrease in particle size dispersity relative to adding the organic solvent to water.<sup>150</sup>

## 2.4.2.1 "Protein Safe" Approaches

The thin film rehydration method is the most common organic solvent-free approach to polymersome formation.<sup>152,159–162</sup> For this method, the block copolymer is fully dissolved in an apolar solvent that is a good solvent for both blocks. The polymer solution is then dried slowly in a glass vessel to form a thin polymer film on the surface. Any remaining solvent is removed using high vacuum and then the film is rehydrated with an aqueous solution of the molecule or protein that is being encapsulated. The exposure of the film to water causes the block copolymer chains to rearrange and self-assemble into large vesicles (5– 20 µm).<sup>159,160</sup> An examination of this process identified several stages in the transition from dry polymer to dispersed polymersomes.<sup>163</sup> When a polymer film is exposed to water, ultra-small pores form in the film. The film then hydrates into lamellae as the hydrophilic blocks swell. Continued swelling then leads to the formation of a sponge-like phase in which the hydrated film contains interconnected pores. The morphology then transforms into hexagonally packed vesicles and eventually into dispersed polymersomes.<sup>163</sup> This approach also tends to result in the suspension of some bulk polymer aggregates, so the resulting polymer suspensions are often extruded through poly(carbonate) membranes with a pore diameter of 200-400 nm to form a final polymersome suspension with an average size of 50-200 nm.<sup>152,159</sup> In addition, heating of the sample is often required to bring the temperature above the melting temperature of the copolymer blocks,<sup>160</sup> which could limit the range of proteins that can be encapsulated in certain polymer systems. Finally, this process is fairly slow with the film hydration phase often requiring 24-48 hours.<sup>159,160</sup>

A recent advancement in this technique is often referred to as direct hydration.<sup>152,153,164</sup> This method was initially demonstrated by Neil *et al.* using a blended mixture of poly(ethylene glycol)-poly(propylene sulphide) diblock copolymers and poly(ethylene glycol) dimethylether ( $M_w$ =500Da) (PEG-DME).<sup>152</sup> The concept is to include an excipient, PEG-DME in this case, which is readily soluble in water and in the polymer. In this approach, the two polymers are melt blended together under solvent-free conditions, cooled to room temperature, and then exposed to an aqueous solution of the encapsulant.<sup>152,153</sup> At this point, the excipient dissolves causing rapid hydration of the blended polymer and formation of polymersomes. This

method is quite promising as it typically results in much higher encapsulation efficiencies than those obtained using conventional thin film hydration.<sup>152</sup>

Finally, detergent removal has recently been proposed as a method of inducing polymersome formation.<sup>151</sup> This technique has been used to induce liposome formation for decades.<sup>165</sup> In this approach, detergents with a relatively high CMC are used to generate mixed micelles containing both the detergent and the desired amphiphile. Detergent removal by dilution or dialysis can then gradually induce the self-assembly of the amphiphile as the detergent concentration approaches the CMC with excellent control over the resulting vesicle size.<sup>165</sup> For lipids, the generation of the initial mixed micelles typically requires between a 2:1 and 5:1 ratio of detergent:amphiphile. While the first example of this approach for polymers used a 2000:1 ratio, it is possible that the ratio could be lowered with different polymers or solubilisation conditions and it is worth further investigation as another protein safe method of inducing polymersomes.

# **Chapter 3**

# **Cinnamoyl and Coumarin Functionalized Aliphatic Polycarbonates**

## **3.1 Preface**

This chapter describes the synthesis of a series of cinnamoyl and coumarin functionalized carbonate monomers, which fulfilled Specific Aim 1. In addition, it explores their homopolymerization and copolymerization with TMC, which contributed to the completion of Specific Aim 2. This chapter is based on a paper that was submitted to Macromolecules on August 8, 2016, with a few modifications to maintain consistency of terminology and formatting within the thesis. I designed, conducted, and analyzed all experiments described in this chapter with some suggestions and feedback provided by my PhD supervisor, Dr. Brian Amsden. In addition, I (first author) prepared the resulting manuscript with editorial input from Dr. Brian Amsden (corresponding author).

## **3.2 Introduction**

Aliphatic polycarbonates are promising biomaterials due to their nonacidic degradation products and customizable properties. By combining specific monomers, the hydrophobicity, degradability, viscoelasticity, and other properties of polycarbonates can be easily tailored<sup>34</sup>. Commercially available monomers include trimethylene carbonate (TMC) and neopentylene carbonate (NPC), while a variety of other carbonate monomers have been synthesized for use in specific applications.<sup>34</sup> One of the primary reasons for the wide assortment of synthesized carbonate monomers is that preparing functionalized cyclic carbonates is generally straightforward. Typically, these carbonates are generated by obtaining or synthesizing a functionalized 1,3-propanediol, which can be converted into the corresponding 6-membered cyclic carbonate using ethyl chloroformate.<sup>35</sup> This relatively simple synthesis process means that custom carbonate monomers are attractive candidates for introducing specific functionality into polyesters and

polycarbonates. For example, carbonate monomers have been synthesized to introduce pendant functional groups, such as protected hydroxyls,<sup>36</sup> azides,<sup>37</sup> and halides,<sup>38</sup> along polycarbonate backbones.

The preparation of polymers that can respond to external stimuli such as light or pH is a growing research area with biomedical applications including responsive drug delivery systems and intraocular lenses.<sup>92,98</sup> Many of these systems employ compounds that can either undergo a reversible isomerization<sup>79,80</sup> or form or cleave bonds<sup>81–85</sup> when photo-irradiated. The reversibility of these bonds is dependent on the compound used, with acrylates<sup>45,86</sup> and *o*-nitrobenzyl<sup>81,82,87,88</sup> resulting in irreversible bond formation or cleavage respectively, while anthracene,<sup>83,92</sup> cinnamoyl,<sup>84,93,94</sup> and coumarin,<sup>85,95–97</sup> are capable of photo-reversible bonding. These groups are often incorporated into polymer backbones<sup>93,96,89</sup> or at the end of polymer chains used to form hydrogels.<sup>81,83,92</sup> Thus, exposure to the appropriate wavelength of light can be used to induce the disintegration of the polymer backbone or hydrogel. Further, the incorporation of these functional groups as pendant chains along the polymer backbone allows the creation of polymer matrices where the degree of crosslinking is dependent on the density of the functional groups along the polymer backbone and can be altered using light.<sup>84,94,95</sup>

Cinnamoyl and coumarin moieties are capable of photo-reversible dimerization through [2+2] cycloaddition,<sup>99</sup> and therefore the crosslinking density of a network prepared from polymers using these functional groups would be reversibly alterable. Consequently, the polymers could be photo-crosslinked using 300-380 nm UV light and the network de-crosslinked using 250-280 nm UV light or 500-560 nm visible light through two-photon absorption, thereby providing photo-switchable control.<sup>93,99,105</sup> However, the efficiency of the photo-reversible dimerization varies considerably among cinnamoyl and coumarin derivatives and is especially sensitive to the substituents on the aromatic rings, which can dramatically affect the UV-Vis absorbance of the compounds and the two-photon absorbance cross-section, especially if they are strongly electron donating or withdrawing.<sup>99,166</sup> In particular, coumarin derivatives substituted at the 7-position appeared to be well suited for this application.<sup>99</sup> Both 7-hydroxy-4-methylcoumarin (4-

methylumbelliferone) (MU) and 7-amino-4-methylcoumarin (AMC) are commercially available dyes that are reactive at the 7-position (Figure 3.1).



Figure 3.1: Chemical structures of MU and AMC

Consequently, in this study, a series of carbonate monomers with pendant cinnamoyl, MU, or AMC moieties were synthesized. As previously discussed, polycarbonates are an excellent choice for this application due to the ease of preparing similar functionalized and non-functionalized monomers. A range of chemical linkages was employed as they affect the ease of monomer preparation, the stability of the monomers and resulting polymers, and the photochemistry of the photoactive groups. For example, ester linkages are simpler to prepare using commercially available reagents, but are more susceptible to hydrolysis than amides or ethers. Accordingly, the ester linkages should be stable *in vivo* for a few months while the amide bonds should be stable for several years.<sup>167</sup> This distinction is important, because hydrolysis of the linkage between the carbonate backbone and photoactive pendant group would lead to premature cleavage of the polymer crosslinks.

Once synthesized, the homopolymerization of the functionalized monomers and their copolymerization with TMC was examined. Copolymerization with TMC, which is commercially available and has been used to prepare biocompatible polymers,<sup>43-46</sup> provides control over the maximum crosslink density of the resulting polymers and lowers the rate of consumption of the functionalized carbonate monomers. Homopolymerizations were initially conducted using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and triazabicyclodecene (TBD) as organocatalysts. Both DBU and TBD have been reported to catalyze the

polymerization of TMC at 25 °C in solution with moderate to excellent control over molecular weight and molar mass dispersity.<sup>42,53,55</sup> Of the two, TBD has been reported to have higher catalytic activity than DBU for TMC with rapid monomer conversion, but poorer control over the polymerization resulting in an increased molar mass dispersity, which may be due to its ability to mediate acyl transfer reactions resulting in chain reshuffling.<sup>42,53</sup> In addition, the homopolymerization of the various monomers and their copolymerization with TMC was explored using catalyst-free conditions, which had previously resulted in excellent end group fidelity for the polymerization of TMC.<sup>168</sup>

## 3.3 Experimental

## 3.3.1 Materials

All reagents were obtained from commercial suppliers and used as received unless otherwise noted. Dimethylsulfoxide-d6, chloroform-d (99.8%D) (CDCl<sub>3</sub>), anhydrous dimethylformamide (DMF) (99.8%), 1,1,1-tris(hydroxymethyl)ethane (99%), cinnamoyl chloride (98%), 4-methylumbelliferone (MU) (98%), *p*-toluenesulfonic acid monohydrate (98%), sodium hydrogen sulfate monohydrate (99%), 2-amino-2-methyl-1,3-propanediol (99%), lithium perchlorate (95%), hexamethyldisilazane (99%), 2,2-bis(hydroxymethyl)-propionic acid (98%), anhydrous 1-octanol (99%), benzyl alcohol (anhydrous, 99.8%), and methyl *tert*-butyl ether (98%) were obtained from Sigma-Aldrich (Oakville, Canada). Toluene (extra dry over molecular sieves), 2,2-dimethoxypropane (98%), ethyl chloroformate (99%), sodium bicarbonate (99.7%), cesium fluoride (99%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (98%), and anhydrous 2-methyltetrahydrofuran were obtained from Acros Organics (New Jersey, USA). Acetonitrile, dichloromethane (DCM), tetrahydrofuran (THF), isopropanol, anhydrous diethyl ether, toluene, acetone, methanol, ethyl acetate, hexanes, potassium carbonate (99%), sodium chloride (99%), sodium hydroxide (97%), anhydrous magnesium sulfate (97%), glacial acetic acid, 48% hydrobromic acid (HBr), concentrated hydrochloric acid (HCl), 85% phosphoric acid, and triethylamine (TEA) (99%) were obtained from Fisher Scientific (Ottawa, Canada). THF and TEA were dried by storage over 4 Å molecular sieves, while DCM

was dried by storage over 3 Å molecular sieves. 3-methyl-3-oxetanemethanol (97%) and 750 g/mol poly(ethylene glycol) methyl ether (mPEG) were obtained from Alfa-Aesar (Ward Hill, USA) and the mPEG wan dried by dissolving in toluene (4 mL/g) and concentrating *in vacuo* 4 times. Trimethylene carbonate (99%) was purchased from Leapchem (Hangzhou, China) and purified by recrystallization from ethyl acetate (3 mL/g). Bis(pentafluorophenyl) carbonate (97%) was obtained from Matrix Scientific (Columbia, USA). 7-amino-4-methylcoumarin (AMC) was obtained from Aapptec (Louisville, USA). 1,1'- carbonyldiimidazole (CDI) (98%) was obtained from ApexBio (Houston, USA).

## 3.3.2 Synthesis

## 3.3.2.1 Synthesis of COM

#### 3.3.2.1.1 (2,2,5-trimethyl-1,3-dioxan-5-yl)methanol

A 500 mL round bottom flask was charged with tris(hydroxymethyl)ethane (15.0 g, 125 mmol), 2,2dimethoxypropane (15.4 ml, 125 mmol), and acetone (125 ml) to form a cloudy white suspension. The addition of *p*-toluenesulfonic acid monohydrate (0.237 g, 1.25 mmol) with stirring instantly resulted in a clear homogeneous solution, which was allowed to stir for 2 h and then quenched by adding potassium carbonate (6.00 g, 43.4 mmol). The fluffy precipitate was removed by filtration and the solvent was evaporated *in vacuo* to obtain a clear viscous liquid. Yield: 94%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 4.55 (t, 10H), 3.57 (d, 2H), 3.42 (d, 2H), 3.36 (d, 2H), 1.30 (d, 6H), 0.76 (s, 3H).

## 3.3.2.1.2 (2,2,5-trimethyl-1,3-dioxan-5-yl)methyl cinnamate

A flame-dried 250 mL round bottom flask was purged with argon and charged with (2,2,5-trimethyl-1,3dioxan-5-yl)methanol (18.7 g, 117 mmol) and anhydrous THF (60 mL) and cooled in an ice bath. Cinnamoyl chloride (29.2 g, 175 mmol) was added and stirred briefly, then TEA (24.7 ml, 177 mmol) was added dropwise over 30 min and the reaction was mixed for 1 h in the ice bath and overnight at room temperature. The TEA·HCl precipitate was removed by filtration and the solvent was evaporated *in vacuo* to obtain a yellow oil that was used immediately without further purification. <sup>1</sup>H NMR (500 MHz, DMSO- d<sub>6</sub>, δ in ppm): 7.67 (m, 2H), 7.63 (d, 1H), 7.38 (m, 3H), 6.61 (d, 1H), 4.27 (s, 2H), 4.14 (dd, 4H), 1.24 (d, 6H), 1.00 (s, 3H).

#### 3.3.2.1.3 3-hydroxy-2-(hydroxymethyl)-2-methylpropyl cinnamate

A 1 L round bottom flask was charged with (2,2,5-trimethyl-1,3-dioxan-5-yl)methyl cinnamate (33.9 g, 117 mmol), DCM (467 mL), and isopropanol (117 mL). Sodium hydrogen sulfate monohydrate suspended on silica<sup>169</sup> (11.7 g, 24.8 mmol) was added as a heterogeneous catalyst and the reaction was stirred for 5 h. The silica was removed by filtration and the solvent concentrated *in vacuo* to obtain a yellow oil, which formed white crystals from diethyl ether (22.5 g). Yield: 77% (two steps). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 7.71 (m, 2H), 7.65 (d, 1H), 7.42 (m, 3H), 6.63 (d, 1H), 4.53 (t, 2OH), 4.01 (s, 2H), 3.32 (dd, 4H), 0.85 (s, 3H).

### 3.3.2.1.4 5-cinnamoyloxymethyl-5-methyl-1,3-dioxan-2-one (COM)

A flame-dried 1 L round bottom flask was purged with argon and charged with 3-hydroxy-2-(hydroxymethyl)-2-methylpropyl cinnamate (18.2 g, 73 mmol) and anhydrous THF (700 mL) and cooled in an ice bath for 20 minutes. Ethyl chloroformate (21.0 mL, 219 mmol) was added dropwise over 12 min and the reaction was allowed to mix for 45 min, then TEA (32.0 mL, 230 mmol) was added dropwise over 25 min to form a cloudy white suspension. The suspension was stirred for 30 min in the ice bath and then 72 h at room temperature. The precipitate (TEA·HCl) was removed by filtration and the solvent was evaporated *in vacuo* to obtain an orange oil. The product was crystallized from 4:1 diethyl ether:THF (20 mL/g) at -20 °C and washed with diethyl ether to obtain 18.32 g of white crystals with a purity > 99%. Yield: 90%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 7.64 (d, *J* = 16.0 Hz, 1H), 7.51 – 7.39 (m, 2H), 7.39 – 7.26 (m, 2H), 6.36 (d, *J* = 16.0 Hz, 1H), 4.29 (d, *J* = 10.9 Hz, 2H), 4.15 (s, 2H), 4.12 (d, *J* = 10.9 Hz, 2H), 1.08 (s, 3H). (Figure A.1)<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 166.30, 147.78, 146.16, 134.01, 130.69, 128.95 (2C), 128.23 (2C), 116.72, 73.75 (2C), 65.35, 32.39, 17.23. (Figure A.2) Melting point: 90 °C.

#### 3.3.2.2 Synthesis of CAM

#### 3.3.2.2.1 2,2,5,8,8-pentamethyl-3,7-dioxa-2,8-disilanonan-5-amine

A 250 mL round bottom flask was charged with 2-amino-2-methyl-1,3-propanediol (4.98 g, 47 mmol), lithium perchlorate suspended on silica (4.80 g, 15 mmol),<sup>170</sup> and DCM (50 mL). Hexamethyldisilazane (15 mL, 72 mmol) was added and the reaction was allowed to mix for 2 h. The silica was removed by filtration and the solvent was evaporated *in vacuo* to obtain a pale yellow oil, which was used without further purification. Yield: ~100%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 4.30 (s, 4H), 4.13 (s, 2H), 0.83 (s, 3H), 0.06 (s, 18H).

## 3.3.2.2.2 N-(2,2,5,8,8-pentamethyl-3,7-dioxa-2,8-disilanonan-5-yl)cinnamamide

A flame-dried 500 mL round bottom flask was purged with argon and charged with 2,2,5,8,8-pentamethyl-3,7-dioxa-2,8-disilanonan-5-amine (11.9 g, 47.6 mmol) and anhydrous DCM (150 mL). TEA (7.6 ml, 55 mmol) was added and the reaction was cooled in an ice bath. Cinnamoyl chloride (8.72 g, 52.3 mmol) dissolved in anhydrous DCM (40 mL) was added dropwise via a cannula and the reaction was allowed to mix for 1 h in the ice bath and overnight at room temperature. The solvent was evaporated *in vacuo* and THF was added to precipitate the TEA·HCl, which was removed by filtration. The solvent was again evaporated *in vacuo* and the product was used immediately in the next step without isolation or purification.

#### 3.3.2.2.3 N-(1,3-dihydroxy-2-methylpropan-2-yl)cinnamamide

A 250 mL round bottom flask was charged with *N*-(2,2,5,8,8-pentamethyl-3,7-dioxa-2,8-disilanonan-5yl)cinnamamide (18.1 g, 47.6 mmol), and methanol (95 mL). Glacial acetic acid (5.5 mL, 95 mmol) was added and the reaction was allowed to stir for 2 h. The solvent was evaporated *in vacuo*. Yield: 33% over two steps. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 7.54 (s, 1NH), 7.54 (d, 2H), 7.42 (m, 4H), 6.81 (d, 1H), 4.97 (s, 2OH), 3.56 (q, 4H), 1.22 (s, 3H).

#### 3.3.2.2.4 5-(cinnamoylamino)-5-methyl-1,3-dioxan-2-one (CAM)

A flame-dried 250 mL round bottom flask was purged with argon and charged with *N*-(1,3-dihydroxy-2-methylpropan-2-yl)cinnamamide (3.01 g, 12.8 mmol) and anhydrous THF (150 mL) and cooled in an ice bath for 20 min. Ethyl chloroformate (3.8 mL, 40 mmol) was added dropwise over 10 min and the reaction was allowed to mix for 45 min, then TEA (5.8 mL, 42 mmol) was added dropwise over 25 min to form a cloudy white suspension. The suspension was stirred for 30 min in the ice bath and then 24 h at room temperature. The precipitate (TEA·HCl) was removed by filtration and the solvent was evaporated *in vacuo*. Finally, the product was obtained by crystallization from 1:1 THF:diethyl ether (40 mL/g) at -20 °C, collected by vacuum filtration, and washed with diethyl ether (1.89 g). Yield: 56%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 8.43 (s, 1NH), 7.56 (d, *J* = 6.9 Hz, 2H), 7.45 (d, *J* = 15.8 Hz, 1H), 7.43 – 7.35 (m, 3H), 6.70 (d, *J* = 15.8 Hz, 1H), 4.67 (d, *J* = 10.1 Hz, 2H), 4.40 (d, *J* = 10.1 Hz, 2H), 1.31 (s, 3H). (Figure A.3) <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 166.36, 147.67, 139.84, 135.14, 130.08, 129.41 (2C), 128.05 (2C), 122.50, 73.18 (2C), 48.40, 17.67. (Figure A.4) Melting point: 122 °C

### 3.3.2.3 Synthesis of MUM

#### 3.3.2.3.1 2-bromomethyl-2-methyl-1,3-propanediol

A 500 mL round bottom flask was charged with 3-methyl-3-oxetanemethanol (21.1 g, 206 mmol) and 2methyltetrahydrofuran (200 mL) and cooled in an ice bath for 20 min. Concentrated HBr (68 mL, 601 mmol) was added dropwise using an addition funnel. The reaction was stirred for 2 h in the ice bath and 16 h at room temperature. The reaction mixture was washed with 100 mL of brine and 3 x 50 mL of saturated sodium bicarbonate solution to remove residual HBr and dried using magnesium sulfate to obtain a clear colorless solution. The solvent was evaporated *in vacuo* to obtain 36.9 g of white crystals. Yield: 98%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 4.11 (br s, 20H), 3.44 (s, 2H), 3.26 (dd, 4H), 0.84 (s, 3H).

### 3.3.2.3.2 5-bromomethyl-2,2,5-trimethyl-1,3-dioxane

A 250 mL round bottom flask was charged with 2-bromomethyl-2-methyl-1,3-propanediol (15.8 g, 86 mmol), 2,2-dimethoxypropane (16 mL, 130 mmol), and acetone (58 mL) to form a yellow solution. Added

*p*-toluenesulfonic acid monohydrate (0.819 g, 4.3 mmol) and let mix for 3 h. Added potassium carbonate (5.97 g, 43.2 mmol), let mix for 5 min, and then removed salts by filtration and evaporated solvent *in vacuo*. Obtained 18.9 g of crystals. Yield: 98%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>, δ in ppm): 3.68 (s, 2H), 3.62 (dd, 4H), 1.33 (d, 6H), 0.85 (s, 3H).

### 3.3.2.3.3 7-2,2,5-trimethyl-1,3-dioxan-5-methoxyl-4-methylcoumarin

A flame-dried 500 mL round bottom flask was purged with argon and charged with 5-bromomethyl-2,2,5trimethyl-1,3-dioxane (27.9 g, 125 mmol), MU (24.2 g, 137 mmol), and anhydrous DMF (200 mL). Potassium carbonate (76 g, 549 mmol) was added and the reaction was heated to 100 °C and allowed to reflux for 24 h. The reaction mixture was then cooled and water (400 mL) was added to dissolve the potassium salts and precipitate the product. The reaction was mixed for 30 min and then filtered to obtain 41.5 g of tan powder. Yield: ~100%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 7.69 (d, 1H), 7.01 (d, 1H), 7.00 (d, 1H), 6.21 (s, 1H), 4.09 (s, 2H), 3.68 (dd, 4H), 2.40 (s, 3H), 1.35 (d, 6H), 0.91 (s, 3H).

#### 3.3.2.3.4 7-(3-hydroxy-2-(hydroxymethyl)-2-methylpropoxy)-4-methylcoumarin

A 500 mL round bottom flask was charged with 7-2,2,5-trimethyl-1,3-dioxan-5-methoxyl-4methylcoumarin (44.9 g, 141 mmol), DCM (142 mL), and methanol (142 mL). Concentrated HCl (23 mL, 289 mmol) was added dropwise and the mixture was stirred overnight. The flask was then cooled in an ice bath and sodium bicarbonate (24.5 g, 296 mmol) was added to quench the reaction and allowed to mix for 10 minutes. The salts were removed by filtration and the solvent was evaporated *in vacuo* to obtain a pale tan powder (36.6 g). Yield: 93%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 7.65 (d, 1H), 6.94 (dd, 1H), 6.92 (d, 1H), 6.19 (s, 1H), 4.53 (s, 20H), 3.88 (s, 2H), 3.36 (dd, 4H), 2.29 (s, 3H), 0.91 (s, 3H).

#### 3.3.2.3.5 5-(4-methylumbelliferyloxymethyl)-5-methyl-1,3-dioxan-2-one (MUM)

A flame-dried 1 L round bottom flask was purged with argon and charged with 7-(3-hydroxy-2-(hydroxymethyl)-2-methylpropoxy)-4-methylcoumarin (35.173 g, 126 mmol) and anhydrous THF (828 mL). TEA (37 mL, 265 mmol) was added and the flask was placed in a water bath. Ethyl chloroformate (25.0 mL, 260 mmol) was added gradually over 20 min to form a cloudy white suspension, which was stirred for 24 h at room temperature. The suspension was then filtered and the solvent was evaporated *in vacuo*. The reaction precipitate was washed with water to remove TEA·HCl salts and combined with the evaporate. The combined solids were dissolved in acetonitrile (10 mL/g) and cooled to 4 °C to precipitate a small amount of fluffy white material (various oligomers), which was removed by vacuum filtration. The filtrate was then crystallized at -20 °C and collected to obtain 30.7 g of white powder. Yield: 80%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 7.50 (d, *J* = 8.7 Hz, 1H), 6.86 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.80 (d, *J* = 2.5 Hz, 1H), 6.13 (s, 1H), 4.49 (d, *J* = 10.9 Hz, 2H), 4.26 (d, *J* = 10.9 Hz, 2H), 4.03 (s, 2H), 2.39 (s, 3H), 1.24 (s, 3H). (Figure A.5) <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 160.95, 160.92, 155.09, 152.39, 147.86, 125.80, 114.33, 112.46, 112.18, 101.70, 73.50 (2C), 69.49, 32.87, 18.62, 17.19. (Figure A.6) Melting point: 143 °C.

### 3.3.2.4 Synthesis of MUC

## 3.3.2.4.1 Pentafluorophenyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate

This reaction was conducted using a procedure by Sanders *et al.*<sup>171</sup> with some modifications. A 250 mL round bottom flask was charged with 2,2-bis(hydroxymethyl)-propionic acid (6.0 g, 45 mmol), bis(pentafluorophenyl) carbonate (43.4 g, 110 mmol), cesium fluoride (1.4 g, 9.3 mmol), and anhydrous THF (140 mL). The reaction mixture was stirred for 20 h during which it transitioned from heterogeneous to homogeneous over the course of the first hour. The solvent was evaporated *in vacuo* and DCM was added to precipitate the pentafluorophenol by-product. The precipitate was removed by filtration and the filtrate was washed sequentially with a saturated sodium bicarbonate solution and brine, dried with magnesium sulfate, and the solvent was evaporated *in vacuo*. The product was recrystallized from 2:1 ethyl acetate:hexanes (30 mL/g). Yield: 69%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 4.85 (d, 2H), 4.37 (d, 2H), 1.56 (s, 3H). <sup>19</sup>F NMR (500 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): -153.9 (d, 2F), -157.1 (t, 1F), -162.3 (t, 2F).

#### 3.3.2.4.2 5-(4-methylumbelliferyloxycarbonyl)-5-methyl-1,3-dioxan-2-one (MUC)

A flame-dried 100 mL round bottom flask was purged with argon and charged with pentafluorophenyl 5methyl-2-oxo-1,3-dioxane-5-carboxylate (3.1 g, 9.4 mmol), MU (1.7 g, 9.4 mmol), cesium fluoride (1.4 g, 9.4 mmol), and anhydrous DMF (30 mL). The reaction was allowed to mix for 24 h, precipitated using diethyl ether, and crystallized from acetonitrile (6 mL/g) at -20 °C. Yield: 77%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm): 7.56 (d, J = 8.6 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.00 (dd, J = 8.6, 2.4 Hz, 1H), 6.22 (d, J =1.0 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.29 (d, J = 11.0 Hz, 2H), 2.37 (d, J = 1.0 Hz, 3H), 1.45 (s, 3H). (Figure A.7) <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,  $\delta$  in ppm) 169.49, 160.11, 154.13, 152.18, 151.76, 147.22, 125.75, 118.50, 117.54, 114.94, 110.09, 72.78 (2C), 40.98, 18.69, 17.41. (Figure A.8) Melting point: 145 °C.

## 3.3.2.5 Synthesis of MAC

#### 3.3.2.5.1 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid

A 250 mL round bottom flask was charged with 2,2-bis(hydroxymethyl)-propionic acid (10.02 g, 74.7 mmol), *p*-toluenesulfonic acid monohydrate (0.719 g, 3.78 mmol), and acetone (50 mL). 2,2-dimethoxypropane (14 mL, 114 mmol) was added and the reaction mixture was stirred for 3 h. TEA (0.60 mL, 4.3 mmol) was added to neutralize the acid and the solvent was evaporated *in vacuo* to obtain a clear oil that crystalized under blowing air. Dissolved crystals in methyl *tert*-butyl ether to remove unreacted 2,2-bis(hydroxymethyl)-propionic acid (insoluble) and recrystallized at -20 °C. Yield: 85%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>61</sub>  $\delta$  in ppm): 4.01 (d, 2H), 3.58 (d, 2H), 1.34 (s, 3H), 1.26 (s, 3H), 1.07 (s, 3H).

#### 3.3.2.5.2 2,2,5-trimethyl-(4-methylcoumarin-7-yl)-1,3-dioxane-5-carboxamide

This reaction was conducted using an amide coupling protocol by Larrivée-Aboussafy *et al.* with some modifications.<sup>172</sup> A flame-dried 250 mL round bottom flask was purged with argon and charged with 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (19.8 g, 114 mmol), 1,1`-carbonyldiimidazole (19.4 g, 120 mmol), and anhydrous THF (114 mL). The reaction resulted in vigorous bubbling as carbon dioxide was produced, so the presence of a pressure relief mechanism was important. The reaction mixture was stirred for 2 h and

then concentrated *in vacuo* to remove any residual carbon dioxide. The round bottom flask containing the residual oil was purged with argon and charged with AMC (20.0 g, 114 mmol), DBU (17.2 mL, 114 mmol), and 2-methyltetrahydrofuran (114 mL). The reaction was heated to 82 °C and allowed to reflux for 24 h. The product precipitated from the reaction mixture upon cooling and was collected by filtration. Yield: 74%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 9.75 (s, 1NH), 7.82 (d, 1H), 7.72 (d, 1H), 7.58 (dd, 1H), 6.27 (s, 1H), 4.17 (d, 2H), 3.76 (d, 2H), 2.40 (s, 3H), 1.40 (s, 3H), 1.31 (s, 3H), 1.18 (s, 3H).

## 3.3.2.5.3 3-hydroxy-2-(hydroxymethyl)-2-methyl-(4-methylcoumarin-7-yl)propanamide

A 500 mL round bottom flask was charged with 2,2,5-trimethyl-(4-methylcoumarin-7-yl)-1,3-dioxane-5carboxamide (27.8 g, 84 mmol), THF (250 mL), and methanol (80 mL). Concentrated HCl (14 mL, 173 mmol) was added dropwise and the mixture was stirred for 5 h. The reaction was then cooled in an ice bath and TEA (25 mL, 177 mmol) was added dropwise to quench the reaction. The precipitate (TEA·HCl) was removed by filtration and the solvent was evaporated *in vacuo* to obtain 13.2 g of product. Yield: 54%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 9.70 (s, 1NH), 7.84 (d, 1H), 7.69 (d, 1H), 7.55 (dd, 1H), 6.25 (s, 1H), 4.96 (t, 2OH), 3.60 (dd, 4H), 2.40 (s, 3H), 1.13 (s, 3H).

#### 3.3.2.5.4 5-(4-methylcoumarin-7-carbamoyl)-5-methyl-1,3-dioxan-2-one (MAC)

A flame-dried 250 mL round bottom flask was purged with argon and charged with 3-hydroxy-2-(hydroxymethyl)-2-methyl-(4-methylcoumarin-7-yl)propanamide (4.05 g, 13.9 mmol) and a 3:1 mixture of anhydrous THF (75 mL) and anhydrous DMF (25 mL). A solution of CDI (2.95 g, 18.2 mmol) in a blend of anhydrous THF (20 mL) and anhydrous DMF (10 mL) was added dropwise and the reaction was allowed to mix for 2 h. A white precipitate formed during the reaction and was collected by vacuum filtration and rinsed with THF to obtain pure product (1.08 g). Yield: 24%. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 10.22 (s, 1NH), 7.90 – 7.68 (m, 2H), 7.60 (dd, J = 8.7, 2.1 Hz, 1H), 6.30 (d, J = 1.1 Hz, 1H), 4.80 (d, J = 10.3 Hz, 2H), 4.42 (d, J = 10.3 Hz, 2H), 2.41 (d, J = 1.1 Hz, 3H), 1.33 (s, 3H). (Figure A.9) <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>,  $\delta$  in ppm): 170.77, 160.38, 153.92, 153.45, 147.81, 142.21, 126.31, 116.48, 116.09, 113.19, 107.19, 72.96 (2C), 41.37, 18.43, 16.96. (Figure A.10) Melting point: 251 °C

## **3.3.3 Polymerization Kinetics**

Solution polymerization kinetics were studied by monitoring the polymerization in real-time using a 500 MHz Bruker NMR spectrometer. The general procedure was to dissolve the monomer (0.60 mmol, 50 eq) in 500  $\mu$ L CDCl<sub>3</sub> (freshly filtered through basic alumina) and add 50  $\mu$ L of benzyl alcohol diluted in CDCl<sub>3</sub> (26 mg/mL). This sample was then used to lock and shim the NMR. 50  $\mu$ L of DBU diluted in CDCl<sub>3</sub> (91.4 mg/mL) was then added and the polymerization kinetics were monitored by collecting <sup>1</sup>H NMR spectra at regular intervals (1-5 min, depending on the monomer).

## 3.3.4 Melt Copolymerizations

All bulk melt copolymerizations were performed in Wheaton 5 mL gold band pre-scored ampules. The ampules were flame-dried, cooled in a 65 °C oven, and purged with argon. The general procedure was to combine monomers (e.g. TMC (146 mg, 1.43 mmol, 22.5 eq), 3 (145 mg, 0.48 mmol, 7.5 eq)) and an initiator (e.g. benzyl alcohol or 1-octanol diluted in anhydrous toluene (8.3 mg, 0.064 mmol, 1 eq, 33% w/w)) in an ampule, purge it with argon, and then evacuate the ampule's atmosphere at a reduced pressure of 28 kPa for 30 s and flame-seal. The ampules were then placed in a 65 °C oven for 5 min to melt the reagents, gently vortexed to ensure even mixing, and placed in an oil bath thermostat-controlled at the appropriate temperature. The polymerizations were halted by transferal from the oil bath to a -20 °C freezer, where they were briefly stored until they could be analyzed.

## **3.3.5 Characterization**

<sup>1</sup>H NMR, <sup>13</sup>C NMR, and <sup>19</sup>F NMR spectroscopy was conducted using a Bruker 500 MHz NMR spectrometer with peak shifts referenced using an internal trimethylsilane standard when conducted in CDCl<sub>3</sub> and the residual DMSO peak when conducted in DMSO-d<sub>6</sub>. Melting points were determined using a Mettler Toledo DSC1 System using a heating rate of 10 °C/min. End group fidelity for homopolymer samples was calculated as previously described.<sup>168</sup> End group fidelity for copolymer samples was calculated

using <sup>1</sup>H NMR spectra collected in DMSO-d<sub>6</sub> by comparing the integration of a non-overlapping chemical shift for the initiator (5.11 ppm for the methylene group in benzyl alcohol and 0.82 ppm for the terminal methyl group in 1-octanol) to the sum of the integration of the chemical shifts for the terminal hydroxyl group of the polymer chain (4.81 ppm for functionalized carbonates and 4.53 ppm for TMC). The molecular weight and molecular weight distribution of the PTMC samples were also characterized by gel permeation chromatography (GPC) using a Waters 2695 GPC apparatus with a guard and 4 columns, a Waters 410 Differential Refractometer, and a Wyatt-Dawn EOS 18 light detecting system. Samples were dissolved in HPLC grade THF at a 2 mg/mL concentration, filtered with a 0.2  $\mu$ m syringe filter, and run at 25 °C using distilled THF as the eluent.

## **3.4 Results and Discussion**

## 3.4.1 Synthesis

A series of cinnamoyl and coumarin functionalized cyclic carbonate monomers were prepared utilizing various functional groups as linkages as shown in Figure 3.2.



*Figure 3.2: Cyclic carbonate monomers with pendant cinnamoyl (COM and CAM) or coumarin (MUM, MUC, and MAC) groups linked by various functional groups.* 

With the exception of COM, this paper is the first reported synthesis of each monomer. The synthesis of COM, which incorporates a cinnamate linkage, was previously reported by Hu et al. with an overall yield of 33%.<sup>84</sup> In this study, the overall yield was nearly doubled to 65% by utilizing a mild acetonide deprotection method reported by Mahender *et al.* to reduce hydrolysis of the ester in the cinnamate group, and optimizing the ethyl chloroformate ring-closing reaction (Figure 3.3).<sup>169</sup> The optimum conditions for the ring-closing reaction were a dilute solution ( $\leq 0.1$  M) and long reaction times ( $\geq 48$  h). The remainder of the monomers were synthesized by incorporating a combination of various published methods and custom modifications. The preparation of CAM (Figure 3.4) was complicated by the need to control the relative reactivity of the hydroxyl and amine groups, while the syntheses of MUM, MUC, and MAC were heavily influenced by the solubility of the coumarin group. In the case of MUM (Figure 3.5), an extra step was required to prepare the 1,3-propanediol precursor as a suitable one is not commercially available and the Williamson ether synthesis required DMF and forcing conditions (elevated temperature and a large excess of potassium carbonate) to achieve desirable yields due to the poor solubility of the potassium coumarin salts in most solvents. However, despite requiring five steps, the overall yield was 71% as several steps were nearly quantitative. MUC was synthesized following a general procedure published by Sanders et al. for the preparation of cyclic carbonates with pendant esters and amides with modifications to address the limited solubility of MU (Figure 3.6).<sup>171</sup>



Figure 3.3: Synthesis of COM



Figure 3.4: Synthesis of CAM



Figure 3.5: Synthesis of MUM



Figure 3.6: Synthesis of MUC

MAC (Figure 3.7) proved especially difficult to synthesize, because aromatic amines, such as AMC, are considerably less nucleophilic than aliphatic amines. Consequently, strongly forcing conditions were required to drive its reaction with an acetonide-protected carboxylic acid. However, the ester bond within the coumarin tended to participate in side reactions if the conditions were too forcing. Consequently, a variety of amide coupling approaches were examined. Heating the reaction to 60 °C resulted in no coupling,

while heating to 100 °C resulted in non-selective reaction of the amine with both the cyclic ester in the coumarin and the carboxylic acid. The use of thionyl chloride<sup>173</sup> to selectively activate the acetonideprotected carboxylic acid resulted in decomposition and a series of side reactions. Using phosphorus oxychloride<sup>174</sup> as a coupling agent resulted in low yield with some acetonide removal observed. The trimethyl phosphite and iodine<sup>175</sup> approach resulted in ring-opening of the coumarin ester being the primary reaction. The limited solubility of the AMC meant that 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4methylmorpholinium chloride (DMT-MM)<sup>176</sup> was a poor choice as there was no mutual solvent and no reaction was observed for a suspension. Using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and Nmethylmorpholine<sup>177</sup> resulted in low 3-10% yields with side reactions at elevated temperatures. The bis(pentafluorophenyl)carbonate<sup>171</sup> method used to make MUC was also unsuccessful with either no reaction observed in the absence of cesium fluoride or side reactions in its presence. The best coupling method identified was a two-step reaction using 1,1'-carbonyldiimidazole (CDI) to activate the carboxylic acid and DBU<sup>172</sup> to catalyze the coupling, which resulted in a 74% yield. Finally, the ring-closing reaction was conducted using CDI rather than the higher yielding ethyl chloroformate as the TEA HCl by-product of that reaction formed a complex with MAC that confounded purification attempts. This impurity was especially undesirable as it has previously been shown to have catalytic/initiatory activity for carbonate polymerizations in trace amounts.<sup>168</sup>



Figure 3.7: Synthesis of MAC

## 3.4.2 Polymerization

Following the synthesis of these cinnamate and coumarin functionalized cyclic monomers, their polymerization kinetics were examined. It was hypothesized that the bulky cinnamoyl and coumarin pendant groups would slow the polymerization rate of the functionalized monomers relative to TMC by slowing the diffusion of the monomers and stabilizing the carbonate ring, as has previously been reported by Matsuo *et al.* for large alkyl substituents.<sup>35</sup> To test this hypothesis, the solution homopolymerization of several of the functionalized monomers was monitored in real-time using <sup>1</sup>H NMR and compared to the homopolymerization of TMC.

Surprisingly, the functionalized monomers COM and MUM polymerized significantly faster than TMC with DBU as a catalyst and benzyl alcohol as an initiator (Figure 3.8). In addition, the polymerization of MUM began to precipitate after 1 h and the monomer conversion plateaued around 75% after 2 h, by which time an insoluble gel had formed in the NMR tube. This result suggests that the solubility of the polymer in CDCl<sub>3</sub> decreased with increasing molecular weight, which would increasingly bias the observed monomer conversion toward the remaining monomer as the longer polymer chains precipitated out of solution and were no longer detectable by <sup>1</sup>H NMR. The insolubility of this material is consistent with the observations of Corkery regarding the insolubility of polystyrene and polymethacrylate homopolymers functionalized with pendant coumarin moieties in common deuterated solvents.<sup>178</sup>



Figure 3.8: Homopolymerization kinetics of various carbonate monomers catalyzed with DBU and initiated with benzyl alcohol (25  $^{\circ}$ C 1 M in CDCl<sub>3</sub>) with a monomer:initiator:catalyst ratio of 100:2:5

The rapid homopolymerization of the functionalized monomers relative to TMC suggested that the pendant groups were playing an unexpected role in the polymerizations as these results were inconsistent with Matsuo *et al.*'s conclusion that the polymerization rate of carbonate monomers decreases with increasing pendant group size.<sup>35</sup> Venkataraman *et al.* reported that carbonate monomers with pendant groups containing amides or carbamates, which are capable of very strong hydrogen bonding, polymerized in 3-5 min using DBU as a catalyst.<sup>179</sup> On this basis, it was hypothesized that the chemical nature of the pendant group is the primary factor in determining a monomer's polymerization rate with groups capable of hydrogen bonding or with an inherent polarity enhancing the polymerization rate. To test this hypothesis, another solution polymerization was conducted using a cyclic carbonate functionalized with an acrylate group (AC) (Figure 3.9), which was prepared by another lab member using a modified version of the procedure by Chen *et al.*<sup>180</sup> This monomer is chemically identical to COM with the exception of a phenyl ring.



Figure 3.9: Homopolymerization kinetics of various carbonate monomers catalyzed with DBU and initiated with benzyl alcohol (25  $^{\circ}$ C 1 M in CDCl<sub>3</sub>) with a monomer:initiator:catalyst ratio of 100:2:5

As hypothesized, AC polymerized significantly faster than TMC, which suggests that the presence of an ester in the pendant group enhanced the polymerization rate. This result is consistent with the observations of Pratt *et al.* regarding the polymerization of carbonate monomers with ester-linked pendant groups using a DBU-thiourea co-catalyst system.<sup>55</sup> In addition, the fact that AC also polymerized faster than COM is consistent with the Matsuo *et al.*'s original observations regarding substituent size.<sup>35</sup> Overall, it appears that the primary rate-determining factor is the nature of the functional groups (alkyl, ester, ether, or amide) present in the pendant group with size exerting an influence only when comparing substituents with the same chemical character.

Attempts to polymerize the remaining cinnamoyl and coumarin functionalized monomers (CAM, MUC, and MAC) under the same conditions proved unsuccessful. The real-time tracking of the polymerization of

CAM revealed a steady rate of monomer consumption and the appearance of a new set of small molecule peaks (Figure A.11). This change revealed that CAM was undergoing an intramolecular rearrangement rather than polymerizing as desired. The rearrangement product is shown in Figure 3.10 and appears to be quite stable. In addition, MUC and MAC proved insoluble at sufficient concentration in common polymerization solvents, so further solution polymerization studies were conducted using solely COM and MUM.



*Figure 3.10: Intramolecular rearrangement of CAM in CDCl<sub>3</sub> with DBU as a catalyst.* 

Unfortunately, for all DBU catalyzed polymerizations examined, the end group fidelity was poor. For MUM and AC, the end group fidelity remained consistently low throughout the polymerization while it decreased steadily during the polymerization of COM and TMC (Table 3.1). The low or decreasing end group fidelity for all of the monomers may be the result of DBU acting as an initiator, which has previously been suggested for a norbornene functionalized carbonate.<sup>181</sup>

*Table 3.1: End group fidelity of polycarbonates polymerized with DBU as a catalyst and benzyl alcohol as an initiator.* 

Monomer	End Group Fidelity (After 10 min) (%)	End Group Fidelity (After 120 min) (%)	End Group Fidelity (After 200 min) (%)
TMC	85	64	58
COM	68	53	51
AC	40	36	36
MUM	31	30	N/A

The polymerization of COM and MUM using TBD was monitored in real-time using <sup>1</sup>H NMR (Figure 3.11). The use of TBD as a catalyst resulted in homopolymerization rates that were closer in magnitude for the various carbonate monomers. Interestingly, COM polymerized faster than MUM with TBD, which was opposite to what was observed with DBU as a catalyst. However, both COM and MUM still polymerized significantly faster than TMC, which is consistent with the theory that the chemical character of these pendant groups enhances the polymerization rate of the functionalized monomers more than pendant group size inhibits it. In addition, the use of TBD as a catalyst resulted in low end group fidelity for all monomers (38-55% after 2 h), which suggested that TBD was also acting as an initiator. To test this possibility, the TBD concentration was increased from 2 to 5 mol% after 125 min for COM and TMC, and a step change in monomer conversion of 3% was observed. This result is consistent with nucleophilic attack on carbonate monomers by the TBD to form an acyl-activated intermediate, thereby initiating additional propagating chains.<sup>42,182</sup> However, the increased TBD concentration and the corresponding increase in propagating chains had no impact on the rate of monomer conversion, which suggests that the polymerization is rate-limited by another factor, such as diffusion.



Figure 3.11: Carbonate polymerizations catalyzed with TBD and initiated with benzyl alcohol (25 % 0.5 *M* in CDCl<sub>3</sub>) with a monomer:initiator:catalyst ratio of 100:2:2 (increased to 100:2:5 after 125 min for COM and TMC).

Given the limited solubility of many of the monomers at 25 °C and the poor end group fidelity achieved for the homopolymerization of those that were soluble, room temperature solution homopolymerization was not feasible under these conditions. Therefore, catalyst-free melt polymerization at elevated temperatures was examined as it had previously been shown to yield high end group fidelity for the polymerization of TMC.<sup>168</sup> While TMC has a comparatively low melting point of 45 °C, the melting points of the functionalized carbonate monomers are significantly higher (Table 3.2), so higher polymerization temperatures were required.

Monomer	Melting Point (°C)		
TMC	45		
COM	90		
CAM	122		
MUM	143		
MUC	145		
MAC	251		

Table 3.2: Melting point of the functionalized carbonate monomers.

While a test homopolymerization of COM achieved > 99% monomer conversion after 18 h at 120 °C, the end group fidelity was poor (50%). A test homopolymerization of CAM at 130 °C was unsuccessful as the monomer decomposed with the production of sufficient gas pressure to rupture the vacuum-sealed ampule. Finally, a test homopolymerization of MUM at 145 °C was once again characterized by the appearance of a precipitate during the polymerization, which proved to be insoluble in a range of organic solvents thereby interfering with characterization. A possible explanation for this result is that the melting point of oligomers of MUM is significantly higher than the monomer and so they freeze out from the polymerization mixture. The insolubility of this homopolymer, however, reduces its potential applications. Melt homopolymerizations of MUC and MAC were not attempted due to their high melting points and the expectation of similar insolubility issues to MUM.

In addition, the copolymerization of the functionalized carbonate monomers with TMC was explored. However, room temperature organocatalyzed solution copolymerizations were heavily influenced by the differences in the polymerization kinetics between the different monomers with the polymerization consisting almost exclusively of the faster polymerizing monomer (Figure 3.12). This finding is consistent with others in the literature that have shown that blocky copolymers are formed with DBU as a catalyst, with the faster propagating monomer polymerizing first.<sup>61,183</sup>



Figure 3.12: Monomer conversion kinetics for a 50:50 copolymerization of TMC and MUM catalyzed with DBU and initiated with benzyl alcohol (25  $\degree$ C 1 M in CDCl<sub>3</sub>) with a monomer:initiator:catalyst ratio of 100:2:5.

Consequently, the catalyst-free melt copolymerization of the functionalized monomers with TMC was examined. Ordinarily, the high melting point for MUM, MUC, and MAC could pose an issue as very high temperatures can lead to uncontrolled polymerization and the potential for side-reactions, such as oxidation. However, melted TMC acted as a solvent for the other carbonate monomers, thereby allowing melt copolymerizations to be conducted at lower temperatures ( $\geq 100$  °C for COM, MUM, and MUC and  $\geq 160$  °C for MAC). Under these conditions, catalyst-free copolymerizations of TMC with COM or MUM were successful using various initiators (Table 3.3). While a difference between the polymerization rate of the functionalized monomers and TMC was still observed, it was less pronounced than for the room temperature copolymerizations catalyzed with DBU. Also, polymerization times decreased markedly at

increased temperatures. Unfortunately, both MUC and MAC proved to be unstable under the melt copolymerization conditions. MUC underwent a rapid transesterification reaction with the initiator at 100 °C resulting in the substitution of benzyl alcohol for MU on the pendant chain (Figure A.12) and the liberated MU was insufficiently nucleophilic to initiate polymerization. MAC underwent a rapid and uncontrolled polymerization in 5 min at 160 °C with considerable bubbling and the formation of a variety of species.

Table 3.3: Catalyst-free melt preparation of  $P(TMC_m\text{-}co\text{-}COM_n)$  and  $P(TMC_m\text{-}co\text{-}MUM_n)$  copolymers (P = poly; m, n = # of repeating units targeted) using various initiators (PEG = 750 g/mol poly(ethylene glycol) methyl ether, Bz = benzyl alcohol, Oct = 1-octanol).

Polymer	Temperature (°ි)	Time (h)	TMC Monomer Conversion (%)	Functionalized Monomer Conversion (%)	End Group Fidelity (%)
$PEG-P(TMC_{12}-co-MUM_4)$	100	0.5	13	51	57
$PEG-P(TMC_{12}-co-MUM_4)$	100	2	77	92	52
$Bz-P(TMC_{22.5}-co-COM_{7.5})$	100	1	97	96	82
$Oct-P(TMC_{22.5}-co-MUM_{7.5})$	135	0.25	94	93	52

In addition, the molecular weight and molecular weight distribution of  $P(TMC_{40}-co-MUM_{10})$  copolymers prepared using DBU-catalyzed or TBD-catalyzed solution polymerizations were characterized by gel permeation chromatography (GPC) and compared to a  $P(TMC_{40}-co-MUM_{10})$  copolymer prepared using catalyst-free melt polymerization in order to compare the polymerization control achieved using each condition. The GPC traces indicated that the molecular weight distributions were monomodal when DBUcatalyzed solution or catalyst-free melt polymerizations were conducted (Figure 3.13). Further analysis indicated that DBU-catalyzed polymerizations produced a much lower molar mass dispersity than the catalyst-free melt polymerization, while TBD-catalyzed polymerizations resulted in a much higher molar mass dispersity (Table 3.4). In addition, both organocatalysts resulted in  $M_n$  and  $M_w$  values that were less than half those observed for the catalyst-free melt polymerization, which is consistent with the poor end group fidelity previously observed with these catalysts.



Figure 3.13: GPC traces for  $P(TMC_{40}\text{-}co\text{-}MUM_{10})$  copolymers prepared using various polymerization conditions

Table 3.4: GPC data for  $P(TMC_{40}\text{-}co\text{-}MUM_{10})$  copolymers prepared using various polymerization conditions.

Condition	M <sub>n</sub> (GPC) (g/mol)	M <sub>w</sub> (GPC) (g/mol)	Ð <sup>a</sup> (GPC)	
Catalyst-Free Melt	7000	10700	1.54	
DBU-Catalyzed Solution	3000	3600	1.23	
TBD-Catalyzed Solution	2100	4300	2.00	
a 1 1 1				

<sup>&</sup>lt;sup>a</sup> molar mass dispersity

Finally, a series of copolymerizations were conducted using various molar ratios of TMC and either COM or MUM at 120 °C using 1-octanol as an initiator while maintaining a consistent number of repeating units (30) in the polymer (Table 3.5). This relatively short polymer length was selected to facilitate the detection of changes in polymer properties due to photo-crosslinking in future studies as even low levels of crosslinking would result in significant increases in molecular weight and the corresponding polymer properties, regardless of whether a crosslinked polymer network was formed. Overall, the copolymerizations were successful, achieving % monomer incorporations close to those targeted, excellent end group fidelity for copolymers with COM, and moderate to good end group fidelity for copolymers with MUM. A calculated end group fidelity > 100% for one sample suggested that a small amount of chain transesterification occurred during the polymerization to generate polymer chains di-functionalized with the initiator. Purification of copolymers containing COM resulted in  $M_n$  values that were consistently lower than targeted with the deviation increasing with the % monomer incorporation. The purified copolymers had a much narrower  $M_n$  than targeted, ranging from 3400-3700 g/mol instead of 3700-4900 g/mol, which was attributed to reduced solubility of the higher molecular weight polymer chains in DCM. In the case of MUM, the M<sub>n</sub> values were all slightly higher than targeted, which is consistent with low molecular weight chains being removed by the polymer purification.

Table 3.5:  $P(TMC_m\text{-}co\text{-}COM_n)$  and  $P(TMC_m\text{-}co\text{-}MUM_n)$  copolymers (P = poly; m, n = # of repeating units targeted) initiated with 1-octanol at 120 °Cafter purification.

Polymer	Targeted % Monomer Incorporation	Actual % Monomer Incorporation (NMR)	Targeted M <sub>n</sub> (g/mol)	M <sub>n</sub> (NMR) (g/mol)	End Group Fidelity (%)
$P(TMC_{27}\text{-}co\text{-}COM_3)$	10	9	3700	3400	90
$P(TMC_{25.5}$ -co- $COM_{4.5})$	15	13	4000	3700	105
$P(TMC_{24}\text{-}co\text{-}COM_6)$	20	18	4200	3600	97
P(TMC <sub>22.5</sub> -co-COM <sub>7.5</sub> )	25	23	4500	3400	100
$P(TMC_{20}\text{-}co\text{-}COM_{10})$	33	33	4900	3700	97
P(TMC <sub>27</sub> -co-MUM <sub>3</sub> )	10	9	3800	4100	81
$P(TMC_{25.5}-co-MUM_{4.5})$	15	15	4100	4400	60
$P(TMC_{24}\text{-}co\text{-}MUM_6)$	20	18	4400	4900	62
$P(TMC_{22.5}-co-MUM_{7.5})$	25	22	4700	4800	84
$P(TMC_{20}\text{-}co\text{-}MUM_{10})$	33	29	5200	6200	69

## **3.5 Conclusions**

A series of photo-reactive cinnamoyl and coumarin functionalized cyclic carbonate monomers were synthesized. The room temperature solution homopolymerization kinetics of these monomers using either DBU or TBD as an organocatalyst were examined and compared to those for TMC under the same conditions. Unfortunately, only the ester-linked cinnamoyl (COM) and the ether-linked coumarin (MUM) monomers proved to be sufficiently soluble in suitable deuterated solvents to allow real-time monitoring using <sup>1</sup>H NMR. However, despite their bulky functional groups, these monomers polymerized significantly faster than TMC. This result suggested that the chemical nature (alkyl, ether, ester, or amide) of the pendant functional groups had a more significant impact on the polymerization kinetics than the size of the pendant group. It was only when comparing monomers with pendant groups containing very similar chemical characteristics that the variations in alkyl substituent size appeared to influence the homopolymerization kinetics. In addition, the catalyst-free melt homopolymerization of the monomers was examined. While this method was successful for the homopolymerization of the monomer with an ester-linked cinnamoyl (COM), the remainder of the monomers either decomposed (amide-linked cinnamoyl, CAM), precipitated
from the melt polymerization as insoluble solids (ether-linked coumarin, MUM) or required infeasible temperatures (ester- and amide-linked coumarin, MUC and MAC, respectively). Finally, it was determined that the monomers with an ester-linked cinnamoyl (COM) and an ether-linked coumarin (MUM) were capable of catalyst-free melt copolymerization with TMC at 100 °C and that this provided better end group fidelity than copolymerizations catalyzed by TBD or DBU and better molecular weight control than copolymerizations catalyzed by TBD.

# **Chapter 4**

# Triethylamine-Based Catalysts for the Melt Polymerization of Carbonate Monomers

# 4.1 Preface

This chapter describes some new catalysts and conditions for the polymerization of carbonate monomers that were identified during the studies described in Chapter 3 and contributed to the completion of the Specific Aim 2. This chapter is based on a paper that was submitted to Polymer Chemistry on July 19, 2016, with a few modifications to maintain consistency of terminology and formatting within the thesis. I designed, conducted, and analyzed all experiments described in this chapter with some suggestions and feedback provided by my PhD supervisor, Dr. Brian Amsden. In addition, I (first author) prepared the resulting manuscript with editorial input from Dr. Brian Amsden (corresponding author).

# **4.2 Introduction**

Aliphatic polycarbonates are promising biomaterials due to their nonacidic degradation products and customizable properties. By combining specific monomers, the hydrophobicity, degradability, viscoelasticity, and other properties of polycarbonates can be easily tailored.<sup>34</sup> Commercially available monomers include trimethylene carbonate (TMC), neopentylene carbonate (NPC), and 5-benzyloxytrimethylene carbonate (BTMC), and a wide assortment of other carbonate monomers have been synthesized for use in specific applications.<sup>34</sup>

Irrespective of which carbonate monomers are used, most aliphatic polycarbonates are best prepared using ring-opening polymerization.<sup>41</sup> This process is convenient, facilitates the incorporation of a wide assortment of end-group functionality and architectures by using various alcohols as initiators,<sup>54,184</sup> and results in higher molecular weights than polycondensations.<sup>41</sup> Initially, ring-opening polymerization of carbonates was

conducted using catalysts that were originally identified for the synthesis of polylactones, including stannous octoate  $(SnOct_2)$ .<sup>48</sup> Although SnOct<sub>2</sub> is widely used, tin complexes are becoming increasingly controversial due to the potential toxicity of tin residues, especially in food or biomedical applications where extensive purification of the resulting polymers is required before they can be used.<sup>49,50</sup>

Consequently, researchers have explored less toxic metal complexes such as zinc, calcium, and magnesium<sup>50,51</sup> and a variety of organic compounds,<sup>49,52</sup> such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),<sup>53</sup> triazabicyclodecene (TBD),<sup>53</sup> and trifluoroacetic acid<sup>54</sup> as catalysts for the preparation of both polyesters and polycarbonates. In addition, thermal catalysis at high temperatures<sup>54,56–58</sup> or using microwave irradiation has been examined.<sup>58–60</sup> These studies have highlighted the fact that catalytic activity varies significantly between catalysts.<sup>53,61</sup> In addition, the choice of catalyst dictates the polymerization rate, polymerization conditions, purification process, polymer dispersity, and end group fidelity and thus warrants careful consideration based on the intended application for the polymer. In this study, two new catalysts for melt polymerizations of carbonate monomers were identified and compared to the widely used SnOct<sub>2</sub> and catalyst-free polymerizations.

# 4.3 Experimental

## 4.3.1 Materials

All reagents were obtained from commercial suppliers and used as received unless otherwise noted. Trimethylene carbonate (99%) was obtained from Leapchem (Hangzhou, China) and Obiter Research (Champaign, USA) and purified by recrystallization from ethyl acetate (3 mL/g). Neopentylene carbonate was obtained from Frinton Labs (Hainesport, USA) as an oligomer and the monomer was obtained by distillation at 180 °C 5-benzyloxytrimethylene carbonate was obtained from Obiter Research (Champaign, USA). Triethylamine hydrochloride (TEA·HCl) (98%), benzyl alcohol (anhydrous, 99.8%), 5,000 g/mol poly(ethylene glycol) methyl ether (mPEG), 350 g/mol mPEG, and chloroform-d (99.8%D) (CDCl<sub>3</sub>) were

obtained from Sigma-Aldrich (Oakville, Canada). Tin (II) 2-ethylhexanoate (stannous octoate) (SnOct<sub>2</sub>) (96%) and 750 g/mol mPEG were obtained from Alfa-Aesar (Ward Hill, USA). 1,000 g/mol mPEG was obtained from TCI America (Portland, USA). Toluene (extra dry over molecular sieves) was obtained from Acros Organics (New Jersey, USA). Triethylamine (TEA) (99%), dichloromethane (DCM), diethyl ether (anhydrous), and tetrahydrofuran (THF) (HPLC grade) were obtained from Fisher Scientific (Ottawa, Canada) and the TEA was stored over 4 Å molecular sieves. mPEGs were dried by dissolving 5 g in 20 mL of toluene and concentrating *in vacuo*. This process was repeated 4 times and the resulting mPEGs were stored under argon.

## 4.3.2 Polymerization of Carbonate Monomers

All polymerizations were conducted as bulk melt polymerizations in Wheaton 5 mL gold band pre-scored ampules. The ampules were flame-dried, cooled in a 65  $^{\circ}$ C oven, and purged with argon. The general procedure was to add TMC (269 mg, 2.64 mmol, 50 eq), followed by benzyl alcohol diluted in anhydrous toluene (5.71 mg, 0.053 mmol, 1 eq, 20 % w/w) as an initiator and either TEA, TEA  $\cdot$ HCl, or SnOct<sub>2</sub> (0.0053 mmol, 0.1 eq) diluted in 15 mg anhydrous toluene as the catalyst (Figure 4.1). Catalyst-free polymerizations were conducted with 15 mg of anhydrous toluene added as a control. The ampules were again purged with argon, then evacuated at a reduced pressure of 28 kPa for 1 min, and flame-sealed while under vacuum. The ampules were then placed in a 65  $^{\circ}$ Coven for 3 min to melt the reagents, gently vortexed to ensure even mixing, and placed in an oil bath thermostat-controlled at the appropriate temperature. At each time point, two ampules were removed from the oil bath for each catalyst condition and halted by transferal to a -20  $^{\circ}$ C freezer where they were briefly stored until they could be analyzed.



Figure 4.1: General pathway for poly(carbonate) synthesis using benzyl alcohol as an initiator.

## 4.3.3 Polymer Characterization

Each polymer sample was dissolved in 1 mL DCM at room temperature. 60  $\mu$ L of the resulting polymer solution was collected and the DCM was evaporated by a continuous flow of air. The polymer residue was then dissolved in 0.55 mL of CDCl<sub>3</sub> and transferred to an oven-dried NMR tube. <sup>1</sup>H NMR spectroscopy was conducted using a Bruker 500 MHz NMR spectrometer with peak shifts referenced using an internal trimethylsilane standard. For TMC, the monomer conversion was determined by integrating the pair of peaks at 4.39 ppm (2 x OCH<sub>2</sub> monomer) and 4.17 ppm (2 x OCH<sub>2</sub> polymer) and the pair of peaks at 2.08 ppm (central CH<sub>2</sub> monomer) and 1.98 ppm (central CH<sub>2</sub> polymer). The polymer peak integration was divided by the sum of the monomer and polymer peak integrations for each pair and these were averaged to determine the monomer conversion. The terminal group ratio ( $\tau$ ) was calculated by dividing the peak integration at 3.67 ppm (terminal  $CH_2$  on the polymer chain) by the integration of the peak at 5.09 ppm (CH<sub>2</sub> on the benzyl alcohol initiator following polymerization). If no chains were initiated by another source, such as residual water,  $\tau$  would be 1.  $\tau$  can be used to calculate end group fidelity ( $\alpha$ ), which represents the number of polymer chains that incorporate the initiator, as shown in Equation 1.<sup>54</sup> The amount by which  $\tau$  exceeds 1 represents the integration contribution from polymer chains without an attached initiator. This quantity is then halved to account for the fact that non-benzyl alcohol initiated polymer chains will have an identical terminal CH<sub>2</sub> group on both chain ends due to decarboxylation of the carbonic ester end.<sup>185,186</sup>  $\alpha$  is then obtained by normalizing the number of benzyl alcohol initiated polymer chains to the total number of polymer chains (initiated plus non-initiated). Monomer conversion and  $\alpha$  for NPC and BTMC were calculated by a similar method using the integration of the pair of peaks at 4.01 ppm (2 x  $OCH_2$  monomer) and 3.90 ppm (2 x  $OCH_2$  polymer) and the pair of peaks at 1.06 ppm (central  $CH_2$ monomer) and 0.94 ppm (2 x  $CH_3$  polymer) and the terminal peak at 3.29 ppm (terminal  $CH_2$  on the polymer chain) for NPC and the integration of the pair of peaks at 4.38 ppm (2 x OCH<sub>2</sub> monomer) and 4.17 ppm (2 x OCH<sub>2</sub> polymer) and the pair of peaks at 3.81 ppm (central CH<sub>2</sub> monomer) and 3.77 ppm (central CH polymer) and the terminal peak at 3.65 ppm (terminal CH<sub>2</sub> on the polymer chain) for BTMC.

The  $M_n$  for PTMC was calculated by two methods: based on the integration (A) of the peaks at 4.17 ppm (2 x OCH<sub>2</sub> polymer) (Equation 2) and 1.98 ppm (central CH<sub>2</sub> polymer) (Equation 3) and the end group fidelity of the polymers, and reported as the average.

$$\alpha = \frac{1}{1 + \frac{\tau - 1}{2}} \cdot 100\%$$
 (1)

$$M_n = 102.089 \cdot \frac{A_{4.17}}{4\left(1 + \frac{\tau - 1}{2}\right)} + 108.138\alpha \quad (2)$$

$$M_n = 102.089 \cdot \frac{A_{1.98}}{2\left(1 + \frac{\tau - 1}{2}\right)} + 108.138\alpha \quad (3)$$

The molecular weight and molecular weight distribution of the PTMC samples were also characterized by gel permeation chromatography (GPC) using a Waters 2695 GPC apparatus with a guard and 4 columns, a Waters 410 Differential Refractometer, and a Wyatt-Dawn EOS 18 light detecting system. Samples were dissolved in HPLC grade THF at a 2 mg/mL concentration, filtered with a 0.2  $\mu$ m syringe filter, and run at 25 °C using distilled THF as the eluent. Number average molecular weight, M<sub>n</sub>, weight average molecular weight, M<sub>w</sub>, and dispersity were calculated from a universal calibration using Mark-Houwink coefficients for PTMC of 2.77x10<sup>-4</sup> and 0.677 for K and a, respectively.<sup>187</sup>

#### **4.3.4 Statistics**

All data is expressed as mean values and where indicated, error bars and  $\pm$  values are given in terms of the standard error about the mean. Results were analyzed for statistical significance using a one-way ANOVA and a Bonferroni post hoc test (p < 0.05) was applied.

## 4.4 Results and Discussion

While examining the copolymerization kinetics of several synthesized carbonate monomers with TMC, unusually rapid polymerization (< 30 min) was observed at 135  $^{\circ}$ C. In order to identify the cause, high purity TMC was obtained from two different commercial suppliers and further purified by recrystallization from

ethyl acetate.<sup>188</sup> The purified TMC was used to conduct a series of polymerizations to determine whether trace impurities from the monomer synthesis might be responsible. These polymerizations included catalyst-free conditions as it has been reported that TMC can polymerize without catalyst at temperatures at or above 120 °C.<sup>54,56,57</sup> Unexpectedly, the addition of triethylamine hydrochloride (TEA·HCl) proved sufficient to catalyze the complete polymerization of TMC in 20 min at 135 °C, as shown in Table 4.1. The absence of literature reports of this catalytic ability is notable since TEA·HCl is a by-product of the ethyl chloroformate ring-closing reaction commonly used to prepare cyclic carbonates.<sup>35</sup> In addition, TEA·HCl is a solid, so it would have a number of advantages as a catalyst including ease of handling and stability.

Table 4.1: Polymerization of TMC at 135 °C after 20 min with varying amounts of TEA·HCl and benzyl alcohol as initiator.

TEA·HCl Loading	Monomer Conversion (%)	α <sup>a</sup> (%)	M <sub>n</sub> (Theoretical) (g/mol)	M <sub>n</sub> (NMR) (g/mol)	M <sub>n</sub> (GPC) (g/mol)	M <sub>w</sub> (GPC) (g/mol)	Ð <sup>b</sup> (GPC)
None	56.5	92.6	2800	2500	3100	4100	1.34
500:1 M:C <sup>c</sup>	92.4	86.6	4800	4400	5500	8400	1.52
100:1 M:C <sup>c</sup>	99.3	71.2	4900	3700	4100	6300	1.52

a end group fidelity

b molar mass dispersity

c monomer:catalyst molar ratio

Both 500:1 and 100:1 monomer:catalyst (M:C) molar ratios of TEA·HCl resulted in a significant increase in the polymerization rate relative to catalyst-free polymerization of TMC. While the higher molar ratio of TEA·HCl resulted in a 7.5% increase in monomer conversion, it also resulted in a 17.8% reduction in end group fidelity, which corresponds to the 16% decrease in  $M_n(NMR)$  and 25% decrease in  $M_n(GPC)$  and  $M_w$ . This result indicates that the TEA·HCl may play a role in both polymer chain initiation and propagation. The similar dispersities suggest that polymer chain initiation occurred relatively early during the polymerization. The apparent lower dispersity for the catalyst-free polymerization is an artefact of the incomplete monomer conversion as the dispersity increased over the course of the polymerization for all catalyst systems examined.

A possible explanation for the polymer chain initiation observed in the presence of TEA·HCl is that TEA·HCl acts as a source of nucleophilic anions, through the dissociation of chloride ions from the salt, which then initiate the ring-opening of TMC (Figure 4.2). This mechanism would be consistent with the reported use of tetrabutylammonium salts as co-catalysts for calcium salen catalyzed polymerizations.<sup>189</sup> In addition, the chloride and fluoride salts of these bulky alkylammonium cations have variable catalytic activity on their own.<sup>189,190</sup>



Figure 4.2: Ring-opening of TMC by TEA HCl to form an activated linear carbonate and TEA.

The potential of TEA·HCl as a catalyst was further explored by conducting several polymerizations at 135  $^{\circ}$ C without an added initiator. The TEA·HCl catalyzed polymerization proceeded significantly faster than the catalyst-free polymerization, achieving 78% and 13% monomer conversion respectively after 20 min. However, the 6-fold increase in monomer conversion with TEA·HCl resulted in only a 50% increase in M<sub>n</sub> (6,900 g/mol vs 4,800 g/mol), which further supports the proposition that TEA·HCl can act as both a catalyst and an initiator. Consequently, further experiments were conducted using only the lower TEA·HCl loading (higher M:C ratio) to minimize the impact of the catalyst on end group fidelity. The partial polymerization of TMC in the absence of a catalyst or added initiator was likely either initiated by trace residual moisture or heterolytic cleavage to form an alkoxide.<sup>188</sup>

To further explore the catalytic ability of TEA·HCl, a series of TMC polymerizations were conducted at 110 °C and control polymerizations were also conducted using either TEA or SnOct<sub>2</sub> as a catalyst or catalyst-free. SnOct<sub>2</sub> has been widely used as a catalyst for ring-opening polymerization,<sup>48</sup> while TEA has previously been reported to catalyze the solution polymerization of TMC in tetrahydrofuran at 55 °C,<sup>191</sup> albeit using a much higher catalyst loading (~20 times) with 1.5-2 equivalents relative to initiator and a M:C ratio of only 10-15:1. TEA has also been reported to enhance the activity of a cationic zinc catalyst for TMC melt and solution polymerizations,<sup>51</sup> but to have no catalytic activity for the melt polymerization of a cyclic carbonate with a norbornene pendant group in the absence of a hydroxyl initiator.<sup>181</sup> The monomer conversion kinetics with the various catalysts at 110 °C is shown in Figure 4.3 and the polymer properties after 2.5 h are summarized in Table 4.2.



*Figure 4.3: TMC polymerization using various catalysts and initiated by benzyl alcohol at 110*  $^{\circ}C(n = 2)$ .

Catalyst	Monomer Conversion (%)	α <sup>a</sup> (%)	M <sub>n</sub> (Theoretical) (g/mol)	M <sub>n</sub> (NMR) (g/mol)	M <sub>n</sub> (GPC) (g/mol)	M <sub>w</sub> (GPC) (g/mol)	Ð <sup>b</sup> (GPC)
None	99±3	81±3	5150	4250	6120	9300	1.52
TEA·HCl <sup>c</sup>	98.4±0.1	70±2	5000	3730	5080	7520	1.48
TEA <sup>c</sup>	98.3±0.1	77±3	5130	4220	6030	9080	1.51
$\operatorname{SnOct}_2^c$	96±1	75±2	5640	4490	6425	9910	1.54

Table 4.2: Polymerization of TMC at 110 °Cafter 2.5 h with various catalysts and using benzyl alcohol as initiator (n = 2).

<sup>a</sup> end group fidelity <sup>b</sup> molar mass dispersity

c 500:1 monomer:catalyst molar ratio

The polymerizations at 110  $^{\circ}$ C revealed that both TEA HCl and TEA can catalyze the polymerization of TMC significantly faster than the widely used SnOct<sub>2</sub> with complete monomer conversion in 1 h rather than 2.5 h. As expected, the M<sub>n</sub> values determined from the <sup>1</sup>H NMR data were lower than the theoretical M<sub>n</sub> values as the  $M_n(NMR)$  uses the end group fidelity to account for polymer chains that were not initiated by the benzyl alcohol. However, the M<sub>n</sub> values determined using GPC were all higher than the theoretical M<sub>n</sub>, which could be due to a deviation between the values calculated using the literature Mark-Houwink coefficients and the actual values at low molecular weights. A significantly lower end group fidelity was once again observed when using TEA HCl as a catalyst relative to the catalyst-free control, which is consistent with the fact that the  $M_n$  (GPC) and  $M_w$  observed for the TEA HCl catalyzed polymerization were significantly lower than in the other polymerization conditions. However, the fact that the molar mass dispersity is nearly identical across the polymerization conditions suggests that the reduction in end group fidelity with TEA HCl is caused by additional initiation rather than chain scission.

The differences in catalytic rate were more pronounced when the TMC polymerization kinetics were subsequently examined at 85  $^{\circ}$ C (Figure 4.4). Once again, the addition of either TEA HCl or TEA resulted in significantly faster polymerization than with SnOct<sub>2</sub>. In addition, the relative catalytic ability of the two TEA-based catalysts became apparent with TEA catalyzing complete TMC conversion in half the time required by TEA HCl. A possible explanation for this difference is that TEA is the active catalyst in both

systems and that a thermally driven dissociation of the TEA·HCl must occur before it can act as a catalyst. TEA is hypothesized to act as a proton transfer agent to catalyze the anionic ring-opening polymerization of TMC (Figure 4.5).



*Figure 4.4: TMC polymerization using various catalysts and initiated by benzyl alcohol at 85*  $^{\circ}C(n = 2)$ .



*Figure 4.5: Catalytic role of TEA as a proton transfer agent in the anionic ring-opening polymerization of TMC.* 

Surprisingly, the catalyst-free polymerization of TMC also proceeded steadily and achieved complete monomer conversion after 32 h. To our knowledge, this temperature is the lowest at which complete

monomer conversion has been reported in a melt polymerization of TMC without the addition of a catalyst. Kricheldorf *et al.* reported the partial catalyst-free polymerization of TMC at 60  $^{\circ}$ C using hematin as an initiator, but only achieved 26% conversion after 48 h and 82% conversion after 144 h; all other reports of catalyst-free polymerizations occurred at 100-120 °C<sup>54,57,58,188</sup> These results were reproduced using TMC sourced from a second commercial supplier.

In addition, the monomer conversion rates for all four catalyst conditions were roughly linear with respect to time, which indicates pseudo-zero order reaction kinetics. This result is likely attributable to the large excess of the TMC monomer with respect to the propagating polymer chain ends. Finally, lowering the temperature to 85 °C from 110 °C resulted in a significant improvement in the end group fidelity obtained for all catalyst conditions (Table 4.3).

Table 4.3: Polymerization of TMC at 85 °C with various catalysts and using benzyl alcohol as initiator (n = 2).

Catalyst	Time (h)	Monomer Conversion (%)	α <sup>a</sup> (%)	M <sub>n</sub> (Theoretical) (g/mol)	M <sub>n</sub> (NMR) (g/mol)	M <sub>n</sub> (GPC) (g/mol)	M <sub>w</sub> (GPC) (g/mol)	${oldsymbol{\mathcal{D}}}^{\mathrm{b}}$
None	32	99.19±0.03	94.3±0.4	5430	5140	7330	11420	$1.56 \pm 0.01$
TEA · HCl <sup>c</sup>	12	98.3±0.8	$80 \pm 4$	4800	4180	5410	7660	$1.42 \pm 0.02$
TEA <sup>c</sup>	6	96.7±0.1	93±1	5360	4820	6220	8610	$1.38 \pm 0.01$
$\operatorname{SnOct}_2^c$	24	95.9±0.6	90±1	5160	4580	6210	9500	$1.53 \pm 0.01$

<sup>a</sup> end group fidelity <sup>b</sup> molar mass dispersity

<sup>c</sup> 500:1 monomer:catalyst molar ratio

As previously observed, the use of TEA HCl as a catalyst resulted in significantly lower end group fidelity than the catalyst-free control and a corresponding decrease in  $M_n(NMR)$ ,  $M_n(GPC)$ , and  $M_w$  relative to the other polymerization conditions. For the other catalyst conditions, the  $M_n(NMR)$  values showed a reasonable correlation with the theoretical  $M_n$  values while the  $M_n$ (GPC) values were higher. Interestingly, the molar mass dispersity was very consistent across the replicates for each catalyst condition.

Consequently, the polymerizations catalyzed with TEA significantly outperformed those catalyzed with  $SnOct_2$  in terms of polymerization rate and final molar mass dispersity, while maintaining end group fidelity. The fact that the TEA·HCl catalyzed polymerization resulted in a lower molar mass dispersity despite a significantly worse end group fidelity relative to the catalyst-free polymerization is further indicative of additional sources of initiation rather than increased chain scission, which would increase the molar mass dispersity.

The melt polymerization of TMC under the various catalyst conditions was subsequently examined at 65  $^{\circ}$ C to further probe the lower limits (Figure 4.6). However, only TEA was able to catalyze complete monomer conversion at 65  $^{\circ}$ C in a reasonable timeframe with all other polymerization conditions having < 15% monomer conversion after 16 h.



*Figure 4.6: TMC polymerization using various catalysts and initiated by benzyl alcohol at 65* C(n = 2).

Table 4.4 compares the efficacy of TEA·HCl, TEA, and SnOct<sub>2</sub> at various temperatures using the turnover frequency (TOF), which is a calculation of the number of moles of monomer converted per mole of catalyst

per hour. This analysis facilitates comparisons between the catalysts used in this work and previously reported catalysts. For example, the calculated TOFs for TEA·HCl and TEA are comparable to the reported TOF of 63.3 h<sup>-1</sup> at 85 °C for tetrabutylammonium chloride,<sup>189</sup> but cannot compete with the 30,000-240,000 h<sup>-1</sup> TOFs reported for some custom synthesized zinc and magnesium catalysts.<sup>51,192</sup> A comparison of these TOFs also highlights that while the activity of all of the catalysts is temperature dependent, TEA·HCl and SnOct<sub>2</sub> are more sensitive to decreases in temperature than TEA. At 110 °C, the catalytic ability of TEA is 1.5-1.8 times that of TEA·HCl and SnOct<sub>2</sub> and this discrepancy increases to 2-4 times at 85 °C and 20-200 times at 65 °C.

Temp. (°C)	Catalyst	Time (h)	Conversion (%)	$TOF(h^{-1})$
	TEA	8	99	62
65	TEA·HCl	16	1.4	0.3
	SnOct <sub>2</sub>	12	7.0	3.3
	TEA	4	85	98
85	TEA·HCl	6	65	49
	$SnOct_2$	18	89	23
	TEA	0.67	96	770
110	TEA·HCl	0.67	95	520
	SnOct <sub>2</sub>	1	83	430

Table 4.4: Comparison of catalyst efficacy at various temperatures.

In addition, a few test polymerizations were conducted to assess the catalytic ability of TEA and TEA·HCl for two other commercially available carbonate monomers, neopentylene carbonate (NPC) and 5-benzyloxytrimethylene carbonate (BTMC). Control polymerizations were conducted with SnOct<sub>2</sub> as a catalyst as well as catalyst-free (Table 4.5). These tests revealed that polymerizations using TEA or TEA·HCl achieved similar monomer conversion to those catalyzed with SnOct<sub>2</sub> and over double the monomer conversion of catalyst-free polymerizations conducted for the same length of time. The

significantly higher melting points of both NPC (110  $^{\circ}C^{193}$ ) and BTMC (142-143  $^{\circ}C^{194}$ ) relative to TMC (46  $^{\circ}C^{188,195}$ ) required the use of much higher polymerization temperatures, which resulted in a corresponding decrease in the end group fidelity achieved during these polymerizations. The NPC polymerizations resulted in no significant differences in end group fidelity between the catalyst conditions. However, the BTMC polymerizations showed an interesting change, with SnOct<sub>2</sub> catalysis resulting in a significantly higher end group fidelity relative to catalyst-free or TEA·HCl catalyzed polymerizations. While the cause of this discrepancy is uncertain, it could be related to the asymmetrical nature of BTMC or the influence of the large pendant group on the stability of the monomer ring towards polymerization or heterolytic cleavage.

Table 4.5: Polymerization of other carbonate monomers with various catalysts and using benzyl alcohol as initiator (n = 2).

Catalwat	NPC <sup>a</sup>	NPC <sup>a</sup>	BTMC <sup>b</sup>	<b>BTMC<sup>b</sup></b>
Catalyst	Conversion (%)	α <sup>c</sup> (%)	Conversion (%)	α <sup>c</sup> (%)
None	37±4	63±2	40±30	47±6
TEA·HCl	88.7±0.4	54±4	90±3	47±1
TEA	90.5±0.2	59.7±0.2	90.4±0.3	57±2
$SnOct_2$	91±1	59±1	89.8±0.6	73±1

<sup>a</sup> NPC polymerized at 130 °C for 2 h using a monomer:initiator:catalyst molar ratio of 50:1:0.1

<sup>b</sup> BTMC polymerized at 150 °C for 0.75 h using a monomer:initiator:catalyst molar ratio of 50:1:0.1 <sup>c</sup> end group fidelity

Finally, given that catalyst-free polymerization of TMC had been observed at lower temperatures than previously reported and that these conditions resulted in the highest end group fidelity observed, catalyst-free polymerizations using poly(ethylene glycol) (PEG) as an initiator were explored. A series of PEG-PTMC diblock copolymers with molecular weights from 870-20000 g/mol were synthesized using 350-5000 g/mol monomethoxy PEG (mPEG) initiators at temperatures ranging from 85-115  $^{\circ}$ C(Table 4.6). The polymerizations proceeded slightly slower than observed using benzyl alcohol as an initiator with a 14.7:1 monomer:mPEG-initiator polymerization achieving 70% conversion after 1.5 h at 115  $^{\circ}$ C while a 50:1

monomer:benzyl alcohol polymerization was complete in 2.5 h at 110 °C. The reduced polymerization rate can partially be attributed to the mPEG initiated polymerizations being conducted on six times the scale, which would have lowered the heat transfer efficiency and delayed the onset of polymerization. Other contributing factors may include slower diffusion of the mPEG initiators due to their much larger size and a potential reduction in accessibility of the hydroxyl group depending on how the mPEG chain is coiled in the melt polymerization. Despite polymerizing slower than with benzyl alcohol as an initiator, complete monomer conversion was achieved, which demonstrates that the hydroxyl groups on macroinitiators can initiate the catalyst-free polymerization of TMC in the same manner as low molecular weight alcohols. This result is of particular interest in biomedical applications where the higher end group fidelity achieved under catalyst-free conditions combined with the absence of a catalyst, reduces the polymer purification requirements.

Table 4.6: Properties of mPEG initiated  $PEG_m$ -PTMC<sub>n</sub> diblock copolymers (m, n = # of repeating units targeted).

Polymer	Temp. (°C)	M <sub>n</sub> (Theoretical) (g/mol)	M <sub>n</sub> (NMR) (g/mol)	M <sub>n</sub> (GPC) (g/mol)	M <sub>w</sub> (GPC) (g/mol)	Đ <sup>a</sup>
PEG <sub>7.2</sub> -PTMC <sub>6.9</sub>	85	1100	1100	1200	1900	1.60
PEG <sub>7.2</sub> -PTMC <sub>5.1</sub>	85	870	920	990	1500	1.53
PEG <sub>16.3</sub> -PTMC <sub>14.7</sub>	115	2200	2300	2900	3600	1.25
PEG <sub>16.3</sub> -PTMC <sub>11.0</sub>	115	1800	1900	2400	3100	1.29
PEG22.0-PTMC19.6	115	3000	3000	3800	5500	1.43
PEG <sub>22.0</sub> -PTMC <sub>14.7</sub>	115	2400	2500	3200	4400	1.36
PEG <sub>112.8</sub> -PTMC <sub>146.9</sub>	100	19800	19800	13600	19900	1.46
PEG <sub>112.8</sub> -PTMC <sub>114.3</sub>	100	16500	16300	9800	15400	1.58
PEG <sub>112.8</sub> -PTMC <sub>97.9</sub>	100	15000	14900	8200	12200	1.49
PEG <sub>112.8</sub> -PTMC <sub>59.9</sub>	100	11000	10900	7600	11600	1.52

<sup>a</sup> molar mass dispersity

The  $M_n$  values determined by <sup>1</sup>H NMR showed excellent correlation with the theoretical  $M_n$  values for the PEG-PTMC polymerizations under all conditions examined. However, the  $M_n$  and  $M_w$  values determined by GPC deviated significantly from the theoretical  $M_n$  with increases in the molecular weight of the mPEG

initiator. This is likely an artefact of the GPC molecular weights being calculated using universal calibration based on the Mark-Houwink parameters for pure PTMC. However, the polymer dispersities determined by GPC will be relatively unaffected by this factor and in fact the polymerizations conducted at 85 °C and 100 °C show a similar dispersity range to each other and to those previously reported for the melt polymerizations initiated by benzyl alcohol. The dispersities for the polymerizations conducted at 115 °C are lower as these samples were analyzed following purification by precipitation from minimal DCM with diethyl ether, which in addition to removing unreacted monomer, would have removed the lowest molecular weight polymer chains.

# 4.5 Conclusions

This study examined the use of TEA, TEA·HCl, SnOct<sub>2</sub>, and no catalyst for the polymerization of carbonate monomers. It is the first reported use of TEA·HCl as a catalyst for carbonate polymerizations and the first reported use of TEA as a catalyst for melt polymerizations. Both proved capable of catalyzing carbonate polymerization and achieved faster TMC conversion than the widely used SnOct<sub>2</sub> catalyst under most conditions tested. However, TEA·HCl also appeared to be capable of initiating polymerization, which resulted in a decrease in end group fidelity when it was used as a catalyst. The combined catalytic and initiatory activity of TEA·HCl is especially noteworthy for two reasons. First, the prevalence of the ethyl chloroformate ring-closing reaction<sup>35</sup> in the synthesis of carbonate monomers means that TEA·HCl is a potential impurity in many carbonate monomers, which could lead to undesired auto-polymerization if monomers are not carefully purified. Second, for applications where the decrease in end group fidelity is acceptable, TEA·HCl has several advantages as a catalyst, including ease of handling and availability. Solid catalysts for carbonate polymerizations are rare, but are easy to mix with the other solid reagents prior to heating and eliminate the safety precautions required to work with volatile toxic or flammable liquids. In addition, TEA proved capable of catalyzing the melt polymerization of TMC at 65 °C, which was too cold for TEA·HCl catalyzed, SnOct<sub>2</sub> catalyzed, or catalyst-free polymerizations to proceed in a reasonable

timeframe. These conditions could allow melt polymerization using thermally sensitive end-groups that cannot survive traditional SnOct<sub>2</sub> catalyzed ring-opening polymerizations at 110+  $^{\circ}$ C.

Finally, the catalyst-free polymerization of TMC, which has previously only been reported after long times at temperatures exceeding 100  $^{\circ}$ C, was observed to proceed smoothly and with high end group fidelity at 85  $^{\circ}$ C. This study also demonstrated that the catalyst-free polymerization of TMC can be used to prepare diblock copolymers using mPEG as an initiator at 85  $^{\circ}$ C. This finding is especially important for biomaterials and some industrial applications as it eliminates the need for an added catalyst and thereby simplifies the polymerization and purification processes.

# Chapter 5

# Reversibly Photo-Crosslinkable Aliphatic Polycarbonates Functionalized with Coumarin and Cinnamate

# 5.1 Preface

This chapter describes an examination of the reversible photo-crosslinking of polycarbonates functionalized with cinnamoyl and coumarin groups, which fulfilled Specific Aim 3. It is based on a paper that is currently being prepared for submission to Macromolecules, with a few modifications to maintain consistency of terminology and formatting within the thesis. The majority of the experiments described in this chapter were conducted in Dr. Timothy Hughes' lab at the CSIRO research facility in Clayton, Australia. I designed, conducted, and analyzed all experiments described in this chapter with some suggestions and feedback provided by Dr. Timothy Hughes and my PhD supervisor, Dr. Brian Amsden. In addition, I (first author) prepared the resulting manuscript with editorial input from Dr. Timothy Hughes (second author) and Dr. Brian Amsden (corresponding author).

# **5.2 Introduction**

The preparation of polymers that can respond to external stimuli such as light or pH is a growing research area with biomedical applications including responsive drug delivery systems and intraocular lenses.<sup>92,98</sup> Many of these systems employ compounds that can either decompose or undergo a reversible change in response to a trigger. In particular, photo-responsive materials have been extensively explored using compounds such as *o*-nitrobenzyl,<sup>81,82,88–90</sup> anthracene,<sup>83,92</sup> cinnamoyl,<sup>84,93,94</sup> and coumarin.<sup>85,95–97</sup> These compounds can be incorporated into polymer backbones,<sup>88,93,96,89</sup> conjugated to the end of polymer chains to form crosslinked hydrogels,<sup>81,83,92</sup> or incorporated as pendant crosslinking nodes along the polymer backbone.<sup>94,95</sup> By exposing these materials to the appropriate wavelength of light, the disintegration of the polymer backbone, crosslinked hydrogel, or polymer network can be induced. The incorporation of these

compounds as pendant groups results in polymer matrices wherein the degree of crosslinking is dependent on their density along the polymer backbone and can be altered using light.<sup>84,94,95</sup> The most convenient way to prepare these materials is through the copolymerization of functionalized and non-functionalized monomers. In particular, a wide variety of functionalized carbonate monomers have been synthesized including ones with pendant cinnamoyl or coumarin groups.<sup>84,196</sup> These monomers have been successfully copolymerized with commercially available non-functionalized monomers such as trimethylene carbonate (TMC) and lactide, which have been used extensively in biomaterials applications.<sup>39–46</sup>

Cinnamoyl and coumarin are both capable of reversible photo-dimerization through [2+2] cycloaddition, with dimerization occurring when irradiated with 300-380 nm UV light and dimer cleavage occurring under 250-280 nm UV light (Figure 5.1).<sup>93,99,105,197</sup> Consequently, polycarbonate copolymers with these compounds as pendant groups should be capable of photo-reversible network formation, which could be used to prepare photo-triggerable biomaterials. However, to the best of our knowledge there no reports of systematic comparisons between of the cinnamoyl and coumarin functionalized polymers. In this study, the reversible photo-crosslinking of polycarbonate copolymers functionalized to varying degrees with either pendant cinnamoyl or coumarin groups (Figure 5.2) was examined and the extent and rate of reaction of the two systems compared.



*Figure 5.1: Schematic of the reversible [2+2] photo-cycloaddition reaction for an example polycarbonate with a pendant cinnamoyl group* 



Figure 5.2: Cinnamoyl or coumarin functionalized polycarbonate copolymers initiated with 1-octanol

# **5.3 Experimental**

# 5.3.1 Materials

All reagents were obtained from commercial suppliers and used as received unless otherwise noted. Dimethylsulfoxide-d<sub>6</sub>, chloroform-d (99.8%D) (CDCl<sub>3</sub>), anhydrous dimethylformamide (DMF) (99.8%), liquid chromatography (LC)-grade acetonitrile, dioxane, 1,1,1-tris(hydroxymethyl)ethane (99%), cinnamoyl chloride (98%), 4-methylumbelliferone (98%), *p*-toluenesulfonic acid monohydrate (98%), sodium hydrogen sulfate monohydrate (99%), anhydrous 1-octanol (99%), and methyl *tert*-butyl ether (98%) were obtained from Sigma-Aldrich (Oakville, Canada). Toluene (extra dry over molecular sieves), 2,2-dimethoxypropane (98%), ethyl chloroformate (99%), sodium bicarbonate (99.7%), and anhydrous 2-methyltetrahydrofuran were obtained from Acros Organics (New Jersey, USA). Acetonitrile, dichloromethane (DCM), tetrahydrofuran (THF), isopropanol, anhydrous diethyl ether, toluene, acetone, methanol, ethyl acetate, hexanes, potassium carbonate (99%), sodium chloride (99%), sodium hydroxide (97%), anhydrous magnesium sulfate (97%), 48% hydrobromic acid (HBr), concentrated hydrochloric acid (HCl), 85% phosphoric acid, and triethylamine (TEA) (99%) were obtained from Fisher Scientific (Ottawa, Canada) and the THF and TEA were stored over 4 Å molecular sieves. 3-methyl-3-oxetanemethanol (97%) was obtained from Alfa-Aesar (Ward Hill, USA). Trimethylene carbonate (99%) was purchased from Leapchem (Hangzhou, China) and purified by recrystallization from ethyl acetate (3 mL/g). 5-cinnamoyloxymethyl-5-methyl-1,3-dioxan-2-one (COM) and 5-(4-methylumbelliferyloxymethyl)-5-methyl-1,3-dioxan-2-one (MUM) were synthesized as described by Chesterman and Amsden.<sup>196</sup>

## 5.3.2 Polymerization

COM and MUM were copolymerized with TMC by catalyst-free melt polymerization and characterized as previously described.<sup>196</sup> Briefly, TMC (1.53 g, 15.0 mmol, 22.5 eq) and either COM (1.52 g, 5.0 mmol, 7.5 eq) or MUM (1.38 g, 5.0 mmol, 7.5 eq) were combined in a flame-dried ampule and 1-octanol diluted in anhydrous toluene (87 mg, 0.67 mmol, 1 eq, 20% w/w)) was added as an initiator. The ampule was purged with argon, evacuated at a reduced pressure of 28 kPa for 30 s, and flame-sealed. The ampules were then placed in an oil bath thermostat-controlled at 120 °C and allowed to polymerize for 18 h. The copolymers were purified by precipitation from 20:1 methanol:DCM. Purified polymers were characterized by <sup>1</sup>H NMR spectroscopy in CDCl<sub>3</sub> using a Bruker 500 MHz NMR spectrometer with peak shifts referenced to an internal trimethylsilane standard. In addition, the glass transition temperature ( $T_g$ ) of the polymers was assessed by differential scanning calorimetry (DSC). DSC was conducted using a Mettler Toledo DSC1

System and the samples were monitored through two heating cycles from -60 °C to 105 °C at a 10 °C/min heating rate. The glass transition temperature was determined from the second heating cycle.

## 5.3.3 Photo-Crosslinking and Cleavage

The photo-crosslinking of various copolymers either solvent-free or as a concentrated solution in dioxane (50 mass% polymer) was conducted using an EXFO Acticure 4000 light source via a liquid light-guide with a maximum intensity of 1.4 W/cm<sup>2</sup> (365 nm) while the photo-cleavage was performed using a UVP Pen-Ray at 0.3 mW/cm<sup>2</sup> (254 nm). Changes in the polymer properties were monitored in real-time using a ARES rheometer (TA Instruments, USA) with parallel plate geometry and a 0.3 mm gap. The top plate was a 20 mm quartz plate with a Peltier plate on the bottom. The photo-crosslinking was conducted at 25 °C using an oscillatory frequency of 10 rad s<sup>-1</sup> and a strain of 1%.

## **5.3.4 Photo-Kinetics**

The reversible photo-crosslinking of various copolymers in a very dilute solution in LC-grade acetonitrile (15-40 mg/L) was monitored in real-time using a UV-Vis spectrometer to determine the photo-crosslinking and photo-de-crosslinking kinetics. A Cary 50-Bio UV-Vis spectrophotometer (Varian) was modified to allow irradiation of samples perpendicular to the instrument beam, thereby allowing real-time tracking of changes in the UV-Vis absorbance of the samples as they were irradiated using either an EXFO Acticure 4000 light source via a liquid light-guide attenuated to 83 mW/cm<sup>2</sup> (365 nm) for crosslinking or a UVP Pen-Ray at 0.3 mW/cm<sup>2</sup> (254 nm) for de-crosslinking.

## 5.4 Results and Discussion

## **5.4.1 Copolymer Properties**

To examine the impact of crosslinking on the mechanical properties of cinnamate and coumarin functionalized polycarbonate copolymers, monomers COM and MUM were copolymerized with TMC at varying monomer ratios (Table 5.1). Relatively low molecular weights were targeted to facilitate the detection of changes in polymer properties during photo-crosslinking. The % monomer incorporations for the resulting copolymers were calculated using <sup>1</sup>H NMR spectroscopy and were close to those targeted. Copolymers containing COM had number average molecular weights,  $M_n$ , that were consistently lower than targeted, with the deviation increasing with the % monomer incorporation. Since the purified copolymers also had a much narrower  $M_n$  range than targeted, ranging from 3400-3700 g/mol instead of 3700-4900 g/mol, this result is likely due to reduced solubility of the higher molecular weight polymer chains in DCM. Conversely, copolymers containing MUM had  $M_n$  values that were all slightly higher than targeted, which is consistent with the removal of some low molecular weight chains during the polymer purification. Finally, the Tg of the copolymers increased as the % functionalized monomer incorporated increased for both cinnamoyl and coumarin functionalization. A comparison of P(TMC<sub>m</sub>-co-COM<sub>n</sub>) and P(TMC<sub>m</sub>-co-MUM<sub>n</sub>) copolymers with similar % functionalization revealed that coumarin functionalization results in a significantly greater increase in the Tg than cinnamoyl functionalization, which may be due to either the larger size of the coumarin pendant group or greater coordination between the coumarin groups than with the cinnamoyl groups.

Table 5.1:  $P(TMC_m\text{-}co\text{-}COM_n)$  and  $P(TMC_m\text{-}co\text{-}MUM_n)$  copolymers (P = poly; m, n = # of repeating units targeted) initiated with 1-octanol at 120 °Cafter purification.

Polymer	Targeted % Functionalized Monomer Incorporated	Actual % Functionalized Monomer Incorporated (NMR)	Targeted M <sub>n</sub> (g/mol)	M <sub>n</sub> (NMR) (g/mol)	T <sub>g</sub> (℃)
$P(TMC_{27}\text{-}co\text{-}COM_3)$	10	9	3700	3400	-23
$P(TMC_{25.5}\text{-}co\text{-}COM_{4.5})$	15	13	4000	3700	-19
$P(TMC_{24}\text{-}co\text{-}COM_6)$	20	18	4200	3600	-15
$P(TMC_{22.5}\text{-}co\text{-}COM_{7.5})$	25	23	4500	3400	-11
$P(TMC_{20}\text{-}co\text{-}COM_{10})$	33	33	4900	3700	-7
$P(TMC_{27}$ -co- $MUM_3)$	10	9	3800	4100	-27
$P(TMC_{25.5}-co-MUM_{4.5})$	15	15	4100	4400	-14
$P(TMC_{24}\text{-}co\text{-}MUM_6)$	20	18	4400	4900	-6
P(TMC <sub>22.5</sub> -co-MUM <sub>7.5</sub> )	25	22	4700	4800	14
$P(TMC_{20}\text{-}co\text{-}MUM_{10})$	33	29	5200	6200	18

#### 5.4.2 Influence of the Photoactive Moiety on Copolymer Photo-Crosslinking

The changes in the storage and loss moduli of copolymers functionalized with either cinnamate or coumarin groups during photo-crosslinking of solvent-free samples was monitored over time (Figure 5.3). The final storage modulus for P(TMC<sub>22.5</sub>-co-MUM<sub>7.5</sub>) (Figure 5.3A) was 13.5 times higher than for P(TMC<sub>22.5</sub>-co-COM<sub>7.5</sub>) (Figure 5.3B), which suggests that the coumarin groups are more effective as crosslinking agents. In addition, the onset of crosslinking (i.e. the time delay before a significant increase in the storage modulus was observed, defined as a storage modulus of 1 kPa) occurred significantly sooner with the coumarin (after 0.3 min) than the cinnamate (after 39 min). This time delay likely represents the length of time required for the polymer crosslinking to increase the molecular weight of the polymer chains sufficiently to influence the viscosity and by extension the storage modulus of the polymer samples. Finally, the storage modulus for P(TMC<sub>22.5</sub>-co-MUM<sub>7.5</sub>) plateaued after 1.5 h, which indicates the formation of a completely crosslinked network, while P(TMC<sub>22.5</sub>-co-COM<sub>7.5</sub>) had not plateaued after 2 h.



Figure 5.3: Comparison of the change in storage modulus of two polycarbonate copolymers during photocrosslinking: (A)  $P(TMC_{22.5}\text{-}co\text{-}MUM_{7.5})$  and (B)  $P(TMC_{22.5}\text{-}co\text{-}COM_{7.5})$  (conditions: neat polymer, 365 nm, 1.4  $W/cm^2$ ).

The relative photo-crosslinking ability of  $P(TMC_{22.5}\text{-co-MUM}_{7.5})$  and  $P(TMC_{22.5}\text{-co-COM}_{7.5})$  was also compared in dilute solution by monitoring changes in real-time using a UV-Vis spectrophotometer. The change in UV-Vis absorption at 317 nm (coumarin) or 276 nm (cinnamoyl) during irradiation was used to calculate the change in concentration of undimerized cinnamoyl and coumarin groups. As observed in the photo-rheometer studies, coumarin groups dimerized significantly faster than cinnamoyl groups with a 64% reduction in free coumarin groups and a 23% reduction in free cinnamoyl groups after 15 min (Figure 5.4).



Figure 5.4: Comparison of the relative change in absorbance of two polycarbonate copolymers ( $P(TMC_{20}-co-COM_{10})$ ) and  $P(TMC_{20}-co-MUM_{10})$ ) during photo-crosslinking (conditions: dissolved in LC-grade acetonitrile, 365 nm, 330 mW/cm<sup>2</sup>).

The slower dimerization of the cinnamoyl groups may be due to their ability to undergo a photochemically induced cis/trans isomerization, which would reduce the quantum yield of the dimerization.<sup>198,199</sup> In addition, the absorbance maximum of cinnamoyl is further from the 365 nm maximum intensity of the UV light source than the absorbance maximum of coumarin, which suggests that there would be a difference in the relative abundance of photons with sufficient energy to activate each compound (Figure 5.5). As a result of these findings, the P(TMC-co-MUM) copolymers were selected for use in the remainder of the reversible photo-crosslinking studies as they were better suited to crosslinking with UV light sources with a wavelength range of 300 to 400 nm and a 365 nm maximum intensity, which are widely used.



Figure 5.5: Comparison of the UV-Vis absorbance spectrums of two polycarbonate copolymers ( $P(TMC_{20}-co-COM_{10})$ ) and  $P(TMC_{20}-co-MUM_{10})$ ) dissolved in LC-grade acetonitrile.

#### 5.4.3 Photo-Crosslinking of P(TMC-co-MUM)

The impact of the % MUM content on the storage modulus of the P(TMC-co-MUM) copolymers during photo-crosslinking was assessed via photo-rheometry (Figure 5.6). The samples were loaded as concentrated solutions in dioxane to facilitate handling as the  $T_g$  of the higher MUM content copolymers is near room temperature (Table 5.1). As the % monomer incorporation of MUM increased from 9% to 29%, the storage modulus after 90 min of irradiation increased from 20 kPa to 310 kPa. Moreover, the time at onset of crosslinking decreased from 39.5 min to 6.5 min. The gelation time of these copolymers (Table 3.2), defined as the length of irradiation time required for the storage modulus to exceed the loss modulus (Figure 5.7), was also dependent on the % MUM in the copolymer. As the MUM content increased, the gelation time decreased sharply with a doubling from 9% to 22% reducing gelation time from 0.75 h to about 20 s. The gelation time was assessed for solvent-free polymer samples as the loss modulus (G<sup>2</sup>) was considerably less noisy for these samples due to the higher quantity of the polymer chains, which facilitated an accurate determination of the modulus crossover point. The pronounced change in the gelation time

reflects the relative ability of the copolymers to efficiently form a crosslinked network. If the number of coumarin dimers formed per polymer chain is less than three, then high molecular weight polymers form as the short polymer chains are linked together in a generally linear fashion. The formation of three or more dimers per polymer chain introduces branching to the growing polymer chains and the potential to form lateral links to other extended polymer chains to form an interconnected network. Since the 9% MUM copolymer had on average just under three pendant coumarin groups per polymer chain, nearly all of the coumarin groups would have needed to dimerize for it to form an interconnected network. This requirement explains the poor final storage modulus and very slow gelation of this copolymer. As the number of coumarin groups per chain increased, the probability of sufficient dimerization to form a highly crosslinked network increased significantly and a corresponding decrease in gelation time and improved final storage modulus were observed.



Figure 5.6: Storage modulus with respect to irradiation time of P(TMC-co-MUM) copolymers with varying MUM content. (conditions: blended with dioxane (50 mass% polymer), 365 nm, 1.4  $W/\text{cm}^2$ ).



Figure 5.7: Changes in storage (G', blue) and loss (G", red) moduli with respect to irradiation time of P(TMC-co-MUM) copolymers (A. 9% MUM, B. 15% MUM, C. 22% MUM) (conditions: neat, 40°C, 365 nm, 1.4 W/cm<sup>2</sup>). The crossover of G' and G" was taken as the gelation time.

Table 5.2: Gelation time of P(TMC-co-MUM) copolymers as a function of % MUM incorporated (conditions: neat, 365 nm, 1.4 W/cm<sup>2</sup>).

MUM Content (%)	Gelation Time (min)
9	45.3
15	20.7
22	0.3

In addition, the effect of the film thickness and the irradiation intensity on the photocuring of the copolymers was assessed. Since the crosslinking is the result of a photo-dimerization process, the intensity of irradiation should have a direct impact on the crosslinking rate, with more rapid crosslinking occurring as the intensity increases due to an increased probability of photons being absorbed by the coumarin groups.<sup>105,107</sup> Conversely, the thickness of the sample should not influence the crosslinking rate as the density of the coumarin groups and the intensity remains constant. A copolymer with 22% MUM dissolved in dioxane (50 mass%) was selected for these studies as it had previously been determined to have sufficient functionalization to form a crosslinked polymer network in a reasonable period of time.

As the film thickness increased, both the final storage modulus of the films and time at onset of crosslinking increased (Figure 5.8A). Similarly, decreasing the irradiation intensity used resulted in an increase in both the final storage modulus and the onset of crosslinking (Figure 5.8B). These changes suggest that the crosslinking rate of the copolymer was dependent on both conditions, with slower crosslinking resulting in stiffer polymer networks, which may be attributable to the chains in the developing polymer networks having more time to rearrange to achieve a higher final crosslinking density. The slower crosslinking of thicker samples could indicate attenuation of the UV intensity as it penetrates through the polymer, thereby allowing the formation of more extensively crosslinked polymer networks. The lower final modulus achieved for the highest intensity could also indicate that some de-crosslinking was occurring with irradiation at this intensity of 365 nm UV, with the plateau in the modulus representing the extent of crosslinking at which the crosslinking and de-crosslinking rates became equal.



Figure 5.8: A) Storage modulus change with respect to irradiation time of  $P(TMC_{22.5}\text{-}co\text{-}MUM_{7.5})$  for various sample thicknesses (conditions: blended with dioxane (50 mass% polymer), 365 nm, 1.4 W/cm<sup>2</sup>). B) Storage modulus change with respect to irradiation time of  $P(TMC_{22.5}\text{-}co\text{-}MUM_{7.5})$  for various UV intensities (conditions: blended with dioxane (50 mass% polymer), 365 nm, 300 µm thick).

## 5.4.4 Photo-Crosslinking Kinetics of P(TMC-co-MUM)

The photo-crosslinking was also monitored in real-time using UV-Vis spectrophotometry in order to characterize the kinetics of the photo-dimerization process and determine whether it was consistent with the photo-dimerization of free coumarin molecules. In order to track the photo-crosslinking in real-time, very dilute polymer solutions (15-40 mg/L) in LC-grade acetonitrile were used. The dilute conditions were required to ensure the maximum absorbance of the coumarin was low enough to be within the UV-Vis spectrometer's detection limits and LC-grade acetonitrile was selected for its low UV cutoff. A representative result for the photo-crosslinking of a copolymer containing 22% MUM over 15 min is illustrated in Figure 5.9. As the samples were irradiated with 365 nm UV, the coumarin groups dimerized, which consumed alkene bonds and reduced the UV-Vis absorbance at 317 nm. The % decrease in absorbance with respect to irradiation time was determined for P(TMC-co-MUM) copolymers of varying compositions (Figure 5.10). As expected, based on the previous results, the rate and extent of the photo-crosslinking increased with increasing % functionalization. In all cases, the observed dimerization did not result in the formation of a crosslinked network due to the dilute conditions.



Figure 5.9: Reduction in UV-Vis absorbance of  $P(TMC_{22.5}$ -co- $MUM_{7.5})$  during crosslinking with each line representing the absorbance after an additional minute of irradiation (conditions: dissolved in LC-grade acetonitrile, 365 nm, 83 mW/cm<sup>2</sup>).



Figure 5.10: Change in UV-Vis absorbance at 317 nm with irradiation time for P(TMC-co-MUM) copolymers with varying MUM content (conditions: dissolved in LC-grade acetonitrile, 365 nm, 83  $mW/cm^2$ ).

These results were converted into changes in the equivalent coumarin solution concentration ([MUM]) using a linear calibration curve for the UV-Vis absorbance of MUM, which was the only source of absorbance at 317 nm, and the photo-dimerization kinetics for the functionalized copolymers were extracted. The photo-dimerization kinetics were hypothesized to be second order with respect to [MUM] as dimerization requires two molecules of coumarin to diffuse into sufficient proximity to dimerize, which would mean that a plot of [MUM]<sup>-1</sup> with respect to time should be linear. The experimental results were consistent with this hypothesis (Figure 5.11) with coefficient of determination (R<sup>2</sup>) values ranging from 0.980 to 0.994. The rate constant for the photo-dimerization kinetics of each copolymer was obtained from the slope of a linear regression of the data in Figure 5.11. Since these calculations were based on the equivalent solution concentration of the coumarin groups, the rate constant should be independent of the % functionalization as it has already been taken into account in the concentration. However, an increase in the photo-dimerization rate constant with respect to the % functionalization was observed (Figure 5.12), which

indicated that the attachment of the coumarin groups to the polymer backbone increases their rate of dimerization relative to what would be expected based on their concentration alone. This phenomenon was likely a result of intra-chain dimerization as the coiling of the polymer chains in solution would result in the coumarin groups being held in close proximity. This effect should increase with the frequency of the coumarin group along the backbone, which is consistent with the effect becoming especially pronounced for the 29% functionalized copolymer.



*Figure 5.11: Photo-dimerization kinetics showing linear relationship between [MUM]<sup>-1</sup> and irradiation time for P(TMC-co-MUM) copolymers. Linear regression curve fits are shown as dotted lines.* 



Figure 5.12: Photo-dimerization rate constants for P(TMC-co-MUM) copolymers with respect to the % functionalization. (Error bars =  $\pm$  SEM)

## 5.4.5 Photo-Reversibility Kinetics of P(TMC-co-MUM)

In addition, the rate and extent of photo-reversibility of the functionalized copolymers was assessed following photo-crosslinking. Attempts to reverse the photo-crosslinking of crosslinked polymer networks by irradiating them with 254 nm UV light resulted in no discernible change in bulk polymer properties, such as the  $T_g$  or the storage modulus. This result was considered to be due to the limited penetration of 254 nm UV into the polymer networks preventing the photo-reversion of the majority of the crosslinks and the limited chain mobility. However, the irradiation of dilute solutions with 254 nm resulted in rapid photo-reversion, which could be tracked in real-time using a UV-Vis spectrophotometer by monitoring the increase in uV-Vis absorbance at 317 nm caused by the regeneration of alkene bonds (Figure 5.13). The % increase in absorbance with respect to 254 nm irradiation time was determined for crosslinked P(TMC-co-MUM) copolymers of varying compositions (Figure 5.14). However, unlike for the photo-crosslinking, the rate and extent of the photo-reversion did not show a consistent trend with respect to the % MUM incorporation.



Figure 5.13: Recovery of UV-Vis absorbance of  $P(TMC_{22.5}$ -co- $MUM_{7.5})$  during de-crosslinking with each line representing the absorbance after an additional minute of irradiation (conditions: dissolved in LC-grade acetonitrile, 254 nm, 0.3 mW/cm<sup>2</sup>).



Figure 5.14: Change in UV-Vis absorbance at 317 nm with irradiation time for crosslinked P(TMC-co-MUM) copolymers of varying MUM content (conditions: dissolved in LC-grade acetonitrile, 254 nm, 0.3  $mW/cm^2$ ).

The photo-reversion kinetics were hypothesized to be first order with respect to the dimer concentration [D] as only a single dimer is required for photo-cleavage to occur. [D] was defined as half the difference
between the current [MUM] and the initial [MUM] prior to photo-crosslinking. A first order mechanism would mean that a plot of  $\ln([D])$  with respect to time should be linear, but this was not the case (Figure 5.15A). Instead, the experimental results more closely resembled a second order mechanism (Figure 5.15B) with excellent linearity for the first 240 s during which time the majority of the photo-reversion occurs (Figure 5.14). This result was both unexpected and inconsistent with conventional dimerization theory, but has been previously reported by Kehrloesser *et al.*,<sup>197</sup> who proposed that competition with the forward photo-crosslinking reaction was responsible. This explanation would also help to explain the incomplete photo-reversion observed with the increase in absorbance plateauing around 85% of the initial absorbance prior to crosslinking (~75% recovery of the initial decrease in absorbance). The rate constants for the photo-reversion kinetics of the copolymers were calculated by linear regression of the first 3.5 min of  $[D]^{-1}$  with respect to time and ranged from 1360-2610 M<sup>-1</sup>s<sup>-1</sup>. Unlike the photo-crosslinking kinetics, there was no discernible trend in the rate constant relative to the % MUM incorporated (Figure 5.16).



*Figure 5.15: Photo-reversion kinetics showing plots of the first-order (A) and second-order (B) relationship between [D] and irradiation time for P(TMC-co-MUM) copolymers.* 



Figure 5.16: Photo-reversion rate constants for P(TMC-co-MUM) copolymers with respect to the % MUM incorporation. (Error bars =  $\pm$  SEM).

# 5.4.6 Applications

These polycarbonates have a wide range of potential applications as the use of functionalized carbonate monomers provides a facile way to incorporate photo-reversibility and allows copolymerization with commercially available monomers such as TMC, which has previously been used to prepare biocompatible polymers.<sup>43–46</sup> This study demonstrated that coumarin significantly outperforms cinnamate as a crosslinking agent as it both dimerized faster and resulted in stronger polymer networks. To the best of our knowledge, the dimerization of these compounds to form polymer crosslinks had not previously been directly compared. Buckup *et al.* reported that coumarin compounds had a higher photo-cleavage efficiency than truxillic acid, which is the dimer of cinnamic acid, but did not quantify the dimerization reaction.<sup>99</sup> In addition, a comparison of individual reports of the synthesis of cinnamate and coumarin dimers indicated that considerably higher irradiation intensities had been reported for the generation of cinnamate dimers.<sup>84,97,200,201</sup> The functionalized carbonate copolymers examined in this study were successfully photocrosslinked both in solution and as solvent-free samples, which means that the photo-crosslinking could be

used to reinforce a variety of polymer architectures, such as electro-spun fibres, polymer films, or 3Dprinted materials. In addition, unlike some photo-crosslinkable groups such as acrylates, cinnamate and coumarin groups are thermally stable and thus that they could survive melt processing without crosslinking.

The photo-reversibility of these copolymers was marginal for crosslinked polymer networks, which limits their potential use as materials with photo-tunable properties. However, they were readily photo-reversible in dilute solution and as such they potentially could be used as the hydrophobic block of a diblock copolymer to form micelles or polymersomes for photo-triggerable encapsulation and delivery of compounds.

## 5.5 Conclusions

The photo-crosslinking of cinnamoyl and coumarin functionalized polycarbonates was examined under various conditions and monitored in real-time using both photo-rheometry and UV-Vis spectrophotometry. Coumarin proved to be the more effective crosslinking agent when using UV light sources with a wavelength range of 300 to 400 nm and a 365 nm maximum intensity, as it dimerized faster and resulted in stronger polymer networks. In addition, the influence of varying the % functionalization, UV intensity, and sample thickness on the storage modulus was successfully demonstrated using photo-rheometry and the photo-dimerization kinetics were analyzed using UV-Vis spectrophotometry. Finally, the photo-reversibility of these polymers was explored. No appreciable change in polymer properties was detected for the photo-cleavage kinetics were determined. Both the photo-dimerization and photo-cleavage reactions were second order with respect to the concentration of the photoactive group. Overall, the photo-reversible crosslinking of cinnamoyl and coumarin functionalized polycarbonates was demonstrated and their potential for use in photo-crosslinking or photo-reversible applications was discussed.

# **Chapter 6**

# Preparation of Photo-Crosslinkable Polymersomes from Poly(Ethylene Glycol)-*b*-Polycarbonate Diblock Copolymers Functionalized with Coumarin

# 6.1 Preface

This chapter describes the preparation of polymersomes from diblock copolymers functionalized with coumarin. In addition, the encapsulation of fluorescent dyes and the impact of membrane stabilization through photo-crosslinking was investigated. The research is this chapter was motivated by Specific Aim 4. I designed, conducted, and analyzed all experiments described in this chapter with some suggestions and feedback provided by my PhD supervisor, Dr. Brian Amsden.

# **6.2 Introduction**

Drug delivery systems composed of polymers that can respond to an external stimulus such as light or pH are a growing research area.<sup>92,202–204</sup> In particular, drug delivery vehicles composed of photo-responsive polymers have been extensively explored as a means of introducing an externally triggerable control mechanism. These systems typically employ compounds, such as *o*-nitrobenzyl,<sup>88–90,132</sup> anthracene,<sup>83,92</sup> cinnamoyl,<sup>93,94</sup> and coumarin,<sup>95,96</sup> that either decompose or undergo a reversible change in response to light. These groups have been used to either reversibly stabilize an existing polymer structure, such as crosslinking the core of micelles,<sup>95</sup> or to introduce a photo-triggerable failure point, such as the connection between polymer blocks.<sup>130,205,206</sup> By exposing these materials to the appropriate wavelength of light, the stabilization of polymer structures can be reversed or the disintegration of the component polymer chains can be induced.<sup>205,207</sup>

The incorporation of photo-reversible compounds as pendant groups along polymer backgrounds is ideally suited for the reversible stabilization of self-assembled polymers.<sup>95</sup> This configuration can be achieved by the copolymerization of functionalized and non-functionalized monomers. For example, a variety of functionalized carbonate monomers have been synthesized, including ones with pendant cinnamoyl or coumarin groups.<sup>84,196</sup> These monomers have been copolymerized with commercially available non-functionalized monomers such as trimethylene carbonate (TMC) and lactide, which have been used extensively in biomaterials applications.<sup>39–46</sup> A comparison of a series of polycarbonate copolymers functionalized with cinnamoyl or coumarin groups demonstrated that the coumarin groups dimerize significantly faster and thus generate a stronger polymer network in a given time.<sup>208</sup> In addition, coumarin can be attached to the cyclic carbonate monomer via an ether linkage, which provides better chemical stability than ester-linked systems due to a lower susceptibility to hydrolysis.<sup>167,209</sup>

A variety of polymer morphologies have been investigated for drug delivery including micelles,<sup>210</sup> nanoparticles,<sup>211</sup> and polymersomes<sup>212</sup> with each one having a variety of advantages and limitations. Polymersomes, which are polymer vesicles with an aqueous core, have been the focus of considerable research as they combine the ability of liposomes to encapsulate proteins and other large hydrophilic drugs with the customizability of a system based on synthetic polymers.<sup>137</sup> By selecting appropriate polymers, polymersome membrane strength, thickness, and chemical stability can be tailored to specific applications.<sup>137</sup> In addition, the incorporation of crosslinkable groups into the polymersome membrane allows the formation of highly robust polymersomes that can withstand de-hydration and re-hydration, high osmotic gradients, and immersion in organic solvents.<sup>135,136</sup> We therefore reasoned that combining the benefits of polymersomes as a delivery vehicle with photo-reversible control over crosslinking in the polymersome membrane (Figure 6.1) could provide an excellent drug delivery vehicle.

In this study, the self-assembly of a poly(ethylene glycol)-*b*-poly(carbonate) (PEG-PC) copolymer incorporating a previously reported coumarin functionalized carbonate monomer<sup>196</sup> was examined to determine its suitability for the preparation of photo-reversible polymersomes. The self-assembly of diblock

copolymers in water is a thermodynamically driven process that is influenced by concentration, temperature, co-solvents, and the copolymer composition.<sup>147</sup> The relative hydrophobicity of the two polymer blocks is one of the most important factors governing self-assembly into polymersomes as opposed to other morphologies such as micelles.<sup>137</sup> Typically, a hydrophilic mass fraction (*f*-factor) of  $35 \pm 10\%$  is necessary to obtain polymersomes,<sup>137</sup> but several studies have found polymersome formation for PEG-PC copolymers occurs at or below 20%.<sup>148,213</sup> Therefore, a series of coumarin functionalized PEG-PC diblock copolymers with *f*-factors ranging from 12-43% were synthesized and their self-assembly was assessed using several induction methods to determine conditions suitable for the formation of polymersomes. Finally, the encapsulation of small molecule dyes and fluorescent proteins was examined under the identified conditions to determine the suitability of these materials for drug delivery.



Figure 6.1: Conceptual illustration of photo-reversible encapsulation and release of a dye (green circles) in polymersomes

# 6.3 Experimental

#### 6.3.1 Materials

All reagents were obtained from commercial suppliers and used as received unless otherwise noted. Dimethylsulfoxide-d<sub>6</sub>, chloroform-d (99.8%D) (CDCl<sub>3</sub>), anhydrous dimethylformamide (DMF) (99.8%), 4-methylumbelliferone (98%), p-toluenesulfonic acid monohydrate (98%), 350 g/mol poly(ethylene glycol) methyl ether (mPEG), 550 g/mol mPEG, 500 g/mol poly(ethylene glycol) dimethyl ether (PEG-DME), sodium cholate, and calcein were obtained from Sigma-Aldrich (Oakville, Canada). Toluene (extra dry over molecular sieves), 2.2-dimethoxypropane (98%), ethyl chloroformate (99%), sodium bicarbonate (99.7%), and anhydrous 2-methyltetrahydrofuran were obtained from Acros Organics (New Jersey, USA). Acetonitrile, dichloromethane (DCM), tetrahydrofuran (THF), isopropanol, anhydrous diethyl ether, toluene, acetone, methanol, ethyl acetate, hexanes, potassium carbonate (99%), sodium chloride (99%), sodium hydroxide (97%), anhydrous magnesium sulfate (97%), 48% hydrobromic acid (HBr), concentrated hydrochloric acid (HCl), 85% phosphoric acid, and triethylamine (TEA) (99%) were obtained from Fisher Scientific (Ottawa, Canada) and the THF and TEA were stored over 4 Å molecular sieves. 3-methyl-3oxetanemethanol (97%) and 750 g/mol mPEG were obtained from Alfa-Aesar (Ward Hill, USA). 1,000 g/mol mPEG was obtained from TCI America (Portland, USA). Trimethylene carbonate (99%) was purchased from Leapchem (Hangzhou, China) and purified by recrystallization from ethyl acetate (3 mL/g). mPEGs were dried by dissolving 5 g in 20 mL of toluene and concentrating in vacuo. This process was repeated 4 times and the resulting mPEGs were stored under argon. 5-(4-methylumbelliferyloxymethyl)-5methyl-1,3-dioxan-2-one (MUM) was synthesized as previously described.<sup>196</sup> A small sample of EGFP was obtained from the Arnold research group at Rutgers University (New Brunswick, NJ, USA).

#### 6.3.2 Synthesis of Diblock Copolymers

Trimethylene carbonate (TMC) and MUM were copolymerized with a series of poly(ethylene glycol) methyl ethers (mPEG) ranging from 0.35-2 kg/mol to prepare a series of diblock copolymers with 1-4

kg/mol polycarbonate blocks and ~ 33% coumarin functionalization. Polymerizations were conducted by catalyst-free ring-opening melt polymerization.<sup>168,196</sup> Briefly, TMC and MUM were combined in a flamedried ampule and an mPEG diluted in anhydrous toluene (66% w/w) was added as an initiator. The ampule was purged with argon, evacuated at a reduced pressure of 28 kPa for 30 s, and flame-sealed. The ampules were then placed in an oil bath thermostat-controlled at 100 °C and allowed to polymerize for 18 h. The copolymers were purified by dissolution in minimal dichloromethane and precipitation using a 10-fold excess of diethyl ether. Purified polymers were characterized by <sup>1</sup>H NMR spectroscopy in CDCl<sub>3</sub> using a Bruker 500 MHz NMR spectrometer with peak shifts referenced to an internal trimethylsilane standard. The hydrophilic mass fraction of the diblock copolymer (*f*-factor) was calculated by dividing the known average M<sub>n</sub> for the mPEG by the average total M<sub>n</sub> for the copolymer calculated from the <sup>1</sup>H NMR spectra by using the ratio of the terminal CH<sub>3</sub> group on the mPEG initiator (3.31 ppm) to the sum of chemical shifts for CH<sub>3</sub> on the MUM (1.08 ppm (repeating) and 1.84 ppm (terminal)) and the sum of chemical shifts for CH<sub>3</sub> on the MUM (1.08 ppm (repeating) and 1.03 ppm (terminal)) to calculate the degree of polymerization (D<sub>p</sub>) for each monomer. The D<sub>p</sub>'s were multiplied by their corresponding monomer molecular weight and combined with the M<sub>n</sub> for the mPEG to determine the total M<sub>n</sub> for the polymer.

#### 6.3.3 Induction of Polymer Self-Assembly

A variety of methods for inducing polymer self-assembly were explored including direct-injection,<sup>148</sup> reverse solvent evaporation,<sup>149</sup> detergent removal,<sup>151</sup> direct hydration,<sup>152,153</sup> and solvent displacement.<sup>150</sup> The resulting polymer suspensions were characterized using dynamic light scattering (DLS) and confocal microscopy. Direct injection was conducted by adding 20 µL of the polymer dissolved in either chloroform or THF (10 mg/mL) into 1 mL of phosphate buffered saline (PBS). Reverse solvent evaporation consisted of adding 150 µL of polymer dissolved in THF (50 mg/mL) to a glass vial with stir bar and diluting to 2 mL of THF. Afterwards, 3 x 1 mL of PBS were added and the THF was removed *in vacuo* to form a polymer suspension. Samples for detergent removal were prepared by adding 0.5 µmol of polymer dissolved in THF (20 mg/mL) to a vial and diluting with various concentrations of sodium cholate in aqueous solution to a

final volume of 1 mL. For direct hydration, the 10 mg of polymer was melt blended with 10 mg of PEG-DME, as a water-soluble excipient, in a 2 mL micro-centrifuge tube and allowed to cool. Water was then added in a series of aliquots (10, 20, 70, and 900  $\mu$ L) with vortexing after each addition. Finally, solvent displacement consisted of dissolving 0.5-5  $\mu$ mol of polymer in 500  $\mu$ L of a water miscible solvent (THF, acetone, or DMSO) and gradually diluting the sample with 1 mL of water with a series of aliquots (2 x 25, 2 x 50, 2 x 75, 2 x 100  $\mu$ L) over the course of 10 min with continuous mixing.

# 6.3.4 Reversible Photo-crosslinking

Polymer suspensions were crosslinked while stirring under 365 nm UV at 100 mW/cm<sup>2</sup> from a Hamamatsu Lightning Cure LC-8. Polymer suspensions were de-crosslinked while stirring under 254 nm UV at 0.3 mW/cm<sup>2</sup> from a UVP Penray.

# 6.3.5 Dynamic Light Scattering

DLS was conducted using a Malvern Instruments Zetasizer Nano ZS equipped with a 633 nm laser, which is capable of measuring particle sizes from 0.6 nm to 6  $\mu$ m. Following self-assembly, aqueous polymer suspensions were placed in a plastic cuvette (1 cm path length) and analyzed using backscatter detection at 173°.

### 6.3.6 Confocal Microscopy

Confocal microscopy was conducted using an Olympus FV-1000 microscope using 40x and 63x (oil immersion) objectives to examine a thin film of the polymer suspension trapped between a cover slip and a glass slide and images were captured at a resolution of 2048 x 2048 pixels. Polymer suspensions were stained by adding  $1.25 \,\mu$ L of a Nile Red (excitation: 543 nm, emission: 600-675 nm) solution in THF (1.5 mg/mL). Nile red only fluoresces when it is in a hydrophobic environment.<sup>214,215</sup> The polymers could also be imaged directly due to the fluorescence of the coumarin moiety (excitation: 405 nm, emission: 425-475 nm). Polymersome formation was validated by examining the encapsulation of the hydrophilic dye calcein

(excitation: 485 nm, emission: 500-525 nm) and the encapsulation of enhanced green fluorescent protein (EGFP) (excitation: 485 nm, emission: 510 nm) to identify the presence of an aqueous core.

## 6.3.7 Encapsulation of Calcein

 $25 \ \mu$ L of an aqueous solution of calcein (1.00 mM) was substituted for the first aqueous aliquot in the selfassembly methods. Following self-assembly, the suspension was centrifuged at 12000 x g for 10 min to pellet the polymersomes and the supernatant containing the unencapsulated dye was removed and replaced with an equal volume of 1x PBS. The concentration of un-encapsulated calcein in the supernatant was determined by measuring the UV absorbance at 490 nm using a Molecular Devices SpectraMax M2 UV-Vis spectrophotometer.

#### 6.4 Results

#### **6.4.1 Copolymer Properties**

A series of PEG-PC diblock copolymers (Figure 6.2) were prepared with a 2:1 ratio of TMC:MUM in the hydrophobic block to ensure a high availability of crosslinking groups. The mPEG initiators were varied to achieve a range of *f*-factors while maintaining relatively low molecular weights and ensuring a minimum of 3 coumarin groups per polymer chain, which is necessary to achieve a crosslinked network.<sup>208</sup> Low molecular weights were targeted as this increases the similarity with lipids and thus might improve the viability of using liposome derived self-assembly techniques, such as detergent removal.<sup>149,165</sup> The copolymerizations achieved degrees of polymerization (D<sub>p</sub>) close to those targeted across a range of mPEG initiators with the MUM content ranging from 29-33%. The resulting diblock copolymers had a range of *f*-factors from 12-43% and were assessed using a variety of techniques for inducing polymer self-assembly.



Figure 6.2: PEG-PC copolymer with a 2:1 ratio of TMC:MUM in the hydrophobic block

Table 6.1: Properties after purification of PEG-P( $TMC_m$ -co- $MUM_n$ ) copolymers (P = poly; m, n = # of repeating units targeted) polymerized catalyst-free at 100 °C

Polymer	M <sub>n</sub> of mPEG Initiator	D <sub>p</sub> of TMC	D <sub>p</sub> of MUM	M <sub>n</sub> (NMR) (g/mol)	<i>f</i> -factor (%)
$PEG-P(TMC_{10}-co-MUM_5)$	350	10.4	4.7	2830	12
$PEG-P(TMC_8-co-MUM_4)$	350	8.3	3.5	2260	15
$PEG-P(TMC_{10}-co-MUM_5)$	550	9.9	5.2	3150	17
$PEG-P(TMC_8-co-MUM_4)$	550	9.4	4.1	2750	20
$PEG-P(TMC_8-co-MUM_4)$	550	7.3	3.7	2430	23
$PEG-P(TMC_{11}-co-MUM_{5.5})$	750	11.2	4.8	3340	22
$PEG-P(TMC_8-co-MUM_4)$	750	9.0	4.3	2980	25
$PEG-P(TMC_8-co-MUM_4)$	750	7.7	3.8	2710	28
$PEG-P(TMC_7-co-MUM_{3.5})$	1000	8.3	4.0	3070	33
$PEG-P(TMC_6-co-MUM_3)$	1000	5.7	2.6	2360	42
$PEG-P(TMC_{16}-co-MUM_{8})$	2000	16.5	7.7	6030	33
$PEG-P(TMC_{13}-co-MUM_{6.5})$	2000	12.8	6.2	5180	39
$PEG-P(TMC_{11}-co-MUM_{5.5})$	2000	10.3	5.3	4650	43

# **6.4.2 Induction Methods**

#### 6.4.2.1 Direct Injection

Direct injection of PEG-PC copolymers dissolved in organic solvents resulted in precipitation/aggregation, which indicated that the rapid dilution of the organic solvent did not allow the polymer chains sufficient

time to orient in the aqueous solution. This method also tends to have very low encapsulation efficiencies,<sup>155</sup> so was not explored further.

#### 6.4.2.2 Direct Hydration

The melt blending of PEG-DME and PEG-PC copolymers was not successful, which was attributed to the high degree of interchain interactions in the PEG-PC copolymers associated with the coumarin groups. Even when fairly uniform blending was achieved, addition of water resulted in rapid dissolution of the PEG-DME leaving the PEG-PC as an undissolved polymer aggregate.

#### 6.4.2.3 Detergent Removal

The presence of sodium cholate or THF was sufficient to facilitate the formation of polymer suspensions. However, the effect of increasing sodium cholate on particle size varied depending on the *f*-factor of the polymer examined (Table 6.2) with a representative change in particle size for a diblock copolymer (f = 33%) shown in Figure 6.3. In order to induce polymersome self-assembly by detergent removal, the detergent must form mixed micelles with the polymer chains.<sup>151,165</sup> For the formation of liposomes, a 2:1 detergent:lipid mass ratio is commonly used.<sup>165</sup> However, detergent:polymer ratios as high as 100:1 and 500:1 failed to produce mixed micelles and instead caused either an increase in particle size (f = 33% or 42%) or a small reduction in particle size (f = 23%). Increasing the detergent ratio further is undesirable as it could affect the stability of proteins during encapsulation and means a high dilution factor would be required to decrease the concentration below the critical micelle concentration (CMC) of the detergent, which would lower encapsulation efficiency and increase the processing required following self-assembly. The failure to form mixed micelles even at high detergent loadings suggests that the detergent was unable to disrupt the interaction between the copolymer chains, which is likely the result of coordination of the pendant coumarin groups. Coumarin group functionalization has previously been reported to heavily influence polymer properties, possibly through  $\pi$ -stacking.<sup>97,208</sup>

Table 6.2: Effect of varying the sodium cholate concentration on the average particle size of PEG-PC copolymers with varying f-factors.

<i>f</i> -factor (%)	Particle Size with 0 mM Sodium Cholate (nm)	Particle Size with 50 mM Sodium Cholate (nm)	Particle Size with 250 mM Sodium Cholate (nm)
23	5560	5330	4650
33	74	288	717
42	45	158	682



Figure 6.3: Particle size intensity distribution obtained from DLS for a f = 33% PEG-PC diblock copolymer using various concentrations of sodium cholate as a detergent.

#### 6.4.2.4 Reverse Solvent Evaporation

The removal of THF from THF:PBS solutions resulted in self-assembly for a wide range of the PEG-PC diblock copolymers. The resulting average particle size was dictated by the *f*-factor of the copolymers with a reduction in particle size as the *f*-factor increased from 12% to 28% (Figure 6.4). Finally, the particles

obtained from the self-assembly of copolymers with *f*-factors ranging from 33-43% were highly disperse in size with some particles that were too large to be measured accurately by the DLS. Therefore, these samples were analyzed by confocal microscopy and a variety of morphologies including unilamellar vesicles were observed (Figure 6.5).



*Figure 6.4: Particle size distributions for PEG-PC copolymers with varying f-factors (0.25% polymer in 3:2 PBS:THF).* 



Figure 6.5: Self-assembly morphologies stained with Nile Red and imaged by confocal microscopy: A. Large unilamellar vesicle (10  $\mu$ m), scale bar = 10  $\mu$ m. B. Smaller unilamellar vesicles (1  $\mu$ m), scale bar = 5  $\mu$ m

#### 6.4.2.5 Solvent Displacement

Solvent displacement also successfully induced polymer self-assembly, but resulted in much larger particles than reverse solvent evaporation, which is consistent with literature reports.<sup>153</sup> The much slower introduction of water in this method allows the polymer chains to gradually coordinate with each other and form larger self-assembly constructs as the interfacial energy between the hydrophobic blocks and the solvent increases. These larger constructs should be quite stable as their formation was thermodynamically rather than kinetically driven.<sup>153</sup> Confocal microscopy revealed that *f*-factors under 30% resulted in the formation of solid microparticles with sizes ranging from 10-50  $\mu$ m (Figure 6.6). The uniform distribution of Nile Red fluorescence throughout these particles provides a clear contrast with the images of particles obtained for an *f* = 33% copolymer by reverse solvent evaporation (Figure 6.5). Solvent displacement of copolymers with *f*-factors between 33% and 43% also resulted in the formation of unilamellar vesicles (Figure 6.7). While a variety of other polymer aggregates were also observed, solvent displacement yielded a much higher percentage of polymersomes than reverse solvent evaporation and a more uniform size distribution. As a result, solvent displacement was selected as the polymersome induction method for the crosslinking and encapsulation studies and some optimization studies were conducted.



Figure 6.6: Cluster of microparticles (10-50  $\mu$ m) stained with Nile Red and imaged by confocal microscopy. Scale bar = 40  $\mu$ m.



Figure 6.7: Confocal microscopy image of polymersomes (1-4  $\mu$ m) and other polymer constructs obtained by solvent displacement of a f = 39% copolymer in THF. Hydrophobic regions are stained with Nile Red. Scale bar = 20  $\mu$ m.

For example, the effect of varying the polymer concentration on particle size obtained by solvent displacement was examined (Table 6.3) and a representative change is shown in Figure 6.8. In all cases, increasing the polymer concentration resulted in an increase in particle size. This result is consistent with literature reports for other polymer systems and has been attributed to the increased number of polymer chains in the proximity of growing polymer constructs and an increase in viscosity slowing the diffusion rate.<sup>150</sup> The effect of varying the initial organic solvent was also examined with DMSO resulting in an increase in polymer aggregates and acetone resulting in a reduction in average particle size (Figure 6.9). In both cases, there was a reduction in polymersomes relative to the use of THF (Figure 6.7). DMSO has previously been reported to reduce both average particle size and particle size dispersity relative to THF due to quicker solvent exchange with water.<sup>150</sup> In this system, the increase in polymer aggregation with DMSO may indicate that the DMSO was being displaced too quickly for controlled self-assembly to occur.

*Table 6.3: Effect of varying the polymer concentration on the average particle size of PEG-PC copolymers with varying f-factors.* 

<i>f</i> -factor (%)	Polymer M <sub>n</sub> (g/mol)	0.5 mM (nm)	2.5 mM (nm)	5 mM (nm)
33	3070	74	2270	4180
33	6030	66	131	278
42	2310	45	163	1410



Figure 6.8: Particle size distributions for PEG-PC diblock copolymers at various polymer concentrations  $(f = 33\%, M_n = 6030 \text{ g/mol})$ 



Figure 6.9: Confocal microscopy images of the self-assembly of a f = 39% copolymer following the displacement of A. DMSO and B. acetone. Hydrophobic regions are stained with Nile Red. Scale bar = 20  $\mu$ m.

#### 6.4.3 Membrane Stabilization (Crosslinking)

Once the polymersomes were formed, the impact of crosslinking on the membrane stability was examined using both confocal microscopy (Figure 6.10) and DLS (Figure 6.11) to monitor changes in particle size and morphology respectively. Membrane stability was tested by subjecting the particles to centrifugation at 12000 x g in the presence of 33% v/v THF after various lengths of irradiation with 365 nm UV. Centrifugation under these conditions in the absence of crosslinking resulted in the destruction of the majority of polymersomes and the formation of solid microparticles (Figure 6.10A versus Figure 6.10B). Irradiation of the polymersomes with 365 nm UV for 5 min significantly reduced aggregation during centrifugation (Figure 6.10C) and irradiation for 10 min allowed the majority of particles to maintain their morphology during centrifugation (Figure 6.10D). These results were consistent with those obtained from DLS where the average particle size decreased as the duration of irradiation increased.



Figure 6.10: Effect of crosslinking on polymersome morphology following centrifugation of a selfassembled copolymer (f = 39%). A. pre-centrifugation. B. No crosslinking post-centrifugation; note the dramatic increase in size of particles due to aggregation and higher intensity of the Nile Red (fluorescence of solid particles is more intense as core is more hydrophobic/more dye in total). C. 5 min crosslinking followed by centrifugation. D. 10 min crosslinking followed by centrifugation. Scale bars = 20  $\mu$ m. White arrows indicate polymersomes.



Figure 6.11: Particle size distributions for PEG-PC polymersomes irradiated with 365 nm UV for varying times. The resulting polymers (f = 39%,  $M_n = 5180$  g/mol) were then centrifuged at 12000 x g in the presence of 33% v/v THF.

#### **6.4.4 Encapsulation**

#### 6.4.4.1 EGFP

Initial encapsulation studies were conducted with EGFP and imaged by confocal microscopy with the aim of confirming the presence of an aqueous core in the polymersomes and assessing the viability of this system for protein delivery. The fluorescence of EGFP is inherent to the protein's structure, which means that it only fluoresces when correctly folded.<sup>216</sup> Thus, the encapsulation of EGFP would also provide a qualitative assessment of the protein compatibility of the polymersome induction method. Unfortunately, EGFP was observed to aggregate extensively during the solvent displacement method of inducing polymersomes (Figure 6.12), which both hindered encapsulation and confirmed that solvent displacement is poorly suited for the encapsulation of proteins. Since "protein safe" polymersome induction methods such as detergent removal and direct hydration were not compatible with these copolymers, their suitability

for protein encapsulation appears poor. However, they could still be used to encapsulate short peptides and small molecule drugs.



Figure 6.12: Confocal microscopy image showing EGFP aggregation (green clusters) with no colocalization with polymersomes (stained with Nile Red) prepared by solvent displacement of THF. Scale  $bar = 20 \ \mu m$ .

## 6.4.4.2 Calcein

Consequently, the encapsulation of the hydrophilic dye calcein into the polymersomes was examined as a model for drug encapsulation. Confocal microscopy demonstrated that the calcein encapsulation was successful. Prior to centrifugation, polymersomes could be identified as areas that had higher or lower fluorescence than the average calcein fluorescence (Figure 6.13A) and these regions were shown to be co-localized with the hollow red spheres observed on the Nile Red channel (Figure 6.13B). Following polymersome crosslinking and centrifugation, calcein encapsulation can be readily observed as regions of high calcein fluorescence (Figure 6.14A), which are co-localized with aqueous environments (no Nile Red fluorescence) and surrounded by a hydrophobic polymer membrane (Nile Red fluorescence) (Figure 6.14B).



Figure 6.13: Calcein encapsulation of a self-assembled copolymer (f = 39%) by solvent displacement of THF prior to centrifugation. A. Nile Red and calcein channels overlapped. Note the two red rings, one with a green centre that is brighter than the surrounding unencapsulated region and one with a dark centre. B. Nile Red channel alone showing the hollow red spherical appearance of the polymersomes.



Figure 6.14: Calcein encapsulation of a self-assembled copolymer (f = 39%) by solvent displacement of THF following 15 min crosslinking and centrifugation. A. Nile Red and calcein channels overlapped showing several bright green spots (areas of high calcein concentration), which are all surrounded by red. B. Nile Red channel alone showing absence of fluorescence at the centre of the locations where calcein is high, thereby confirming they have an aqueous core. Scale bars = 10 µm.

#### 6.4.4.3 Quantification

In order to quantify the encapsulation efficiency of the polymersomes for calcein, the supernatant from the centrifugation of the polymersomes was collected and its UV-Vis absorbance was measured in a UV-Vis spectrophotometer and converted to concentrations using a previously prepared calibration curve. The difference between this measured concentration and the original concentration during loading represents the amount of calcein that was successfully encapsulated. The change in calcein absorbance was compared with pure calcein solutions that had been irradiated under the same conditions as a control for photobleaching. However, due to a combination of background absorbance from coumarin groups on residual polymer chains and some photobleaching of the calcein, no change in calcein concentration greater than that observed for the control was detected. Consequently, the calcein encapsulation efficiency for the polymersomes was very low (< 1%).

# 6.5 Conclusion

A series of PEG-PC diblock copolymers that incorporated a coumarin functionalized carbonate monomer were synthesized with *f*-factors ranging from 12-43%. The self-assembly of the diblock copolymers was examined using a range of different induction techniques and the resulting polymer constructs were characterized using DLS and confocal microscopy. Direct injection and direct hydration were not suitable for these diblock copolymers as they both induced polymer aggregation. The detergent removal method resulted in the formation of polymer suspensions, but no evidence of mixed micelle formation was detected even at a detergent:polymer ratio of 500:1. Finally, reverse solvent evaporation and solvent displacement both induced the self-assembly of these diblock copolymers with *f*-factors of 12-28% resulting in the formation of solid microparticles and nanoparticles and *f*-factors of 33-43% resulting in the formation of polymer resulted in larger particles and more uniform size distributions than reverse solvent evaporation due to the more gradual introduction of water allowing the polymer chains more time to arrange themselves, so this method was used for the remainder of the studies.

In addition, stabilization of the polymersome membranes through photo-crosslinking was demonstrated with a decrease in the level of polymer aggregation and average particle size observed as the length of irradiation increased. The crosslinking of the polymersomes' membranes allowed them to withstand centrifugation at 12,000 x g. However, encapsulation of EGFP as a model protein was not possible due to aggregation caused by the organic solvents necessary for polymersome self-assembly. Calcein encapsulation as a model small molecule drug was successfully demonstrated using confocal microscopy, although the encapsulation efficiency was too low to be measured. Overall, this study demonstrated the preparation of a series of coumarin functionalized PEG-PC diblock copolymers, the impact of the *f*-factor on the self-assembly of the resulting copolymers, the stabilization of the polymersome membranes though photo-crosslinking of the coumarin groups, and the encapsulation of calcein.

# Chapter 7

# **Conclusions and Recommendations**

# 7.1 Conclusions

As outlined in the introduction, the current gold standard for ocular drug delivery is to intravitreally inject the drugs. However, the short half-life of many ocular drugs necessitates regular injections every 4-6 weeks, which leads to both patient discomfort and the potential for complications, such as vitreous hemorrhage or retinal detachment.<sup>9</sup> Recently, several sustained drug delivery devices, such as Iluvien and Ozurdex, have been approved for the treatment of specific ocular conditions, but the drug release rate of these devices cannot be altered.<sup>78</sup> Thus, there is a currently unmet need for new drug delivery vehicles that can provide tunable drug delivery in response to an external stimulus.<sup>78</sup>

The original motivation for this research was to contribute to the development of an externally controllable biodegradable intraocular drug delivery system. Thus, this project aimed to develop photo-triggerable biomaterials that could be used for drug delivery by preparing a series of reversibly photo-crosslinkable polymers and examining their suitability for drug encapsulation and release. Specifically, the synthesis of aliphatic polycarbonates with photo-responsive functionality was selected, as they have non-acidic degradation products,<sup>33</sup> can be functionalized with comparative ease,<sup>34</sup> and have shown promise as biomaterials.<sup>43,45,46</sup>

# 7.1.1 Synthesis of Cyclic Carbonate Monomers with Photo-Reversible Functionality

Therefore, the first research aim was to prepare cyclic carbonate monomers functionalized with photoactive groups that could be used to introduce photo-responsive properties to aliphatic polycarbonates. As described in Chapter 3, a series of cyclic carbonate monomers with pendant cinnamoyl and coumarin groups were synthesized. Both of these compounds are known to undergo a photo-reversible dimerization through a [2+2]-cycloaddition,<sup>99</sup> so their incorporation as pendant groups on a polymer would introduce a means of

photo-reversible crosslinking. The synthesis of the COM monomer (with an ester-linked cinnamoyl) had been previously reported,<sup>84</sup> but the modified synthesis procedure reported here nearly doubles the overall yield from 33% to 65%. The other four monomers (with an amide-linked cinnamoyl or ester, amide, or ether-linked coumarin) were all synthesized for the first time. In order to prepare these monomers, a variety of challenges were overcome including the limited solubility of certain coumarin intermediates and the poor nucleophilicity of 7-amino-4-methylcoumarin, which necessitated the investigation of a wide range of amide coupling techniques. By synthesizing monomers with two different photoactive functional groups and a range of chemical linkages, this research aim was completed successfully.

#### 7.1.2 Synthesis of Polycarbonate Homopolymers and Copolymers with Photo-

#### **Reversible Functionality using Various Initiators.**

The second research aim was to prepare aliphatic polycarbonates using the newly synthesized cyclic carbonates to introduce photo-responsive functionality. As a result, the homopolymerization of the functionalized monomers and their copolymerization with a commercially available carbonate monomer (TMC) were examined under a variety of conditions. The monomer conversion rates, percent functionalized monomer incorporated, and end group fidelity were analyzed and used to assess the polymerization dynamics under various conditions.

As described in Chapter 3, the room temperature solution homopolymerization of these monomers were examined using either DBU or TBD as an organocatalyst and the polymerization kinetics were compared to those for TMC under the same conditions. Only the ester-linked cinnamoyl (COM) and the ether-linked coumarin (MUM) monomers were sufficiently soluble in deuterated solvents for real-time monitoring using <sup>1</sup>H NMR. However, despite their bulky functional groups, these monomers polymerized significantly faster than TMC. This unexpected result suggested that the chemical nature (alkyl, ether, ester, or amide) of the pendant functional group on the carbonate monomer had a more significant impact on its polymerization kinetics than the size of the pendant group. Variations in alkyl substituent size only appeared to influence

the homopolymerization kinetics when comparing pendant groups with a very similar chemical nature. However, these DBU and TBD catalyzed polymerizations resulted in poor end group and the difference in polymerization rate between the functionalized monomers and TMC was too large to achieve random copolymerization.

Therefore, the melt polymerization of carbonate monomers was investigated. It was during these studies that unusually rapid melt polymerizations were observed, which lead to the studies reported in Chapter 4. An investigation of potential causes revealed that TEA·HCl can both catalyze and initiate the melt polymerization of carbonate monomers, which is especially noteworthy as it is a by-product of the ethyl chloroformate ring-closing reaction commonly used to synthesize carbonate monomers.<sup>35</sup> Thus, insufficient purification of synthesized monomers could lead to undesired auto-polymerization catalyzed by residual TEA·HCl. Alternatively, if high end group fidelity is not required, TEA·HCl has several advantages as a solid catalyst, including ease of handling and availability.

Further studies revealed that TEA can also catalyze the melt polymerization of TMC and is effective at temperatures as low as 65  $^{\circ}$ C, which is lower than possible with the commonly used SnOct<sub>2</sub> and could allow the incorporation of thermally sensitive end groups. Finally, the catalyst-free polymerization of TMC, which has previously only been reported after long times at temperatures exceeding 100  $^{\circ}$ C, was observed to proceed smoothly and with high end group fidelity at 85  $^{\circ}$ C. This study also demonstrated that the catalyst-free polymerization of TMC can be used to prepare diblock copolymers using mPEG as an initiator at 85  $^{\circ}$ C. This finding is especially important for biomaterials and some industrial applications as it eliminates the need for an added catalyst and thereby simplifies the polymerization and purification processes.

Therefore, the catalyst-free melt homopolymerization of the cinnamoyl and coumarin functionalized monomers was examined (Chapter 3). While this method was successful for the homopolymerization of the COM monomer, the remainder of the monomers either decomposed (amide-linked cinnamoyl), precipitated from the melt polymerization as insoluble solids (MUM) or required infeasible temperatures

(ester- and amide-linked coumarin). Finally, it was determined that the COM and MUM monomers were capable of catalyst-free melt copolymerization with TMC at 100 °C and that this provided better end group fidelity than copolymerizations catalyzed by TBD or DBU and better molecular weight control than copolymerizations catalyzed by TBD.

While the polymerization potential of three of the functionalized monomers investigated proved limited, the successful copolymerization of the COM and MUM monomers with TMC meant that polycarbonate copolymers functionalized with either cinnamoyl or coumarin groups could be prepared for use in future studies. In addition, the catalyst-free melt copolymerization process provides high end group fidelity and is compatible with a range of initiators including macromolecules. Thus, this research aim was successfully achieved.

# 7.1.3 Characterization of Extent and Reversibility of Polycarbonate Photo-

## Crosslinking

The third research aim was to investigate the reversible photo-crosslinking of cinnamoyl and coumarin functionalized aliphatic polycarbonates. Hence, the photo-crosslinking of P(TMC-co-COM) and P(TMC-co-MUM) copolymers prepared by catalyst-free melt polymerization was examined under various conditions and monitored in real-time using both photo-rheometry and UV-Vis spectrophotometry as described in Chapter 5. These studies demonstrated that coumarin functionalization was more effective for crosslinking with UV light sources with a wavelength range of 300 to 400 nm and a 365 nm maximum intensity, as it dimerized faster and resulted in stronger polymer networks. In addition, the influence of varying the % functionalization, UV intensity, and sample thickness on the storage modulus of P(TMC-co-MUM) copolymers was successfully demonstrated using photo-rheometry and the photo-dimerization kinetics were analyzed using UV-Vis spectrophotometry. Finally, the photo-reversibility of these copolymers was explored. While no appreciable change in polymer properties was detected for the photo-reversion of crosslinked polymer networks, rapid photo-reversion was observed in dilute solution and the

photo-cleavage kinetics were determined. Both the photo-dimerization and photo-cleavage reactions were second order with respect to the concentration of the photoactive group. Overall, this research aim was satisfied through the demonstration and characterization of reversible photo-crosslinking of the coumarin functionalized polycarbonates.

#### 7.1.4 Preparation of Reversibly Photo-Crosslinkable Polymersomes

The final research aim was to generate polymersomes using a diblock copolymer capable of reversible photo-crosslinking and assess their potential for drug delivery. Since coumarin functionalization had been demonstrated to provide superior crosslinking in Chapter 5, a series of PEG-PC diblock copolymers that incorporated the MUM monomer were synthesized by catalyst-free polymerization with *f*-factors ranging from 12-43% and their self-assembly was examined using a range of induction techniques as described in Chapter 6. Reverse solvent evaporation and solvent displacement were both found to induce self-assembly with *f*-factors of 12-28% resulting in the formation of solid microparticles and nanoparticles and *f*-factors of 33-43% resulting in the formation of polymersomes. Solvent displacement resulted in larger particles and more uniform size distributions than reverse solvent evaporation due to the more gradual introduction of water allowing the polymer chains more time to arrange themselves, so this method was used for the remainder of the studies.

Stabilization of the polymersome membranes through photo-initiator-free photo-crosslinking was demonstrated with the crosslinking allowing polymersomes to withstand centrifugation at 12,000 x g. In addition, the encapsulation of calcein, as a model small molecule drug, in stabilized polymersomes was successfully demonstrated using confocal microscopy. However, the encapsulation efficiency was too low to be quantified by UV-Vis spectrophotometry. The encapsulation of EGFP, as a model protein, was unsuccessful as the organic solvents used to induce polymersome self-assembly also induced protein aggregation. Finally, the inability to accurately quantify the encapsulation of calcein into the polymersomes prevented the characterization of the photo-reversibility of the encapsulation, so this research aim was only

partially met. Overall, the preparation and stabilization of polymersomes composed of coumarin functionalized diblock copolymers and the encapsulation of a model small model drug into them were successfully demonstrated.

#### 7.2 Contributions to the Literature

A number of the results obtained during the pursuit of this research represent new contributions to the scientific literature and some of the key findings are summarized here. Chapter 3 discusses the synthesis of four novel cyclic carbonate monomers functionalized with cinnamoyl or coumarin groups and an improved synthesis for a fifth monomer. In addition, it explores the polymerizability of these monomers under a variety of conditions including catalyst-free melt polymerizations with TMC where the TMC doubled as a solvent to allow polymerizations to occur below the melting point of the functionalized monomers. Chapter 4 discusses the previously unreported ability of triethylamine and triethylamine hydrochloride to catalyze the melt polymerizations of cyclic carbonates. It also demonstrates that the catalyst-free melt polymerization of TMC occurs faster and at lower temperatures than previously reported. Chapter 5 is the first report of a direct comparison between cinnamoyl and coumarin groups as crosslinking agents. Finally, Chapter 6 includes the first report of polymersomes that can be stabilized by coumarin dimerization, which means that the membrane can be stabilized by photo-crosslinking without the need for a photo-initiator.

## 7.3 Summary

The overall objective of this research project was to develop photo-reversibly crosslinked polymersomes that could be used for ocular drug delivery. This thesis describes a number of advances towards this goal. First, the preparation of novel carbonate monomers with pendant cinnamoyl and coumarin pendant groups provides a convenient method of incorporating photo-reversible functionality into polycarbonates. Second, a variety of polymerization conditions and catalysts were analyzed in order to identify which methods resulted in the highest end group fidelity, which is essential for the preparation of diblock copolymers for polymersomes. Third, the photo-reversible crosslinking of functionalized polycarbonate copolymers was observed in dilute solution, which suggests that photo-control of these polymer networks may be possible. Fourth, the formation of polymersomes and photo-stabilization of their membranes was successfully achieved. Finally, the encapsulation of calcein, which is a hydrophilic, small molecule dye, into the polymersomes as a model drug was demonstrated by confocal microscopy. However, a number of challenges were also encountered including the limited solubility of the functionalized carbonates, the significant variation in polymerization rates for the various carbonate monomers, the inability to detect photo-reversibility of the bulk properties for the functionalized carbonate copolymers, and the calcein encapsulation being too low to quantify. Thus, while significant progress has been made, further research or modifications to the proposed approach would be required in order to achieve the desired objective.

# 7.4 Potential Future Studies and Recommendations

#### 7.4.1 Additional Characterization of Polymerization Kinetics

The novel cinnamoyl and coumarin functionalized cyclic carbonate monomers introduced a variety of solubility and polymerization challenges. In particular, the preparation of homopolymers of the functionalized monomers proved to be challenging, which necessitated their copolymerization with other monomers. The incorporation of other monomers carries a number of advantages, including tuning the properties of the copolymers, modifying the density of the crosslinkable groups, and lowering the cost of the preparing the polymers by using monomers that are commercially produced in bulk. However, the polymerization kinetics of carbonate monomers appear to vary widely depending on the catalyst and temperature conditions used, which makes the preparation of random copolymers challenging. While using a catalyst-free melt polymerization reduced the difference in polymerization rates relative to DBU and TBD catalyzed solution polymerizations, it is likely that a gradient rather than random copolymer was achieved. Thus, a more comprehensive study of the copolymerization kinetics using a variety of different catalyst systems and temperatures would be advisable prior to using these polymers in systems where the

randomness of the monomers is important. However, tracking many of these polymerizations in real-time would not be possible by <sup>1</sup>H NMR and time point sampling of polymerizations conducted at high temperature under vacuum requires a separate polymerization for each time point as any attempt to sample the polymerization would halt or significantly affect the polymerization, which is impractical for these monomers due to the time and monetary cost required to synthesize them. Therefore, the challenge is how to assess the randomness of the copolymer following polymerization. The chemical shifts for the terminal units of these monomers by <sup>1</sup>H NMR is distinct from TMC, but a comparison of the integration of these shifts would only provide a measure of the relative distribution of the terminal unit, which can be influenced by factors such as auto-initiation, chain scission, or trans-esterification. A possible alternative would be a detailed study using <sup>13</sup>C NMR to determine whether the chemical shifts of the carbonyl groups are dependent on the nature of the neighbouring repeating unit.

#### 7.4.2 Improved Encapsulation of Polymersome System

While successful encapsulation of calcein into polymersomes was qualitatively demonstrated by confocal microscopy, the encapsulation efficiencies were too low to quantify. In addition, EGPF encapsulation was not possible due to the use of an organic solvent to induce the polymersome formation. However, the self-assembly of polymersomes is a complex thermodynamic process that can be influenced by a wide range of conditions including the rate of induction, the concentration of the polymer, the molecular weight of the copolymer, and the *f*-factor of the copolymer. In this study, the presence of the pendant coumarin groups was also observed to shift the required *f*-factor for self-assembly relative to what had previously been reported for the self-assembly of PEG-PC copolymers.<sup>148,213</sup> Therefore, it is possible the self-assembly of the polymersomes could be improved by varying these conditions or factors such as the percent incorporation of the MUM monomer or the monomer with which it is copolymerized. For example, increasing the total molecular weight of the diblock copolymer would allow the % MUM incorporation to be significantly lowered while still maintaining 3 crosslinking sites per polymer chain. Some of these

changes might also improve the compatibility of the diblock copolymers with polymersome induction methods that have higher encapsulation efficiencies or that are organic solvent-free.

# 7.4.3 Demonstration of Drug Release from the Polymersome System

If the encapsulation efficiency of the polymersome system could be improved sufficiently for quantification of the encapsulation efficiency, then it would also be desirable to examine the reversibility of the coumarin stabilization and quantify the release of a fluorescent dye from the system. While photo-reversibility in the properties of polymer networks crosslinked via coumarin dimers was not observed, the rapid photoreversibility of the dimerization in dilute solutions suggests that the crosslinking of the polymersome membranes should be reversible. However, the extent to which reversing the membrane stabilization by cleaving the photo-crosslinks would disrupt the membrane is unknown and cannot be properly examined without an accurate means of quantifying the amount of dye encapsulated.

## 7.4.4 Change Photo-Responsive Species

In terms of the long-term objective of developing an ocular drug delivery vehicle, there is a strong argument for changing the photo-responsive species employed. Both cinnamoyl and coumarin systems are typically reversed using 254 nm UV irradiation, which is not safe for use in biological applications due to its ability to cause DNA mutation.<sup>217</sup> In addition, while the two-photon cleavage of coumarin dimers has previously been proposed as a possible method to use visible light to activate these systems,<sup>85,105,107</sup> the process is very inefficient with 100 min of irradiation required to achieve a 3  $\mu$ M (1%) change in the concentration of the coumarin dimer using 70 mJ/pulse at a 20 Hz repitition.<sup>107</sup> Consequently, the intensities required to achieve efficient two-photon cleavage would easily exceed the thresholds at which damage to the surrounding tissue could occur.<sup>218</sup> Photo-responsive species that can undergo single photon cleavage in response to visible light, such as perylene-3-yl linkers,<sup>131,219</sup> would likely be a better choice for this application.

#### 7.4.5 Change How the Photo-Responsive Species is Incorporated

The incorporation of bulky photo-responsive groups, such as coumarin, has a pronounced effect on both the synthesis and the self-assembly of diblock copolymers. As previously discussed, the bulky substituents can significantly alter the polymerization rate of functionalized monomers, which makes achieving random copolymerization of the monomers challenging and could potentially alter the nature of the hydrophobic block by introducing a gradient in hydrophobicity depending on the monomers used. Further, the presence of the pendant groups changes the hydrophobic balance of the diblock copolymer with a shift in the f-factor required to prepare polymersomes from 20% to about 40% observed in this study. It is also possible that these pendant groups could influence the packing of the polymer chains within the membrane or otherwise alter the thermodynamics of the self-assembly process. Finally, while the ability to stabilize the polymersome membrane through photo-reversible crosslinking is novel, the drug release potential of this system is uncertain as cleaving the crosslinks may be insufficient to disrupt the membrane. Conversely, polymersome systems that incorporate the photo-responsive group between the copolymer blocks have a well-defined release mechanism. Therefore, changing how the photo-responsive group is incorporated into the diblock copolymer could simplify the preparation of the polymers, significantly reduce the change in self-assembly behaviour relative to non-photo-responsive systems, and provide more precise control over the drug release.

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## Appendix A



Figure A.2: <sup>13</sup>C NMR spectrum for COM





Figure A.4: <sup>13</sup>C NMR spectrum for CAM



Figure A.6: <sup>13</sup>C NMR spectrum for MUM



Figure A.8: <sup>13</sup>C NMR spectrum for MUC



Figure A.9: <sup>1</sup>H NMR spectrum for MAC (limited solubility in DMSO-d<sub>6</sub>)



Figure A.10: <sup>13</sup>C NMR spectrum for MAC (limited solubility in DMSO-d<sub>6</sub>)



Figure A.11: Product of DBU catalyzed rearrangement of CAM



Figure A.12: <sup>1</sup>H NMR showing transesterification of MUC during an attempted melt copolymerization. The full conversion of benzyl alcohol to an ester (5.22 ppm) and the appearance of the chemical shifts for MU (10.59 ppm, 7.60 ppm, 6.81 ppm, 6.71, and 6.13 ppm) can be seen.