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ABSTRACT

The work described in this thesis is divided into three major parts, and all of which involve the exploration of the chemistry of polyphosphazenes. The first part (chapters 2 and 3) of my research is synthesis and study polyphosphazenes for biomedical applications, including polymer drug conjugates and injectable hydrogels for drug or biomolecule delivery. The second part (chapters 4 and 5) focuses on the synthesis of several organic/inorganic hybrid polymeric structures, such as diblock, star, brush and palm tree copolymers using living cationic polymerization and atom transfer radical polymerization techniques. The last part (chapters 6 and 7) is about exploratory synthesis of new polymeric structures with fluorinated side groups or cycloaliphatic side groups, and the study of new structure property relationships.

Chapter 1 is an outline of the fundamental concepts for polymeric materials, as such the history, important definitions, and some introductory material for to polymer chemistry and physics. The chemistry and applications of phoshazenes is also briefly described.

Chapter 2 is a description of the design, synthesis, and characterization of development of a new class of polymer drug conjugate materials based on biodegradable polyphosphazenes and antibiotics. Poly(dichlorophosphazene), synthesized by a thermal ring opening polymerization, was reacted with up to 25 mol% of ciprofloxacin or norfloxacin and three different amino acid esters (glycine, alanine, or phenylalanine) as cosubstituents via macromolecular substitutions. Nano/microfibers of several selected polymers were prepared by an electrospinning technique. The hydrolysis rate and the antibiotic release profile can be well tuned by either the polymer compositions, or the surface area monitored by a six week in vitro hydrolysis experiment. All the polymers gave a near-neutral hydrolysis environment with the pH ranging from 5.9–6.8. In an in
antibacterial test against *E. coli*, the antibacterial activity of the hydrolysis media was maintained as long as the polymer hydrolysis continued.

Chapter 3 is concerned with the development of a class of injectable and biodegradable hydrogels based on water-soluble poly(organophosphazenes) containing oligo(ethylene glycol) methyl ethers and glycine ethyl esters. The hydrogels can be obtained by mixing α-cyclodextrin aqueous solution and poly(organophosphazenes) aqueous solution in various gelation rates depending on the polymer structures and the concentrations. The rheological measurements of the supramolecular hydrogels indicate a fast gelation process and flowable character under a large stain. The hydrogel system also exhibits structure-related reversible gel-sol transition properties at a certain temperature. The formation of a channel-type inclusion complex induced gelation mechanism was studied by DSC, TGA, $^{13}$C CP/MAS NMR and X-ray diffraction techniques. *In vitro* bovine serum albumin release of the hydrogel system was explored and the biodegradability of poly(organophosphazenes) was studied.

Chapter 4 outlines the preparation of a number of amphiphilic diblock copolymers based on poly[bis(trifluoroethoxy)phosphazene] (TFE) as the hydrophobic block and poly(dimethylaminoethylmethacrylate) (PDMAEMA) as the hydrophilic block. The TFE block was synthesized first by the controlled living cationic polymerization of a phosphoranimine, followed by replacement of all the chlorine atoms using sodium trifluoroethoxide. To allow for the growth of the PDMAEMA block, 3-azidopropyl-2-bromo-2-methylpropanoate, an atom transfer radical polymerization (ATRP) initiator, was grafted onto the endcap of the TFE block via the ‘click’ reaction followed by the ATRP of 2-(dimethylamino)ethyl methacrylate (DMAEMA). Once synthesized, micelles were formed by a standard method and their characteristics were examined using fluorescence techniques, dynamic light scattering, and transmission electron microscopy. The critical micelle concentrations of the diblock copolymers
as determined by fluorescence techniques using pyrene as a hydrophobic probe were between 3.47 and 9.55 mg/L, with the partition equilibrium constant of pyrene in these micelles ranging from $0.12 \times 10^5 - 1.52 \times 10^5$. The diameters measured by dynamic light scattering were 100-142 nm at 25°C with a narrow distribution, which were also confirmed by transmission electron microscopy.

Chapter 5 is a report on the design and assembly of polyphosphazene materials based on the non-covalent “host–guest” interactions either at the terminus of the polymeric main-chains or the pendant side-chains. The supramolecular interaction at the main chain terminus was used to produce amphiphilic palm-tree like pseudo-block copolymers via host-guest interactions between an adamantane end-functionalized polyphosphazene and a 4-armed β-cyclodextrin (β-CD) initiated poly[poly(ethylene glycol) methyl ether methacrylate] branched-star type polymer. The formation of micelles of the obtained amphiphiles was analyzed by fluorescence technique, dynamic light scattering, transmission electron microscopy, and atomic force microscopy. The supramolecular interactions involving polymer side-chains were achieved between polyphosphazenes with β-CD pendant units and other polyphosphazene molecules with adamantyl moieties on the side-chains. These interactions worked as physical crosslinks which were responsible for the formation of a supramolecular hydrogel. The results of this work demonstrated the synthetic possibilities for these novel polymeric structures. These materials show potential for applications as smart drug delivery micro-vehicles, responsive hydrogels, and self-healing materials.

Chapter 6 is an investigation of the influence of bulky fluoroalkoxy side groups on the properties of polyphosphazenes. A new series of mixed-substituent high polymeric poly(fluoroalkoxyphosphazenes) containing trifluoroethoxy and branched fluoroalkoxy side groups was synthesized and characterized by NMR and GPC methods. These polymers contained 19–29 mol% of di-branched hexafluoropropoxy groups or 4 mol% of tri-branched tert-
perfluorobutoxy groups, which serve as regio-irregularities to reduce the macromolecular microcrystallinity. The structure–property correlations of the polymers were then analyzed and interpreted by several techniques: specifically by the thermal behavior by DSC and TGA methods, the crystallinity by wide-angle X-ray diffraction, and the surface hydrophobicity/oleophobicity by contact angle measurements. Ultraviolet crosslinkable elastomers were prepared from the new polymers through the incorporation of 3mol% of 2-allylphenoxy and photo-irradiation. The mechanical properties and the elastomeric deformation–recovery behavior were then monitored by varying the time of ultraviolet irradiation. Side reactions detected during the synthesis of the high polymers, such as side group exchange reactions and alpha-carbon attack, were analyzed via use of a cyclic trimer model system.

Chapter 7 is an outline of the exploratory synthesis of a new series of phosphazene model cyclic trimers and single- and mixed- substituent high polymers containing cyclic aliphatic rings, $-C_nH_{2n-1}$ (where $n = 4–8$). The cyclo-aliphatic side group containing phosphazenes expand the structural and property boundaries of phosphazene chemistry, and suggest additional approaches for studying slow macromolecular substitution reactions and substituent exchange reactions. Polymer structure–property relationships are interpreted and correlated to glass transition temperatures, thermal decomposition temperatures, hydrophobicity, and membrane mechanical properties. Films prepared from these polymers are low cost, tough and non-adhesive. They can be used in variety of applications especially where transparency is important.
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PREFACE

Portion of this dissertation have been adapted for publication. Chapter 2 was adapted for publication in *Polymer Chemistry* and was co-authored by Harry R. Allcock, Yufan Zhang, Xiao Liu, Chen Chen and Mark J. Guiltinan. Chapter 3 was adapted for publication in *Macromolecules* and was co-authored by Harry R. Allcock and Chen Chen. Chapter 4 was adapted for publication in *Macromolecules* and was co-authored by Harry R. Allcock, Xiao Liu and Chen Chen. Chapter 5 was adapted for publication in *Macromolecules* and was co-authored by Harry R. Allcock and Chen Chen. Chapter 6 was adapted for publication in *Macromolecules* and was co-authored by Harry R. Allcock and Chen Chen. Chapter 7 was submitted to *Macromolecules* and was co-authored by Harry R. Allcock, Andrew Hess, Christopher R. Fellin, and Hunaid Hulwala.
ACKNOWLEDGEMENTS

I would like to thank Prof. Harry R. Allcock for his support throughout my PhD study. He encouraged me to generate my own research ideas and guided me to solve the problems. The scientific skills that I have developed under his tutelage will undoubtedly help me establish my future career in industry. I would like to thank my graduate committee, Prof. John Badding, Prof. Benjamin J. Lear, and Prof. Mike Hickner for their guidance throughout my graduate work. I would also like to thank Noreen Allcock for her help and encouragement during my time at Penn State. I am also thankful to The Pennsylvania State University, Department of Energy, and PN Leadership Endowment for funding my research.

I would also like to express my gratitude to several past and present group members for their generous assistance throughout my graduate career. Some of them greatly influenced my research and some of them even became good friends. These people include Dr. Xiao Liu, Dr. Chen Chen, Dr. Zhongjing Li, Dr. Nicole Morozowich, Dr. Jessica Nichol, Dr. Andrew Hess, and Dr. Cuiyan Tong, Tomasz Modzelewski, and Chris Fellin. The thesis would not have been done without their help or advice, and many of them were co-authors on my papers. I would also want to acknowledge my research collaborators including Prof. Mark J. Guiltinan (Huch Institute of the Life Science, Penn State), Prof. Ayusman Sen (Department of Chemistry, Penn State), Prof. David Cormode (Perelman School of Medicine, University of Pennsylvania), Prof. Hunaid Nulwala (Department of Chemistry, Carnegie Mellon University), and Prof. Christopher Siedlecki (College of Medicine, Penn State Hershey). There are some other people I want to thank for helping me with instrumental characterizations. They are James Miller (MS), David
Shelleman (Instron), Dr. Alan Benesi and Dr. Wenbin Luo (NMR), Dr. Hemant P. Yennawar (XRD), Meng Zhang (UV-Vis) and Gang Ning (TEM).

Finally, I would like to thank my family and friends for their never-ending love, support, and encouragement throughout these years. As a professor in Physics, my father, Hongzhi Tian, instilled my curiosity in science, and inspired me to pursue a career in Chemistry. My mother, Lihong Zhao always believes in my choices. The only things she cares are my health and happiness. I would like to thank my lovely wife, Bohui Lv, for helping me through the good and the bad times. She is the world best cook for me, and always knows what I want to eat. I am lucky to meet so many great friends and basketball buddies in State College, especially Xiao Liu, Yufan Zhang, Yanqi Qu, Kuo Han and Wei Zhao.
Chapter 1

Introduction

1.1 Introduction to Polymer Chemistry

1.1.1 History of Polymers

The word polymer is derived from the Greek words *poly* and *meros*, meaning many and parts. Naturally occurring polymers such as cotton, cellulose, and proteins have been discovered and utilized by mankind for centuries. It has been discovered that ancient Mayan civilizations in Central American developed playing balls for children that were made from local rubber trees. Ancient Egyptians and Chinese also used natural polymers such as wool and silk as materials for clothing.\(^1\) It was not until the 19\(^{th}\) century that the existence of macromolecules started to be noticed by scientists, and some techniques for altering natural polymers were developed. Charles Goodyear discovered vulcanization in 1839, by introducing sulfur to natural rubber at 130 °C, which became the first successfully commercialized polymer.\(^2,3\) After that, more discoveries were reported for modifications of natural polymers to change their properties, including artificial silk Rayon and flash cotton by nitrating cellulose.\(^4\) The major breakthroughs in polymer science started in the early 20\(^{th}\) century. In 1907, Leo Baekeland invented the first synthetic polymer, phenol-formaldehyde resin, called Bakelite.\(^5\) Despite the wide-spread usage of these new materials, a complete understanding of the structure of polymers remained elusive. People still believed that they were clusters of small molecules attracted to each other by an unknown force. This situation persisted until German scientist Hermann Staudinger firstly proposed the concept of “macromolecular theory” in the paper “Uber Polymerization” in 1920.\(^6\) He proposed that
polymers have long chains of atoms held together by covalent bonds. X-ray studies of many natural and synthetic materials were used as structural proof that polymers existed.\textsuperscript{7} Later, Staudinger’s concept was further supported by Carothers, who demonstrated that polyesters could be prepared by well-understood organic reactions using diacids and diols.\textsuperscript{8,9} Unlike other areas of chemistry, most of the development in polymer science and technology was in industry. Some pioneer companies, including Dupont, Dow, ICI \textit{etc.}, developed a large variety of new materials, and many of them were commercialized, an advance that had a great impact on household materials, and the food, biomedicine, automobile, electric and military industries.\textsuperscript{10,11} A list of some selected early commercialized polymers is shown in \textbf{Table 1-1}. From the evolution of computer and information science, nano technology, and new energy, the development of polymers shifted to specialized and functional characteristics, such as the invention and manufacture of polysulfone (1965), liquid crystals (1966), polyacetylene (1970), and carbon nanotubes (1991).

\textbf{Table 1-1:} Commercialization of selected polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Year</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakelite</td>
<td>1909</td>
<td>General Bakelite Corp.</td>
</tr>
<tr>
<td>Rayon</td>
<td>1910</td>
<td>American Viscose Company</td>
</tr>
<tr>
<td>Poly(vinyl chloride)</td>
<td>1927</td>
<td>Goodrich</td>
</tr>
<tr>
<td>Styrene-Butadiene copolymer</td>
<td>1929</td>
<td>I.G. Farben</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>1929</td>
<td>I.G. Farben &amp; Dow</td>
</tr>
<tr>
<td>Neoprene</td>
<td>1931</td>
<td>Dupont</td>
</tr>
<tr>
<td>Poly(methyl methacrylate)</td>
<td>1936</td>
<td>Rhom and Hass</td>
</tr>
</tbody>
</table>
Polymers or macromolecules are giant molecules with large dimensions and ultra-high molecular weight. They are composed of many similar repeat units called monomers. The accumulation of physical interactions between polymers, such as hydrogen bonding, dipole interaction, dispersion force, and chain entanglement, provides them with unique physical properties, including toughness, viscoelasticity, and the tendency to form glasses and semicrystalline structures.

1.1.3 Polymer Architectures

The shapes of polymers are intimately connected to their properties. Most of the polymers synthesized and studied are linear. Homopolymers are linear polymers that possess a backbone with a single type of monomer. Copolymers are obtained when two or more different monomer
units polymerize. Monomers within a copolymer may be organized along the linear backbone in a variety of ways, such as random copolymers, alternating copolymers, periodic copolymers, di-block or tri-block copolymers (Figure 1-1).\textsuperscript{12}

\begin{align*}
\text{AAAAA} & \text{AAAAAA} & \text{AAAABBB} & \text{BBBBAABAABAAAAABB} \\
\text{Homopolymer} & & \text{Random copolymer} \\
\text{ABABABABAABABABABAB} & \text{AAAAAAAA} & \text{BBBBBBAAAAABBBAAAAA} & \text{Periodic copolymer} \\
\text{Alternating copolymer} & & & \\
\text{AAAAAA} & \text{BBBBBBBB} & \text{AAAAAABBBBBBCCCC} & \text{Tri-block copolymer} \\
\text{Di-block copolymer} & & & \\
\end{align*}

Figure 1-1: Different types of linear polymeric structures.

Star-shaped polymers have a simple branched structure consisting of several linear chains connected to a central core, while dendrimers have a tree-like hyper-branched structure with expanding density in the outer layers. Comb or brush polymers are segmented copolymers with a linear backbone and polymeric side chains covalently linked. There are other more complex architectures, such as the palm tree, ring block, and dumbbell structures (Figure 1-2).\textsuperscript{12}
1.1.4 Molecular Weight

Unlike small molecules, the molecular weight of most high polymers cannot be measured precisely. Polymers are mixtures of long chains with different chain length, distribution of branching, and lengths of individual branches. The average molecular weights are statistically described as number average ($M_n$) and weight average molecular weights ($M_w$). $M_n$ corresponds to a measurement of chain length average, while $M_w$ determines how much each molecule or chain contributes to the measured result relative to its size. $M_w$ is always larger than $M_n$, and the ratio between $M_w$ and $M_n$ is called the polydispersity index (PDI). The PDI indicates the uniformity of the chain length distributions.\textsuperscript{11}
1.1.5 Thermal Transition Temperatures

Small molecules such as water can exist in three phases: solid, liquid, and gas. However, high polymers degrade before boiling, so they do not exist in the gaseous state. Even so, polymers generally undergo several major thermal transitions (Figure 1-3). At low temperature, polymers are brittle, like glasses, since there is not sufficient energy present to encourage local or segmental chain movement. As the temperature increases, more energy is available to allow for certain chain mobility. The mobile chains behave like “snakes” moving in the unoccupied space. The temperature which polymers start to transform from glassy materials to rubbery materials is called the glass transition temperature ($T_g$). As the temperature increases further, there is sufficient energy to overcome the forces present in the crystalline portion of the polymers (if the polymers have certain degrees of crystallinity), allowing a disruption of the crystallinity. Nearly all the polymer chains have freedom in motion, and the materials are soft. This temperature is often referred to as the crystalline transition temperature ($T_c$), which is directly related to the melt transition temperature ($T_m$). When the temperature is high enough to break chemical bonds, polymers start to decompose. That temperature is called the ceiling temperature, or the decomposition temperature of the polymers ($T_d$).\(^{11}\)

Figure 1-3: Demonstration of thermal transition temperatures.
1.1.6 Polymer Crosslinking

A crosslinked polymer is one in which the individual chains are connected to each other to form a 3D network. Crosslinked polymers have different properties compared to uncrosslinked polymers. Based on the nature of these interactions, the types of crosslinking that occur can be categorized as either chemical or physical crosslinks (Figure 1-4). Chemical crosslinking is obtained via the formation of covalent or ionic bonds between adjacent chains. Physical crosslinking can be generated in various ways, such as chain entanglements, crystallinity, or hydrogen bonding. Chemical crosslinking permanently restricts chain mobility, while physical crosslinking is reversible. The crosslinking process significantly increases a material’s stability, mechanical strength, and life-time.13

Figure 1-4: Polymer crosslinking.
1.2 Polymer Synthesis

1.2.1 Step Growth Polymerization

Step growth polymerization is exemplified by condensation polymerization. This method involves the reactions between functional groups on di-functional or multi-functional monomers with or without byproducts. Repeat units are covalently connected by the newly formed functional groups. Typical examples of polymers that are prepared using such technique are polyamides, polyurethanes, polycarbonates, and polyesters (Figure 1-5).\textsuperscript{13}

![Polymer Structures]

Figure 1-5: Selected polymers synthesized by step growth polymerization.
1.2.2 Chain Growth Polymerization

In contrast to the relatively slow step growth polymerization, chain growth polymerization is usually rapid. The kinetic chain reaction typically consists of three steps: initiation, propagation, and termination. The majority of polymers produced every year are synthesized using chain growth polymerization. Vinyl compounds with unsaturated bonds are common monomers (Figure 1-6).

Free radical polymerization is industrially the most widespread method to produce polymeric materials such as thermoplastics, rubber and fibers. The process can be carried out using bulk, solution, suspension, or emulsion polymerization conditions. Most of the commercial polymers are prepared by the free radical polymerization method due to their simple, low cost, and convenient characteristics. Typical examples include poly(vinyl chloride), poly(vinyl acetate), poly(methyl methacrylate), and polystyrene. However, free radical polymerization usually gives uncontrolled polymer architecture and poor molecular uniformity.\textsuperscript{14}

Ionic polymerizations including living cationic and living anionic polymerizations are also commonly used chain growth techniques. Cationic polymerizations occur with vinyl compounds that contain electron-donating groups using Lewis acids as the initiator. Monomers with electron-withdrawing groups can undergo anionic polymerization in the presence of anionic initiators, usually n-butyl lithium. Common products produced by cationic and anionic polymerizations are polyisobutylene and thermoplastic olefin elastomers respectively.\textsuperscript{15,16}

Since the development of the Ziegler-Natta catalyst, many linear and stereoregular polymers have been synthesized via complex coordination polymerizations.\textsuperscript{14} These materials, such as less branched high density polyethylene (HDPE) and stereoregular polypropylene (PP), show higher degrees of crystallinity and improved mechanical properties.
1.2.3 Atom Transfer Radical Polymerization (ATRP)

A relatively new method to synthesize well-defined polymers and copolymers is controlled radical polymerization (CRP). Several methods have been developed recently that allow for the preparation of many previously unattainable well-defined structures. The most widely used CRP techniques include nitroxide mediated polymerization (NMP), reversible addition-
fragmentation chain transfer polymerization (RAFT), and atom transfer radical polymerization (ATRP). 

Developed by Matyjaszewski and coworkers, ATRP is one of the most robust CRP techniques with a diversity of monomers, desirable molecular weight control, and narrow polydispersity. This comes about because the low radical concentrations present during the polymerization reduce the contribution of inter- and intramolecularly terminated chains. ATRP is controlled by an equilibrium between propagating radicals and dormant species (Figure 1-7). The initiation step usually involves the presence of an initiator containing halogenated species, commonly chlorine or bromine, and a small amount of copper (I) as the catalyst. Electron rich ligands are used to alter the solubility and reactivity of the catalyst. First, the halogenated initiator reacts with a lower oxidation state copper catalyst to form an activated radical species R·. Then, the radical species start to propagate in the presence of vinyl type monomers generating longer polymer chains. At the same time, the deactivated catalyst, Cu (II), in the higher oxidation state can reversibly react with the propagating polymer chain to terminate the free radicals. The reformation of the dormant polymer chain can again be activated by the copper (I). The equilibrium between activation and deactivation greatly reduces the radical concentration, and prevents extensive chain transfer and chain coupling. 

Polymer chains with halogen ends or side groups can be prepared as macroinitiators. This significantly decreases the synthetic difficulty of some complex structures, such as block, brush or star copolymers. The monomers grow from the initiation site at the presence of the ATRP catalyst system with controlled lengths and uniform distributions. ATRP also allows multiple monomers to grow simultaneously or asynchronously. This method has been utilized in several aspects of this thesis.
1.3 Polymer Characterizations

1.3.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is a powerful and probably the most frequently used tool for polymer structure characterization. The chemical shifts from $^1$H NMR indicate the structure and purity of polymer products, while the integrations describe the compositions. $^{13}$C NMR and $^{31}$P NMR are sometimes used for specific polymers. Two dimensional NMR techniques of nuclear overhauser effect spectroscopy (NOESY) and correlation spectroscopy (COSY) can also be used to determine the distance and conformation of protons attached to adjacent atoms or nearby chains.\textsuperscript{26}

1.3.2 Gel Permeation Chromatography (GPC)

GPC separates polymers based on their size or hydrodynamic volume. Separation occurs via the use of porous beads packed in a column. Polymers with smaller hydrodynamic volumes or

---

Figure 1-7: Atom transfer radical polymerization.
with lower molecular weight pass through the column slower. They can enter the pores more easily and therefore have longer retention times. On the contrary, polymers with higher molecular weight usually possess bigger hydrodynamic volumes. They spend less time in these pores, and pass through the column faster yielding shorter retention times. GPC equipment is usually filtered with a refractive index detector, and is pre-calibrated by a series of standard linear polystyrenes. Therefore, GPC only indicates a relative molecular weight instead of an absolute molecular weight.

1.3.3 Differential Scanning Calorimetry (DSC)

The major instrument involved with the analysis of thermal properties of polymers, such as \( T_g \), \( T_c \), and \( T_m \), is called DSC. DSC measures the heat flow by maintaining a thermal balance between the reference and sample pans by changing a current passing through the heaters. It is programmed to give a constant temperature increase between the reference and the sample pans. Any thermal transitions in the sample pan, either endothermic or exothermic, will change the energy that the sample needs in order to maintain the same temperature increase. The amount of energy is recorded, and the corresponding temperature is a thermal transition of the polymer sample.

1.3.4 Thermal Gravimetric Analysis (TGA)

TGA is equipped with a sensitive balance to measure the weight change of a polymer as a function of temperature. The temperature where a polymer starts to massively lose weight is the decomposition point. It is important to remember that TGA cannot detect when a polymer actually begins to decompose. Chain cleavage and the resulting decline in molecular weight, is
only evident when a polymer begins to lose mass by expelling smaller, volatile decomposition products. Therefore, the actual ceiling temperature of all polymers may be lower than the results derived from TGA measurements.

1.3.5 Tensile Tests

Tensile tests are usually performed using an instrument called an Instron. A “dog-bone” shaped polymer specimen is stabilized by two clamps on two sides. The clamps start to pull away from each other at a constant rate, and the instrument measures the ability of the specimen to withstand the pulling stresses. The stress and strain of the specimen is recorded, and a profile is made. Different kinds of materials give different types of stress–strain curves (Figure 1-8). The behavior of all classes of polymers before the yield point fulfill Hook’s law. The deformation in this range is reversible and recoverable. The stress is called the yield strength, the deformation is called the yield elongation, and the region is called the elastic region. The highest stress the polymer can tolerate is called the tensile strength, and the break elongation is the maximum deformation a polymer can deal with. However, the deformation after the yield point is from irreversible slippage of polymer chains, which is not recoverable.

The same polymer can have different mechanical properties at different temperatures (Figure 1-9). Semi-crystalline polymers are glassy when the temperature is below their T_g. When the temperature is increased to above their T_g, but is still lower than the temperature necessary to break the crystallinity, the polymers become leathery. The polymers become viscous liquids when the temperature is increased to over their T_m. Amorphous polymers become significantly weaker after reaching their T_g since there are no crystalline domains to maintain good mechanical properties. Most elastomers are amorphous polymers with T_g's lower than the application temperatures.
The ratio of stress to strain within the elastic region is called Young’s modulus. It is also called the modulus of elasticity or tensile modulus. Polymers with a larger Young’s moduli mean they are rigid and resistant to elongation and stretching.

\[
\text{Young’s modulus (Pa)} = \frac{\text{Stress (Pa)}}{\text{Strain (mm/mm)}}
\]

Figure 1-8: Typical stress–strain curves for four different kinds of polymers.
1.3.6 Other Characterization Techniques

Some other techniques will be mentioned and are used to characterize polymers in the later chapters of the thesis. Polymers are viscoelastic materials meaning they can act as liquids and also as solids. The rheology measurement of polymers is usually carried out using dynamic mechanical analysis (DMA). The storage modulus (E’) measures the stored energy, representing the elastic portion, and the loss modulus (E”) measures the energy dissipated as heat, representing the viscous portion.

X-ray diffraction (XRD) is used to identify semi-crystalline polymers and recognize crystalline phases, such as the degree of crystallinity, crystallite size, and crystalline orientation. Infrared spectroscopy (IR) is sometimes used to identify specific functional groups and UV-Visible spectroscopy can detect the presence of certain molecules. Scanning electron microscopy
(SEM) and transmission electron microscopy (TEM) are used to observe polymer morphology in nano- or micro-scales. Water or hexadecane contact angle measurements can study the hydrophobicity or oleophobicity of the surface of polymeric films. Dynamic light scattering (DLS) studies the hydrodynamic size of polymers or polymer aggregates.\(^\text{13}\)

1.4 Introduction to Polyphosphazenes

1.4.1 Definition

Conventional polymers typically contain carbon-based backbones, and the monomers are derived from petroleum. Due to their petrochemical-based nature, they are usually called organic polymers. There exist two unique families of polymers that contain both organic and inorganic components. Polysiloxanes and polyphosphazenes fall into this category. Polyphosphazenes are linear polymers that contain an inorganic backbone with alternating phosphorus and nitrogen atoms. Two side groups, usually organic, are covalently attached on the phosphorus atom at every repeat unit. The inorganic backbone is inherently flexible and fire resistant, and the side groups impart other properties and alter their applications.\(^\text{27}\)

1.4.2 Discovery

The beginning of phosphazene science can be traced back to the middle of the 19\(^{\text{th}}\) century. In 1834, Liebig, Wohler, and Rose discovered that phosphorus pentachloride could react with ammonia to yield a white, crystalline compound.\(^\text{28,29}\) Later, Gerhardt and Laurent analyzed the compound and reported the empirical formula as NPCl\(_2\).\(^\text{30}\) Later work confirmed the structure as [NPCl\(_2\)]\(_3\), now called hexachlorocyclotriphosphazene.\(^\text{31,32}\) Stokes discovered an elastomeric
material after heating the cyclic \([\text{NPCl}_2]_3\), and he realized the material is very sensitive to moisture forming an insoluble rubber.\(^33\)\(^34\) Hence, the material was called “inorganic rubber”. After the emergence of Staudinger’s macromolecular concept, people started to suspect that the elastomer discovered by Stokes was also a polymer. This speculation was then confirmed by later studies showing that the “inorganic rubber” contains linear high polymeric chains.\(^35\) However, attempts to modify this polymer by chlorine replacement were not successful leading to insoluble or hydrolyzed materials until the mid-1960s when Allcock, Kugel, and Valan reported a method to produce a soluble form of this polymer.\(^36\)\(^38\) The good solubility of this polymer makes it a remarkable macromolecular reactant, which can further react with organic nucleophiles in various solvents such as benzene, toluene, THF, and dioxane. After that, the field of polyphosphazene progressed rapidly, and more than 700 different polyphosphazenes have been synthesized thus far.\(^27\)

### 1.4.3 Thermal Ring Opening Polymerization

The reactive poly-intermediate poly(dichlorophosphazene) or \([\text{NPCl}_2]_n\), is the starting material for all macromolecular substitutions. Poly(dichlorophosphazene) is prepared by thermal ring opening polymerization of recrystallized and sublimed \([\text{NPCl}_2]_3\) in a sealed and evacuated Pyrex tube (Figure 1-10).\(^39\) The viscosity of the polymer in the tube is monitored, and is used to determine when to terminate the polymerization process. Hence, experience is important, and there are usually differences from batch to batch. The polymerization can be carried out directly at 250 °C. The resulting polymers usually possess a high molecular weight (>1 million, g/mol) with more than 1000 repeat units. The yield is low to medium based on the degree of polymerization, usually 20–40%. Crosslinking tends to occur when the polymer tube is over exposed to heat. The polymerization process can also be conducted in the presence of a Lewis
acid as a catalyst, such as BCl$_3$. This catalyzed polymerization route generates polymers with medium to high yield (>70%), but lower molecular weight (~0.5 million, g/mol). Thermal ring opening polymerization does not allow for precise control over molecular weight or polymer chain length distribution.

![Figure 1-10: Thermal ring opening polymerization.](image)

### 1.4.4 Living Cationic Polymerization

Living cationic polymerization is a relatively new method to produce linear poly(dichlorophosphazenes). The process was developed by Allcock, Manners et al. in the mid-1990s.$^{41,42}$ The process involves the synthesis of chlorophosphoramidine as a monomer. Polymerization is initiated at the presence of a catalytic amount of phosphorus pentachloride in dichloromethane at room temperature (Figure 1-11). More monomers are added to maintain chain growth with the release of a byproduct, trimethylsilyl chloride. Since the conversion of monomers can reach 100%, the number of repeat units of final polymer can be manipulated by the ratio of reactants. The polymer chains show high uniformity with PDIs usually lower than 1.2. During chain propagation, the chain remains living unless reagents are added to terminate the living ends. Living cationic polymerization allows for the construction of complex polymeric structures, such as block copolymers with two different phosphazene blocks or with other conventional organic polymers.$^{43-47}$
1.4.5 Macromolecular Substitution

One of the most unique features that distinguishes polyphosphazenes from most other polymer systems is the modification of polymers via macromolecular substitution reactions.\(^{27}\) The replacement of the labile chlorine atoms on the poly(dichlorophosphazene) can be achieved by using different nucleophilic reagents as side groups, such as alkoxides, aryloxides, or amines (Figure 1-12).\(^{48,49}\) When the chlorine replacement reactions are conducted using one type of side group, single-substituent homo-polymers are obtained. Different side groups can be linked to phosphazene backbone giving mixed-substituent polymers, which usually possess more complex properties. The key advantage of this process is that it provides a method to introduce a wide range of side groups using the same pathway, resulting in various but predictable properties. Alcohols are usually converted to their sodium salt to increase the reactivity, while amines are substituted at the presence of an organic base to neutralize the evolved hydrochloride acid.\(^{31}\) \(^{31}\) P NMR techniques can be utilized to monitor this substitution process. A completion of chlorine

Figure 1-11: Living cationic polymerization.
replacement is important, since any remaining chlorine atoms on the phosphazene backbone will hydrolyze or crosslink the polymer when exposed to water.\textsuperscript{27}

![Macromolecular substitution diagram](image)

**Figure 1-12:** Macromolecular substitution.

### 1.4.6 Substitution Patterns

In principle, macromolecular substitution of side groups could proceed geminally, non-geminally cis, non-geminally trans, or randomly (Figure 1-13).\textsuperscript{27,50} If one side group is used, only geminal structure could be obtained. However, when two or more nucleophiles are used, the substitution patterns rely highly on the property of the reagents. When the side group is a strong electro-withdrawing unit with a small size, the linkage of one side group tends to activate that phosphorus atom making it more electro-deficient. As a consequence, the incoming reagents prefer to attack at the phosphorus atoms with one side group already attached leading to geminal substitutions. Non-geminal substitutions are usually harder to obtain. Bulky reagents are used first in the substitution reaction to increase the steric hindrance around the phosphorus atoms. Then,
unreacted nucleophiles will avoid the highly hindered phosphorus center by reacting with the non-substituted phosphorus atoms. However, it is extremely hard to control gemical cis and geminal trans substitution patterns due to the flexible nature of polymer backbone. Meanwhile, it is hard to carry out any effective characterization to analyze the differences. Random substitution occurs when two reagents with similar reactivity are added to the reaction medium simultaneously. Meanwhile, the substitution patterns are also controlled by the addition ratio of different side groups, the addition speed and temperature, and the concentration of side groups. Thus, it is difficult to precisely control the distribution of side groups along the phosphazene backbone for the mixed-substituent polymers.⁵¹

![Substitution patterns of polyphosphazenes.](image)

**Figure 1-13**: Substitution patterns of polyphosphazenes.

### 1.4.7 The Cyclic Model Compound Concept

Macromolecules are inherently more difficult to synthesize, purify, and characterize than small molecules. This problem could be exacerbated when studying the substitutions of new and
complex side groups. Thus, hexachlorocyclotriphosphazene is used as a model system for exploratory substitutions before any studies are carried out with high polymers (Figure 1-14).\textsuperscript{52}

The information derived from the trimer reactions can then be applied to high polymer reactions. Over 300 trimer compounds have been synthesized, and some of them are not only valuable as model molecules but also show significant applications potentials.\textsuperscript{53-56} Moreover, cyclic trimers can also be components in polymer structures such as polymers that have trimer units linked to the backbone,\textsuperscript{57} or as part of the main chain.\textsuperscript{58} Phosphazene cyclic tetramers, while less common, can also be used as model compounds. They are inherently more similar to the high polymers because of their higher skeletal flexibility.\textsuperscript{59,60}
1.4.8 Hydrolytic Sensitivity

Hydrolytically sensitive polymers can be synthesized using labile side groups such as amino acid esters. The degradation process starts with the release of a parent amino acid ester side group and continuous with the formation of rearranged phosphazane products. Then the phosphazane can release the other amino acid ester, or undergo cleavage of the backbone. The
continuity of this process finally leads to the formation of phosphate and ammonium residues (Figure 1-15). There are several advantages to the availability of degradable polyphosphazenes. First, the hydrolysis can be finely tuned by the size of the unit at the alpha carbon of the amino acid; second, the degradation process gradually releases the side groups; the degradation residues are a non-toxic buffer pair, which can maintain a relatively neutral pH value.

Figure 1-15: Hydrolysis of polyphosphazenes containing amino acid side groups.
1.5 Synthetic Challenges of Polyphosphazenes

1.5.1 Solubility

Although macromolecular substitution provides a convenient pathway to alter the polymer properties, it may generate a solubility problem. Bulky side groups reduce the flexibility of the polymers causing the products or intermediates to precipitate from the organic solvent. This may prevent the completion of chlorine replacement.\textsuperscript{64} As mentioned above, any remaining chlorine atoms will generate crosslinks by hydrolysis during the purification step. Soluble co-substituents are required to increase the overall solubility in order to complete the substitution.\textsuperscript{65}

1.5.2 Synthetic Degradation

The substitutions of amines onto a phosphazene backbone generate hydrochloride acid which would cause chain cleavage of the polymers in solution.\textsuperscript{66} In order to minimize this situation, triethylamine is used to complex with the acid by forming an insoluble salt. However, the polymers are still susceptible to long time reaction since the dissociation of the salt releases hydrochloride acid which in turn will cause backbone degradation. Therefore, reaction times need to be carefully controlled when synthesizing amino acid ester substituted polyphosphazenes.

1.5.3 Substituent Exchange Reactions

Sometimes, the substitution reactions can take place at the phosphorus atoms that already have side groups attached (Figure 1-16).\textsuperscript{67} Mixed-substituent polymers can also be prepared by treating a single-substituent polymer with a second nucleophile via the substituent exchange
reactions. However, this usually yields polymers with low molecular weights. Moreover, exchange reactions may cause the final ratios of the two side groups to deviate from the target ratios. Several rules determine the tendency of the exchange reaction. First, small and good leaving groups are easy to replace. Second, larger side groups are relatively more difficult to replace since they can better protect the backbone phosphorus atoms. Third, strong electron-withdrawing side groups can activate the adjacent phosphorus atoms, which favors exchange reactions. Hence, reaction conditions and the addition sequence must be finely controlled during the synthesis to minimize exchange reactions.\textsuperscript{68,69}

\[
\begin{align*}
\text{OR}_1 & \quad \text{P=N} \quad \text{OR}_1 \\
\text{NaOR}_2 & \quad \rightarrow \\
\text{OR}_2 & \quad \text{P=N} \quad \text{OR}_1 \\
\end{align*}
\]

Figure 1-16: Substituent exchange reactions.

1.6 Applications of Polyphosphazene

1.6.1 Fluoroelastomers

The most widely identified application for polyphosphazene related materials are high-performance elastomers. These materials are usually chemically crosslinked amorphous polymers with different fluoroalkoxy groups as side units (Figure 1-17).\textsuperscript{70} The elastomers were first discovered by Rose in 1968.\textsuperscript{71,72} Fluoroalkoxyphosphazene elastomers have important advantages over other elastomers at low operational temperatures due to the ultra-low glass transition temperatures of these polymers. This property is particularly valuable for extreme conditions,
especially for military applications. Usually, small amount of crosslinkable functional groups such as allyl phenoxy groups, are linked to the phosphazene backbone. These groups are susceptible to undergo crosslinking reactions at the presence of heat or UV irradiation with or without radical initiators.

\[
\begin{align*}
\text{OCH}_2\text{CF}_3 & \quad \text{OCH}_2\text{CF}_3 \\
\text{P=O} & \quad \text{P=O} \\
\text{OCH}_2(\text{CF}_2)_x\text{CF}_2\text{H} & \quad \text{OCH}_2(\text{CF}_2)_y\text{CF}_3
\end{align*}
\]

Figure 1-17: Mixed-substituent fluoroalkoxyphosphazene elastomers.

### 1.6.2 Biomedical Materials

Polyphosphazenes offer a number of crucial advantages for biomedical applications. The macromolecular substitution process enables the synthesis of polymers with multiple properties. The co-substitution concept can also tune the glass transition temperatures of the polymers over a broad range. Depending on specific applications, polymers can be elastomeric, leathery, or glassy. Polyphosphazenes with amino acid esters are biocompatible and biodegradable. They degrade into a non-toxic residue, and the side groups are released during the degradation process. The degradation rates can be well controlled by the structure and hydrophobicity of the side groups with half lives in a physiological environment from months to years.

Research on polyphosphazenes for biomedical applications constitutes a high percentage of all phosphazene related research (Figure 1-18). Biodegradable polyphosphazenes containing amino acid esters have been prepared in the form of porous scaffolds for tissue regenerations.
Biostable phosphazene elastomers have been studied for use in prosthetic blood vessels, artificial heart membranes, and dental applications. Biodegradable polyphosphazenes are physically blended with other FDA-approved implant biomaterials, such as poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) to modify their degradation rate, and to neutralize their acidic degradation products.

Polyphosphazenes also show great potential for drug delivery applications. Polymers with co-substitutions of amino acid esters can gradually release drugs, such as antibiotics, vitamins or steroids over the period of their hydrolysis. Polyphosphazenes with crosslinkable aromatic carboxylic acid side groups can form microsphere particles at the presence of Ca$^{2+}$. These capsules are capable of trapping living cells, drugs, and vaccines for targeted delivery and controlled release. Moreover, water soluble polyphosphazenes can form hydrogels after crosslinking treatment which can also localize drugs or other bioactive molecules.

Figure 1-18: Selected polyphosphazenes for biomedical applications.
1.6.3 Membranes

Polyphosphazenes have been studied as gas transport membranes, liquid separation membranes, and ion transport membranes (Figure 1-19). Although many conventional organic polymers have been developed as membrane materials, polyphosphazenes have also generated a significant interest for several reasons. The versatility of macromolecular substitution process offers many opportunities to tune glass transition temperature over a wide range. The capability of incorporation of multiple side groups can endow materials with multiple properties needed for membrane function. Their thermal and chemical stability allows polymers to be used in various environments. There is an important type of gas transport membranes specifically designed for the separation of CO$_2$. These membranes have stronger physical interactions with CO$_2$ than with other gases such as N$_2$ or CH$_4$. This oxophilic behavior leads to a higher selectivity for CO$_2$.

![Figure 1-19](image-url): Selected polymers for membrane applications.
1.6.4 Micelles

Amphiphilic block copolymers can lead to a self-assembly behavior that is similar to small molecular surfactants. Typical spherical micelles are formed which in water have a core of a hydrophobic block and a corona of a hydrophilic segment (Figure 1-20). Micelles have the capability to incorporate hydrophobic molecules to increase their solubility or, the stability of those hydrophobic compounds in water. They can also be used as vehicles to localize or deliver biomolecules. Polyphosphazenes synthesized via the living cationic polymerization allow for chain-end modifications to link to another polymeric block, such as another polyphosphazene, polystyrene, poly(ethylene oxide), etc. 

Figure 1-20: Micelle formation and phosphazene block copolymers.
1.6.5 Electrospin

Electrospin is a straightforward method for producing fibrous polymer mats with fiber diameters in the range of nanometers to micrometers. In an electrospinning process, a strong electrostatic field is applied to a polymer solution held in a syringe with a capillary outlet (Figure 1-21). When the voltage surpasses a threshold value where the electric force overcomes the surface tension of the droplet, a charged jet of the solution is ejected from the tip of the Taylor cone. As the jet moves toward a collecting screen, solvent evaporates and a non-woven fabric mat is formed on the collector. The diameter and morphology of the receiving fibers can be controlled by several parameters, including molecular weight of the polymer, solution concentration, electric potential, solvent, and working distance. Electrospun nanofiber matrices have ultrafine continuous fibers, high surface-to-volume ratio, and high porosity. Some biodegradable polyphosphazenes have been electrospun into porous fiber mats for tissue engineering applications. Super-hydrophobic surfaces have been prepared by electrospinning poly[bis(trifluoroethoxy)phosphazene] into nano-fibers.

Figure 1-21: Electrospinning of a polymer solution.
1.7 References

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Chapter 2

Biodegradable Polyphosphazenes Containing Antibiotics: Synthesis, Characterization, and Hydrolytic Release Behavior

2.1 Introduction

Antibiotics, such as penicillin, tetracycline, and fluoroquinolines are among the most effective means for treating bacterial infections.\(^1,^2\) However, traditional antibiotic administration by oral ingestion or injection of solutions have disadvantages especially when local dosage is required. Large and repeated doses must be given to maintain a sufficient plasma concentration of antibiotics for local effectiveness due to the dilution of the drugs in the systemic circulation.\(^3^-^5\) This may lead to side effects including systemic toxicity with associated renal and liver complications due to the toxicity of most antibiotics.\(^6,^7\) Localized delivery methods based on physical stabilization of antibiotics in a polymer matrix such as a hydrogel or self-eluting polymer can release antibiotics at the target region to maintain a high local concentration without exceeding systemic toxicity limits.\(^8^-^12\) However, due to the weak nature of the physical interactions between drugs and polymer matrices, most antibiotics are released quickly with short term activities and suffer from initial bursts which limits their applicability. Alternatively, chemically linking the antibiotics to a free-standing polymer can provide long-term continuous local release when the linkages are hydrolysable.\(^13^-^15\) Immobilization of drugs on polymers can increase the lifetime of the drug since the molecules that are normally free to be carried throughout the body are now bound to a support.\(^16\) These materials are more desirable for treating local infections such as the osteomyelitis, or for preventing post-surgery infections caused by either temporary or permanent implants. However, the available range of these materials is
limited due to the strict requirements which include facile modifications with tunable antibiotic loading, biocompatibility, biodegradability, and nontoxic degradation products. Polyphosphazenes are a class of highly tunable synthetic polymers that could be ideal candidates for this application.

Polyphosphazenes are hybrid polymers with an inorganic backbone of alternating phosphorus and nitrogen atoms with two side groups attached to each phosphorus. Their synthesis is based on the reactions of poly(dichlorophosphazene) which is commonly synthesized through a thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 250°C in a sealed system. This reactive polymeric intermediate can then undergo substitution reactions through the replacement of chlorine atoms by various nucleophiles such as alkoxides, aryloxides, or primary or secondary amines. Hydrolytically sensitive polyphosphazenes are formed when amino acid ethyl ester groups are linked to the polymer backbone via the amine terminus. The nontoxic hydrolysis products are the parent amino acids, ethanol, phosphates, and ammonia, a mixture that results in a near-neutral pH. The secondary amine functionality on the piperazinyl group of ciprofloxacin and norfloxacin provides a reaction site for the linkage to poly(dichlorophosphazene). These FDA-approved synthetic broad spectrum antibiotics inhibit the growth of bacteria by interfering with the DNA gyrase (gram-negative) and type IV topoisomerase (gram-positive).

In this work, hydrolytically sensitive polyphosphazenes containing ciprofloxacin or norfloxacin and various amino acid ethyl esters (glycine, alanine, and phenylalanine) were synthesized (Figure 2-1). Hydrolysis behavior over a six week period was studied using different polymers as films and as nano/microfiber mats for in vitro experiments based on their mass lost and the pH of the hydrolysis media. Meanwhile, the antibiotic release profiles were monitored quantitatively via the characteristic ultraviolet absorptions of the antibiotics in the hydrolysis media. The presence of antibiotics in the media was further confirmed by mass spectrometry. In vitro antibacterial tests
were performed against *E. coli* using the hydrolysis solution directly to verify the retained antibacterial capability of the antibiotics.

![Chemical structure with text: Antibiotics: Ciprofloxacin or Norfloxacin, Amino Acid Ethyl Esters.](image)

**Figure 2-1:** Designing of polymer structures

### 2.2 Experimental Section

#### 2.2.1 Reagents and Equipment.

Tetrahydrofuran (THF), dichloromethane (CH$_2$Cl$_2$), dimethylformamide (DMF) and triethylamine (Et$_3$N) were purchased from EMD and dried using solvent purification columns. Ciprofloxacin (Sigma-Aldrich), norfloxacin (Sigma-Aldrich), glycine ethyl ester hydrochloride (Chem Impex), alanine ethyl ester hydrochloride (Chem Impex), phenylalanine ethyl ester hydrochloride (Chem Impex), morpholine (Sigma-Aldrich), tetrakis(triphenylphosphine)palladium(0) (Sigma-Aldrich), di-tert-butyl dicarbonate (Sigma-Aldrich), and sodium bicarbonate (Sigma-Aldrich) were used as received. Allyl bromide (Sigma-Aldrich) was freshly distilled before use. Poly(dichlorophosphazene) was prepared by the thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical, Japan) in evacuated Pyrex tubes at 250°C. All synthesis reactions were carried out using standard Schlenk line techniques and a dry argon atmosphere. The glassware
was dried overnight in an oven at 120°C before use. $^1$H and $^{31}$P NMR spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 or 145MHz, respectively. $^1$H NMR spectra were referenced to solvent signals, while $^{31}$P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10mm and AM gel 10mm 104Å columns, and calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40°C with a 0.1wt% solution of tetra-n-butylammonium nitrate (Sigma-Aldrich) in THF. Glass transition temperatures were measured with a TA Instruments Q10 differential scanning calorimeter using a sample size of ca. 10mg with a heating rate of 10°C/min under nitrogen. Mass spectrometric analysis data were collected using a turbospray ionization technique with an Applied Biosystems API 150EX LC/MS mass spectrometer. The ultraviolet absorptions of the hydrolysis media were measured with use of a CARY 50 Bio UV-Visible spectroscopy (VARIAN). The pH values were measured using a VWR Symphony SB70P pH meter at room temperature. The pH meter was calibrated to pH=7 and pH=4 buffer solutions before use. The optical densities of the in vitro antibacterial tests were measured by LKB BIOCHROM ULTROSPEC 4050 spectrophotometer with a wavelength of 600nm at room temperature. The visual images were captured by a Canon EOS REBEL XS digital camera.

2.2.2 Allyl Protection of Ciprofloxacin (1) and Norfloxacin (2).

The allyl protection of ciprofloxacin and norfloxacin followed procedures reported previously, with some minor changes. $^{31}$ The protection of ciprofloxacin is used as an example.

Allyl Protection of Ciprofloxacin (1). A mixture of ciprofloxacin (5g, 15.1mmol), di-tert-
butyl dicarbonate (4.025g, 18.4mmol), and sodium bicarbonate (6.27g, 74.6mmol) in 100mL of anhydrous DMF was stirred at room temperature for 5h under an argon atmosphere. Allyl bromide (6mL, 69.5mmol) was added and the reaction mixture was heated at 90°C for 24h. After the mixture was cooled to room temperature, the solvent was removed in vacuum. The residue was dissolved in a mixture of chloroform (100mL) and dichloromethane (50mL), and was then washed with water (3×100mL) and brine (2×100mL). The organic layer was collected and dried over magnesium sulfate. After removal of all the solvent, the material was then redissolved in 150mL of ethyl acetate (AcOEt) in a 500mL Erlenmeyer flask. Concentrated HCl (~20mL) was added dropwise, and the yellowish solution was stirred for 30min. The solvent was removed in vacuum to give yellowish solids 1 (Yield: 74%). $^1$H NMR (D$_2$O): δ 7.96 (s, 1H), δ 7.05 (d, 1H), δ 6.76 (d, 1H), δ 5.95 (m, 1H), δ 5.31 (t, 2H), δ 4.54 (s, 2H), δ 3.36-3.20 (bm, 9H), δ 1.27-0.85 (bm, 4H). MS (ES+): m/z 372.2 ([M+H]).

**Allyl Protection of Norfloxacin (2).** Yellowish solids (Yield: 78%). $^1$H NMR (D$_2$O): δ 8.03 (s, 1H), δ 6.92 (d, 1H), δ 6.76 (d, 1H), δ 5.84 (m, 1H), δ 5.22 (t, 2H), δ 4.53 (s, 2H), δ 4.02 (q, 2H), δ 3.29 (m, 8H), δ 1.17 (t, 3H). MS (ES+): m/z 360.1 ([M+H]).

### 2.2.3 Synthesis of Polymers.

All the syntheses followed a similar pattern, and the differences are emphasized in the following descriptions. The synthesis of polymer AC is demonstrated in detail.

**Synthesis of Polymer AC.** Poly(dichlorophosphazene) (0.5g, 4.32mmol) was dissolved in anhydrous dichloromethane (50mL). A suspension of alanine ethyl ester hydrochloride (0.66g, 4.32mmol) and triethylamine (3.6mL, 25.92mmol) in dry CH$_2$Cl$_2$ (30mL) was stirred at room temperature for 6h and then added to the polymer solution. The mixture was allowed to stir at room temperature for 6h to give a partially substituted polymer. Ciprofloxacin allyl ester (1)
(0.88g, 2.16mmol) and triethylamine (3.6mL, 25.92mmol) were dissolved in anhydrous CH₂Cl₂ (30mL) in a separate Schlenk flask, and stirred for 6h at room temperature. After that, the solution of (1) was added to the partially substituted polymer solution, and the reaction mixture was stirred for another 8h at room temperature. An additional 1.5 equivalents of alanine ethyl ester hydrochloride (1.0g, 6.48mmol) was stirred with triethylamine (3.6mL, 25.92mmol) in anhydrous CH₂Cl₂ (30mL) in an another Schlenk flask for 6h at room temperature, and it was then added to the polymer solution to complete the substitution reaction. The final reaction mixture was stirred at 60–70°C for 16h. The solution was concentrated and dialyzed versus MeOH/CH₂Cl₂ 20/80 for 3d, and MeOH/hexanes/CH₂Cl₂ 20/40/40 for another 2d. The product was dried under vacuum to give AC as yellowish solids. Polymers AC’ and GC were also yellowish solids.

**Synthesis of Polymer PC.** All the procedures were the same as above, except that this reaction mixture was stirred at 60–70°C for 24h after addition of an excess of phenylalanine ethyl ester in the last step. A yellowish product remained after drying under vacuum.

**Synthesis of Norflaxacin Containing Polymers.** The synthesis of polymers AN and GN were the same as for polymers AC and GC, and the synthesis of polymer PN was the same as polymer PC.

### 2.2.4 Polymer Deprotection Reactions.

The deprotection reactions followed the same procedure as reported in previous literature. The deprotection of polymer AC is given as an example. Briefly, polymer AC (0.2g, 0.539mmol) was dissolved in 200mL anhydrous CH₂Cl₂. To this was added morpholine (0.11g, 1.3mmol) and tetrakis(triphenylphosphine)palladium(0) (0.031g, 0.027mmol). The solution was stirred at room temperature for 4h during which time the yellowish polymer precipitated from solution. Methanol
(20mL) was added to redissolve the polymers and the solution was purified by dialyzing against 20/80 MeOH/CH₂Cl₂ intensively for 5d. Solvent was removed, and the polymer was dried under vacuum to yield yellowish solids.

2.2.5 Electrospinning.

The electrospinning apparatus consisted of a 1mL syringe fitted with an 18 gauge needle. The fiber collector was a grounded aluminum foil target at a predetermined distance from the needle. The flow of the polymer solution was maintained with a syringe pump (Kent Scientific Corporation, Torrington, CT) at a fixed rate (1mL/h) for each run. A high voltage power supply ES40P-20W with a low current output (0–40KV, 20W, Gamma High Voltage Research, Ormond Beach, FL) was used as a power source. A positive voltage was applied to the solution in the syringe by attaching an alligator clip to the needle from the positive lead of the high voltage supply. Electrospinning conditions were adjusted to obtain bead-free fibers for polymers AC, GC, AN, and GN by varying the concentration of the solution (grams of polymer per milliliter of solvent in percentage, w/v) at ambient temperature. The distance from the needle to the collecting foil was maintained at 12cm, the voltage was 20kV and the flow rate was 1mL/h. MeOH/CHCl₃ 20/80 was used as the solvent. The nano/microfiber mats produced were stored in vacuum before use.

2.2.6 Environmental Scanning Electron Microscopy.

Environmental scanning electron microscopy samples were prepared by affixing the dried electrospun fiber sample to conductive carbon tape. The samples were viewed and recorded at various magnifications with ESEM FEI Quanta 200.
2.2.7 In vitro Hydrolysis Studies and Drug Release Profiles.

For preparing polymer films, the polymers were dissolved in MeOH/CHCl$_3$ 20/80 (100mg/1mL). The solutions were cast into square films (3×3cm) on a Bytac (PTFE) surface. The films were allowed to stand in the ambient environment for 1 day, and were then dried further under reduced pressure for 2 days. The dried films were divided into 18 samples (~5×5mm, ~10mg each). The nano/microfiber mats were peeled from the aluminum foils and divided into 18 samples (~10mg each). All the samples were placed in 18 different vials with 3mL deionized water (pH=6.70) each. The vials were secured in a shaker bath maintained at 37°C for six weeks. After each week, three vials were removed from the shaker bath. The aqueous media were decanted and the pH of each sample was monitored, while the solid samples were dried under vacuum for one week and weighed. The drug release profiles were characterized by UV-Vis spectroscopy in a 1×1cm quartz cuvette at room temperature. The aqueous media were diluted by standard dilution techniques if the characteristic absorption at 275nm was higher than 1.5. The molar extinction coefficients for ciprofloxacin and norfloxacin in deionized water were found to be 34,500 and 34,000L·mol$^{-1}$·cm$^{-1}$ respectively from a linear Beer-Lambert plot of absorbance at 275nm versus concentration. Mass spectrometry was used to confirm the molecular weight of the antibiotics in the hydrolysis media.

2.2.8 In vitro Antibacterial Tests of the Hydrolysis Media.

The bioactivities of the antibiotics left in the media by the hydrolysis of the polymers were studied by standard in vitro antibacterial assays using E.coli as a model bacterium. Start Lysogeny Broth (LB) liquid culture (30mL) was inoculated with 50µL of Top10 strain E.coli from glycerol stock bacterium (in -80°C) in a 150mL flask. The culture was grown at 37°C in a
rotary shaker at 200rpm overnight. The \textit{E.coli} of \(\text{OD}_{600}=1.0\) was used for the following functional tests. \textit{E.coli} from the start culture (10µL) was inoculated into 2mL LB liquid media in 15mL culture tubes containing the hydrolysis media from polymers from different weeks (1:1 mixture of LB and hydrolysis media). For visual comparison, two additional media were prepared, a homogeneously mixed LB solution and hydrolysis media (GC) without inoculation with \textit{E.coli} (a) and an inoculation of \textit{E.coli} in LB solution without the addition of hydrolysis media (b). In a parallel experiment, the antibacterial tests of the hydrolysis media of homosubstituted amino acid ethyl ester polyphosphazenes (synthesized previously\textsuperscript{22,23}) were followed the same method as above. All these samples were incubated at 37°C in a rotary shaker (200rpm) for 18h. Bacterial growth was studied by visually inspecting the media for turbidity (turbid LB solutions indicate bacterial growth). Further quantifications were obtained by measuring the optical density of the samples using a spectrophotometer with a wavelength of 600nm (\(\text{OD}_{600}\)) and sample (a) was calibrated as \(\text{OD}_{600}=0.000\). Because the \(\text{OD}_{600}\) is proportional to the absorbance value of the samples, the lower the \(\text{OD}_{600}\), the less is bacterial growth. The minimum inhibitory concentrations (MIC) of the antibiotics in the hydrolysis media were determined by a series double dilutions.

### 2.3 Results and Discussion

Generally speaking, the substitution reactions of poly(dichlorophosphazene) become more challenging when the structures of the nucleophiles are more complex. However, in order to achieve better polymer properties or more functions for biological applications, some relatively complex bioactive agents need to be linked to the polyphosphazene backbone regardless of their structural complexity. For example, the introduction of anesthetics,\textsuperscript{34} vitamins,\textsuperscript{35} and antioxidants\textsuperscript{32} endow polyphosphazenes with various new biomedical properties. In these cases, multi-step protections and deprotections of those side groups may have to be conducted in
order to prevent crosslinking or side reactions during polymer synthesis. The steric hindrance of large side groups is another concern which can increase the synthetic difficulty. For example, the polyphosphazenes containing steroid pendant groups can reach no more than 20% steroid substitution, and the solubility of the polymers decreased significantly with greater substitution.\textsuperscript{36} Previous studies showed that the hydrolysis of polyphosphazenes can release side groups linked to the polymer backbone during polymer breakdown.\textsuperscript{37} Hence, the idea of attaching drugs to a biodegradable polyphosphazene backbone for local continuous release is quite logical.\textsuperscript{38-40} Fluoroquinolines such as ciprofloxacin and norfloxacin are widely accepted broad-spectrum antibiotics with relatively simple structures. However, the free carboxylic acid units on these antibiotics have to be protected before polymer reactions to prevent side reactions and uncontrollable crosslinking during synthesis. Therefore, the carboxylic acid units were protected by allyl groups instead of ethyl or propyl groups due to their milder deprotection conditions (Figure 2-2). The reactions of a similar molecule, piperidine, with both hexachlorocyclotriphosphazene\textsuperscript{41} and poly(dichlorophosphazene)\textsuperscript{42,43} have been studied before. Even though the success in synthesizing these compounds supports the feasibility of the reactions between ciprofloxacin (norfloxacin) and poly(dichlorophosphazene), the solubility issue needed to be addressed in this work due to the considerably larger size of these two antibiotics.

\begin{figure}[h]
\centering
\includegraphics{allyl_protection_reactions.png}
\caption{Allyl protection reactions.}
\end{figure}
2.3.1 Synthesis of Polymers.

Macromolecular substitution reactions were carried out in a two-step process. First, poly(dichlorophosphazene) was synthesized by the ring-opening polymerization of hexachlorocyclophosphazene at 250°C in a sealed Pyrex tube. Second, replacement of chlorine atoms was attempted based on macromolecular substitutions by the secondary amino unit of the piperazinyl group of the two antibiotics. The reactions were first carried out in boiling THF by adding (1) or (2) together with triethylamine to poly(dichlorophosphazene) solution. The use of a large excess of triethylamine to complex the free hydrochloride acid generated during macromolecular substitution reaction prevents polymer backbone degradation that can occur in an acidic environment. However, the polymers precipitated from solution immediately after the addition of the antibiotics due to the poor solubility of the partially substituted polymers in THF.

To circumvent this problem, amino acid esters were introduced in the first step to increase the polymer solubility, and the antibiotics were introduced in the second step to reach an appropriate amount of antibiotic loading. Additional amino acid esters were than introduced in the last step to replace all the remaining chlorine atoms on the backbone. Even though the addition of amino acid esters in the first step increased the solubility of the polymer in boiling THF, the polymers still precipitated after the addition of (1) or (2) in the second step unless more than 80% of the chlorine had been replaced by amino acid esters in the first step. The intensive shielding of the polymer backbone by amino acid esters made it difficult for the bulkier antibiotics to replace the remaining chlorine even after extensive refluxing. From 1H NMR spectra, less than 8mol% of the antibiotic was linked to the polymer backbone after four days’ reaction. Meanwhile, the polymers showed significant degradation to species with molecular weights below 30,000.

The solubility issue was solved by using CH2Cl2 as a solvent instead of THF since the antibiotics showed better solubility in this medium. Replacement of 50% of the chlorine by amino
acid ester units in the first step was enough to keep the polymer in solution during the reactions in
the second step. This allowed up to 25mol% of the antibiotics to be attached during only several
hours’ reaction time at ambient temperature. However, no higher percentage of antibiotic
attachment could be obtained in this step even after longer reaction times or when using higher
equivalents of antibiotic. Finally, additional amino acid esters were added to replace all the
remaining chlorine atoms at 60–70°C (Figure 2-3). All the polymers can be synthesized within
two days, and 31P NMR spectroscopy was used to monitor the reaction process. Figure 2-4 shows
an example of the spectra from the synthesis of polymer AC, and all the other polymers gave
similar spectra during synthesis. The single peak at -8.49ppm after the addition of 50% of amino
acid esters represents a mono-substitution at each phosphorus atom along the polymer backbone.
Following this step, another peak at -0.79ppm appeared when 25% of the antibiotic was added,
which represents the phosphorus atoms that bear one amino acid ester and one antibiotic unit.
Additional amino acid esters were then added in the last step, and the peak at -8.49ppm
disappeared as the other 25% of mono-substituted phosphorus centers reacted with amino acid
esters. The resultant polymers were insoluble in THF when the mole percent of antibiotics was
higher than 12%. All the polymers were soluble in CH₂Cl₂, CHCl₃, and DMF. The
characterization data were summarized in Table 2-1.

The GPC data for polymer AC’ with low ciprofloxacin content (soluble in THF) showed a
much lower molecular weight (82,300g/mol) compared with the unreacted
poly(dichlorophosphazene) (~500,000g/mol, calculated from
poly[trifluoroethoxy]phosphazene]. No molecular weight data could be obtained for the
polymers with higher antibiotic content due to their insolubility in THF. Because the reaction
conditions are similar for those polymers compared with polymer AC’, no higher molecular
weights were expected. Meanwhile, all the polymers gave medium yields around 60%.

Hydrochloric acid formed in the replacement reaction normally complexes with excess
triethylamine. However, it can also attack the nitrogen atoms in the polyphosphazene backbone causing P–N bond cleavage, which was probably responsible to the lower molecular weights and yields. All antibiotic containing polymers have higher glass transition temperatures ($T_g$) than the amino acid ester homosubstituted polymers due to the introduction of bulkier side groups which hinder the skeletal motion of polymer backbone. Polymer AC with a higher antibiotic loading showed a higher $T_g$ (14.2 °C) than polymer AC’ (12.1 °C), and this supports the effect of the bulky antibiotics in increasing the $T_g$. No melting temperatures were detected for any of these polymers up to 250 °C.

![Chemical structure](image)

Ciprofloxacin ($R_1=C_3H_7$)

- AC: $R_2=CH_3$, $x=0.5$, $y=1.5$
- GC: $R_2=H$, $x=0.48$, $y=1.52$

Norfloxacin ($R_1=C_2H_5$)

- AN: $R_2=CH_3$, $x=0.44$, $y=1.56$
- PN: $R_2=CH_2C_6H_5$, $x=0.48$, $y=1.52$

*Symbols represent substitution patterns

<table>
<thead>
<tr>
<th>Ciprofloxacin (C)</th>
<th>Norfloxacin (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine ethyl ester (A)</td>
<td>AC' (12%), AC</td>
</tr>
<tr>
<td>Glycine ethyl ester (G)</td>
<td>GC</td>
</tr>
<tr>
<td>Phenylalanine ethyl ester (P)</td>
<td>PC</td>
</tr>
</tbody>
</table>

Figure 2-3: Macromolecular substitution reactions.
Figure 2-4: Change of $^{31}$P NMR spectra during the synthesis of polymers.

Table 2-1: Replace this with table caption above the table.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$^{31}$P NMR (ppm)</th>
<th>$^1$H NMR (ppm)</th>
<th>Tg ($^\circ$C)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>-0.81</td>
<td>8.44 (s, 1H, cipro), 7.88-7.01 (br, 2H, cipro), 5.92 (s, 1H, cipro), 5.33 (m, 2H, cipro), 4.73 (s, 2H, cipro), 4.62-3.89 (br, 2H, ala), 3.72-3.03 (br, 9H, cipro, 1H ala), 1.50-0.82 (br, 4H, cipro, 6H, ala)</td>
<td>12.1</td>
<td>61%</td>
</tr>
<tr>
<td>AC</td>
<td>-0.47</td>
<td>8.44 (s, 1H, cipro), 7.92-6.92 (br, 2H, cipro), 5.98 (s, 1H, cipro), 5.40 (m, 2H, cipro), 4.73 (s, 2H, cipro), 4.62-3.89 (br, 2H, ala), 3.77-3.02 (br, 9H, cipro, 1H ala), 1.52-0.89 (br, 4H, cipro, 6H, ala)</td>
<td>14.2</td>
<td>65%</td>
</tr>
<tr>
<td>GC</td>
<td>-1.19</td>
<td>8.46 (s, 1H, cipro), 7.81-7.02 (br, 2H, cipro), 5.97 (s, 1H, cipro), 5.31 (m, 2H, cipro), 4.75 (s, 2H, cipro), 4.39-4.05 (br, 2H, gly), 3.79-3.01 (br, 9H, cipro, 2H gly), 1.62-0.84 (br, 4H, cipro, 3H, gly)</td>
<td>9.4</td>
<td>60%</td>
</tr>
<tr>
<td>PC</td>
<td>-1.47</td>
<td>8.37 (s, 1H, cipro), 7.86-7.42 (br, 1H, cipro), 7.32-6.68 (br, 1H, cipro, 5H, phe), 6.02 (s, 1H, cipro), 5.32 (m, 2H, cipro), 4.77 (s, 2H, cipro), 4.48-4.02 (br, 2H, phe), 3.98-2.54 (br, 9H, cipro, 3H, phe), 1.37-0.78 (br, 4H, cipro, 3H, phe)</td>
<td>35.4</td>
<td>52%</td>
</tr>
<tr>
<td>AN</td>
<td>-1.03</td>
<td>8.62 (s, 1H, nor), 7.73-7.03 (br, 2H, nor), 6.04 (s, 1H, nor), 5.36 (m, 2H, nor), 4.73 (s, 2H, nor), 4.52-3.96 (br, 2H, nor, 2H, ala), 3.91-2.63 (br, 8H, nor, 1H ala), 1.42-0.87 (br, 3H, nor, 6H, ala)</td>
<td>19.0</td>
<td>65%</td>
</tr>
<tr>
<td>GN</td>
<td>-0.97</td>
<td>8.42 (s, 1H, nor), 7.78-6.88 (br, 2H, nor), 6.07 (s, 1H, nor), 5.34 (m, 2H, nor), 4.72 (s, 2H, nor), 4.48-3.92 (br, 2H nor, 2H, gly), 3.90-2.63 (br, 8H, nor, 2H gly), 1.48-0.77 (br, 3H, nor, 3H, gly)</td>
<td>18.4</td>
<td>58%</td>
</tr>
</tbody>
</table>
2.3.2 Polymer Deprotection Reactions.

The deprotection reactions were carried out under mild conditions using morpholine and tetrakis(triphenylphosphine)palladium(0) for 4h at room temperature (Figure 2-5). Initially, polymers dissolved well in anhydrous CH2Cl2. After 4h, yellow polymers precipitated from CH2Cl2 due to the exposure of the carboxylic acid unit which introduced strong hydrogen bonding and decreased their solubility in CH2Cl2. Hence, a small amount of the more polar solvent, methanol, was added to the reaction mixture to break the chain-chain hydrogen bonding. The yellow polymers then redissolved with mild stirring. These solutions were then concentrated, and dialyzed versus 20/80 MeOH/CH2Cl2 for 4d to remove all tetrakis(triphenylphosphine)palladium(0). The 1H NMR characterizations confirmed the removal of the allyl groups on the antibiotic units by the disappearance of the peaks at around 5.89ppm, 5.33ppm, and 4.73ppm. 31P NMR spectra showed no evidence of polymer degradation since the same spectra patterns were obtained before and after deprotection. The characterization data for deprotected polymers are summarized in Table 2-2. The polymers showed increases in Tg after deprotection due to hydrogen bonding generated by the carboxylic acid groups on the antibiotics. After deprotection, polymers were soluble only in DMSO or solvent mixtures such as 20/80 MeOH/CH2Cl2 or MeOH/CHCl3.
Figure 2-5: Deprotection reaction of polymers.

Table 2-2: Replace this with table caption above the table.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$^3$P NMR$^a$ (ppm)</th>
<th>$^1$H NMR$^a$ (ppm)</th>
<th>Tg (°C)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC$^+$</td>
<td>-0.81</td>
<td>8.44 (s, 1H, cipro), 7.88-6.91 (br, 2H, cipro), 4.62-3.89 (br, 2H, ala), 3.72-3.03 (br, 9H, cipro, 1H, ala), 1.50-0.82 (br, 4H, cipro, 6H, ala)</td>
<td>20.0</td>
<td>82%</td>
</tr>
<tr>
<td>AC</td>
<td>-0.47</td>
<td>8.44 (s, 1H, cipro), 7.82-7.03 (br, 2H, cipro), 4.62-3.89 (br, 2H, ala), 3.77-3.02 (br, 9H, cipro, 1H, ala), 1.52-0.89 (br, 4H, cipro, 6H, ala)</td>
<td>21.8</td>
<td>84%</td>
</tr>
<tr>
<td>GC</td>
<td>-1.19</td>
<td>8.46 (s, 1H, cipro), 7.81-6.85 (br, 2H, cipro), 4.39-4.05 (2H, gly), 3.79-3.01 (br, 9H, cipro, 2H, gly), 1.62-0.84 (br, 4H, cipro, 3H, gly)</td>
<td>19.2</td>
<td>80%</td>
</tr>
<tr>
<td>PC</td>
<td>-1.47</td>
<td>8.37 (s, 1H, cipro), 7.86-7.48 (br, 1H, cipro), 7.32-6.88 (br, 1H, cipro, 5H, phe), 4.48-4.02 (br, 2H, phe), 3.98-2.54 (br, 9H, cipro, 3H, phe), 1.37-0.78 (br, 4H, cipro, 3H, phe)</td>
<td>47.5</td>
<td>87%</td>
</tr>
<tr>
<td>AN</td>
<td>-1.03</td>
<td>8.62 (s, 1H, nor), 7.73-7.03 (br, 2H, nor), 4.52-3.96 (br, 2H, nor, 2H, ala), 3.91-2.63 (br, 8H, nor, 1H, ala), 1.42-0.87 (br, 3H, nor, 6H, ala)</td>
<td>25.8</td>
<td>85%</td>
</tr>
<tr>
<td>GN</td>
<td>-0.97</td>
<td>8.42 (s, 1H, nor), 7.68-6.92 (br, 2H, nor), 4.48-3.92 (br, 2H, nor, 2H, gly), 3.90-2.63 (br, 8H, nor, 2H, gly), 1.48-0.77 (br, 3H, nor, 3H gly)</td>
<td>23.2</td>
<td>80%</td>
</tr>
<tr>
<td>PN</td>
<td>-1.26</td>
<td>8.52 (s, 1H, nor), 8.01-7.86 (br, 1H, nor), 7.62-6.67 (br, 1H, nor, 5H, phe), 4.52-3.88 (br, 2H, nor, 2H, phe), 3.82-2.59 (br, 8H, nor, 3H, phe), 1.55-0.75 (br, 3H, nor, 3H, phe)</td>
<td>49.8</td>
<td>90%</td>
</tr>
</tbody>
</table>

$^a$All the NMR spectra were measured in DMSO-d6. $^b$wt% of antibiotics was calculated from the mol% of antibiotics based on $^1$H NMR.
2.3.3 Electrospinning.

Electrospinning is a straightforward method for producing fibrous polymer mats with fiber diameters in the range of nanometers to micrometers.\textsuperscript{45} In an electrospinning process, a strong electrostatic field is applied to a polymer solution held in a syringe with a capillary outlet.\textsuperscript{46} The fibers generated are accumulated on an oppositely charged collector which is usually aluminum foil. The diameter and morphology of the receiving fibers are affected by several parameters, including the molecular weight of the polymer, solution concentration, electric potential, solvent, working distance, and other environmental parameters such as temperature and humidity.\textsuperscript{47} With an increase of concentration of polymers AC, GC, AN and GN from 5\% (w/v) to 20\% (w/v), the morphology of the collected mats changed from beads, beads and fibers, to fine fibers as observed by ESEM. The concentration was optimized at 20\% (w/v) for all these four polymers, and the nano/microfibers showed average diameters of approximately 500nm to 2\,\mu m (Figure 2-6). Unfortunately, polymers PC and PN could not be electrospun into fiber mats even though various conditions were attempted including changing the voltage (5–35kV), working distance (5–20cm), and concentration (5–40\% w/v), or changing the solvent to DMSO. Because the solvent is a vital factor determining the formation of fibers, the limited solubility of these polymers reduces the possibility of successful electrospinning.\textsuperscript{48}

The surface areas (SA, m\textsuperscript{2}/g) of the fiber mats were estimated by the following equations:\textsuperscript{49}

\[ SA = \frac{\text{Area}(m^2)}{\text{Weight}(g)} = \frac{\text{Area}(m^2)}{\text{Volume} \times \text{Density}} = \frac{2\pi r L + 2\pi r^2}{\pi r^2 L \rho} = \frac{2\pi r L}{\pi r^2 L \rho} + \frac{2\pi r^2}{\pi r^2 L \rho} = \frac{2}{r \rho} + \frac{2}{L \rho} \]

where, \( r \) is the average radius of the nanofibers, \( L \) is the length of the nanofibers and \( \rho \) is the density of the polymers (assume as poly[bis(ethyl glycinate-N-yl)phosphazene], 1.16g/mL).\textsuperscript{50} Since the fiber length is significantly larger than the radius, \( 2/L\rho \) approximates to zero. The equation can be rewritten as:
Hence, the fiber mats of polymers AC/AN and GC/GN were calculated as ~3.5 and 0.9 m$^2$/g respectively. The surface areas of AC/AN and GC/GN as fiber mats were about hundreds times larger than the corresponding films (~5×$10^{-3}$ m$^2$/g for each hydrolysis sample).

![Figure 2-6: Selected ESEM images of polymers AC (left) and GC (right) as nanofibers and microfibers. The scale bars are 20µm.](image)

2.3.4 *In vitro* Hydrolysis Studies and Drug Release Profiles.

All the polymers synthesized in this work were sensitive to hydrolysis. The hydrolytic degradation of polymers and the nano/microfiber mats in deionized water (pH=6.70) was monitored over a six week period at 37°C. The percent remaining was calculated as the average of three samples for each week, with the error bar representing the standard deviation. As shown in Figure 2-7 (ciprofloxacin containing polymers as examples), the degradation speed of the polymers followed a trend of GC>AC>PC. This is due to the increased shielding effect of the bulkier and more hydrophobic amino acid ethyl esters as their α-carbon substituent varies from hydrogen to methyl and benzyl for glycine, alanine, and phenylalanine ethyl esters respectively.
Thus, the bulkier substituents more effectively shield the polyphosphazene backbone from access to water. About 87% and 82% percent by weight of the polymers were left for AC and GC as films after the six week study. However, the weights remaining as films for polymers PC were more than 95%. Although polymers AC’ and AC contain different amounts of ciprofloxacin (12% and 25%), their films showed similar degradation patterns, with AC degrading only slightly slower than AC’. This can be explained by a better protection of the polymer backbone generated by the larger number of bulky ciprofloxacin groups in AC. The corresponding polymers AC and GC as nano/microfibers mats showed significantly faster hydrolysis rates (only about 55-60% of mass was left after the six week study). The faster degradation rate of the nano/microfiber mats is due to their significantly higher surface area, as calculated above. Polymers AN, GN, and PN containing norfloxacin in the form of films and as nano/microfibers showed similar degradation patterns compared with the corresponding polymers of AC, GC, and PC, as $\text{GN} > \text{AN} > \text{PN}$.

Figure 2-7: Hydrolysis data of ciprofloxacin containing polymers as films and as fiber mats.
The cumulative drug release profiles were monitored by UV-Vis spectroscopy. The percent of ciprofloxacin or norfloxacin released was calculated by:

\[
\text{Released} \% = \frac{m_{\text{released}}}{m_{\text{drug}}} \times 100\% = \frac{A \times M \times V}{\varepsilon \times m_{\text{initial}} \times \text{wt}\%} \times 100\%
\]

where \( A \) is the absorption at 275nm from UV-Vis spectra of the aqueous media, \( M \) is the molecular weight of the drug, \( V \) is the volume of water in the vials (3mL), \( \varepsilon \) is the molar extinction coefficient, \( m_{\text{initial}} \) is the mass of a sample before hydrolysis study, and \( \text{wt}\% \) is the weight percent of the drug on the polymer backbone. 

Figure 2-8 (ciprofloxacin containing polymers as examples) showed that about 15% of the drugs were released from polymers AC, and about 25% for polymers GC, while less than 5% were released for polymers PC as films. Polymers AC and GC as nano/microfibers showed much more rapid drug release profiles indicate that about 45% of drugs released. The correlated patterns between the hydrolysis and the drug release profiles show the drugs were released coincident with the hydrolysis of the polymers. Mass spectrometry was used to analyze the hydrolysis media from AC, GC and PC in detail to further understand the release patterns of these polymers. For polymers GC as films, ciprofloxacin ([M+H]=331.1) was detected from the first week, which agreed with the UV-Vis results. For polymers AC as films, the antibiotics were detected after the second week based on the mass spectrometry data at the second week which also corresponded to the UV-Vis analysis. For polymer PC as films, the antibiotics were only released after week six. Neither mass spectrometric nor UV-Vis results showed any detectable signals for the presence of antibiotics in the media before this time. We reasoned that at the early stage of the hydrolysis, the polymer backbone was randomly cleaved into shorter chains without the release of any side groups. After that, the increasing accessibility of water to the polymer structure caused the release of the antibiotics. This process is governed by the hydrophobicity of the polymer: the more hydrophobic the polymer, the slower is the process. Polymers containing norfloxacin showed similar release
patterns as GN>AN>PN. The hydrolysis media from all the fiber mats showed the release of antibiotics from the first week as detected by both mass spectrometry and UV-Vis spectroscopy. A bulk degradation mechanism is supported by three items of evidence: (1) a significant molecular weight decrease (AC’ films from 82,000 to <30,000g/mol); (2) a retention of the films’ dimension throughout the hydrolysis study, but embrittlement; (3) an increase in porosity of the polymer films as indicated by the surface images obtained by ESEM (Figure 2-9).51,52

![Figure 2-8: The cumulative release of antibiotics of ciprofloxacin containing polymers as films and as fiber mats.](image1)

![Figure 2-9: ESEM images of polymer AC’ as films for hydrolysis in week 1, 3 and 6.](image2)
The pH analysis of the hydrolysis media showed the presence of near-neutral degradation products ranging from pH 5.9–6.8 for different polymers and time (Figure 2-10, ciprofloxacin containing polymers as examples). The buffering character of this environment is attributed to the phosphates and ammonia produced by the hydrolysis of the polyphosphazene backbone. Meanwhile, the near-neutral pH also showed that no residual chlorine was present along on the polymer backbone. Otherwise hydrochloride acid generated during hydrolysis would lead to a more acidic environment. This relatively constant near-neutral environment also would prevent adverse physiological complications caused by the degradation products.

Figure 2-10: pH values of the hydrolysis media.
2.3.5 *In vitro* Antibacterial Tests of the Hydrolysis Media.

The samples from hydrolysis at week one showed that only the hydrolysis media from polymers **GC** ($C_{antibiotics}=24.5 \mu g/mL$) and **GN** ($C_{antibiotics}=21.4 \mu g/mL$), the nano/microfibers ($C_{antibiotics}=38.3, 44.5, 36.5, 53.1 \mu g/mL$ for **AC**, **GC**, **AN**, **GN** respectively) had antibacterial activity. Hydrolysis media from polymers **AC’** ($C_{antibiotics}=24.6 \mu g/mL$), **AC** ($C_{antibiotics}=28.9 \mu g/mL$), and **AN** ($C_{antibiotics}=37.3 \mu g/mL$) began to show antibacterial character from week two. The hydrolysis media from polymers **PC** ($C_{antibiotics}=22.5 \mu g/mL$) and **PN** ($C_{antibiotics}=19.6 \mu g/mL$) were antibacterial only from week six (**Figure 2-11**). The visual observations matched the optical densities derived from spectrophotometry (**Table 2-3**) and all the results corresponded well to the UV-Vis analyses. The antibacterial activity of these media was detected as long as the antibiotic was being released into the hydrolysis media. Ciprofloxacin and norfloxacin released showed minimum inhibitory concentration values of (MIC)$\leq 0.45$ and $0.58 \mu g/mL$ respectively ($OD_{600}<0.02$) as determined by consecutive double dilutions. The MICs were close to the previous reports of the behavior of ciprofloxacin and norfloxacin against *E.coli*. 28,53,54 This suggests that the hydrolysis environment of the polymers had little affect on the effectiveness of these antibiotics. Meanwhile, in a parallel experiment, polyphosphazenes with homosubstituted amino acid esters (side groups from glycine, alanine, or phenylalanine ethyl esters without antibiotics) showed no antibacterial effects during the six-week’s hydrolysis study because bacterial growth was observed for all of those media ($OD_{600}>0.8$). This suggests that the release of antibiotics from the polyphosphazenes provided the antibacterial character while the amino acid ester groups only functioned to tune the hydrolysis profile.
Polyphosphazenes bearing the fluoroquinoline antibiotics, ciprofloxacin or norfloxacin, were designed and synthesized. Polymers with ~12mol% to 25mol% antibiotics and ~88mol% to 75 mol% amino acid esters, including alanine, glycine, phenylalanine, were synthesized by macromolecular substitutions using allyl-protected ciprofloxacin or norfloxacin together with different amino acid esters, followed by the removal of the allyl group under mild conditions. The hydrolysis behavior and drug release profiles of all the polymers as films and several selected polymers as nano/microfiber mats were studied during a six week in vitro hydrolysis test at 37°C. The hydrolysis rates of the polymer films were dependent on the different amino acid esters linked to the polymer backbone, and these rates were characterized by from 5% to 23% mass loss.

2.4 Conclusions
over the six-week period. The hydrolysis rates for the correspondent polymer nano/microfibers were characterized by much faster degradation rates, with 40% to 45% mass loss over the same time period. The pH analyses revealed a near neutral degradation environment. The amount of antibiotics released in the hydrolysis media was monitored by a UV-Vis spectroscopy, and this showed degradation-dependent releases of about 4% to 30% from polymer films and 45% to 50% from nano/microfibers. Mass spectrometric analysis of the aqueous hydrolysis media also confirmed the release of ciprofloxacin or norfloxacin. Moreover, based on in vitro antibacterial tests performed against E.coli, the hydrolysis media showed antibacterial capabilities for as long as the antibiotics were being released. These results give an indication of the design parameters for the development of devices for the release of antibiotics. The rate of release in this system can be well tuned by either different polymer compositions or via the surface area of the materials. Overall, polymers that contained alanine ethyl ester or glycine ethyl ester cosubstituents showed faster degradation rates and antibiotic release profiles than the polymers that contained phenylalanine ethyl ester cosubstituents. These polymers could be used as antibacterial biomaterials for tissue engineering, as coatings and blends with other implantable biomaterials to prevent post-surgery infections, or as pro-drugs for local chronic infection treatments. The faster antibiotic release profiles of the nano/microfiber mats make them particularly promising materials for advanced wound dressings.55-57

2.5 References

45. Huang, Z. M.; Zhang, Y. Z.; Kotaki, M.; Ramakrishna, S. Compos. Sci. Technol. 2003, 63, 2223.
Chapter 3

Injectable and Biodegradable Supramolecular Hydrogels by Inclusion Complexation Between Poly(organophosphazenes) and α-Cyclodextrin

3.1 Introduction

Hydrophilic hydrogels are three-dimensional crosslinked networks derived from water-soluble polymers. They are of special interest due to their unique properties and potential applications, such as tissue engineering matrixes, drug delivery, and cell immobilization.\textsuperscript{1-5} Water-soluble polymers can be crosslinked in various ways. Chemical crosslinks can be generated either by a reaction between functional polymers or by the addition of small molecule crosslinking agents.\textsuperscript{6,7} Physical non-covalent crosslinking of polymer chains can be achieved by physicochemical interactions including hydrophobic interactions, charge condensation, hydrogen bonding, or stereocomplexation.\textsuperscript{8-11} Although physical interactions are weaker than chemical crosslinking, they allow useful properties such as processibility and reversibility. Cyclodextrins (CDs), usually α-, β-, and γ-CD, which consist of 6, 7, and 8 glucose units respectively, have been widely studied to form inclusion complexes with oligomers or polymers including poly(ethylene glycol),\textsuperscript{12} poly(propylene glycol),\textsuperscript{13} poly(ε-caprolactone),\textsuperscript{14} polyisobutylene,\textsuperscript{15} poly(ε-lysine),\textsuperscript{16} etc.\textsuperscript{17} Supramolecular hydrogels can be obtained in water when α-CD interacts with poly(ethylene glycol)-grafted hydrophilic polymers (heparin or dextrin),\textsuperscript{18,19} or poly(ethylene glycol)-containing block copolymers such as poly(ε-caprolactone)-\textit{b}-poly(ethylene glycol)-\textit{b}-poly(ε-caprolactone) or poly(ethylene glycol)-\textit{b}-poly(ε-caprolactone)-\textit{b}-poly[2-(dimethylamino)ethyl methacrylate].\textsuperscript{20,21}

In these cases, the hydrogen bonding generated by inclusion complexation functions as a physical crosslinking process. Since these hydrogels show desirable properties, it is interesting to develop
other alternative approaches with facile syntheses, tunable properties, and good biological behavior, especially biocompatibility and tunable biodegradability.

Polyphosphazenes are hybrid polymers with a backbone of alternating phosphorus and nitrogen atoms with two side groups attached to each phosphorus.\textsuperscript{22} Their synthesis is based on the reactions of poly(dichlorophosphazene) which is prepared by a thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 250 °C in a sealed system.\textsuperscript{23} This reactive polymeric intermediate can then undergo facile and tunable substitution reactions through the replacement of chlorine atoms by various nucleophiles such as alkoxides,\textsuperscript{24} aryloxides,\textsuperscript{25} or amines,\textsuperscript{26} and various combinations of the above. Hydrolytically sensitive polyphosphazenes are formed when amino acid ethyl ester groups are linked to the polymer backbone via the amino terminus.\textsuperscript{27} The nontoxic hydrolysis products are the parent amino acids, ethanol, phosphates, and ammonia, a mixture that results in a buffer system of near-neutral pH.\textsuperscript{28,29} Polyphosphazenes have long been proposed for hydrogel applications, and several repeats have been published previously. For example, hydrogels were prepared by γ-radiation crosslinking of hydrophilic methoxyethoxyethoxy containing polyphosphazene (MEEP) or MEEP derivatives.\textsuperscript{30,31} Poly(organophosphazenes) containing isoleucine ethyl ester and oligo(ethylene glycol) substituents showed a reversible sol-gel transition at tunable temperatures depending on the polymer composition.\textsuperscript{32,33} Ionically crosslinked hydrogels were achieved by serine- or threonine-containing hydrophilic polyphosphazenes when the carboxylic acid groups were exposed to Ca\textsuperscript{2+}.\textsuperscript{34} pH-Responsive hydrogels were also generated from hydrophilic polyphosphazenes that bear aryl-carboxylate and methoxyethoxyethoxy substituents.\textsuperscript{35} By combining the advantages of polyphosphazenes and supramolecular hydrogels, a novel hydrogel system can be visualized with desirable properties for biomedical applications.

In the work described here, biodegradable poly(organophosphazenes) have been synthesized by grafting poly(ethylene glycol) methyl ether (mPEG) and about 20% of glycine ethyl ester to
the phosphazene backbone. After mixing aqueous solutions of the polymers and α-CD, supramolecular hydrogels were formed rapidly by the aggregation of polyrotaxane-like side chains resulting from the inclusion complexations (IC) between mPEG grafts and α-CD (Figure 3-1). Rheological measurements were then performed to characterize the hydrogels which show a fast gelation process and an injectable rheological behavior. It is interesting that the hydrogels obtained exhibit a unique thermo-reversible gel-sol transition based on supramolecular assembly and dissociation. The evidence of IC was explored by various techniques, such as TGA, DSC, $^{13}$C CP/MAS NMR and X-ray diffraction. In vitro model protein release was studied using bovine serum albumin (BSA). Both the stability of the hydrogel and the biodegradability of the polymers were studied at 37 °C by monitoring the mass loss and the molecular weight decrease respectively.

Figure 3-1: Schematic illustration of the supramolecular poly(organophosphazene) hydrogels.
3.2 Experimental Section

3.2.1 Reagents and Equipment.

Tetrahydrofuran (THF) and triethylamine were purchased from EMD and were dried using solvent purification columns. Glycine ethyl ester hydrochloride (GlyEE, Chem Impex), α-cyclodextrin (Chem Impex), and NaH (60% in paraffin, Sigma-Aldrich) were used as received. Poly(ethylene glycol) methyl ethers (mPEGs, Sigma-Aldrich) with average $M_n$=350, 550, 750, 2000, and 5000 g/mol were dried under vacuum extensively before use. Bovine serum albumin (BSA) was purchased from EMD Millipore Biosciences (M.W.~66,000 g/mol). Poly(dichlorophosphazene) was prepared by the thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical, Japan) in evacuated Pyrex tubes at 250°C. All synthesis reactions were carried out using standard Schlenk line techniques and a dry argon atmosphere. The glassware was dried overnight in an oven at 120 °C before use. $^1$H and $^{31}$P spectra were recorded with use of a Bruker WM-360 NMR spectrometer operated at 360 or 145 MHz, respectively. $^1$H NMR spectra were referenced to solvent signals, while $^{31}$P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns, and calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt% solution of tetra-n-butylammonium nitrate (Sigma-Aldrich) in THF. The ultraviolet absorptions of BSA release were measured with a CARY50 Bio UV-Visible spectrophotometer (Varian). The IC rates were determined by a LKB Biochrom Ultrospec
4050 spectrophotometer with a wavelength of 700 nm at room temperature. The visual images were captured by a Canon EOS Rebel XS digital camera.

### 3.2.2 Synthesis of Cyclic Trimer Models

The synthesis of mPEG350 hexa-substituted cyclic phosphazene trimer \( \text{(N}_3\text{P}_3\text{(mPEG350)}_6 \) is representative of the procedures used to synthesize all the small molecule trimer models. To a THF solution (50 mL) of hexachlorocyclotriphosphazene (1 g, 4.04 mmol), was added the sodium salt of mPEG350 prepared by stirring mPEG350 (11.3 g, 32.3 mmol) and NaH (1.3 g, 32.3 mmol) in THF (100 mL) overnight at room temperature. The reaction mixture was stirred for 1 day at room temperature, and was then dialyzed versus methanol for 4 days and methanol/acetone/hexanes (40/20/40) for 1 day (Spectra/Por dialysis membrane, MWCO: 1,000). Solvent was removed, and the product was dried under vacuum overnight (yield: 45–74%). For the syntheses of \( \text{N}_3\text{P}_3\text{(mPEG2000)}_6 \) and \( \text{N}_3\text{P}_3\text{(mPEG5000)}_6 \), the mPEG2000 and mPEG5000 were pre-dissolved in hot THF before treatment with NaH. The substitution of mPEG2000 and mPEG5000 were completed under reflux for 2 days. \(^{31}\)P NMR (CDCl\(_3\)): \( \delta \) 19.01 (s). \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 4.02 (s, 2H, -OCH\(_2\)CH\(_3\)-), \( \delta \) 3.63 (m, 22H, -OCH\(_2\)CH\(_2\)-), \( \delta \) 3.36 (s, 3H, -OCH\(_3\)).

### 3.2.3 Synthesis of Polymers

The syntheses of all the polymers followed a similar pattern, and the preparation of PmPEG350 is demonstrated in detail. Briefly, poly(dichlorophosphazene) (0.5 g, 4.32 mmol) was dissolved in anhydrous THF (100 mL). Meanwhile, mPEG350 (2.42 g, 6.90 mmol) and NaH (0.28 g, 6.90 mmol) suspensions were stirred in anhydrous THF (100 mL) at room temperature overnight to give a brownish solution. It was then added to the polymer solution dropwise and
allowed to react at room temperature for 1 day to give a partially (80 mol%) substituted polymer. In a separate Schlenk flask, a suspension of glycine ethyl ester hydrochloride (0.60 g, 4.32 mmol) and triethylamine (5.0 mL, 25.89 mmol) in dry THF (50 mL) was stirred at room temperature for 6 h to remove the hydrochloride component. It was then added to the polymer flask by filter addition to complete the substitution reaction overnight at room temperature. The reaction medium was then concentrated and dialyzed versus water for 2 days, then methanol for 2 days, and methanol/acetone/hexanes (40/20/40) for 1 day (Spectra/Por dialysis membrane, MWCO: 12–14,000). The product was dried under vacuum to give PmPEG350 as a pale yellow viscous polymer (yield: 52%–80%). For the syntheses of PmPEG2000 and PmPEG5000, mPEG2000 and mPEG5000 needed to be pre-dissolved in hot THF before addition to NaH suspension in THF. The substitutions of mPEG2000 and mPEG5000 required 2 and 5 days under reflux, followed by completing the substitution with glycine ethyl ester for 1 and 2 days under reflux. \(^{31}\text{P NMR (CDCl}_3\):} \(\delta -1.11\) (br), \(\delta -6.63\) (br). \(^1\text{H NMR (CDCl}_3\):} \(\delta 4.07\) (s, 2H, GlyEE, -OCH\(_2\)CH\(_3\)), \(\delta 3.98\) (br, 2H, mPEG, -OCH\(_2\)CH\(_2\)), \(\delta 3.69\) (s, 2H, GlyEE, -CH\(_2\)COO\(^-\)), \(\delta 3.61-3.49\) (br, 22H, mPEG, -OCH\(_2\)CH\(_2\)), \(\delta 3.34\) (s, 3H, mPEG, -OCH\(_3\)), \(\delta 1.20\) (t, 3H, GlyEE, -CH\(_2\)CH\(_3\)).

3.2.4 Supramolecular Hydrogelation.

The gelation of mPEG2000 grafted polymer (PmPEG2000) with \(\alpha\)-CD is given as an example. PmPEG2000 (80 mg) was dissolved in water (8 wt%) as a hydrogel precursor, and then mixed with aqueous \(\alpha\)-CD (175 mg) solution (10 wt%) at room temperature. The resultant mixture was shaken mechanically during the gelation. The gelation times of all the hydrogels were estimated by a vial-tilting method. The timing was started immediately after mixing two components until no flow was observed for at least 30 s when a vial containing the hydrogel was inverted.\(^{21,36}\) All the hydrogels were prepared in a similar manner, and depending on the molar ratio between
ethylene glycol repeating units and α-CD, the resultant mixtures could be in the form of precipitates, hydrogels, viscous liquids, or solutions. To investigate the gelation kinetics, time sweep tests were performed at a constant oscillatory frequency of 1 rad/s under a strain of 1% by an Advanced Rheometric Expansion System (Rheometric Scientific). The samples were loaded on a parallel plate (25 mm diameter, 1mm gap) at 25 °C immediately after mixing. The measurement started at 60 s after mixing, and the results were recorded every 10 s. The gel-sol transition temperature (T_{trans}) and the reverse sol-gel transition (T'_{trans}) were determined by a vial inversion method with a monotonic temperature increase or decrease of 1 °C per step. Thus, the vials of hydrogel samples were immersed in a water bath at each temperature for 10 min, the T_{trans} and T'_{trans} were monitored visually according to whether the hydrogels flowed when inverting the vials for 30 s. To further characterize the IC samples, they were freeze-dried and stored under vacuum.

3.2.5 Characterization of the Inclusion Complexations.

The evidence for IC formation between mPEG grafts and α-CD to form polyrotaxane-like side chains linked polyphosphazenes was collected by various means, including the following:

**Thermal Property Analysis.** Thermal characteristics of samples were measured with a TA Instruments Q10 differential scanning calorimeter (DSC) and a Perkin-Elmer thermogravimetric analyzer (TGA). About 10mg of freeze-dried sample was used for each test. A heating rate of 10 °C/min with a temperature range from -100 to 200 °C was used for DSC, while a heating rate of 20 °C/min from 25 to 800 °C was applied for TGA. Both instruments used dry nitrogen as the purge gas.
^{13}C CP/MAS NMR. Solid-state $^{13}$C CP/MAS (cross polarization/magic angle spinning) NMR spectra were measured at 75.5 MHz on a Bruker AV-300 NMR spectrometer at room temperature. The amount of sample used was 100 mg and the accumulated scans were 18K.

**Powder X-ray Diffraction.** X-ray diffraction was recorded on powdered samples of freeze dried hydrogels using a wide-angle setup of a Bruker D8 Advance diffractometer. The radiation source used was Ni-filtered, CuKα radiation with a wavelength of 1.54 Å. The voltage was set to 40 kV and the current was set to 40mA. Samples were mounted on a circular sample holder, and the data were collected at a rate of $2\theta = 5^\circ \text{min}^{-1}$ over the range $2\theta = 5^\circ$–$40^\circ$.

3.2.6 *In Vitro* Bovine serum albumin (BSA) Release and Hydrogel Dissociation.

For *in situ* encapsulation of BSA, 8mg of BSA was dissolved in a pre-dissolved PmPEG2000 solution (80 mg, 8 wt%) in a phosphate buffered saline (PBS, 0.0027 M, pH=7.4). At the same time, an appropriate amount of α-CD (175 mg) was dissolved in PBS in a separate vial (10 wt%). The α-CD solution was then added to the polymer solution to induce supramolecular gelation at room temperature. To study the *in vitro* release behavior, the BSA-loaded hydrogel was immersed in PBS (10 mL) and secured in a shaker bath maintained at 37 °C. At predetermined time points, 3 mL aliquots of the solution were taken out, filtered through a 0.45 μm syringe filter before UV-Vis measurements, and then added back to maintain the same solution volume. The cumulative percent release of BSA was determined by UV-Vis spectroscopy at 279 nm in a 1×1 cm quartz cuvette at room temperature. The molar extinction coefficient of BSA in PBS solution was 43,800 Lmol$^{-1}$cm$^{-1}$ calculated from a linear Beer-Lambert plot of absorbance at 279 nm versus concentrations. The dissociation of the hydrogels was studied by the weight loss of the supramolecular hydrogels in PBS solution. Briefly, a series of supramolecular hydrogels with known weights were prepared as in the method above. Then, they were immersed in 10 mL PBS,
and placed in a shaker bath at 37 °C. Samples were taken out at predetermined times. The upper aqueous medium was decanted and the remaining hydrogels were freeze-dried. The percent mass loss of the supramolecular hydrogel was recorded based on the mass of freeze-dried hydrogel left and the total mass of polymer and α-CD used initially. All the studies above were carried out in triplicate with the error bars representing the standard deviations. The degradability of the polymers was determined by monitoring the molecular weight decrease in PBS solution. The dried polymers (∼10 mg each) were dissolved in PBS solution (5 mL) in 10 mL glass vials maintained at 37 °C. Each time, a sample was dried and the remaining solid was redissolved in THF (2 mL) for GPC analysis.

3.3 Results and Discussion

3.3.1 Polymer Synthesis and Characterization.

The syntheses of mPEG-containing phosphazene small molecule cyclic trimers were attempted first as model systems as a prelude to polymer syntheses. This provided data for the greater synthetic challenges of the macromolecular substitution reactions. As monitored by $^{31}$P NMR, full substitutions of mPEG350, mPEG550, and mPEG750 on the trimers were obtained within 1 day in THF at room temperature, while the full substitutions with mPEG2000 and mPEG5000 required 2 days under reflux. Even though mPEG2000 and mPEG5000 have longer chain lengths, the full substitution on the phosphazene cyclic trimers was still achieved, probably due to the high flexibility of mPEGs. However, the lower reactivity was expected due to the increased steric hindrance and the shielding of reaction sites by the longer mPEGs.

The success of the cyclic trimer reactions demonstrated the feasibility of linking mPEGs to the linear high polymers, and the substitution conditions for the trimers were used to guide the
polymer syntheses. All the polymers in Figure 3-2 were prepared by a simple two-step synthetic route in which 80% of mPEG was attached by macromolecular substitution in the first step, followed by the reaction of an excess of GlyEE in the second step to ensure complete replacement of the chlorine atoms. The objective was to link about 20% of glycine ethyl ester (GlyEE) to the phosphazene polymer backbone to create biodegradability. The rationale for this addition sequence is two-fold. First, the replacement of chlorine by mPEGs in the first step (P–O linkage) protects the polymer backbone from degradation when the amino acid is added in the second step (P–N linkage). Even though the hydrochloric acid formed in the replacement reaction by the amino acid normally complexes with added triethylamine, it can also attack the nitrogen atoms in the polyphosphazene backbone causing P–N bond cleavage, especially during long reaction times. Second, the addition of smaller side groups in the last step can facilitate the replacement of the remaining chlorine atoms due to their better accessibility to the polymer backbone. In this case, the steric bulk of GlyEE is substantially less than that of mPEG, which can ensure complete halogen replacement if this reagent is added last. During the macromolecular substitutions, similar reactivities were found to those of the cyclic trimer model reactions. The introduction of 80% of mPEG350, mPEG550, and mPEG750 could be completed at room temperature within 1 day as shown by the peak at ~-7ppm (O-P-O) and ~-12ppm (O-P-Cl) in the $^{31}$P NMR spectra. However, 2 days and 5 days in boiling THF were required for mPEG2000 and mPEG5000 respectively. Another important synthetic consideration is that during the substitution of mPEG5000, the THF solution of mPEG5000 sodium salt was added slowly to the vigorously stirred polymer solution at 50 °C over a period of 1 hour to prevent the possible gelation caused by the entanglement between poly(dichlorophospahzene) and mPEG5000. The substitution reaction became considerably slower after the attachment of 50% of mPEG5000 to the polymer backbone. The reaction of GlyEE in the second step was completed within 1 day for PmPEG350, PmPEG550 and PmPEG750 at room temperature, while it took 1 and 2 days in boiling THF for
PmPEG2000 and PmPEG5000 respectively. The completion of the reaction was monitored by the appearance of a new peak at $\sim$1 ppm representing the co-substitution pattern of GlyEE and mPEGs (O-P-N), and the disappearance of the peak at $\sim$12 ppm in the $^{31}$P NMR spectrum. With the increasing length of the mPEG segments, the resultant polymers changed from adhesive elastomers to solid powders. In the $^1$H NMR spectra, the integration at 3.59 ppm (-OCH$_2$CH$_2$-) increases as the number of repeat units of mPEG increases. The percentage of GlyEE on the polymer was calculated from the peaks of 1.20 ppm (-CH$_3$, GlyEE) and 3.34 ppm (-OCH$_3$, mPEG). The polymers show a decreasing solubility in THF with increasing length of the mPEG segments, but all the polymers are soluble in chloroform and water. PmPEG350, PmPEG550, PmPEG750 showed $M_n$=3.2×10$^5$, 4.2×10$^5$, and 9.1×10$^5$ g/mol from GPC, while no molecular weight information could be obtained for PmPEG2000 and PmPEG5000 due to their insolubility in THF.

3.3.2 Supramolecular Hydrogelation.

It is well known that low molecular weight poly(ethylene glycol)s form white precipitates of inclusion complexes (IC) with $\alpha$-CD with a stoichiometry of 2:1 (ethylene glycol unit: $\alpha$-CD) in

![Figure 3-2: Synthesis of mPEG grafted poly(organophosphazenes).]
aqueous solution.\textsuperscript{37} This inclusion effect depends on the molecular weight of the poly(ethylene glycol)s.\textsuperscript{38} In this work, precipitations were observed for PmPEG550, PmPEG750, PmPEG2000, and PmPEG5000 when mixed with \( \alpha \)-CD solution at a stoichiometry of 2:1 in water. The IC rates were monitored by the optical density at 700 nm as shown in Figure 3-3. With increasing length of the mPEG side chain up to mPEG2000, the IC rate increased which suggests that stable \( \alpha \)-CD complexes could form when the pendent mPEG was sufficiently long. However, after further length increases, the efficiency of IC decreased.

![Figure 3-3: Replace this with figure caption below the figure.](image)

Supramolecular hydrogels were obtained by decreasing the amount of \( \alpha \)-CD used (to increase the ratio to 3:1 or higher) leaving an appropriate amount of mPEG repeating units unthreaded. As a consequence, the complexed mPEG units worked as physical crosslinking units, while the uncomplexed units maintain their hydrophilicity (Figure 3-1). As the ratio increased to 4:1–6:1 for PmPEG5000, 4:1–5:1 for PmPEG2000 or 3:1 for PmPEG750, hydrogels were obtained instead of precipitates (Figure 3-4). After mixing, the polymer solution first became white, then
the turbid mixture gradually became more viscous, and finally formed a white hydrogel. The gelation time for different polymers, as shown in Table 3-1, corresponded well with the data in Figure 3-3: the α-CD first threaded onto the mPEG side chain (turned white), followed by supramolecular gelation as the threaded side chains aggregated (gel). However, further increases in the molar ratio only produced viscous mixtures due to weak physical crosslinks, a consequence of insufficient aggregation. Moreover, the gelation required a longer time for the polymers at lower concentrations or lower molar concentrations of α-CD used. However, for PmPEG550, the polymer solution slowly turned white with only a slight increase in viscosity. In this case, the side chain aggregations were not enough for effective physical junction formation due to the shorter length of the mPEG grafts. For PmPEG350, no change in viscosity was detected within 30 min.

Table 3-1: Preparation of Supramolecular Hydrogels.

<table>
<thead>
<tr>
<th>Gel precursor</th>
<th>Gel compositiona</th>
<th>Results</th>
<th>Ttrans (Ttrans')</th>
<th>°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PmPEG 5000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4:1</td>
<td>G1</td>
<td>5</td>
<td>60(42)</td>
</tr>
<tr>
<td>8</td>
<td>5:1</td>
<td>G2</td>
<td>10</td>
<td>58(40)</td>
</tr>
<tr>
<td>4</td>
<td>6:1</td>
<td>G3</td>
<td>5</td>
<td>56(40)</td>
</tr>
<tr>
<td>4</td>
<td>5:1</td>
<td>G4</td>
<td>12</td>
<td>55(39)</td>
</tr>
<tr>
<td>4</td>
<td>6:1</td>
<td>G5</td>
<td>6</td>
<td>54(38)</td>
</tr>
<tr>
<td>4</td>
<td>4:1</td>
<td>G6</td>
<td>20</td>
<td>52(38)</td>
</tr>
<tr>
<td>PmPEG 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4:1</td>
<td>G7</td>
<td>3</td>
<td>46(35)</td>
</tr>
<tr>
<td>4</td>
<td>5:1</td>
<td>G8</td>
<td>10</td>
<td>46(33)</td>
</tr>
<tr>
<td>4</td>
<td>5:1</td>
<td>G9</td>
<td>5</td>
<td>45(33)</td>
</tr>
<tr>
<td>4</td>
<td>3:1</td>
<td>G10</td>
<td>20</td>
<td>42(32)</td>
</tr>
<tr>
<td>PmPEG 750</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3:1</td>
<td>G11</td>
<td>6</td>
<td>40(29)</td>
</tr>
<tr>
<td>4</td>
<td>3:1</td>
<td>G12</td>
<td>15</td>
<td>38(28)</td>
</tr>
</tbody>
</table>

aThe concentration of α-CD solution was kept at 10 wt% for all the studies.
bStoichiometry of ethylene glycol repeating unit: α-CD. cStudies limited to 60 min.
Figure 3-4: Photographs of supramolecular gelation of G1 and G7 (bottom up).

Figure 3-5: Rheological measurements of the selected supramolecular gelations (frequency, 1.0 rad/s; stain, 1.0%).
Time sweep measurements were conducted for the viscoelastic behavior of the supramolecular gelation system. Storage modulus (G’) and loss modulus (G”) were monitored as a function of time after the mixing of the polymer and α-CD solutions. Data for selected hydrogels are shown in Figure 3-6 as examples. The measurement started from 60s after mixing the two components due to the time needed for sample loading and instrument setup. As the gelation proceeded, both moduli increased and the buildup rate of G’ was higher than G” due to the formation of a physical crosslinked network by the IC. Generally speaking, a crossover point (G’=G”) of G’ and G” curves was detected for a gelation process (from G’<G” to G’>G”). In this work, no crossover point was found for the gelation processes in the time range investigated (from 60 s after mixing) since G’ was higher than G” from the beginning of the measurement, implying a fast gelation, and the crossover point that was less than 60s.

Figure 3-6: Dynamic step strain tests of selected supramolecular hydrogels.
To confirm the recovering property of the supramolecular hydrogel system which is critical for injectable applications, a dynamic step strain amplitude test was performed on the selected gels (Figure 4). In this measurement, the initial G’ after gelation was found to be about $1.5 \times 10^4$ Pa, $1.8 \times 10^4$ Pa, $9.7 \times 10^3$ Pa, and $5.1 \times 10^3$ Pa respectively for G1, G2, G7, and G8 at a small strain (1%). When a large strain (100%) was applied to the hydrogel, G’ immediately decreased dramatically to 6 Pa, 5 Pa, 10 Pa, and 14 Pa. Meanwhile, G’ decreased to less than G” suggesting that the gelled systems were disrupted. When the strain was returned to the small value (1%) again, G’ of all the systems recovered rapidly to above G” as the gel network reformed, and the values finally recovered to about their original moduli within 300s. This result is explained by the nature of supramolecular hydrogel systems, since the crosslinks in such systems are transformable and recoverable. To visually confirm the rheological tests, the supramolecular hydrogel (G1) was allowed to form in a 5 mL syringe fitted with a d=0.9 mm needle. The hydrogel could be easily extruded out from the needle as a white viscous liquid, which re-gelled immediately. Such rheological behavior of the supramolecular hydrogel is desirable as an injectable hydrogel matrix for drug or protein delivery.

The hydrogels exhibited a reversible gel-sol transition with increasing or decreasing temperatures (Table 3-1). They became mobile above a certain temperature ($T_{trans}$), and returned to an opaque gel after cooling to a certain temperature ($T_{trans}$). For hydrogels G1–G10, the immobile opaque gel turned into an opaque sol when $T > T_{trans}$. The inter-chain physical crosslinking was interrupted by the dissociation of the polymeric guests from $\alpha$-CD hosts at higher temperatures. However, a complete dethreading of $\alpha$-CDs was not achieved for the mPEG5000 and mPEG2000 side chains because a semi-opaque mixture remained even after further increases in the temperature. For hydrogels G11–G12, the opaque gels also turned into opaque sols when $T > T_{trans}$, and the opaque sols became clear solutions with further increases in
the temperature to above 70 °C since most α-CDs dethreaded from the mPEG750 chains and dissolved in water. This observation is consistent with other studies as the dethreading of α-CD from a polymer chain occurs when the polymer chain is more mobile at higher temperatures. The dethreading process becomes more difficult for mPEGs with a longer chain length due to the presence of more chain distortions and entanglements. All the hydrogels showed reversible gel-sol transitions. When the T\textless T_\text{trans}', the opaque hydrogels were formed again, and the inconsistent temperature of T_\text{trans} and T_\text{trans}' can be explained by the hysteresis effect.

It is interesting that the addition of an excess molar amount of sodium benzoate aqueous solution brings about rapid dissolution of the gel to yield a semi-clear solution. Small molecule guests such as sodium benzoate can compete with mPEG to form IC with α-CD, thus causing the dissociation of α-CD from mPEG strands. This result also supports the mechanism that the supramolecular gelation is induced by the supramolecular interaction of α-CD and mPEG side chains. Meanwhile, the addition of β-CD solution at any molar ratio to the polymer solutions showed no gelation or precipitation. Based on the previous works by Harada and Kamachi, even though β-CD can complex with some linear short chain polymers such as poly(propylene glycol), the tendency for them to complex with poly(ethylene glycol) is nearly nonexistent due to the substantially smaller cross-sectional area of poly(ethylene glycol) compared with the cavity of β-CD.

Hydrogels could also be prepared using mPEG hexa-substituted star-like phosphazene cyclic trimers with α-CD. However, the resulting gels showed less desirable properties such as long gelation time, low gel strength, and low dissociation temperature even with the substitution of mPEG5000. Thus, certain chain entanglements that serve as additional physical crosslinks are essential for maintaining a strong gel network. Thus, hydrogels with mPEG-linked star-structured phosphazene trimers are not discussed in detail in this thesis.
3.3.3 Characterization of the Inclusion Complexations.

PmPEG2000 is given as an example for the evidence of IC formation. The thermal properties of PmPEG2000 and the freeze-dried α-CD threaded precipitate (PmPEG2000 IC) were studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). As shown in Figure 3-7, a strong melting peak was detected for the graft copolymer PmPEG2000 at around 60°C, resulting from the melting of the mPEG2000 chains. However, for PmPEG2000 IC, there was no observed melting peak corresponding to the melting of mPEG because, as long as the mPEG chains are included, they are isolated in the α-CD inner core with the disappearance of any melting characteristics. Meanwhile, α-CD showed no peak in the temperature range. TGA revealed that α-CD had a higher decomposition temperature (T_d=290 °C) after threading on mPEG2000 side chains than pure α-CD (T_d=275 °C) implying that the polymer chains included inside the α-CD molecules improve the thermal stability of the α-CD (Figure 3-7). The two thermal characterizations corresponded well with previous studies on IC of α-CD and polymers.44
Pure α-CD showed a less symmetrical conformation when no mPEG guests were threaded in the cavity. In this case, the $^{13}$C CP/MAS NMR spectrum showed multiple resolved C1 and C4 resonances of the glucose units (1,4-linked, conformationally strained C1 and C4) at ~102 and 83 ppm in Figure 3-8. When polymer guests were introduced into the internal cavity of α-CD to form a channel structure (PmPEG2000 IC), C1 and C4 showed as broad single resonances and the shoulder of C1 in pure α-CD with multiple peaks (95–98 ppm) disappeared. The result indicates that α-CD was in a symmetrical conformation and each glucose unit of α-CD was in a similar environment.\textsuperscript{12,16,45}

Figure 3-7: Thermal Characterizations of pure PmPEG2000, freeze-dried PmPEG2000 IC, and α-CD: (a) DSC study, (b) TGA study.
Results from X-ray diffraction are more persuasive in proving the inclusion complex between α-CD and the poly(organophosphazene). As shown in Figure 3-9, like other studies on CD inclusion complexes,\textsuperscript{46-50} the PmPEG2000 IC showed two characteristic diffraction peaks at 2θ=20.2° and 22.7° which were not detected in the diffraction patterns of either PmPEG2000 or α-CD. These two diffractions are typical peaks observed for the IC of α-CD with a channel type structure (necklace-like) such as the IC between α-CD and valeric acid.\textsuperscript{51,52}

Figure 3-8: $^{13}$C CP/MAS NMR spectra of PmPEG2000 IC, and pure α-CD.

Figure 3-9: X-ray diffraction pattern of pure α-CD, pure PmPEG2000, and freeze-dried PmPEG2000 IC.
3.3.4 In Vitro BSA Release and Hydrogel Dissociation.

The new prospective injectable drug delivery system based on biodegradable poly(organophosphazene) supramolecular hydrogels has potential applications for the encapsulation, localization, and sustained release of bioactive compounds. BSA was used as a model protein for this study. The release behavior of the hydrogel was studied in physiological-mimic conditions in PBS solution at 37 °C. The in vitro cumulative percentage of BSA released was calculated based on Beer-Lambert’s law:

\[
\text{\% Released} = \left( \frac{m_{\text{released}}}{m_{\text{initial}}} \times 100 \% \right) = \left( \frac{A \times M \times V}{\varepsilon \times m_{\text{initial}}} \right) \times 100 \% \quad (1)
\]

where \( A \) is the absorption at 279 nm from UV-Vis spectra of the PBS media, \( M \) is the molecular weight of the BSA (66,000 g/mol), \( V \) is the volume of PBS solution in the vials (10 mL), \( \varepsilon \) is the molar extinction coefficient (43,800 L·mol⁻¹·cm⁻¹), and \( m_{\text{initial}} \) of BSA is 8 mg.

According to Figure 3-10, systems G11 and G7 showed burst releases in 2–5 days, while G2 and G1 gave longer release times over 10–12 days. The release patterns are explained by combining the dissociation profiles of the hydrogels (Figure 3-11). G11 and G7 showed significant structural dissociations suggesting that the BSA release was not diffusion-controlled, and the morphological change of the matrix was abrupt. The drastic erosion of the supramolecular hydrogels was induced by a significant dethreading of the \( \alpha \)-CD from mPEG750 and mPEG2000 side chains when the hydrogels are exposed to a large amount of water at 37 °C, and the physical interactions, which are essential for maintaining the hydrogel structure, fall apart.\(^5\) G2 and G1 prepared by the polymers containing mPEG5000 showed a better stability and a longer release profile since the longer length of mPEG prevents the dethreading of \( \alpha \)-CD by more entanglement. The hydrogels (G1) with higher polymer concentrations showed better stability and a slower release profile than G2. But dissociation of hydrogels G2 and G1 were still detected (Figure 3-11)
at a slow and steady rate suggesting that the release mechanism is a combination of the structural relaxation of the hydrogels and the diffusion of BSA. A calculation based on equation (2) was applied to verify the release mechanism of G2 and G1:\textsuperscript{19,53}

\[
\frac{M_t}{M_\infty} = K t^n \quad \text{(for } \frac{M_t}{M_\infty} \leq 0.6) \tag{2}
\]

where \(M_t\) and \(M_\infty\) are the cumulative amount of the BSA released at a time \(t\) and equilibrium, respectively. \(K\) is the rate constant relating to the properties of the hydrogel matrix and the drug, and \(n\) is the release exponent characterizing the transport mechanism. By transforming equation (2) to a logarithmic equation:

\[
\log\left(\frac{M_t}{M_\infty}\right) = n \log(t) + \log K \tag{3}
\]

the value of \(n\) can be obtained by plotting \(\log(M_t/M_\infty)\) versus \(\log(t)\). The \(n\) values for G2 and G1 were calculated to be 0.83±0.05 and 0.78±0.05 respectively, suggesting an anomalous transport process (0.5<\(n<1\), Case III) as the structure change and the diffusion are comparable.\textsuperscript{54,55}

All the polymers synthesized in this work were sensitive to hydrolysis. For example, the molecular weight of PmPEG750 (soluble in THF) declined rapidly to almost 1/5 (from 9.1×10\(^5\) g/mol to 1.9×10\(^5\) g/mol) after one week in PBS solution at 37°C. The glycine ethyl ester units on the polyphosphazene backbone worked as water sensitive points to cleave the polymer backbone into shorter sections. After that, the molecular weight decreased gradually at a stable rate as the polymer backbone continued to be broken down into oligophosphazene products (molecular weight <4.0×10\(^4\) g/mol) and small molecules (glycine, ethanol, ammonium, and phosphates).\textsuperscript{28} A time–status relationship of this new supramolecular hydrogel system in a physiological environment can be expected. While releasing any encapsulated biomolecules, the hydrogels gradually dissociate and dissolve in an aqueous environment. Then, the water soluble poly(organophosphazenes) gradually degrade into shorter oligomer chains and small molecules which can be excreted through the renal filtration system.\textsuperscript{56,57}
3.4 Conclusions
Novel injectable supramolecular hydrogels were obtained by IC formation between mPEG modified biodegradable polyphosphazenes and α-CD. In this system, the aggregation of α-CD complexed mPEG side chains serve as physical crosslinks, while the uncomplexed mPEG side chains serve as hydrophilic segments. The gelation time varied from several minutes to over twenty minutes depending on the polymer structures and concentrations. The rheological measurements showed a fast gelation process. The thermoreversible gel-sol transitions were observed and studied for all the hydrogels prepared. The IC induced gelation mechanism was studied by DSC, TGA, $^{13}$C CP/MAS NMR, and X-ray diffraction techniques. The in vitro release of BSA and the dissociation of hydrogels showed higher stabilities and longer release profiles for polymers with longer mPEG side chains. The biodegradability of the polymers was monitored based on the molecular weight decrease. Such a biodegradable and biocompatible hydrogel system is a promising candidate for many biomedical applications, especially in the area of injectable drug delivery.

3.5 Acknowledgements

The authors thank Dr. Quan Chen, Dr. Siwei Liang and Professor Ralph Colby for assistance with the rheological measurements. The authors also want to thank Dr. Wenbin Luo for the solid state $^{13}$C NMR and Dr. Hemant P. Yennawar for the wide-angle X-ray diffraction.

3.6 References

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Chapter 4

Synthesis and Micellar Behavior of Novel Amphiphilic Poly[bis(trifluoroethoxy)phosphazene]-co-poly(dimethylamino)ethylmethacrylate Block Copolymers

4.1 Introduction

Amphiphilic diblock copolymers consisting of a hydrophobic and a hydrophilic segment have received considerable attention over the last few decades not only because of their unique self-aggregation properties in selective solvents to form micelles, but also because of their potential applications in separation technologies, nanolithography, and drug/gene delivery.\textsuperscript{1-6} In an aqueous environment the hydrophilic blocks will interact with the water and form the outer shell of a micelle allowing the hydrophobic blocks to aggregate in the core to lower their interaction with the environment. By contrast, reverse micelles may be generated in a hydrophobic organic medium where the hydrophobic blocks form the shell and hydrophilic block the core.\textsuperscript{7} The cores of those micelles can then serve as microcontainers for various substances including drugs, dyes, and nanoparticles, while the outer shells determine the solubility and the interactions with the external environment.\textsuperscript{8,9} Once formed, these micelles are thermodynamically stable with sizes that range from tens to a few hundreds of nanometers in diameter.\textsuperscript{10} Because of their wide-spread utility, it is to develop new amphiphilic diblock copolymer structures with novel chemical and physical characteristics that can be easily tailored for specific applications.\textsuperscript{11}

Polyphosphazenes are hybrid polymers that consist of an inorganic backbone of alternating phosphorus and nitrogen atoms with two side groups (usually organic) attached to each phosphorus.\textsuperscript{12} These structures are synthesized via macromolecular substitution, specifically by the replacement of chlorine atoms in poly(dichlorophosphazene) by various nucleophiles such as
alkoxides,\textsuperscript{13} aryloxides,\textsuperscript{14} and primary or secondary amines.\textsuperscript{15} The common synthetic route to poly(dichlorophosphazene) is through the thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 250°C in a sealed vacuum tube.\textsuperscript{16} This route permits the facile synthesis of the poly(dichlorophosphazene), but it provides little control over molecular weight, polydispersity, and chain architecture.\textsuperscript{17} These issues have been overcome by the recent development of an ambient-temperature living cationic via the PCl\textsubscript{5}-induced polymerization of a phosphoranimine as reported by Allcock, Manners et al.\textsuperscript{18} This polymerization allows for the preparation of a variety of polymeric phosphazene architectures with lower but controllable molecular weights, and narrower polydispersities. This process has been used to synthesize a number of polyphosphazene-containing block copolymers.

Amphiphilic diblock copolyphosphazenes bearing two different types of side groups on two different polyphosphazene blocks, one hydrophobic and one hydrophilic, were first synthesized and their micelle properties tested in 1997 and 2001.\textsuperscript{19,20} However these initial diblock copolyphosphazenes had relative high critical micellar concentrations compared with other polymeric micelles.\textsuperscript{8,9,11} To overcome these issues, unique hybrid block copolymers with a combination of polyphosphazene and organic polymer blocks were synthesized, and their ability to self-assemble into micelles in an aqueous environment was thoroughly studied. In previous work in our program, various organic blocks such as polystyrene,\textsuperscript{21} PPG,\textsuperscript{22} and PEO\textsuperscript{23} were linked to the polyphosphazene. These phosphazene-organic block copolymers showed advantageous micellar properties. However, they were synthesized following a ‘block-to’ strategy, which involved the coupling of two individual polymers via reaction sites at the polymer ends. Although this method was successful, it incurred disadvantages including low coupling efficiency, and the segment that were left unreacted after the coupling was hard to remove.\textsuperscript{24}

In order to overcome the shortcomings of the ‘block-to’ method, a new ‘block-from’ approach has been widely utilized to synthesize diblock copolymers, especially after the development of
In this approach, one block is synthesized first and the endcap is modified to introduce a halogen-containing endgroup capable of acting as a macroinitiator to polymerize various monomers, including styrenes, acrylates, acrylamides, and acrylonitriles in the presence of Cu(I). The resultant diblock copolymers usually have well-defined molecular weights and good chain uniformity. In this present work, we report a new synthetic route to make polyphosphazene-organic amphiphilic diblock copolymers using the ‘block-from’ method by combining the living cationic polymerization with ATRP (Figure 4-1). The hydrophobic segment poly[bis(trifluoroethoxy)phosphazene] (TFE), which is produced via the living cationic polymerization, has been end-functionalized with an ATRP initiating site via an azide-alkyne ‘click’ reaction. The subsequent macroinitiator was used to polymerize 2-(dimethylamino)ethyl methacrylate via ATRP to form the hydrophilic block. Once synthesized, the micellar behavior in an aqueous environment was investigated by fluorescence techniques, dynamic light scattering, and transmission electron microscopy.
Figure 4-1: Synthetic route of diblock copolymers.
4.2 Experimental Section

4.2.1 Reagents and Equipment.

**Materials.** Lithium bis(trimethylsilyl)amide (Aldrich), propargylamine hydrochloride (TCI America), N,N,N’,N”’,N”’-pentamethyldiethylenetriamine (PMDETA) (TCI America), CuBr (Aldrich), NaN₃ (Aldrich), 3-bromopropanol (Aldrich), and NaH (Aldrich) were used without further purification. Phosphorus pentachloride (Aldrich) was purified by sublimation under vacuum before use. 2,2,2-Trifluoroethanol was dried over calcium hydride and was distilled before use. Sulfuryl chloride (Aldrich) and phosphorus trichloride (Aldrich) was purified by distillation. Bromophosphoramine (Br(CF₃CH₂O)₂P=NSiMe₃) was synthesized and purified by literature procedures.¹⁸,²⁰ Dimethylaminoethylmethacrylate (DMAEMA) (Aldrich) was purified via an Al₂O₃ column to remove inhibitor. All the glassware was dried overnight at 120 °C before use. The synthesis reactions were carried out under an atmosphere of dry argon or nitrogen.

**Equipment.** ¹H and ³¹P NMR spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 and 145 MHz, respectively. ¹H NMR spectra were referenced to solvent signals while ³¹P NMR chemical shifts were relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10mm and AM gel 10 mm 104 Å columns, and calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt% solution of tetra-n-butylammonium nitrate (Aldrich) in tetrahydrofuran (THF).
4.2.2 Synthesis of Alkyne Functionalized Trifluoroethoxyphosphoranimine (3).

A mixture of propargylamine hydrochloride (0.10g, 1.07mmol) and triethylamine (0.11g, 1.07mmol) was suspended in THF (15mL). The mixture was stirred under an inert atmosphere at 60°C for 2 hours while a white precipitate formed. The reaction mixture was then cooled to room temperature, and bromophosphoranimine 2 (0.28g, 0.71mmol) and another 1 eqv. of triethylamine (0.11g, 1.07mmol) were added to the mixture. The reaction mixture was then stirred at room temperature overnight. The white precipitate was filtered off, and all volatiles were removed under reduced pressure to give a colorless liquid 3. Yield: 87%. $^1$H NMR (CDCl$_3$): $\delta$ 4.23 (s, 4H), $\delta$ 3.45 (d, 2H), $\delta$ 2.26 (t, 1H), $\delta$ 0.13 (s, 9H). $^{31}$P NMR (CDCl$_3$): $\delta$ -2.65.

4.2.3 Synthesis of Chlorophosphoranimine (Cl$_3$P=NSiMe$_3$).

The synthesis of the chlorophosphazene monomer followed previously reported procedures with some modifications.$^{18,20,30}$ Briefly, PCl$_3$ (46.25g, 0.33mol) was added dropwise to LiN(SiMe$_3$)$_2$ (56.93g, 0.33mol) in ether (500mL) at 0 °C over 30 min. The mixture was allowed to remain at 0 °C and was stirred for another 1 hour. SO$_2$Cl$_2$ (45.22g, 0.33mmol) was then added slowly over 30 min and the reaction mixture was stirred at 0 °C for 2 hours. After completion of the reaction, the salt was removed by filtration. The crude product was condensed to one third of its volume, and was purified by vacuum distillation at room temperature to yield a colorless liquid. Yield: 63%. $^1$H NMR (CDCl$_3$): $\delta$ 0.16 (s, 9H). $^{31}$P NMR (CDCl$_3$): $\delta$ -57.08.

4.2.4 Synthesis of Alkyne Functionalized Poly[bis(trifluoroethoxy)phosphazene] (6).

Compound 3 (0.26g, 0.71mmol) was redissolved in CH$_2$Cl$_2$ (15mL) along with PCl$_5$ (0.25g, 1.19mmol) to initiate polymerization, and the reaction mixture was stirred for 1 hour. The
chlorophosphoranime (4.00g, 17.18mmol) was then added rapidly and the mixture was stirred for another 4 hours under an inert atmosphere at room temperature. The solvent was removed under reduced pressure to yield a viscous liquid. The product was redissolved in THF (50mL) and treated with an excess amount of CF₃CH₂ONa which was pre-prepared by treating CF₃CH₂OH (3.78g, 37.80mmol) with NaH (1.51g, 37.80mmol) in THF (20mL). The reaction mixture was stirred at room temperature overnight, followed by concentration of the solution under reduced pressure and then precipitation of the polymer into water (500mL×3) and hexane (200mL×2) to isolate a white product 6. Yield: 52%. ¹H NMR (acetone-d₆): δ 4.53 (180H), δ 3.60 (2H), δ 2.18 (1H). ³¹P NMR (D₂O): δ -7.88.

4.2.5 Synthesis of 3-Azidopropyl-2-bromo-2-methylpropanoate 7.

Sodium azide (3.60g, 55.40 mmol) was dissolved in water (80mL), followed by the addition of 3-bromopropanol (3.84g, 37.60 mmol) dropwise to the solution. The reaction mixture was stirred at 80°C for 18 hours. The solution was then extracted with ethyl acetate (100mL×3) and the organic layer was washed with saturated brine (100mL×3), and then dried over MgSO₄ overnight. The solvent was removed under reduced pressure to yield a colorless liquid. The liquid was then redissolved in CH₂Cl₂ (20mL) and chilled to 0°C before the addition of triethylamine (1.68g, 16.61 mmol). In a second vial, 2-bromoisobutyl bromide was dissolved in CH₂Cl₂ (20mL) before being added dropwise to the mixture at 0 °C over 1 hour. The reaction mixture was then allowed to warm to room temperature and was stirred overnight. The precipitate was filtered off, and the solvent was removed under reduced pressure. The crude product was purified by passing through a silica column using CH₂Cl₂:hexane (2:1) as the mobile phase, and the sample was isolated as a light yellowish liquid 7. Yield: 20.23%. ¹H NMR (CDCl₃): δ 4.23 (t, 2H), 3.41 (t, 2H), 1.93 (m, 2H), 1.89 (s, 6H).
4.2.6 Synthesis of Bromo-functionalized Polyphosphazene Macroinitiator 8.

Polymer 6 (1.13g, 0.06mmol) and 3-azidopropyl-2-bromo-2-methylpropanoate (7) (0.156g, 0.63mmol) were dissolved in THF (10mL), along with PMDETA (0.04g, 0.22mmol). Nitrogen gas was bubbled through the solution for 20 minutes to remove any dissolved oxygen. A trace amount of CuBr (0.03g, 0.22mmol) was weighed in a vial and the oxygen was removed by purging the system with nitrogen gas. Once the system was free from oxygen, the CuBr was added to the reaction solution rapidly, and the solution was stirred at 60°C for 1 day under an inert atmosphere. The sample was forced through a silica plug using THF to remove the solids. The crude product was then precipitated from THF into water (500mL × 3) and hexane (200mL × 5), and was further purified by dialysis against acetone:methanol (2:1) for 2 days to remove any remaining compound 7. The solvent was then removed under reduced pressure and the resulting product was dried under vacuum overnight to yield a white powder (8). $^1$H NMR (DMSO-d6): δ 7.79 (1H), δ 4.45 (180H), δ 4.32 (2H), δ 4.20 (2H), δ 3.91 (2H), δ 2.41 (2H), δ 1.91 (6H). $^{31}$P NMR (DMSO-d6): δ -7.88.

4.2.7 Synthesis of Poly[bis(trifluoroethoxy)phosphazene]-co-poly(dimethylamino)ethyl methacrylate (10) (TFE-b-PDMAEMA).

2-(Dimethylamino)ethyl methacrylate (DMAEMA) was used as a monomer to grow a second block from the terminus of the polyphosphazene. Macroinitiator 8 (0.50g, 0.03mmol) was dissolved in THF (5mL), followed by the addition of DMAEMA (1.97g, 12.50mmmol) and PMDETA (0.017g, 0.10mmol) to the solution. Nitrogen gas was bubbled through the solution for 20 minutes to remove any dissolved oxygen. Copper(I) bromide (7.20mg, 0.05mmol) was weighed in a small vial and then purged with nitrogen to remove oxygen. It was then added to the solution and the mixture was stirred for 6 hours at room temperature (as an example for TFE-b-
PDMAEMA-4). To terminate the polymerization, the catalysts were removed by passing the sample through a silica plug using THF as mobile phase, and the isolated crude product was dialyzed against acetone:methol (2:1) for two days. All solvent was removed under reduced pressure to yield the diblock copolymer 10 as a yellowish powder. \(^1\)H NMR (acetone-d6): \(\delta 4.55 (-\text{CH}_2\text{CF}_3), \delta 4.09 (-\text{OCH}_2-), \delta 2.62 (-\text{CH}_2\text{N}), \delta 2.23 (-\text{N(CH}_3)_2), \delta 1.88 (-\text{CH}_2-), \delta 1.10 (-\text{CH}_3)\).

\(^{31}\)P NMR (acetone-d6): \(\delta -7.88\).

4.2.8 Micelle Preparation.

To prepare micellar solutions, nanopure water (20mL) with a conductivity of 18.2 MΩ/cm was added dropwise to a mildly stirred solution of the diblock copolymer (200mg) in THF (5mL). Once the water was added, all the THF was removed under reduced pressure as monitored by \(^1\)H NMR. The resulting solution was then diluted to obtain a micelle concentration in the range of 5 to 1\(\times\)10\(^{-4}\) g/L. For fluorescence measurements, a pyrene solution in THF (1.2\(\times\)10\(^{-3}\)M) was added to nanopure water to give a final pyrene concentration of 12\(\times\)10\(^{-7}\)M. Following dilution, the THF was removed under reduced pressure, and its removal was confirmed by \(^1\)H NMR spectroscopy. The pyrene solution was then mixed with the diblock copolymer solutions to obtain copolymer concentrations ranging from 2.5 to 5\(\times\)10\(^{-5}\)g/L, while the pyrene concentration of the samples was maintained at 6\(\times\)10\(^{-7}\)M. All the samples were sonicated for 10 min and were allowed to stand for 1 day before fluorescence measurements.
4.2.9 Micelle Characterizations.

**Fluorescence Measurements.** Excitation spectra of pyrene were measured using a Photon Technology International (PTI) fluorescence spectrometer using an 814 photomultiplier detection system. For the excitation spectra, the emission wavelength was set at 391 nm. All the samples were measured in a 1×1 cm quartz cuvette at room temperature.

**Light Scattering Measurements.** The sizes and size distributions of the diblock copolymer micelles were evaluated by dynamic light scattering using a particle size analyzer (Zetasizer Nano S, Malvern Instruments Ltd.) at room temperature (25°C) with a scattering angle of 90°. Samples were filtered through a 0.45μm syringe filter before measurement of particle size for each sample. The hydrodynamic radius \( R_h \) of the micelles was calculated by using the Stokes-Einstein equation \( R_h = k_B T / 6 \pi \eta D \), where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the solvent viscosity, and \( D \) is the diffusion coefficient. The polydispersity factor of micelles, represented as \( \mu_2 / \Gamma^2 \), where \( \mu_2 \) is the second cumulant of the decay function and \( \Gamma \) is the average characteristic line width, was calculated by the cumulant method.

**Transmission Electron Microscopy.** Transmission electron microscopy (TEM) was performed using a KEOL 2010 unit, operated at an acceleration voltage of 200kV. For observation of the size and distribution of the micellar particles, a drop of sample solution (concentration=1 g/L) was placed onto a 400-mesh copper grid coated with carbon. About 1 min after deposition, the grid was tapped with a filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 2.5 wt% uranyl acetate solution. The samples were air-dried at room temperature overnight.
4.3 Results and Discussion

4.3.1 Synthesis of Block Copolymers.

Conventional free radical polymerization lacks control over the polymer structure due to the slow initiation, fast propagation and subsequent transfer or termination, and makes it unsuitable for synthesizing well-defined block copolymer structures. The development of atom transfer radical polymerization (ATRP) is one of the most robust controlled/living radical polymerization techniques with a diversity of monomers, desirable molecular weight control, and narrow polydispersity. This comes about because the low radical concentrations present during the polymerization reduce the contribution of inter- and intramolecularly terminated chains. In this work, ATRP was chosen to form the organic block linked to a polyphosphazene backbone, and it is the first report to apply ATRP to synthesize polyphosphazene-containing diblock copolymers. In order to introduce the ATRP initiation site to the end of polyphosphazene backbone, azide-alkyne ‘click’ chemistry was applied due to its excellent reaction efficiency, high functional group tolerance, and solvent compatibility. In this work, a small molecule linker (7), an ATRP initiation site, was ‘click’ coupled onto the polyphosphazene block and used for subsequently growing the organic block at the end of the polymer chain (‘block-from’). This avoids the preparation of two distinct polymers with azide or alkyne functionality on the two segments and then coupling them together (‘block-to’). This ‘block-from’ method showed a higher reaction efficiency than the ‘block-to’ method due to the better diffusion of small molecules to the reaction site at the polymer endcap in solution as compared to the reaction of two polymer chains.

A series of phosphazene-organic block copolymers were prepared by the synthetic procedures illustrated in Figure 4-1. The phosphoranimine readily underwent bromine replacement reactions
in the presence of amines to produce an alkyne functionalized initiator 3. To produce the cationic species 4, the initiator 3 was treated with 2 eqv. of PCl₅ at room temperature in CH₂Cl₂. Once formed, the chloromonomer Cl₃P=NSiMe₃ was added to propagate the living cationic polymerization to give poly(dichlorophosphazene) with a predetermined chain length. The resultant poly(dichlorophosphazene) was then treated with an excess amount of NaOCH₂CF₃ in THF to yield the hydrophobic poly[bis(trifluoroethoxy)phosphazene] block with a pendent triple bond attached to the end of the chain. The entire process was carried out in an inert anhydrous atmosphere to prevent uncontrollable crosslinking.

Compound 7 with an azide and bromine at its opposing ends was prepared as both the linker between the phosphazene and organic blocks and the initiator for growing the organic block. Initial attempts with CuSO₄·5H₂O/sodium ascorbate as the catalytic system for the ‘click’ reaction in THF at room temperature were unsuccessful probably due to the poor solubility of the catalyst in THF and the donor coordinating nature of the polyphosphazene backbone. Thus, a stronger catalyst CuBr/PMDETA complex in THF was chosen at an elevated temperature of 60 °C for 1 day to yield the ATRP macroinitiator 8. Unlike the CuSO₄·5H₂O/sodium ascorbate catalyst system, which is tolerant to limited amounts of oxygen in solution by in situ reduction of Cu²⁺, the CuBr/PMDETA catalyzed ‘click’ reaction in this work required the careful removal of all the oxygen dissolved in the solution by exchanging it with nitrogen to prevent the oxidation of Cu⁺. In order to remove all of the uncoupled initiator 7 from the polyphosphazene polymer, the sample was purified extensively by precipitation from THF into hexane 5 times, followed by dialysis against acetone:methanol (2:1) for two days. DMAEMA was selected as the second monomer due to its high reactivity, and CuBr/PMDETA was used as the catalyst in this polymerization. ATRP is usually carried out by bulk polymerization by directly dissolving the initiators in the pure monomers without utilizing solvents. However, in this work, THF was
used as a solvent at a sacrifice of reaction efficiency due to the poor solubility of the macroinitiator 8 in DMAEMA.

The existence of both the trifluoroethoxyphosphazene block and PDMAEMA signals in the $^1\text{H}$ NMR (Figure 4-2) confirmed the structure, together with the $^{31}\text{P}$ NMR (Figure 4-3) of the diblock copolymer. The molecular weight increased from 21,100 to 36,000 (varying by ATRP reaction time) as determined by gel permeation chromatograph (GPC). All these items of evidence support the diblock copolymer structure. The length of both the hydrophobic and hydrophilic blocks can be tuned by controlling the ratio of initiator to monomer and the reaction time. In this work, the length of the hydrophobic block remained constant, and the length of the hydrophilic segment was controlled by the varying reaction times at the same monomer/initiator ratio. Table 4-1 shows the structural characterization of a series of TFE-$b$-PDMAEMA diblock copolymers. The molecular weights calculated by $^1\text{H}$ NMR spectroscopy were estimated by comparing peak integration ratios of the end group on propargylamine (-CH$_2$-, 3.60ppm), the trifluoroethoxy groups on the polyphosphazene (-CH$_2$-, 4.55ppm), and the DMAEMA block (-CH$_2$-, 4.09ppm). The significant difference of $M_n$ between the GPC measurement and the $^1\text{H}$ NMR calculation as shown in Table 4-1 was attributed to i) error caused by integration from the $^1\text{H}$ NMR peaks if the peak intensities of the end groups were too low; and ii) the difference of hydrodynamic radius between TFE-$b$-PDMAEMA and polystyrene standards which were used to calibrate the GPC. The final block copolymers were soluble in THF, acetone, DMSO, and DMF, but were insoluble in hexane, and toluene.
Figure 4-2: $^1$H NMR of TFE-$b$-PDMAEMA-3 block copolymer at ambient temperature referenced to acetone-d6.

Figure 4-3: $^{31}$P NMR of TFE-$b$-PDMAEMA-3 block copolymer at ambient temperature referenced to acetone-d6.
4.3.2 Self-association of Block Copolymers in the Aqueous Phase.

The TFE-\textit{b}-PDMAEMA block copolymer consists of the hydrophilic PDMAEMA and hydrophobic trfluoroethoxy-substituted polyphosphazene segments (TFE), which imparts the ability to form organized micellar structures in an aqueous environment (Figure 4-4). Generally, micelles can be formed in an aqueous environment by one of these three ways: i) direct addition of block copolymers into stirred water; ii) dissolution of the block copolymer in an organic solvent, followed by dialysis against water; or iii) dissolution of the block copolymer in an organic solvent, followed by the addition of water dropwise to the solution using a mild stirring.\textsuperscript{39,40} The last two methods are preferred, as the gradual exchange of the organic solvent with water can give more uniform micelle structures.\textsuperscript{22} However, the organic solvent has to be

Table 4-1: Characterization of TFE-\textit{b}-PDMAEMA Block Copolymers.

<table>
<thead>
<tr>
<th>Block copolymers</th>
<th>ATRP time (h)</th>
<th>Block ratio(n:m) \textsuperscript{a}</th>
<th>TFE Wt%</th>
<th>\textit{M}_n (g/mol)</th>
<th>\textit{M}_n (g/mol) (GPC) \textsuperscript{b}</th>
<th>\textit{M}_n (g/mol) (NMR) \textsuperscript{c}</th>
<th>PDI\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFE-\textit{b}-PDMAEMA-1</td>
<td>12</td>
<td>1:1.23</td>
<td>44</td>
<td>36,000</td>
<td>19,800</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>TFE-\textit{b}-PDMAEMA-2</td>
<td>10</td>
<td>1:0.95</td>
<td>61</td>
<td>33,200</td>
<td>17,800</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>TFE-\textit{b}-PDMAEMA-3</td>
<td>8</td>
<td>1:0.71</td>
<td>66</td>
<td>30,500</td>
<td>16,100</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>TFE-\textit{b}-PDMAEMA-4</td>
<td>6</td>
<td>1:0.63</td>
<td>71</td>
<td>28,900</td>
<td>15,500</td>
<td>1.18</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} The ratio of the hydrophobic block to the hydrophilic block was calculated from the integration obtained from \textsuperscript{1}H NMR.

\textsuperscript{b} The number-average molecular weight (\textit{M}_n) was calculated from \textsuperscript{1}H NMR.

\textsuperscript{c} \textit{M}_n was measured by gel-permeation chromatography (GPC).

\textsuperscript{d} Polydispersity index (PDI) = \textit{M}_w/\textit{M}_n.
removed completely from the aqueous medium to prevent it from influencing the micelle characterization. In this work, the third route was selected, and $^1$H NMR was utilized to monitor for THF residues in the micelle solutions. The micellar behavior of the amphiphilic block copolymers was monitored by a fluorescence technique, dynamic light scattering, and TEM. The critical micelle concentrations (cmcs) of the diblock copolymers in an aqueous phase were determined by a fluorescence technique using pyrene as a probe. Previous studies have shown that pyrene has distinct fluorescence spectra depending on the environment utilized, aqueous or organic.\textsuperscript{41,42} Figure 4-5 shows the excitation fluorescence spectra of polymer and pyrene sample (TFE-$b$-PDMAEMA-3 as an example), in which the concentration of the pyrene was kept constant while varying the concentrations of the polymer. In the spectra, the symmetry-forbidden (0,0) band shifted from 332 to 337 nm and the intensity gradually increased as the pyrene transferred from the aqueous environment to the hydrophobic micelle cores. Meanwhile, the pyrene fluorescence spectrum obtained in pure water is identical to the one obtained from a pyrene solution with low concentrations of block copolymers in Figure 4-5, and the block copolymers themselves give no fluorescence signals in this region. The ratios of the peak intensities at 337 and 332nm were utilized to determine the cmc value.\textsuperscript{41} Figure 4-6 shows the intensity ratios ($I_{337}/I_{332}$) of the pyrene excitation spectra versus the logarithm of concentrations of TFE-$b$-PDMAEMA-3(Log C). At low concentrations of the diblock copolymer, the change in the intensity ratio ($I_{337}/I_{332}$) was negligible since the concentrations of the block copolymer were insufficient to self-aggregate and form micelles. But at a threshold concentration of the diblock copolymer, the intensity ratios began to show a substantial increase with an increase in the concentration of the diblock copolymer. This reflects the shift of the pyrene probe from an aqueous environment to a hydrophobic one. This threshold indicated the minimum concentration of the diblock copolymer needed for the formation of micelles, the inner cores of which were able to act as hydrophobic containers to incorporate the pyrene. Using these data, the cmc values can
be determined from the turning point of the curve as shown in Figure 4-5. The cmc values of the block copolymers were in the range of 3.47-9.55 mg/L depending on the block composition (Table 4-2). The results showed that the cmc values decreased with an increase in the proportion of the hydrophobic segment, which is in agreement with other studies on micelles.\textsuperscript{11,42} These values are much lower than those of low molecular weight surfactants (e.g., 2.3 g/L for sodium dodecyl sulfate) and diblock copolypophosphazenes (e.g., 80 mg/L for methoxyethoxyethoxy and phenyl containing species),\textsuperscript{20,41} but comparable to those of other polymeric amphiphiles.\textsuperscript{1,5,39}

Table 4-2: Properties of TFE-b-PDMAEMA Micelle.

<table>
<thead>
<tr>
<th>Block copolymers</th>
<th>cmc (mg/L)</th>
<th>Diameter (nm)</th>
<th>$\mu_2/\Gamma^2$</th>
<th>$K_v (\times 10^{-7})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFE-b-PDMAEMA-1</td>
<td>9.55</td>
<td>100</td>
<td>0.29</td>
<td>0.12</td>
</tr>
<tr>
<td>TFE-b-PDMAEMA-2</td>
<td>6.31</td>
<td>130</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>TFE-b-PDMAEMA-3</td>
<td>5.46</td>
<td>134</td>
<td>0.16</td>
<td>0.48</td>
</tr>
<tr>
<td>TFE-b-PDMAEMA-4</td>
<td>3.47</td>
<td>142</td>
<td>0.20</td>
<td>1.52</td>
</tr>
</tbody>
</table>
Figure 4-4: Excitation spectra of pyrene as a function of TFE-\textit{b}-PDMAEMA-3 concentration in water.

Figure 4-5: Plot of $I_{337}/I_{332}$ (from pyrene excitation spectra) vs log C for TFE-\textit{b}-PDMAEMA-3.
Dynamic light scattering (DLS) was carried out to determine the diameters of the micelles. **Figure 4-6** shows an example of the size distribution of TFE-\(b\)-PDMAEMA-3 at 25°C. The diameters of the micelles for the four polymers investigated were in the range of 100 to 142 nm with a narrow size distribution in an aqueous phase as summarized in **Table 4-2**. The diameters increased slightly with the increase in the proportion of the hydrophobic block. The polydispersity factors (\(\mu_2/\Gamma^2\)) of the micelles are fairly low (0.16-0.29), which suggests a narrow size distribution.\(^{31,32}\)

The size and shape of the micelles were also examined by TEM. In order to amplify the contrast of the micelles and background, negative staining was performed by using a uranyl acetate solution to make the background dark gray.\(^{33}\) **Figure 4-7** shows the micelles formed from a solution with a polymer (TFE-\(b\)-PDMAEMA-3) concentration of 1 g/L. Although the average size of the micelles was about 100 nm, micelles with a size range of several hundreds of nanometers were often observed. This resulted from intermicellar aggregates that form a multicore structure through the association of individual micelles.\(^{44,45}\) Most of the micelles had a spherical shape, and the average diameter from TEM was in agreement with the mean diameter measured from dynamic light scattering.
4.3.3 Self-association of Block Copolymers in the Aqueous Phase.

Figure 4-6: Diameters of TFE-b-PDMAEMA-3 from dynamic light scattering.

Figure 4-7: TEM image of TFE-b-PDMAEMA-3.
The hydrophobicity of the micelle core was estimated by measuring the equilibrium constant $K_v$ for the partitioning of pyrene between the micelle core and the aqueous media. A higher $K_v$ indicates a higher hydrophobicity of the microdomain (micelle cores) constructed by the hydrophobic segments.\textsuperscript{22,23} In this work, the equilibrium constant $K_v$ was calculated following the approach reported by Wilhelm.\textsuperscript{41} A simple equilibrium between pyrene in the bulk aqueous environment and pyrene incorporated into the micelle was assumed. The ratio of the pyrene concentration inside the micelle to that of pyrene dissolved in the bulk water phase ($\left[ {Py} \right]_m/\left[ {Py} \right]_w$) can be correlated to the ratio of the volumes of each phase as expressed in equation 1. The $K_v$ here is the partition equilibrium constant of pyrene between the micelle core phase and water phase.

\[
\frac{[Py]_m}{[Py]_w} = K_v \frac{V_m}{V_w}
\]

Equation 1 can be rewritten as

\[
\frac{[Py]_m}{[Py]_w} = K_v x (c - cmc) / 1000 \rho
\]

where $x$ is the weight fraction of the hydrophobic polyphosphazene block, $c$ is the concentration of the block copolymer, and $\rho$ is the density of the core of the micelles, which is assumed to be the bulk density of the poly[bis(trifluoroethoxy)phosphazene] (1.10 g/mL). In the intermediate range of polymer concentration with a substantial increase of intensity ratio ($I_{337}/I_{332}$), $\left[ {Py} \right]_m/\left[ {Py} \right]_w$ can be written as

\[
\frac{[Py]_m}{[Py]_w} = \left( F - F_{\text{min}} \right) / \left( F_{\text{max}} - F \right)
\]

where $F_{\text{max}}$ and $F_{\text{min}}$ correspond to the average magnitude of $I_{337}/I_{332}$ in the flat region of the high and low concentration ranges respectively in Figure 4-5, and $F$ is the intensity ratio ($I_{337}/I_{332}$) in the intermediate concentration range of the block copolymers. Combing equation 2 and 3, $K_v$ values for pyrene can be determined as the slope by using a plot of $(F-F_{\text{min}})/(F_{\text{max}} - F)$ versus block copolymer concentrations as shown in Figure 4-8.
The $K_v$ values, as summarized in Table 4-2, were in the range of $0.12 \times 10^5$ to $1.52 \times 10^5$ for the TFE-$b$-PDMAEMA system, which are much larger than those of amphiphilic diblock copolyphosphazenes (e.g. $7 \times 10^3$ for a methoxyethoxyethoxy and phenoxy containing copolyphosphazene).\textsuperscript{20} The data also showed that, as the proportion of the hydrophobic blocks in the amphiphilic block copolymers increased, the $K_v$ value also increased, suggesting an increase in the hydrophobic characteristic of the micelle cores.

![Figure 4-8: Plots of $(F-F_{\text{min}})/(F_{\text{max}}-F)$ vs. concentration of block copolymers.](image)

4.4 Conclusions

A series of amphiphilic TFE-$b$-PDMAEMA diblock copolymers was synthesized via the controlled living cationic polymerization of Cl$_3$N=PSiMe$_3$, azide-alkyne ‘click’ chemistry, and atom transfer radical polymerization of DMAEMA. The length of each block was well controlled
and the polydispersity index was relatively low. The block copolymers self-aggregated into organized micelle structures in an aqueous environment. The micelles which were formed were characterized by the use of fluorescence techniques, dynamic light scattering, and transmission electron microscopy. The critical micelle concentrations of the block copolymers were determined from fluorescence spectra using pyrene as a probe. The cmc values depended on the proportion of the hydrophobic blocks in the copolymer and were in the range of 3.47-9.55 mg/L. TEM and dynamic light scattering results indicated that the spherical micelle aggregates were formed with an average diameter of 100-142nm. The hydrophobicity of the micellar core was estimated by measurement of the partition equilibrium constant of pyrene in the micelle solution, and the values were in the range of $0.12 \times 10^5$ to $1.52 \times 10^5$. The combination of ‘click’ reaction chemistry and ATRP has opened a new facile route (‘block-from’) for synthesizing well-defined hybrid phosphazene-organic block copolymer structures with high synthetic tunability. The properties of the micelles can be tailored by changing either the phosphazene block or the organic block following the synthetic procedures described above. This can be achieved by varying the nucleophiles during the substitution of poly(dichlorophosphazene) or by varying the organic monomers during ATRP. For instance, to synthesize block copolymers containing biodegradable substituents, similar synthetic procedures can be followed except the side group nucleophiles used could be changed to amino acid esters instead of the trifluoroethoxy group to confer biodegradability to the micelles.\(^{15}\)

4.5 References

1994, 263, 1600-1603.
Chapter 5

Synthesis and Assembly of Novel Poly(organophosphazene) Structures Based on Non-covalent “Host–Guest” Inclusion Complexation

5.1 Introduction

The assembly of new polymeric structures by non-covalent connections such as hydrogen bonding, ligand–metal coordination, or “host–guest” inclusion complexation has attracted considerable recent interest.\(^1\)-\(^4\) Such connections at polymer chain-ends or through units on the side chains may respond to various stimuli including changes in temperature, pH, or irradiation, and allow the separation and re-combination of each connection.\(^5,^6\) \(\beta\)-Cyclodextrin (\(\beta\)-CD), a macrocycle with seven glucose units, has long been recognized as a natural host for various small molecules.\(^7\) Chemical attachment of \(\beta\)-CD to polymers has generated considerable interest due to the unique properties of these macromolecules in supramolecular chemistry, analytical studies, separation technology, and pharmaceutical applications.\(^8,^9\) Adamantane is one of the most important guests for \(\beta\)-CD due to its effective inclusion entrapment and high binding affinity.\(^1\(^0,^1\)\(^1\)\) Several possibilities have been reported using the above “host–guest” inclusion complexation to construct non-covalent polymeric structures. For example, host–guest interactions have been used as a bridge to construct amphiphilic pseudo-block copolymers.\(^3\) Main-chain supramolecular polymers have been obtained by alternating host and guest moieties along the backbone.\(^1\)\(^2\) Self-healing materials have been prepared by mixing \(\beta\)-CD modified polymers with guest molecule modified polymers.\(^1\)\(^3\) However, polymers that contain \(\beta\)-CD present many synthetic challenges including the chemical complexity associated with the seven glucose units, the substantial size of the cyclic molecule, and the potential insolubility of the resultant polymers.\(^1\)\(^4\)
Polyphosphazenes possess a backbone of alternating phosphorus and nitrogen atoms with two (usually organic) side groups attached to each phosphorus. Several hundred poly(organophosphazenes) with different side groups and architectures have been reported in the past several decades. Most of the syntheses of these polymers utilize the replacement of the chlorine atoms in poly(dichlorophosphazene) by alkoxides, aryloxides, or amines. As a unique class of organic–inorganic hybrid polymers, polyphosphazenes possess numerous properties including facile and tunable side group substitution, biocompatibility, and in some cases controllable biodegradability. Construction of β-CD containing polyphosphazenes could expand the category of useful polymers, enable the study of structure–property relationships of novel species and, most important, endow the polymer with new properties which would be of benefit in challenging applications.

In this work, palm tree-like pseudo-block organophosphazene copolymers were prepared by “host–guest” inclusion complexation between a phosphazene polymer chain with a terminal adamantyl group and a tetra-branched β-CD functionalized organic polymeric block. The micellization of the prepared amphiphiles was also studied. In a related system, poly(organophosphazenes) with 10% of the side groups in the form of β-CD pendant units and 10% of adamantane guest units on the side-chains of a second polyphosphazene were synthesized. The capability of this configuration to participate in supramolecular gelation of the mixed polymer solutions was investigated (Figure 5-1).
**Experimental Section**

**5.2 Reagents and Equipment.**

**Materials.** Tetrahydrofuran (THF), dimethylformamide (DMF), dichloromethane (DCM), triethylamine (TEA), and diethyl ether were purchased from EMD were dried using solvent purification columns. Lithium bis(trimethylsilyl)amide (Sigma–Aldrich), N,N,N′,N″,N‴–pentamethyltriethylenetriamine (PMDETA) (TCI America), CuBr (Sigma–Aldrich), 1-aminoadmantane (TCA America), 1-adamantyl isocyanate (Sigma–Aldrich), 2-bromoisobutyryl...
bromide (Sigma–Aldrich), β-CD (Sigma–Aldrich) and NaH (Sigma–Aldrich, 60% in mineral oil), diethylene glycol (Alfa Aesar), p-toluenesulfonic acid monohydrate (Alfa Aesar), p-toluenesulfonyl chloride (Sigma–Aldrich), 3,4-Dihydro-2H-pyran (Acros), sodium hydroxide (Sigma–Aldrich), sodium azide (EMD), trifluoroacetic acid (Alfa Aesar), propargyl bromide (TCI America, 80% in toluene), copper sulfate pentahydrate (Sigma–Aldrich), sodium ascorbate (Sigma–Aldrich), 1-bromoadamantane (Alfa Aesar) were used without further purification. Phosphorus pentachloride (Sigma–Aldrich) was purified by sublimation under vacuum before use. 2,2,2-Trifluoroethanol was distilled over sodium metal before use. Sulfuryl chloride (Aldrich) and phosphorus trichloride (Sigma–Aldrich) was purified by distillation. Poly(ethylene glycol) methyl ether methacrylate (mPEGMA MW~300 g/mol, Sigma–Aldrich) was purified via a flash alumina column to remove inhibitor. Bromophosphoranimine (1) (Br(CF3CH2O)2P=NSiMe3), chlorophosphoranimine (Cl3P=NSiMe3), and trifluoroethoxyphosphoranimine (3) ((CF3CH2O)3P=NSiMe3) for the living cationic polymerization were prepared using the methods published elsewhere.1,2 β-CD-Based Macroinitiator (6) (4Br-β-CD), Mono-6-deoxy-6-(p-tolylsulfonyl)-β-cyclodextrin (9) (β-CD-OTs), and Mono-6-deoxy-6-azido-β-cyclodextrin (10) (β-CD-N3) were synthesized following previous procedures.3,4 High molecular weight poly(dichlorophosphazene) (11) was prepared by the thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical Co., Japan) in evacuated Pyrex tubes at 250 °C.1 All glassware was dried overnight at 120 °C before use. All syntheses were carried out under an atmosphere of dry argon.

Equipment.1 H and 31P NMR spectra were recorded on a Bruker WM–360 NMR spectrometer operated at 360 and 145 MHz, respectively. 19F NMR spectra were collected using a Bruker CDPX–300 spectrometer operated at 282 MHz. 2D NOESY NMR spectra were obtained using a Bruker AVANCE 400 spectrometer at room temperature with 20K accumulated scans. Molecular
weights were estimated using a Hewlett–Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns, and calibrated versus polystyrene standards (Polysciences). Thermal characteristics of samples were measured with a TA Instruments Q10 differential scanning calorimeter (DSC) and a Perkin-Elmer thermogravimetric analyzer (TGA) using dry nitrogen as the purge gas. A heating rate of 10 °C/min with a temperature range from -100 to 200 °C was used for DSC, while a heating rate of 20 °C/min from 25 to 800 °C was applied for TGA. Rheological measurements were performed at a constant oscillatory frequency of 1 rad/s under a strain of 1% using an Advanced Rheometric Expansion System (Rheometric Scientific). The samples were loaded on a parallel plate (25 mm diameter, 1 mm gap) at 25 °C.

2D NOESY NMR. Equimolar quantities of the β-CD-PmPEGMA (0.10 g, 0.005 mmol) and the Ad-PTFE (0.06 g, 0.005 mmol) polymeric samples were fully dissolved in 2 mL DMSO-$d_6$. Then, two drops of D$_2$O (roughly 0.1 mL) were added to the polymer solution. The solution was stirred for 2 h at room temperature, and was allowed to stand for 30 min before measurements. 2D NOESY NMR spectra were obtained using a Bruker AVANCE 400 spectrometer at room temperature with 20K accumulated scans.

Fluorescence Measurements. Excitation spectra of pyrene were measured using a fluorescence spectrometer (Photon Technology International) using an 814 photomultiplier detection system. For the excitation spectra, the emission wavelength was set at 391 nm. All samples were measured in a 1×1 cm quartz cuvette at room temperature.

Light Scattering Measurements. The sizes and size distributions of the polymeric micelles were evaluated by dynamic light scattering using a particle size analyzer (Zetasizer Nano S, Malvern Instruments Ltd.) at room temperature (25 °C) with a scattering angle of 90°. Samples were filtered through a 0.45 µm syringe filter before measurement of the particle size for each sample.
Transmission Electron Microscopy (TEM). Transmission electron microscopy (TEM) was performed using a JEOL 2010 unit, operated at an acceleration voltage of 200 kV. For observation of the size and distribution of the micelle particles, a drop of sample solution (concentration=1 g/L) was placed onto a 400-mesh copper grid coated with carbon. About 1 min after deposition, the grid was blotted with a filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 2.5 wt% uranyl acetate solution. The samples were air-dried at room temperature overnight.

Atomic Force Microscopy (AFM). The AFM imaging was performed in contact mode using a Nanopics 2100 AFM (KLA-Tencor, San Jose, CA). A drop of micelle solution (10 µL) was spin-cast onto a 5 mm × 5 mm Si wafer (Ted Pella Inc., Redding, CA) which was pre-cleaned by absolute ethanol. The sample was dried in a dry cabinet overnight at room temperature before measurement.

5.2.2 Synthesis of 1-Aminoadamantane Functionalized Fluoroethoxyphosphoranimine (2) (AdamantaneNH(CF₂CH₂O)₂P=NSiMe₃).

To a tetrahydrofuran (THF) solution of 1-aminoadamantane (0.06 g, 0.38 mmol) and triethylamine (TEA) (0.04 g, 0.38 mmol) was added bromophosphoranimine 1 (Br(CF₂CH₂O)₂P=NSiMe₃) (0.10 g, 0.25 mmol). The reaction mixture was then stirred at room temperature overnight. The white precipitate was filtered off, and all volatiles were removed under reduced pressure to yield a colorless liquid (2). $^{31}$P NMR (CDCl₃): δ (ppm) -4.43. $^{19}$F NMR (CDCl₃): δ (ppm) -75.67 (s, –OCH₂CF₃).
5.2.3 Synthesis of Adamantyl End-Functionalized Poly[bis(trifluoroethoxy)phosphazene] (5) (Ad-PTFE).

Phosphorus pentachloride (0.10 g, 0.50 mmol) was dissolved in 20 mL DCM, and trifluoroethoxyphosphoranimine 3 ((CF$_3$CH$_2$O)$_3$P=NSiMe$_3$) (0.10 g, 0.25 mmol) was added to the solution. The initiation reaction mixture was stirred at room temperature for 1 h, and the chlorophosphoranimine (Cl$_3$P=NSiMe$_3$) (2.24 g, 10.00 mol) was added rapidly to the reaction medium. This mixture was stirred at room temperature for 4 h to give living poly(dichlorophosphazene). The 1-aminoadamantane functionalized fluoroethoxyphosphoranimine 2 (0.25 mmol) was redissolved in 10 mL of dichloromethane (DCM) in a separate vial, and was added rapidly to the living polymer solution. The reaction mixture was then stirred at room temperature overnight. Solvent was removed under reduced pressure to yield 4 as a colorless viscous liquid. The freshly prepared polymer 4 was then redissolved in 30 mL of anhydrous THF. The solution was treated with an excess of NaOCH$_2$CF$_3$, prepared by the reaction of HOCH$_2$CF$_3$ (2.20 g, 22.00 mmol) with NaH (0.88 g, 22.00 mmol) in 50 mL THF, and the reaction mixture was stirred at room temperature for 12 h to complete the chlorine replacement reaction (monitored by $^{31}$P NMR spectroscopy). The medium was concentrated to about 20 mL, and the solid product was precipitated from THF into water (3×500 mL), then from THF into hexanes (1×500 mL). This product was isolated by centrifugation as a white powder. The product was further purified by re-dissolving in 50 mL acetone, and dialyzing the solution versus acetone/methanol 80/20 for 3 d (Spectra/Por dialysis membrane, MWCO: 1,000). The solvent was removed and the resultant white powder was dried under vacuum (yield: 59%). $^1$H NMR (acetone-$d_6$): δ (ppm) 4.36–4.81 (br, 120H, –OCH$_2$CF$_3$), 1.97–1.68 (br, 15H, adamantane). $^{31}$P NMR (acetone-$d_6$): δ (ppm) -2.71 (s, 1P), -3.75 (s, 1P), -7.01–8.12 (br, 25P). $^{19}$F NMR (acetone-$d_6$): δ (ppm) -75.22 (s, –OCH$_2$CF$_3$).
5.2.4 Synthesis of β-CD End-Functionalized Poly[poly(ethylene glycol) methyl ether methacrylate] by ATRP (7) (β-CD-PmPEGMA).

4-Bromo-β-cyclodextrin (0.20 g, 0.12 mmol) was dissolved in 20 mL of anhydrous DMF, followed by addition of mPEGMA (33.6 g, 96 mmol) and PMDETA (80 mg, 0.46 mmol). Argon gas was bubbled through the solution for 20 min to remove any dissolved oxygen. Meanwhile, CuBr (34 mg, 0.24 mmol) was weighed in a small vial and air in the vial was removed by three 10 min purge/backfill cycles of vacuum and argon. The degassed CuBr was added to the solution, and the mixture was stirred for 2, 4, or 6 h under argon at room temperature to give polymers with various molecular weights. To terminate the polymerization, air was bubbled into the reaction medium for 10 min, and the copper catalyst was removed by passing the sample through a flash alumina column. Products in the collected clear solution were then precipitated into diethyl ether (200 mL). The precipitates were isolated by centrifugation as colorless adhesives. Each sample was then redissolved in 50 mL methanol, and the solution was dialyzed versus methanol for 3 d (Spectra/Por dialysis membrane, MWCO: 6,000–8,000). Solvent was removed under reduced pressure to give colorless adhesive polymers (Conv%=6.5%, 12%, and 17%). 1H NMR (CDCl3): δ (ppm) 6.06 (s, β-CD), 5.51 (s, β-CD), 4.24 (s, β-CD), 4.02 (s, PmPEGMA), 3.78 (s, β-CD), 3.74–3.48 (br, PmPEGMA and β-CD), 3.31 (s, PmPEGMA and β-CD), 1.89 (s, β-CD), 1.74 (s, PmPEGMA), 0.96 (br, PmPEGMA).

5.2.5 Synthesis of 2-[2-(Tetrahydropyran-2-yl)ethoxy]ethanol (8).

3,4-Dihydro-2H-pyran (21.27 g, 0.25 mol) was added over a period of 30 min to a mixture of p-toluenesulfonic acid (0.05 g, 0.267 mmol) in diethylene glycol (201.24 g, 1.89 mol) at 0 °C.
The reaction mixture was stirred for 2 h at 0 °C. It was then allowed to warm to room temperature, and was stirred for 1 d. After that, the mixture was poured into 500 mL of 1 M NaOH(aq) and was extracted with DCM (5×200 mL). The collected organic layers were dried over MgSO₄ overnight. The solvent was removed and the crude product was distilled at 40–50 °C under reduced pressure (4–5×10⁻¹ mbar) to collect the colorless liquid product, 8 (yield: 69%). ¹H NMR (CDCl₃): δ 4.55 (t, 1H), 3.80–3.43 (m, 10H), 1.72–1.44 (m, 6H).


To a THF solution of poly(dichlorophosphazene) (3 g, 25.89 mmol) was added the sodium salt of 10 prepared by the reaction of (tetrahydropyranyloxy)ethanol (12.3 g, 64.7 mmol) with NaH (2.90 g, 72.5 mmol, 60% in mineral oil). The mixture was stirred under reflux for 2 d. After that, the reaction medium was concentrated, and dialyzed against methanol for 2 d, and then against methanol/acetone/hexanes 50/20/30 for 2 d (Spectra/Por dialysis membrane, MWCO: 12–14,000). Solvent was removed by rotary evaporation at 40 °C, and a pale yellow adhesive product was obtained after drying under vacuum (yield: 71%). ³¹P NMR (CDCl₃): δ -8.12 (s). ¹H NMR (CDCl₃): δ 4.59 (s, 1H), 4.07 (s, 2H), 3.90–3.24 (bm, 8H), 1.92–1.47 (bm, 6H).

5.2.7 Synthesis of Poly[bis[2-(2-hydroxyethoxy)ethoxy]phosphazene] (13).

Compound 12 (2 g, 5.96 mmol) was dissolved in 40 mL trifluoroacetic acid in 40mL water. The solution was stirred at room temperature for 3 h, followed by neutralization of the solution with 5 M aqueous NaOH. The solution was then dialyzed against water for 2 d, then against methanol for 2 d (Spectra/Por dialysis membrane, MWCO: 12–14,000). The solution was dried,
and a brownish adhesive product was obtained (yield: 62%). $^{31}$P NMR (D$_2$O): $\delta$ -8.03 (s). $^1$H NMR (D$_2$O): $\delta$ 4.11 (s, 2H), 3.72–3.48 (bm, 6H).

5.2.8 Synthesis of Poly[bis[2-(2-hydroxyethoxy)ethoxy]phosphazene] (13).

Compound 13 (0.50 g, 1.96 mmol) was dissolved in 20 mL of anhydrous DMF. A suspension of NaH (0.118g, 2.94 mmol, 60% in mineral oil) in 10 mL anhydrous DMF was added to the polymer solution. The mixture was stirred for 30 min at room temperature. Then, propargyl bromide (0.17 g, 0.78 mmol, 80% in toluene) in 10 mL anhydrous DMF was added dropwise to the reaction mixture. The mixture was stirred at 80 °C for 1 d, and was then dialyzed against methanol/acetone/hexanes 50/20/30 for 2d (Spectra/Por dialysis membrane, MWCO: 12–14,000). The solvent was removed to give pale brown adhesive product (yield: 74%). $^{31}$P NMR (D$_2$O): $\delta$ -5.92 (s). $^1$H NMR (D$_2$O): $\delta$ 4.23 (s, 2H), 4.16 (s, 2H), 3.74-4.58 (br, 6H), 2.86 (s, 1H).

5.2.9 Synthesis of $\beta$-CD Containing Poly(organophosphazene) (15) (PPhos-$\beta$-CD).

Polymer 14 (0.36 g, 1.33 mmol) was dissolved in 10 mL DMF. To this solution was added $\beta$-CD-N$_3$ (0.47 g, 0.40 mmol) in a mixture of 10 mL DMF and 4 mL of DMSO, sodium ascorbate (8 mg, 0.04 mmol), and copper sulfate pentahydrate (10 mg, 0.04 mmol). The mixture was stirred at 100 °C under an argon atmosphere for 3 d. The copper catalyst was removed by means of a short neutral alumina column. The collected solution was precipitated into acetone (500 mL), and the suspension was stirred overnight. The pale brown precipitate was isolated by centrifugation, re-dissolved in 50 mL water, and dialyzed versus H$_2$O for 3 d (Spectra/Por dialysis membrane, MWCO: 12–14,000). Solvent was removed under reduced pressure at 50 °C, and the remaining
pale brown solid was freeze-dried (yield: 40%). $^{31}$P NMR (DMSO-$d_6$): $\delta$ -8.01 (s). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.26 (s, 1H, triazole), 5.92–5.68 (s, 14H, $\beta$-CD), 4.89–4.76 (s, 7H, $\beta$-CD), 4.48–4.36 (s, 6H, $\beta$-CD), 4.23 (s, 2H, diethylene glycol), 4.18 (s, 2H, diethylene glycol), 3.89–3.17 (br, 42H, $\beta$-CD; 6H, diethylene glycol).

5.2.10 Synthesis of Partially Adamantane Functionalized Poly(organophosphazene) (16) (PPhos-Ad).

To an anhydrous DMF solution (30 mL) of polymer 13 (0.40 g, 1.57 mmol) was added an anhydrous DMF solution (5 mL) of 1-adamantyl isocyanate (0.06 g, 0.31 mmol). Then a trace amount of TEA (0.04 mL, 0.31 mmol) was added. The mixture was stirred at 90 °C for 2 d. The polymer solution was then purified by dialysis versus methanol/aceton (40/60) for 3 d (Spectra/Por dialysis membrane, MWCO: 12–14,000). The solution was concentrated to 1/3 of its original volume, and precipitated into diethyl ether (200 mL). The pale brown adhesive precipitate was isolated and dried under vacuum to give polymer 16 (yield: 67%). $^{31}$P NMR (D$_2$O): $\delta$ -7.21 (s). $^1$H NMR (D$_2$O): 4.14 (s, 2H, diethylene glycol), 3.89–3.47 (bm, 6H, diethylene glycol), 2.08–1.53 (bm, 15H, adamantane).

5.2.11 Micelle Preparation.

To prepare micelle solutions, nanopure water (80 mL) with a conductivity of 18.2 MΩ/cm was added dropwise to a mildly stirred solution of the 1:1 host (7) (0.10 g, 0.005 mmol) and guest (5) (0.06 g, 0.005 mmol) polymers in THF (5 mL). Once the host–guest pseudo–block copolymer was formed, all the THF was removed under reduced pressure to give a 2 g/L of micelle aqueous
solution. Then, the solution was diluted with water to obtain a micelle concentration in the range of 2 g/L to $1 \times 10^{-5}$ g/L. For fluorescence measurements, a pyrene solution in THF ($1.2 \times 10^{-3}$ M) was added to nanopure water to give a final pyrene concentration of $12 \times 10^{-7}$ M. Following dilution, the THF was removed under reduced pressure. The pyrene solution was then mixed with the pseudo–block copolymer solutions to obtain copolymer concentrations ranging from 1 g/L to $5 \times 10^{-6}$ g/L, while the pyrene concentration of the all samples was maintained at $6 \times 10^{-7}$ M. The samples were sonicated for 10 min and were allowed to stand for 1 day before fluorescence measurements.

5.2.12 Supramolecular Gelation.

PPhos-β-CD (15) (200 mg) was dissolved in 2 mL of nanopure water (10% w/v) as a macromolecular host component. Then, PPhos-Ad (16) (200 mg) was dissolved in 2 mL of nanopure water (10% w/v) as a macromolecular guest component. The two components were mixed and shaken mechanically at room temperature to allow the formation of the supramolecular gels (18). To study the competitive dissociation of the obtained gels, 1 mL of a saturated β-CD aqueous solution was added to the gel and the system was shaken mechanically for 10 min.
5.3 Results and Discussion

5.3.1 Polymer Synthesis and Characterizations.

*Synthesis of Adamantane Terminated Linear Phosphazene (Ad-PTFE).* The development of the PCl\textsubscript{5}–induced living cationic polymerization of a phosphoranimine for preparing poly(dichlorophosphazene) allows the generation of polymers with controllable molecular weights and narrow polydispersity.\textsuperscript{19,20} More important, the modifiable chain-ends provide accessibility to various polyphosphazene–containing polymeric structures including di- or tri-block copolymers,\textsuperscript{21,22} dendrimers,\textsuperscript{23} and graft copolymers.\textsuperscript{24} As shown in Figure 5-2, polymerization was initiated uni-directionally with a target of 30 repeating units. Then, the adamantyl modified phosphoranimine was added to quench the living chain-end. Subsequently, the resultant polymer was treated with an excess of NaOCH\textsubscript{2}CF\textsubscript{3} to replace the chlorine atoms and yield the hydrophobic phosphazene guest block. The final product was soluble in acetone, DMF and THF, but insoluble in CHCl\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, methanol or water. The chain-end phosphorus signal was detected by \textsuperscript{31}P NMR, and the adamantyl moiety appeared in the \textsuperscript{1}H NMR spectrum (Figure 5-3). The calculation of the number of the repeating units from the \textsuperscript{31}P NMR spectra (from integrations of end-phosphorous -2.71 ppm and repeating unit phosphorous -7.74 ppm, 27 repeating units) and from \textsuperscript{1}H NMR (from integrations of OCH\textsubscript{2}CF\textsubscript{3} 4.36–4.81 ppm and adamantane 1.97 ppm, 34 repeating units) were close to the target of 30 repeating units. The difference in the number of repeating units calculated by GPC (\(M_n\sim12,300\) g/mol, repeating units~50, PDI~1.23) is due to the different hydrodynamic radii between Ad-PTFE and polystyrene standards (for the GPC calibration curve).
Figure 5-2: Synthesis chart of Ad-PTFE (5).
Synthesis of Branched-star Shape Organic Polymers Containing β-CD at the Chain-end (β-CD-PmPEGMA). Atom transfer radical polymerization (ATRP) is one of the most robust controlled/living radical polymerization techniques as it functions with a diversity of monomers, molecular weight control, and narrow polydispersity. Since its development, this technique has stimulated research and exploration of the synthetic boundaries of new polymeric structures, especially for synthesizing block copolymers. In this work, a β-CD based macroinitiator with four bromo-initiation sites on the primary face of β-CD (primary hydroxyl groups) was obtained following a well established method. With an increasing degree of substitution of 2-bromoisobutyryl bromide units, the solubility of the modified β-CD decreased dramatically in water, but increased distinctly in acetone. Tetra-substituted β-CD is at the boundary of solubility which makes it slightly soluble in both water and acetone. Hence, if treated with four equivalents of the bromo-substituent, the esterification reaction products can be purified by repeatedly
washing the crude product with water and acetone. ATRP was carried out using the CuBr/PMDETA complex as a catalyst, with poly(ethylene glycol) methyl ether grafted methyl methacrylate as a monomer (Figure 5-4). A small amount of DMF was added to dissolve the macro-initiator, and the polymerization conditions were optimized for the control of molecular weight and polydispersity. Because the presence of trace quantities of oxygen could oxidize the Cu(I) catalyst and quench the living radical, oxygen was carefully excluded from all reagents and the reactions were carried out under an inert atmosphere. In this work, the molar ratio of monomer to one initiation site (4 initiation sites on each ring) was 200:1. This large excess of monomer was intended to produce a narrower polydispersity by lowering radical concentrations and minimizing chain terminations. A series of guest blocks (β-CD-PmPEGMA) with increasing molecular weights were prepared by varying the polymerization time from 2 h to 6 h (Figure 5-5). The proton resonances of both the chain-end β-CD and four armed PmPEGMA units were detected in the $^1$H NMR spectrum (Figure 5-6).

![Figure 5-4: Synthetic chart of β-CD-PmPEGMA (7).]
Synthesis of Graft Phosphazenes with Pendent $\beta$-CD Units: Macromolecular Hosts ($\text{PPhos-}\beta$-CD). Generally speaking, the substitution reactions of poly(dichlorophosphazene) become more challenging as the structures of the chlorine-replacement nucleophiles become more complex. Large side groups generate more steric hindrance with a corresponding decrease in

Figure 5-5: GPC traces of a series of $\beta$-CD-PmPEGMA.

Figure 5-6: $^1$H NMR of $\beta$-CD-PmPEGMA (7).
reactivity. Meanwhile, the solubilities of the polymers usually decrease after the attachment of large or complex side-groups. This tends to hinder their synthesis, characterization and processibility. Complex or multi-functional side groups also necessitate multi-step protection and deprotection reactions in order to prevent crosslinking or side reactions during the macromolecular substitutions. Thus, linkage of $\beta$-CD units to polyphosphazene side-chains is a challenging prospect because the multiple hydroxyl functional groups on $\beta$-CDs may require serious synthetic efforts to prevent unwanted crosslinking. Moreover, the possibility also exists that the resultant polymers will have poor solubility, or that sterically induced low reactivity will be experienced during the secondary macromolecular modifications. In order to solve these problems, two strategies were employed for designing the polymeric structure and the synthesis protocols. First, one of the 21 hydroxyl groups on the $\beta$-CD must be converted to a different functional group, leaving the remaining hydroxyl groups un-modified. The new functional group should be able to undergo a distinct reaction while the other hydroxyl groups are inert under the reaction conditions. Second, a long and flexible spacer group must connect the polymer backbone to the $\beta$-CD to increase the accessibility of the functional groups in the bulky $\beta$-CDs. Meanwhile, the spacer groups should not change the water solubility or increase the potential toxicity of the resultant polymers depending on their applications.

Progress on modification techniques for CDs, especially for mono-functionalization, has greatly accelerated the diversity of compounds based on $\beta$-CD containing polymers in recent years. Also, since Sharpless and coworkers introduced the “click” chemistry concept in 2001, this copper–catalyzed azide–alkyne cycloaddition (CuAAC) has proved to be an extremely useful tool for construction of polymer networks due to its mild reaction conditions, high reactivity, solvent and functional group tolerance, and lack of by-products. Thus, based on earlier work, $\beta$-CD was mono-azidified (10) on the primary face to selectively react with alkyne functional groups introduced as side units on the polyphosphazene backbone. In order to enhance the
reactivity of the “click” reaction, diethylene glycol groups were used as spacer units to increase the side chain flexibility and lower steric hindrance. Mono-protection of the hydroxyl groups on diethylene glycol (8) was carried out before macromolecular substitution to prevent polymer crosslinking (Figure 5-7). Dihydropyran was selected as the protecting group due to the ease of macromolecular deprotection under acidic conditions.

![Monoprotection of Diethylene Glycol](image)

**Figure 5-7**: Synthesis chart of small molecules for macromolecular reactions.

Thus, as shown in Figure 5-8, poly(dichlorophosphazene), prepared by the thermal ring-opening polymerization of hexachlorocyclotriposphazene, was treated with the sodium salt of mono-protected diethylene glycol to give polymer 12 with a molecular weight (g/mol) of 520,000 (~2720 repeat units). The side group deprotection reaction was carried out in aqueous trifluoroacetic acid to release the free hydroxyl groups for further reaction (13). The success of the deprotection reaction was easily verified by the disappearance of the peaks at 1.92–1.47 ppm (dihydropyran) in the $^1$H NMR spectrum (Figure 5-9). Ten percent of the hydroxyl groups were activated to alkyne group 14 to further react with $\beta$-CD-N$_3$. A new peak at 2.8 ppm from $^1$H NMR indicated the success of the alkyne activation at the terminus of the polymer side-chains. A
maximum of 10% of $\beta$-CD could be “clicked” to the side chains even though the alkynel-activation in the prior step was more than 10%. Because the size of $\beta$-CD is significantly larger than the repeating units of the polyphosphazene backbone, a substantial increase in steric hindrance occurred after the $\beta$-CD units were introduced. This sheltered the remaining functional groups on the polymer from access to additional $\beta$-CD. Meanwhile, the azide functional group on the bulky $\beta$-CD can only interact effectively with alkyn functional groups on the polymer from one direction (at the primary face), which further decreases the overall reactivity. New peaks assigned to $\beta$-CD from the $^1$H NMR spectra indicated the presence of the $\beta$-CD in the polymer. The peak of one end-proton of the alkyn at 2.8 ppm shifted to around 8.3 ppm and represented the formation of a triazole bridge. The new polymer, PPhos-$\beta$-CD, shows no obvious shift in the $^{31}$P NMR spectra (-7 ppm) because the modifications were too distant from the backbone phosphorous atoms (Figure 5-10). Meanwhile, $^{31}$P NMR spectra of 12–15 showed similar signal peaks without significant broadening. This implies that degradation of the polymer was minimal during the multi-step macromolecular side-chain modifications. PPhos-$\beta$-CD (15) showed no evidence of crosslinking since it was soluble in DMF, DMSO (room temperature), and water (heated), but insoluble in less polar organic solvents.
Figure 5-8: Synthetic scheme leading to PPhos-β-CD (15).

Figure 5-9: $^1$H NMR of polymers 12–15.
DSC measurements provide additional evidence of the successful linkage of β-CD to the polyphosphazene backbone (Figure 5-11). Polymer 14 with flexible diethylene glycol side chains had a low glass transition temperature ($T_g$) at around -47 °C. After clicking 10% of β-CD onto the polymer side-chains, the $T_g$ of PPhos-β-CD (15) increased substantially to about 50 °C. This major increase in $T_g$ is attributed to an increase in side chain steric hindrance which prevents network movement, together with assisting the formation of additional hydrogen bonding from the multiple hydroxyl groups on the β-CD. The significant change in $T_g$ could also be detected directly by the different textures of 14 (adhesive gum) and 15 (glassy powder) at room temperature. No melting behavior was detected for 15 up to 220 °C. Above that temperature, intense signal fluctuation was detected from the DSC experiments due to the thermal decomposition.
Synthesis of Graft Phosphazene Containing Adamantane Units at the Termini of the Side-chains: Macromolecular Guests (PPhos-Ad). For the macromolecular guests, around 10% of the adamantyl moieties were linked to the polyphosphazene side-chain. The diethylene glycol spacer groups were required to utilize more flexible side-chains in order to better interact with the host component during “host-guest” complexations. Initial attempts following the same method for synthesizing polymer 14, by reacting polymer 13 with NaH and 1-bromoadamantane, were not successful, probably due to the low reactivity of bulky 1-bromoadamantane. Later, 1-adamantyl isocyanate was used for the formation of a carbamate linkage (Figure 5-12). New peaks appeared at 2.08−1.53 ppm indicating the successful introduction of the adamantyl group by this method (Figure 5-13). The resultant adhesive polymer 16 was soluble in water and DMSO.
5.3.2 Assembly of Organo–Phosphazene Structures by Host–Guest Interactions.

*Host–Guest Interactions at the terminus of the Main-chain.* Palm tree-like pseudo-block copolymer structures (PmPEGMA-\(pb\)-PTFE, 17) were obtained by exploiting the supramolecular interaction between adamantane guest 5 and \(\beta\)-CD host 7 in water. Previous studies have showed that the association constant for adamantane and \(\beta\)-CD is high in the presence of water (\(10^4–10^5\) M\(^{-1}\)).\(^{34}\) Meanwhile, the host-guest interaction between \(\beta\)-CD and oligo(ethylene glycol) is
minimal due to the inappropriate size. The 2D $^1$H NOESY spectrum was obtained after mixing equimolar quantities of the host block and the guest block in DMSO-$d_6$ with a few drops of D$_2$O (Figure 5-14). The cross-peaks at around 3.5 ppm and 1.9 ppm are assigned to the inner protons of $\beta$-CD and the protons of the adamantyl moiety respectively. This indicates the close proximity of these protons, which is direct evidence in favor of the host–guest interactions.

Micelles were prepared by dissolving host and guest blocks in THF. Then a large amount of water was added dropwise to reach a target block copolymer concentration of 2 g/L, followed by the removal of THF at reduced pressure. This stock micelle solution was further diluted to various lower concentrations. The formation of micelles was demonstrated by four methods, fluorescence, dynamic light scattering, TEM, and AFM. The critical micelle concentrations (cmc) of **PmPEGMA-pb-PTFE** (17) were studied by a standard fluorescence technique using pyrene as a fluorescence probe. Although pyrene is a guest molecule for $\beta$-CD as well, the partition of pyrene in the $\beta$-CD cavity is negligible due to the presence of the adamantyl moiety since adamantane is a better guest with a much higher association constant. In the excitation fluorescence spectra of pyrene, the peak initially at 333 nm at low polymer concentrations red-shifted to 336 nm when the polymer concentrations increased to a certain point. At the same time, the intensity of the pyrene fluorescence increased substantially. This turning point of the pyrene’s fluorescence behavior is due to the inclusion of pyrene from aqueous solution into the hydrophobic core of the micelles, which indicates the lowest concentration of the pseudo-block copolymer required for the formation of micelles (cmc). The cmc value can then be calculated by plotting the ratios of the peak intensities at 336 nm to 333 nm ($I_{336}/I_{333}$) versus the logarithm of the polymer concentrations (Figure 5-15). The threshold concentration at the turning point of $I_{336}/I_{333}$ reflected the shift of pyrene from an aqueous environment into the hydrophobic environment in the micelle core. The cmc values summarized in Table 5-1 showed that higher cmc values were obtained with an increase in the molecular weight of the hydrophilic block.
Figure 5-14: 2D $^1$H NOESY spectrum of the mixture of $\beta$-CD-PmPEGMA host and Ad-PTFE guest (1:1) in DMSO-$d_6$ with a few drops of D$_2$O at room temperature. The cross-peaks in the circles indicate the host–guest interaction.

Figure 5-15: Plot of $I_{350}/I_{335}$ (from pyrene excitation spectra) vs log C for PmPEGMA-pb-PTFE
Dynamic light scattering (DLS) suggested an absence of self-aggregation for any of the three host blocks in water. However, when Ad-PTFE was introduced as a guest block (PmPEGMA-pb-PTFE), self-aggregations were detected for all of variations (1 g/L) due to the formation of amphiphilic pseudo-block copolymers (Figure 5-16). Meanwhile, the hydrodynamic radius of the aggregations increased with an increase in molecular weight of β-CD-PmPEGMA. The sizes and shapes of the micelles were also examined by TEM and AFM as shown in Figure 5-16. The average micelle diameter measured by TEM and AFM was in agreement with the mean diameter measured by DLS. The existence of larger micelles with diameters in excess of several hundred nanometers resulted from inter-micellar aggregations with the resultant formation of multi-core micelle structures. Because of the non-covalent connection between the hydrophilic and the hydrophobic blocks, the micelles showed competing guest-responsive self-disassembly behavior. Thus, the addition of pure β-CD host to the micelle solutions induced the dissociation of the micelle structure. β-CD competed with β-CD-PmPEGMA to associate with the adamantyl group on the phosphazene block, thus breaking the non-covalent connections. The TEM results in Figure 5-16 showed the contours of the micelle becoming diffuse, and the spherical shape evident in image (b) no longer existed after the addition of β-CDs. DLS also confirmed the

<table>
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<tr>
<th>Entry</th>
<th>$M_n$ (g/mol) (Ad-PTFE)</th>
<th>$M_n$ (g/mol) (β-CD-PmPEGMA)</th>
<th>cmc (mg/L)</th>
<th>r (nm)</th>
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<td>PmPEGMA-pb-PTFE-1</td>
<td>12,300</td>
<td>19,450</td>
<td>4.6</td>
<td>32</td>
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<tr>
<td>PmPEGMA-pb-PTFE-2</td>
<td>12,300</td>
<td>34,660</td>
<td>8.1</td>
<td>44</td>
</tr>
<tr>
<td>PmPEGMA-pb-PTFE-3</td>
<td>12,300</td>
<td>49,310</td>
<td>28.2</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 5-1: Summary of PmPEGMA-pb-PTFE micelles.
dissociation since the previous narrow size distribution became broad, with a decrease of average radius.

Figure 5-16: AFM (a), TEM (b), and DLS (c) of micelles formed by PmPEGMA-pb-PTFE-3; TEM (d) after the addition of pure β-CD guests.

**Host–Guest Interaction on the Side-Chain.** Pyrene was introduced as a probe guest molecule to study the interaction between the hydrophobic guest and the hydrophobic cavity of β-CD on the side-chains. As shown in the excitation spectra (Figure 5), the fluorescence peak of pyrene was red shifted with an increased intensity in the presence of PPhos-β-CD. Meanwhile, the intensity ratio of the first and third vibrational bands ($I_1/I_3$) of pyrene from the emission spectra changed substantially from $I_1>I_3$ to $I_1<I_3$, and the overall fluorescence intensity increased after the
addition of PPhos-β-CD. This indicated that the pyrene was transferred from the aqueous environment to the hydrophobic β-CD cavities pendent to the polyphosphazene backbone.

A supramolecular gel was obtained by mixing the “guest component” PPhos-Ad and the “host component” PPhos-β-CD. Host–guest interactions between the side chains of the two polyphosphazenes formed non-covalent crosslinks. As shown in Figure 5-18, a gel (18) could be obtained within one hour at higher host polymer and guest polymer concentrations (a, 10% w/v), while only a viscous solution was generated if the concentrations of the two components were lower. Rheological measurement demonstrated the formation of a soft gel, since the storage modulus ($G'$ = 5.1 kPa) surpassed the loss modulus ($G''$ = 1.8 kPa). The soft gel (18) showed no injectable behavior due to a high association constant between adamantane and β-CD. The soft gel (a) turned into viscous solution (b) if a pure β-CD host aqueous solution was added with mechanical shaking for a certain time. The small-molecule host, β-CD, competes with macromolecular host PPhos-β-CD to interact with PPhos-Ad leading to dissociation of some of the physical crosslinks. Moreover, the formation of gel was also inhibited if the macromolecular guest component PPhos-Ad was pre-mixed with pure β-CD before mixing with PPhos-β-CD.
5.4 Conclusions

The assembly of new polyphosphazene structures based on non-covalent “host–guest” interactions (β-CD and adamantane) was achieved both at the termini of the phosphazene main-chain and on the side-chains. Amphiphilic palm-tree like pseudo-block copolymers were obtained through the host-guest coupling of an adamantane end-functionalized polyphosphazene and 4-armed β-CD initiated poly[poly(ethylene glycol) methyl ether methacrylate]. The micelle properties of the non-covalent amphiphiles were studied by various techniques. β-CD or adamantane were also introduced onto the side-chains of polyphosphazenes to form macromolecular hosts or macromolecular guests. The β-CD containing polyphosphazene functions as a macromolecular host demonstrated its capability to carry hydrophobic molecules and the ability to form supramolecular gel when mixed with a macromolecular guest. The success of constructing of these new poly(organophosphazene) structures not only extends the synthetic
boundary of phosphazene related materials but, more important, could endow the new materials aggregative, stimulus-responsive, guest–carrying/releasing, and gelation properties which could extend the areas of application for this system.

5.5 Acknowledgements

The authors thank Dr. Quan Chen and Professor Ralph Colby for assistance with the rheological measurements, and Kan Shen for assistance with AFM.

5.6 References

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32. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem., Int. Ed. 2002, 41, 2596–2599.
Chapter 6
New Mixed-Substituent Fluorophosphazene High Polymers and Small Molecule Cyclophosphazene Models: Synthesis, Characterization, and Structure Property Correlations

6.1 Introduction

Fluoropolymers have been particularly interesting and attractive materials since their first discovery in the late 1930s. They have been utilized extensively in various applications including building materials, the petrochemical and automotive industries, aerospace and aeronautics, optics, fabrics, textiles, and electronics, etc.¹,² Fluoroelastomers are especially important because of their high thermal and chemical stability, resistance to aging, and good weather resistance, low flammability, and inertness to solvents and hydrocarbons.³ Meanwhile, they are often flexible over a broad range of operational temperatures. Currently, most fluoroelastomers are based on fluorocarbon or fluorosilicone systems, such as copolymers of hexafluoropropylene, tetrafluoroethylene, and vinylidene fluoride, or trifluoropropylsilox and dimethylsilox monomers.

Table 6-1: Definitions for abbreviations used.

<table>
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<tr>
<th>Polymer structures</th>
<th>Polymer names</th>
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</thead>
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<tr>
<td>[ N=P(OCH CF\textsubscript{2}CF\textsubscript{3})\textsubscript{3} ] _</td>
<td>Poly[tris(trifluoroethoxy)cyclotriphosphazene]</td>
</tr>
<tr>
<td>[ N=P(OCH\textsubscript{2}CF\textsubscript{3})\textsubscript{1.54} (OCH\textsubscript{2}CF\textsubscript{3})\textsubscript{0.46} ] _</td>
<td>Poly[(trifluoroethoxy)\textsubscript{1.54} (hexafluoropropoxy)\textsubscript{0.46}]</td>
</tr>
<tr>
<td>[ N=P(OCH\textsubscript{2}CF\textsubscript{3})\textsubscript{1.34} (OCH\textsubscript{2}CF\textsubscript{3})\textsubscript{0.66} ] _</td>
<td>Poly[(trifluoroethoxy)\textsubscript{1.34} (hexafluoropropoxy)\textsubscript{0.66}]</td>
</tr>
<tr>
<td>[ \text{N}_3\text{P}[OCH(CF\textsubscript{3})\textsubscript{3}]\textsubscript{6} ]</td>
<td>Hexakis(hexafluoropropoxy)cyclotriphosphazene</td>
</tr>
<tr>
<td>[ \text{N}_3\text{P}[O(CF\textsubscript{3})\textsubscript{3}]Cl ]</td>
<td>Mono(tert-perfluorobutoxy)penta(chloro)cyclotriphosphazene</td>
</tr>
<tr>
<td>[ \text{N}_3\text{P}[OCH(CF\textsubscript{3})\textsubscript{3}] ]</td>
<td>Hexakis(trifluoroethoxy)cyclotriphosphazene</td>
</tr>
</tbody>
</table>
Poly[bis(trifluoroethoxy)phosphazene] (I), was one of the first stable organophosphazene polymers, synthesized by Allcock and Kugel in 1964.\textsuperscript{4,5} It remains today one of the most intriguing fluoropolymers and, at the same time, is the single most heavily studied organophosphazene high polymer. This polymer has attracted attention because of its facile synthesis and purification procedures, solubility in some common organic liquids, good film and fiber forming capability, relatively good thermal and radiation stability, and its microcrystallinity. Species I has been proposed for a variety of applications including inert biomaterials,\textsuperscript{6,7} drug delivery,\textsuperscript{8,9} gas or liquid separation membranes,\textsuperscript{10-12} and super-hydrophobic surface coatings.\textsuperscript{13,14} The presence of other fluorinated cosubstituents in addition to trifluoroethoxy groups lowers the macromolecular symmetry and disrupts the crystalline domains. Such mixed-substituted polyphosphazenes are high-performance rubbery elastomers, known by their trade names as PN-F or Eypel-F.\textsuperscript{15,16} These phosphazene fluoroelastomers are significant technologically due to their low-temperature flexibility, oil-, fuel-, and hydraulic fluid resistance, thermo-oxidative stability, non-flammability, and biological inertness.\textsuperscript{4,17-20} Apart from use of conventional fluoro-cosubstituents, such as the \(2,2,3,3,4,4,5,5\)-octafluoro-1-pentoxy group, several attempts have also been made to study different side group combinations and investigate the corresponding structure property correlations (Figure 6-1).\textsuperscript{4}

<table>
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<th>Structure</th>
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<td>A</td>
<td>(\text{OCH}_2\text{CF}<em>3\text{N}(</em>\text{CF}_2\text{O})_x\text{CF}_3) (x=1-4)</td>
</tr>
<tr>
<td>B</td>
<td>(\text{OCH}_2\text{CF}<em>3\text{N}(</em>\text{CF}_2\text{O})_y\text{CF}_3) (y=1-2)</td>
</tr>
<tr>
<td>C</td>
<td>(\text{OCH}_2\text{CF}<em>3\text{N}(</em>\text{CF}_2\text{O})_2\text{CF}_3) (x=1-2)</td>
</tr>
<tr>
<td>D</td>
<td>(\text{OCH}_2\text{CF}<em>3\text{N}(</em>\text{CF}_2\text{O})_5\text{P}<em>3\text{O}(</em>\text{CF}_2\text{O})_y\text{CF}_3) (y=5-7)</td>
</tr>
<tr>
<td>E</td>
<td>(\text{OCH}_2\text{CF}<em>3\text{N}(</em>\text{CF}_2\text{O})_3\text{CF}_3) (x=1-3)</td>
</tr>
</tbody>
</table>

Figure 6-1: Structures of mixed-substituted poly(fluoroalkoxyphosphazenes).
Earlier studies of the structure–property relationships of mixed-substituent poly(fluoroalkoxyphosphazenes) emphasized four variations of cosubstituents all with trifluoroethoxy as one of the substituents (Figure 6-1). First, Type A fluoroelastomers use a fluoroalkoxy unit with a terminal –CF₂H unit in the cosubstituent but with variations in the number of –CF₂ units between the oxygen and the terminus. This type of mixed-substituent poly(fluoroalkoxyphosphazene) is soluble in a wide range of organic solvents and therefore has good processibility.¹⁵,¹⁷ In addition, they are less expensive to produce than some of the more complex analogues and have been developed commercially. In Type B fluoroelastomers, terminal –CF₂H group in the cosubstituent are replaced by a –CF₃ unit. This type of poly(fluoroalkoxyphosphazene) usually has limited solubility in solvents other than specialized fluoroethers.²¹,²² More recently, an increase in the number of –CH₂ units between the oxygen atom and the fluoroalkyl tails, as in Type C fluoroelastomers, has been investigated.²³ With the change in the structure of the cosubstituents, the newer poly(fluoroalkoxyphosphazenes) have different glass transition temperatures, hydrophobicity, solubility, or stability. Some of them are promising candidates as low-temperature elastomers or adhesives, while others are leathery or rigid materials. These species expand the structural boundary of the fluoropolymers, and suggested additional applications and possibilities. Recently another variant has been prepared in our laboratory (Type D) in which trifluoroethoxy-substituted phosphazene rings were linked to the phosphazene polymer chains in addition to trifluoroethoxy cosubstituents linked to the main chain.²⁴ Type D systems show elastomeric properties due to the intermolecular non-covalent interactions between the cyclophosphazene side groups.

One factor is common for types A, B, and C poly(fluoroalkoxyphosphazenes): linear fluoroalkyloxy groups were used as cosubstituents together with trifluoroethoxy groups. However, it has never been ascertained if branched cosubstituents, ie. with one or two additional –CF₃
groups attached to the \(\alpha\)-carbon atom as \textit{iso-} or \textit{tert-} type branched alkoxy units, can be employed in these polymer synthesis reactions and what the polymer properties would be. Chemical attachment of such branched side groups (with higher fluorine content and larger dimensions) is an important prospect, since such species could significantly influence the properties of poly(fluoroalkoxyphosphazenes) via the glass transition temperature, thermal stability, hydrophobicity, and elastomeric properties. The synthesis of such polymers is more challenging than for the earlier macromolecules given the possible complications of steric hindrance, and perhaps limited solubility.

Thus, in this work a new group of fluoroalkoxyphosphazenes, Type E systems, was synthesized and studied both at the small-molecule model compound level of phosphazene cyclic trimers and as linear high polymers (Table 6-1). The structures were examined by \(^{31}\text{P}, \quad ^{19}\text{F} \) and \(^1\text{H} \) nuclear magnetic resonance (NMR) spectrometry. The molecular weights were determined by gel-permeation chromatography (GPC), and the thermal properties by differential scanning calorimetry (DSC) and thermo-gravimetric analysis (TGA). The crystallinity was examined by X-ray diffraction techniques, and the hydrophobicity/oleophobicity was studied by contact angle measurements using water or \(n\)-hexadecane. Lightly crosslinked elastomers were prepared by the introduction of 2-allyl phenoxy cosubstituent side groups for ultraviolet crosslinking (polymers 5 and 6). For the crosslinked polymers, preliminary tensile tests and deformation recovery tests were carried out to study the mechanical and elastomeric properties for comparison with the other variants. The possibility of substituent exchange reactions and alpha-carbon attack on side groups during the synthesis processes were also monitored and studied at the small molecule cyclic trimer level to understand and address some challenges experienced during the preparation of the high polymer.
6.2 Experimental Section

6.2.1 Reagents and Equipment.

Reagents. Tetrahydrofuran (THF) was purchased from EMD and was dried using solvent purification columns. Sodium hydride (NaH, 60% in mineral oil, Sigma-Aldrich) and sodium metal (Sigma-Aldrich) were stored in an inert atmosphere and used as received. Trifluoroethanol (Sigma-Aldrich), hexafluoropropanol (Matrix Scientific), and tert-perfluorobutanol (Sigma-Aldrich), 2-allyl phenol (Sigma-Aldrich) were distilled and stored over 4A molecular sieves (EMD) in an argon atmosphere. All synthesis reactions were carried out using standard Schlenk line techniques and a dry argon atmosphere. The glassware was dried overnight in an oven at 120 °C before use.

Structural Characterizations. $^1$H and $^{31}$P spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 or 145 MHz, respectively. $^1$H NMR spectra were referenced to solvent signals, while $^{31}$P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. $^{19}$F NMR spectra were collected using a Bruker CDPX-300 spectrometer operated at 282 MHz with trifluoroacetic acid as an internal standard.

Molecular Weights and Distributions. Polymer molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns, calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt% solution of tetra-n-butylammonium nitrate (Sigma-Aldrich) in THF.

Thermal Analysis. Thermal characteristics of samples were measured with a TA Instruments Q10 differential scanning calorimeter and a Perkin-Elmer thermogravimetric analyzer. About 10
mg of dried sample was used for each test. A heating rate of 10 °C/min with a temperature range from -100 to 200 °C was used for DSC, while a heating rate of 20 °C/min from 25 to 800 °C was applied for TGA. Both instruments used dry nitrogen as the purge gas.

X-ray Diffraction. X-ray diffraction data were recorded for polymer films using a wide-angle configuration of a Bruker D8 Advanced diffractometer. The radiation source used was Ni-filtered, CuKα radiation with a wavelength of 1.54 Å. The voltage was set to 40 kV and the current to 40mA. The data were collected at a rate of 2° = 5° min⁻¹ over the range 2θ = 5°–60°.

Contact Angle Measurements. Advancing contact angles were determined with use of a Ramé-Hart automated goniometer/tensiometer (Succasunna, NJ) with DROPimage Advanced v2.6 at room temperature. Polymer films were solution cast from THF on glass slides (25 × 75 mm) and air-dried before measurements. A drop volume of 2 µL deionized water or n-hexadecane was used for each test and the result was the average based on 10 measurements.

Tensile Tests. The tensile tests were carried out using an Instron 5866 unit at a fixed crosshead speed of 50 mm/min equipped with a 100 N load cell at an ambient temperature (~20 °C). The specimens were prepared by a standard solvent-casting technique using THF solvent on a Teflon substrate. After a crosslinking treatment, dog-bone shapes were cut using a Pioneer Dietecs die according to ASTMD-1708. The thickness of each dog-bone sample was measured by a caliper (Mitutoyo, Japan) before testing. The data were collected on at least 6 different samples. For the cyclic tensile deformation and recovery tests, 4 recycles were tested on each dog-bone sample with a maximum testing elongation of 60% of its elongation-at-break. A crosshead speed of 50 mm/min was applied for all the deformation and recovery tests.
6.2.2 Synthesis of Hexakis(hexafluoropropoxy)cyclotriphosphazene (7).

Sodium metal (Na) (1.06 g, 46.02 mmol) was stirred with hexafluoropropanol (7.73 g, 46.02 mmol) in 50 mL THF at room temperature for 30 min to allow the formation of the sodium salt. Later, the sodium salt was added to 50 mL of a THF solution of hexachlorocyclotriphosphazene (2.00 g, 5.75 mmol). The chlorine replacement was allowed to progress at room temperature for 4 hours to complete the reaction. After that, the reaction solvent was removed under reduced pressure. The residue was redissolved in ethyl acetate (100 mL) and extracted versus water 3 times. The organic layer was collected and the solvent was removed. The crude product was further purified by column chromatography using hexanes: ethyl acetate (5: 1) as an eluting solvent. The trimer after purification was dried under vacuum as a white crystalline product (yield=79%). $^{31}$P NMR (acetone-$d_6$) δ (ppm): 14.42 (s). $^{19}$F NMR (acetone-$d_6$) δ (ppm): -75.65 (s, –OCH(CF$_3$)$_2$). $^1$H NMR (acetone-$d_6$) δ (ppm): 5.82 (s, –OCH(CF$_3$)$_2$, 1H).

6.2.3 Preparation of Poly(dichlorophosphazene) ([NPCl$_2$]$_n$).

Poly(dichlorophosphazene) was prepared by the BCl$_3$-catalyzed thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical Co., Japan) in evacuated Pyrex tubes at 220 °C (yield=60%).$^{25}$ $^{31}$P NMR (acetone-$d_6$) δ (ppm): -17.26 (s).

6.2.4 Preparation of Poly(dichlorophosphazene) ([NPCl$_2$]$_n$).

Poly(dichlorophosphazene) (1.0 g, 8.63 mmol) was dissolved in anhydrous THF (100 mL). Meanwhile, trifluoroethanol (2.16 g, 21.58 mmol) and NaH (0.86 g, 21.58 mmol, 60% in mineral
oil) were stirred in anhydrous THF (100 mL) in a separate Schlenk flask at room temperature for 1 hour. This mixture was then added to the polymer solution dropwise and was allowed to react at room temperature for 1 day. The reaction medium was concentrated and precipitated into water twice (2 L), and hexanes twice (2 L). The collected product was dried under vacuum as a white fibrous material (yield = 88%). $^1$H NMR (acetone-$d_6$) $\delta$ (ppm): 4.58 (s, –OC$_2$H$_2$CF$_3$, 2H). $^{19}$F NMR (acetone-$d_6$) $\delta$ (ppm): -75.79 (s, –OCH$_2$CF$_3$). $^{31}$P NMR (acetone-$d_6$) $\delta$ (ppm): -7.81 (s).

6.2.5 Synthesis of Mixed-Substituent Poly(fluoroalkoxyphosphazenes) (2 and 3).

The synthesis of 2 is described in detail as an example. A THF solution (100 mL) of sodium trifluoroethoxide (NaOCH$_2$CF$_3$) prepared by the reaction of NaH (1.76 g, 44.01 mmol, 60% in mineral oil) with trifluoroethanol (4.40 g, 44.01 mmol) was added to a THF solution (300 mL) of poly(dichlorophosphazene) (3.00 g, 25.89 mmol). The reaction mixture was stirred at room temperature for 6 hours. Then, a solution of sodium hexafluoropropoxide, NaOCH(CF$_3$)$_2$, prepared by the reaction of NaH (0.62 g, 15.53 mmol, 60% in mineral oil) with hexafluoropropanol (2.61 g, 15.53 mmol) in 100 mL THF in a separate flask was added to the polymer reaction. The reaction mixture was further stirred for 8 hours at room temperature to complete the chlorine replacement. The reaction medium was then concentrated to one third of its original volume at 40 °C, followed by precipitation of the polymer from THF into water (3 L) twice, and from THF into hexanes (2 L) twice. The product obtained was then dried under vacuum to yield a rubbery material (2). $^{31}$P NMR (acetone-$d_6$) $\delta$ (ppm): -8.02 (br). $^{19}$F NMR (acetone-$d_6$) $\delta$ (ppm): -74.35 (s, –OCH(CF$_3$)$_2$), -76.47 (s, –OCH$_2$CF$_3$). $^1$H NMR (acetone-$d_6$) $\delta$ (ppm): 5.44 (s, OCH(CF$_3$)$_2$, 1H), 4.53 (s, OCH$_2$CF$_3$, 2H).
6.2.6 Synthesis of Mixed-Substituent Poly(fluoroalkoxyphosphazene) (4).

Poly(dichlorophosphazene) (1.00 g, 8.62 mmol) was dissolved in 100 mL THF. To the polymer solution was added a solution of sodium tert-perfluorobutoxide prepared from tert-perfluorobutanol (1.02 g, 4.32 mmol) and NaH (0.17 g, 4.32 mmol, 60% in mineral oil) in 50 mL THF. In the first step, the reaction mixture was stirred at 66 °C for 14 days. After that, sodium trifluoroethoxide, prepared from trifluoroethanol (1.90 g, 18.99 mmol) and NaH (0.76 g, 18.99 mmol, 60% in mineral oil) in 50 mL THF was added to the reaction mixture. The mixture was then stirred at room temperature for 1 day to complete the substitution. This polymer was purified by precipitation from THF into water, and from THF into hexanes three times respectively. The collected product was dried intensively under vacuum to give a waxy polymer. $^{31}$P NMR (acetone-$d_6$) δ (ppm): -8.33 (br). $^{19}$F NMR (acetone-$d_6$) δ (ppm): -75.51 (s, −OC(CF$_3$)$_3$), -76.12 (s, −OCH$_2$CF$_3$). $^1$H NMR (acetone-$d_6$) δ (ppm): 4.51 (s, OCH$_2$CF$_3$, 2H).

6.2.7 Substituent Exchange Reactions and Alpha-carbon Attack on Model Phosphazene Trimers.

The use of excess trifluoroethoxide to exchange side groups in 7 is described in detail. Species 7 (0.20 g, 0.176 mmol) was dissolved in THF (30 mL). In a separate flask, trifluoroethanol (0.21 g, 2.11 mmol) was stirred with sodium metal (0.05 g, 2.11 mmol) for 12 hours at room temperature before being added to the trimer solution. The reaction mixture was stirred at 66 °C for 2 days and the progress of any exchange reactions was monitored by $^{31}$P NMR spectroscopy. The presence of the etheric side products was established by a mass spectrometric analysis of the reaction mixtures.
6.2.8 Preparation of Thermosetting Elastomers.

Crosslinkable polymers 5 and 6 were synthesized using similar procedures to those described above. For example, polymer 5 was synthesized on a larger scale from poly(dichlorophosphazene) (15.00 g, 129.46 mmol) by the introduction of 3 mol% of sodium 2-allyl phenolate first prepared by 2-allyl phenol (1.04 g, 7.77 mmol) and NaH (0.31 g, 7.77 mmol, 60% in mineral oil). Then, 80 mol% of sodium trifluoroethoxide (trifluoroethanol (20.71 g, 207.14 mmol), NaH (8.39 g, 207.14 mmol, 60% in mineral oil)) was added second. An excess mol% of the sodium hexafluoropropoxide was introduced in the last step by using the reaction of hexafluoropropanol (13.05 g, 77.68 mmol) with NaH (3.11 g, 77.68 mmol, 60% in mineral oil). The polymers were purified by the same precipitation technique as described above and were characterized by NMR techniques (yield = 74%–79%). $^{31}$P NMR (acetone-$d_6$) $\delta$ (ppm): -7.89 (br), -12.54 (br). $^1$H NMR (acetone-$d_6$) $\delta$ (ppm): 7.28 (br, 4H), 6.01 (s, 1H), 5.41 (br, 1H), 5.11 (s, 2H), 4.55 (s, 2H), 3.53 (s, 2H). Once synthesized, these polymers were dissolved in THF (40% w/v), and the polymer solutions were cast on a Bytac surface (PTFE), and were allowed to dry slowly at room temperature (air-dried for 4 days, vacuum-dried for 2 days). After that, the polymer films were exposed to ultraviolet irradiation under a nitrogen atmosphere (the wavelength was 254 nm from an EFOS Ultracure 100ss Plus, Mississauga, Ontario). At certain time intervals, films were taken from the irradiation chamber and ten dog-bone samples were prepared for each tensile test.

6.3 Results and Discussion

6.3.1 Cyclic Trimer Model Reactions.

Macromolecular substitutions are inherently more complex and challenging with respect to reactivity, purification, and characterization than those of the corresponding small molecule
model compounds. One solution is to study the behavior of analogous small molecule compounds first, and then utilize that information for the analogous polymer substitution reactions. Hexachlorocyclophosphazene is a good model reactant for this approach due to its reaction similarity to the corresponding high polymers, although the cyclic trimeric ring is more rigid than the linear counterparts (Scheme 1). Thus, the hexafluoropropoxy containing trimer (7) was prepared by the addition of the sodium salt of hexafluoropropanol at room temperature to the cyclic trimer \([\text{NPCl}_2]_3\). This reaction is fast and clean, and is similar to the introduction of trifluoroethoxy groups (Figure 2). Product 7 is soluble in many organic solvents such as THF, acetone, dichloromethane, hexanes, and methanol. However, the reaction of sodium tert-perfluorobutoxide with the cyclic trimer was substantially more difficult. The maximum degree of substitution was only one tert-perfluorobutoxy group per ring even after long reaction times in boiling THF (8). Therefore, it seemed likely that this restriction could also prevail in the synthesis of the corresponding linear high polymers.

![Scheme 1: Synthesis of cyclic trimers 7 and 8.](image)

7 \(R^1 = R^2 = \text{OCH(CF}_3\text{)}_2\)  
Condition: 8 eq HOCH(CF\(_3\))\(_2\); 8 eq Na metal; THF, rt, 3h

8 \(R^1 = \text{OC(CF}_3\text{)}_3\) \(R^2 = \text{Cl}\)  
Condition: 8 eq HOCC(F\(_3\))\(_3\); 8 eq Na metal; THF, 66 °C, 3d

Figure 6-2: Synthesis of cyclic trimers 7 and 8.
6.3.2 Mixed-Substituent Phosphazene High Polymers.

In the initial experiments, the hexafluoropropoxy groups were linked to the polymer first, followed by completing the replacement of chlorine atoms with an excess of the trifluoroethoxy groups. As with the behavior of the cyclic trimer models 7, the initial reaction of the hexafluoropropoxide with poly(dichlorophosphazene) was rapid. However, a significant decrease in solubility was encountered as the halogen replacement proceeded, and this limited the degree of substitution. Specifically, the polymers precipitated from the reaction media prematurely when the mole percentage of hexafluoropropoxy group in the macromolecule reached more than 30%. Moreover, the final mixed-substituent polymers with 70 mol% of trifluoroethoxy side groups suffered from a probable degradation reaction with low yields (30–50%) and low molecular weights (<3×10^5 Da). Calculations based on the final 1H NMR measurements for the mixed-substituent polymers showed that fewer hexafluoropropoxy groups were present than expected compared to the target structures. Previous studies indicated that polyphosphazenes with linear fluorinated side groups can undergo substituent exchange reactions and alpha-carbon attack in the presence of some nucleophiles. The substituent exchange reactions usually result in a
deviation of the final side group ratios from the calculated values, while alpha-carbon attack could sensitize the polymer backbone to main-chain cleavage later in the process when the polymers are exposed to water during polymer purification steps.

This problem was overcome by the technique of introducing the trifluoroethoxy groups first, and then completing the chlorine replacement with hexafluoropropoxy groups (Figure 6-4). The trifluoroethoxy groups introduced first generated negligible steric hindrance for the subsequent introduction of the hexafluoropropoxy groups, and the completion of each reaction was accomplished in several hours at a room temperature. The polymers were purified by a standard precipitation technique from THF into water and from THF into hexanes. Polymer 1 is a white fibrous semi-crystalline polymer, while polymers 2 and 3 are gum-like materials, with 3 tougher than 2. All three polymers had similar solubilities in THF, acetone, and ethyl acetate, but were insoluble in methanol, chloroform or dichloromethane. The ratio of two side groups calculated from $^1$H NMR spectra was close to the targeted values. The sharp $^{31}$P NMR signal and the GPC results indicated no significant polymer degradation (Figure 6-5). The higher molecular weight obtained via this synthetic protocol can certainly generate extensive inter-chain entanglements, which favor elastomeric behavior. The broad polydispersities are typical of polymers prepared by the thermal ring-opening polymerization route.$^{28}$ Selected characterization data are summarized in Table 6-2.
Figure 6-4: Synthesis and the final compositions of poly(fluoroalkoxyphosphazenes).

For crosslinkable polymers 5 or 6:

\[ R^3 = ONC\text{-pentane} \quad (3 \text{ mol}%) \]

is attached in the first step.

Table 6-2: Characterization data of mixed-substituent poly(fluoroalkoxyphosphazenes).

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Yield</th>
<th>MW (10^5 Da)</th>
<th>PDI</th>
<th>Repeat units</th>
<th>WCA a</th>
<th>HCA b</th>
<th>Td onset (°C)</th>
<th>Char% at 800°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91%</td>
<td>6.1</td>
<td>1.8</td>
<td>2,490</td>
<td>104°</td>
<td>53°</td>
<td>445</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>77%</td>
<td>4.4</td>
<td>2.0</td>
<td>1,690</td>
<td>107°</td>
<td>51°</td>
<td>395</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>68%</td>
<td>4.1</td>
<td>1.6</td>
<td>1,320</td>
<td>110°</td>
<td>56°</td>
<td>391</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>49%</td>
<td>2.4</td>
<td>2.3</td>
<td>930</td>
<td>111°</td>
<td>55°</td>
<td>330</td>
<td>8.7</td>
</tr>
</tbody>
</table>

a. Contact angle to deionized water. b. Contact angle to n-hexadecane
However, like the cyclic trimer reactions, the linkage of \textit{tert}-perfluorobutoxy groups to the high polymer was significantly more difficult. The sequential addition technique involved the introduction of \textit{tert}-perfluorobutoxy groups first, followed by the trifluoroethoxy groups to replace any residual chlorine (4 in \textbf{Figure 6-4}). However, the \textit{tert}-perfluorobutoxide showed an extremely low reactivity with poly(dichlorophosphazene) even after prolonged reaction in refluxing THF. The substitution ceased at around 4 mol\% chlorine replacement. Thus, the large size of the \textit{tert}-perfluorobutoxy group appears to shield both nearby and distant reaction sites along the chains and retards nucleophilic attack. This result is consistent with the previous studies on the introduction of \textit{tert}-butoxy groups, which also generate a low \textit{tert}-butoxy loading\textsuperscript{,29} As discussed, the linear \textit{n}-perfluorobutoxy groups gave a maximum of 30 mol\% substitution when reacting with poly(dichlorophosphazene), which is additional evidence that supports the extreme steric restrictions responsible for the low reactivity of the \textit{tert}-perfluorobutoxy groups\textsuperscript{,23,30} The polymer 4 is a semi-crystalline waxy material. Thus, the amount of \textit{tert}-perfluorobutoxy side groups along the backbone was insufficient to disrupt the macromolecular symmetry and generate elasticity. Reactions attempted using other higher boiling solvents such as dioxane did not increase the degree of chlorine replacement. Polymer 4 was soluble in THF and acetone. The characterization data are summarized in \textbf{Table 6-2}.

### 6.3.3 Structure–Property Correlations.

The properties of polyphosphazenes usually depend on the presence of different side groups as well as on the ratios of them. An understanding of several crucial properties such as crystallinity,
glass transition temperature, thermal decomposition and stability, hydrophobicity, mechanical properties, and elastomeric properties are important for the prediction of possible materials’ applications. In this work, the structure property correlations were studied by DSC, TGA, X-ray diffraction, water contact angle, tensile tests, and deformation–recovery tests.

**Thermal Characterizations.** The control polymer 1 is a semi-crystalline material with a $T_g$ at $-64 \, ^\circ C$ and a crystalline-to-mesophase transition ($T_1$) at $80 \, ^\circ C$. Random cosubsitution with 19 mol% of hexafluoroproxy groups (2) disrupts the macromolecular symmetry typical of 1 and results in the absence of the $T_1$ transition (Figure 6-6). As a consequence, 2 is a gum-like polymer. Compared to polymer 1, species 2 showed an increase in the $T_g$ to $-50 \, ^\circ C$, which is consistent with the presence of the bulkier, branched structure of the hexafluoroproxy groups, and their influence in restricting backbone flexibility and internal motion (Figure 6-6). Further increases in the percentage of hexafluoroproxy groups to the level of 29 mol% significantly increase the steric restrictions and result in a $T_g$ of 3 that is roughly $25 \, ^\circ C$ higher than for 2. This generates a tougher rubbery texture of polymer 3. When the hexafluoroproxy side group loading was over 30 mol%, the polymer showed a drastic decrease in solubility, probably connected with its limited molecular flexibility. By contrast with traditional **Type A**, **Type B**, and **Type C** poly(fluoroalkoxyphosphazene) elastomers with linear fluoroalkyloxy groups, the branched structures and their closer proximity along the polymer backbone more effectively influence the $T_g$ behavior. With only a small differences in mole percentage of hexafluoroproxy groups, the $T_g$ can be tuned over a wide temperature range from around $-54 \, ^\circ C$ to $-26 \, ^\circ C$. The $T_g$ of polymer 4, with only 4 mol% of the tri-branched side groups, showed a $10 \, ^\circ C$ increase in $T_g$ compared to polymer 1, and is similar to the $T_g$ of a polymer containing 30 mol% of linear $n$-perfluorobutoxy groups plus 70 mol% trifluoroethoxy groups ($-53 \, ^\circ C$). The $T_1$ transition was still detected in polymer 4 due to the fact that only a small percentage of tert-perfluorobutoxy groups was present, but with a decrease to $53 \, ^\circ C$ compared with polymer 1 at
80 °C. Hence, product 4 is a wax instead of a gum. The polymers showed a decrease in thermal stability with the increased loading of hexafluoropropoxy side groups (Table 6-2). The decomposition onset temperature was lowered by about 50 °C compared to polymers 1 to 3 probably due to a tendency to release the high backbone steric tension generated by these bulkier side units. Product 4 showed an even lower decomposition temperature at 330 °C, but with a slightly increased char yield at 800 °C.

Figure 6-6: DSC overlay curves of polymers 1–4.

**X-ray Diffraction.** X-ray diffraction studies, as shown in Figure 6-7, were consistent with the DSC measurements discussed above. As a semi-crystalline polymer, 1 generated a sharp major diffraction peak at $2\theta = 21.4^\circ$. Polymer 4 with only 4 mol% of tert-perfluorobutoxy groups still showed a moderately strong diffraction signal but with a significant decrease in intensity which indicated a reduction in the degree of crystallinity. As indicated by the DSC results, the amount of
tert-perfluorobutoxy groups was insufficient to generate enough structural defects to completely disrupt the macromolecular organization. For polymer 2, with 19 mol% of hexafluoropropoxy groups, the diffraction peak at $2\theta = 21.4^\circ$ became very weak, meaning that the material was amorphous and the polymer showed no well-defined ordering (the diffraction result for polymer 3 is similar to that of 2). Thus, the X-ray diffraction data together with DSC results explain the differences between the macroscopic properties of polymers 1–4.

**Contact Angle Measurements.** Surface hydrophobicity/oleophobicity is one of the most important parameters that determine polymer utility. Fluoropolymers usually possess excellent hydrophobicity, with water contact angles near or above $100^\circ$. Fluorinated materials are useful in many applications such as inert biomaterials, surface patterning, environmentally resistant coating, and low friction devices. Hexafluoropropoxy groups contain one more −CF$_3$ branch than in the trifluoroethoxy group by replacing one −H group at the alpha-carbon position. This greater exposure of −CF$_3$ groups should endow the surface with greater water repulsion. This is consistent with the results in Table 6-2, where polymers 2 and 3 generated slightly higher contact
angles than 1. The tert-perfluorobutoxy containing polymer (4), with three –CF₃ branches, showed even higher hydrophobicity than the hexafluoropropoxy containing polymers. n-Hexadecane was used to study the surface oleophobicity. All the polymers showed contact angles around 50 to 60 degree. These numbers are higher than for trimethylsiloxy surfaces, and are comparable to previously reported phosphazene fluoropolymers, and Teflon.

**Tensile Tests.** Polymers 2 and 3 are amorphous gums, from which elastomeric properties can be generated by the introduction of chemical crosslinks. Therefore, about 3 mol% of 2-allyl phenoxy groups were linked to the polymer backbone as a crosslinkable functionality to obtain polymers 5 and 6. Thermosetting fluoroelastomers were then obtained by exposing the polymer films to ultraviolet irradiation under nitrogen. The stress–strain curves are illustrated in Figure 6-8. For both polymers, the mechanical properties improved with an increased time of crosslinking. Crosslinked polymer 6 showed a higher tensile strength than crosslinked 5 at any irradiation time due to the higher Tₜ of polymer 6. Polymers with insufficient irradiation treatment, such as polymers 5 and 6 ultraviolet crosslinked for only four hours, showed obvious necking effects after the yield point. These materials still remained soft, gum-like substances with long maximum elongations. The tensile strength increased significantly and the elongation-at-break decreased when sufficient inter-chain crosslinks were formed through increasing the irradiation dose, since the bonds formed between neighboring chains now impede chain slippage under tension. At this stage, all the tested specimens showed “strain hardening” as the tensile stress increased rapidly prior to break. The mechanical properties showed no obvious changes after ultraviolet irradiation for 28 hours, probably due to most of the available unsaturated bonds having reacted in the earlier stages. Mechanical property data are summarized in Table 6-3. Thus, depending on specific property targets, the mechanical properties can be tuned either by the number of hexafluoropropoxy groups in the polymers or through the degree of crosslinking.
Elastomeric Properties. The elastomeric properties of the ultraviolet crosslinked elastomers were studied by deformation–recovery tests (Figure 6-9). The elongation standard was selected to be 60% of the break elongations, and four continuous cyclic tests were conducted. Selected results are summarized in Table 6-4. Chain slippage dominated when the existence of inter-chain crosslinks was insufficient. Thus, the polymers with shorter irradiation exposure showed poor
elastic recovery with about 50% to 65% permanent deformation. The elastomeric behavior varied significantly from the first cycle to the fourth cycle, with more than a 10% decrease in elastic recovery. The elastomeric properties improved for samples subjected to longer crosslinking times as in other polymer systems. Thus, increasing numbers of inter-chain crosslinks formed networks that prevent chain translation and thus limited the viscous flow. This generated better elastomeric behavior. The elastic curves showed much improved similarities between the first run and the fourth run, and the recycling patterns gradually became more constant which indicated that the materials retained their elastic performance. However, roughly 24%–32% permanent deformation was still detected. The permanent deformation might be induced by chain re-arrangement under stress, or it could result from an insufficient time for entropy–driven chain relaxations. These thermostetting elastomers with sufficient crosslinking showed improved deformation–recovery properties compared with the recently reported physically crosslinked polyphosphazene elastomers with fluorinated cyclic trimers units on the side-chains (Type D)\textsuperscript{24} due to the strength of the covalent crosslinks in the polymer described here.

It is difficult to make a direct comparison in mechanical properties with commercial elastomers since nearly all of these are further processed by the use of additives or reinforcement agents. The polymers reported in this work are soft elastomers, but are comparable with other pristine phosphazene fluoroelastomers,\textsuperscript{4,17,23} silicone elastomers, or vulcanized natural rubber due to the ultra-low glass transition temperatures of all these materials.\textsuperscript{38} They show lower Young’s modulus or tensile strength than most urethane or vinyl elastomers,\textsuperscript{38} but they break at longer elongations. The properties of these elastomers can be further tuned by the incorporation of a higher loading crosslinkable 2-allylphenoxy groups (>3 mol%). An increase in the degree of crosslinking would certainly enhance the tensile properties of these elastomers.
Mixed-substituted polyphosphazenes are usually prepared through the sequential introduction of two or more different side groups. Alternatively, they can also be prepared by replacement of organic side units already linked to a polyphosphazene backbone. The extent of such substituent exchange reactions depends on the nucleophilicity, size, and leaving ability of the initial side groups. Such exchange reactions can also occur in sequential synthesis addition steps if two or more different nucleophiles are used. Moreover, the second side group added may also attack any labile alpha-carbon atoms on the first linked groups, resulting in the cleavage of the
first side groups and the formation of etheric by-products.\textsuperscript{22,23} Polymers sensitive to these side reactions usually undergo molecular weight decline and are isolated in low yields. They may also deviate considerably from the planned substituent ratios. The key method for reducing such side reactions is to use the correct addition sequence of the different nucleophiles.

![Diagram](image)

Figure 6-10: Process of the substituent exchange reaction and the alpha-carbon attack for cyclic trimers 7 and 9.

In this work, two problems were detected during the polymer syntheses. If hexafluoropropoxy groups were introduced first and trifluoroethoxy groups second, the ratios of the two groups showed significant deviations from the feed ratios and the polymers suffered from degradation after purification. Cyclic trimer models were again used to study the side reactions in order to bypass the complexity of the high polymer systems (Figure 6-10). As shown in Figure 6-11, hexakis(hexafluoropropoxy)cyclotriphosphazene (7) gives a $^{31}$P NMR shift as a sharp singlet at
14.37 ppm. After exposure to twelve equivalents of sodium trifluoroethoxide (two equivalents per existing side group) until no further change occurs, the main signal shifted to 17.84 ppm which indicated a major formation of hexakis(trifluoroethoxy)cyclotriphosphazene (9). Thus, the trifluoroethoxide ion attacked the phosphorus atoms and replaced all of the originally-attached hexafluoropropoxy groups. As in previous work, alpha-carbon attack was also detected with the formation of product a (Figure 6-10). The etheric by-product c was detected in the reaction medium by mass spectrometric analysis (m/z=251.02 [M+H]+). Multiple \(^{31}\text{P}\) NMR peaks appeared in the range of 14 ppm to 17 ppm which reflect the existence of mixed-substituent trimers arising from incomplete substituent exchange and from the alpha-carbon attack by-products a. Alpha-carbon attack can be prevented if the incoming trifluoroethoxide concentrations are equal to or lower than two equivalents per trimer unit. In the alternative procedure, use of hexafluoropropoxy groups to replace side groups in trimer 9, both the exchange reactions and the alpha-carbon attack were suppressed (using the same amounts of hexafluoropropoxide). From the \(^{31}\text{P}\) NMR spectra shown in Figure 6-11, it is clear that there were two major singlet peaks when the reaction reached equilibrium. These represent species 9 (17.87 ppm) and 7 (15.04 ppm) respectively. Similarly, the multiple peaks between 15 ppm and 17 ppm represent the incomplete exchange products and products from alpha-carbon attack b. No etheric by-products (c) were detected if the amount of hexafluoropropoxide was decreased to four equivalents per trimer unit.
Several conclusions can be derived from these cyclic trimer model reactions. First, the hexafluoropropoxy group in trimer 7 is a better leaving group in the presence of trifluoroethoxide. Second, trifluoroethoxide can also attack the alpha-carbon atom of hexafluoropropoxy groups in 7 rather than the other way around. These results can be explained by the structure of hexafluoropropanol. The branched hexafluoropropoxy group, with its two strong electron-withdrawing \(-\text{CF}_3\) functionalities, makes the alpha-carbon more electro-positive than in the trifluoroethoxy structure. As a consequence, hexafluoropropoxy groups in 7 are more likely to suffer alpha-carbon attack in the presence of nucleophiles. The backbone phosphorus atoms are also activated by hexafluoropropoxy group more than by trifluoroethoxy groups. This backbone phosphorus electron deficiency makes compound 7 more liable to nucleophilic exchange reactions than 9. Meanwhile, the tendency of 7 to reduce the steric tension by being replaced by smaller trifluoroethoxy groups works as a synergistic effect. Thus, the reagent addition sequence plays a key role in the synthesis of the mixed-substituent species. It is for these reasons that polymers 2 and 3 in this work were synthesized by adding the trifluoroethoxy groups first and hexafluoropropoxy groups in a second step.

Figure 6-11: \(^{31}\)P NMR signal shifts during exchange reactions.
6.4 Conclusions

A series of new mixed-substituent poly(fluoroalkoxyphosphazenes), defined as Type E fluorophosphazene elastomers, was synthesized and studied. Novel branched fluorinated side groups with two or three –CF₃ groups, specifically up to 29 mol% of hexafluoropropoxy or 4 mol% of tert-perfluorobutoxy units, were utilized as cosubstituents together with trifluoroethoxy groups. The mixed-substituent polymers containing hexafluoropropoxy groups are amorphous with low glass transition temperatures and good thermal stabilities. Elastomers are formed after the incorporation of crosslinkable side units and exposure to ultraviolet radiation. Tensile tests showed significant changes in mechanical properties with an increase in the crosslinking time. Around an 80% deformation–recovery capability was achieved for polymers with sufficient crosslinks. Side group exchange reactions monitored via cyclic trimer models show that hexafluoropropoxy groups are more labile to the exchange reaction in the presence of trifluoroethoxide nucleophiles. These new Type E fluoroelastomers expand the structural boundaries of polyphosphazene-related materials and reveal new structure–property relationships. The corresponding crosslinked polymers may be promising candidates as high performance, low temperature elastomers, O-rings, hydrophobic coatings, or medical or dental materials.

6.5 Acknowledgements

The authors thank Tomasz Modzelewski for help with the tensile tests, and Hemant P. Yennawar for assistance with the wide-angle X-ray diffraction experiments.

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Chapter 7

Phosphazene High Polymers and Models with Cyclic Aliphatic Side Groups: New Structure–Property Relationships

7.1 Introduction

The study of structure–property relationships is one of the key pillars of polymer science since it provides insight and guidance that suggest paths to future advances. This is especially true for advanced polymer systems with unusual structures. Polyphosphazenes are different from other polymers both in structure and method of synthesis. The backbone consists of alternating phosphorus and nitrogen atoms with two organic side groups attached to each phosphorus. This backbone has some unique and valuable properties that include combinations of molecular and materials flexibility, fire-resistance, near-ultraviolet and visible transparency, and radiation stability. The broad synthetic utility of poly(organophosphazenes) is based on access to macromolecular chlorine replacement reactions carried out on poly(dichlorophosphazene) using a wide variety of nucleophiles including alkoxides, aryloxides, and amines. By careful selection of substituents it is possible to tune the properties of the polymers to optimize, for example, elasticity, stability to biological attack, electro-optical behavior, or membrane character. This approach has generated an impressive portfolio of macromolecules. Although the influence of numerous linear aliphatic chains, aromatic rings, and even the rigid adamantyl cage side units on polyphosphazene properties have been reported, a study of the synthesis and properties of cyclic aliphatic side units has been neglected save for a brief report of a cyclohexanoxy substituted small molecule cyclic trimer by Yamada and Yokoyama in 1982.

A number of classical organic polymers are known with saturated carbocycles as part of their structure. For example, polynorbornene has its a five membered ring as part of the backbone. This polymer is useful for shock-absorbing applications because of the high loss-modulus which
provides mechanical damping. Cycloalkanoxy groups are also important pendent side units in organic polymers. For instance, poly(vinylcyloalkyl) species, such as poly(cyclohexylethylene) have been studied.\textsuperscript{15} Polystyrene has been hydrogenated to yield a polymer with cyclohexyl side groups. It has unique properties such as a high glass transition temperature, high rigidity, and environmental stability, low moisture uptake and exceptional optical properties.\textsuperscript{16,17} Moreover, cyclohexyl side groups have also been linked to other backbone systems, including polysiloxanes,\textsuperscript{18} polymethacrylates,\textsuperscript{19} polyvinyltriazoles,\textsuperscript{20} and their copolymers.\textsuperscript{21}

However, the influence of cycloalkyl pendent groups on the properties of polyphosphazenes was unknown. In principle, these saturated cyclic side groups could generate new sets of properties either in single-substituent polymers or in mixed-substituent derivatives in order to tune glass transition temperatures and mechanical or biological properties. The fields of polymeric membranes and elastomers are two areas of particular interest. Thus, in this study we aimed to elucidate the properties of the cycloalkanoxy single-substituent phosphazene polymers, as well as mixed substituent species in which other co-substituent groups such as trifluoroethoxy are present. Cyclopropoxy groups were not studied due to the inherent instability of the highly strained three-carbon rings. Moreover, rings containing more than eight carbon atoms were not investigated due to their limited availability. However, cycloalkanoxy groups with from four to eight carbon atoms were linked initially to model phosphazene cyclic trimer rings and, then to phosphazene high polymers, followed by the synthesis of mixed substituent polymers with trifluoroethoxy groups as the co-substituent. The properties of these new polymers were examined by DSC, TGA, and water contact angle measurements. Films were prepared by solution casting techniques and appropriate mechanical properties were studied. This is the first study of poly(organophosphazenes) containing a series of saturated carbocyclic rings, and it allows direct comparisons to be made with polyphosphazenes with linear alkoxy or aryloxy side groups and provides a further understanding of the properties of these materials.
7.2 Experimental Section

7.2.1 Reagents and Equipment.

Reagents. Tetrahydrofuran (THF) was purchased from EMD and was dried using solvent purification columns. Sodium hydride (NaH, 60% in mineral oil, Sigma-Aldrich) was stored in an inert atmosphere and was used as received. 2,2,2-Trifluoroethanol (Sigma-Aldrich), cyclobutanol (Alfa Aesar), cyclopentanol (Sigma-Aldrich), cyclohexanol (Alfa Aesar), cycloheptanol (Sigma-Aldrich), cyclooctanol (Alfa Aesar), cyclopentanemethanol (Alfa Aesar), cyclohexanemethanol (TCI America), and 2-cyclohexyl ethanol (Sigma-Aldrich) were distilled under reduced pressure and stored over 4Å molecular sieves (EMD) in an argon atmosphere. All synthesis reactions were carried out using standard Schlenk line techniques and a dry argon atmosphere. The glassware was dried overnight in an oven at 120 °C before use.

Structural Characterizations. $^1$H and $^{31}$P spectra were recorded with use of a Bruker WM-360 NMR spectrometer operated at 360 or 145 MHz, respectively. $^1$H NMR spectra were referenced to solvent signals, while $^{31}$P NMR chemical shifts are relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference.

Molecular Weights and Distributions. Polymer molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns, calibrated versus polystyrene standards (Polysciences). The samples were eluted at 40 °C with a 0.1 wt% solution of tetra-n-butylammonium nitrate (Sigma-Aldrich) in THF.

Thermal Analysis. Thermal characteristics of samples were measured with a TA Instruments Q10 differential scanning calorimeter and a Perkin-Elmer thermogravimetric analyzer. About 10 mg of dried sample was used for each test. A heating rate of 10 °C/min with a temperature range
from -100 to 200 °C was used for DSC, while a heating rate of 20 °C/min from 25 to 800 °C was applied for TGA. Both instruments used dry nitrogen as the purge gas.

**X-ray Diffraction.** X-ray diffraction data were recorded for polymer films using a wide-angle configuration of a Bruker D8 Advanced diffractometer. The radiation source used was Ni-filtered, CuKα radiation with a wavelength of 1.5418 Å. The voltage was set to 40 kV and the current to 40mA. The data were collected at a rate of 2θ = 5° min⁻¹ over the range 2θ = 5 – 60°.

**Water Contact Angles.** Advancing water contact angles were determined using of a Ramé-Hart automated goniometer/tensiometer (Succasunna, NJ) with DROPimage Advanced v2.6 at room temperature. Polymer films were solution cast from THF on glass slides (25 × 75 mm) and air-dried before measurements. A drop volume of 2 µL deionized water was used for each test and the reported results are the average of 10 measurements. All the polymers swell in hexadecane.

**Tensile Tests.** The tensile tests were carried out using an Instron 5866 unit at a fixed crosshead speed of 50 mm/min equipped with a 100 N load cell at an ambient temperature (~20 °C). The specimens were prepared by a standard solvent-casting technique using THF solvent (40% w/v) on a Teflon tray (8 cm × 8 cm). The films were slowly dried at room temperature (air-dry for 3 days, vacuum-dry for 3 days). The “dog-bone” shapes were cut using a Pioneer Dietecs die according to ASTMD-1708. The thickness of each dog-bone sample was measured by a caliper (Mitutoyo, Japan) before testing. The data were collected on at least 6 different samples.

**3D Structure Rendering.** The ball-and-stick and space-filling models in Figure 3 were obtained using ChemBio3D Ultra 11.0, utilizing the standard MM2 minimization function to generate the lowest energy structures. Three phosphazene repeat units in the middle were presented, selected from a total drawing of six repeat units.
7.2.2 Synthesis of Hexakis(cycloalkanoxy)cyclotriphosphazene 1–8.

The cyclic trimer reactions all followed a similar procedure, the synthesis of hexakis(cyclohexanoy)cyclotriphosphazene (3) is typical and is demonstrated in detail. To a 50 mL dioxane suspension of sodium hydride (NaH) (2.07 g, 51.84 mmol, 60% in mineral oil), pre-extracted by 100 mL diethyl ether to remove mineral oil, was added cyclohexanol (5.20 g, 51.84 mmol). The reaction was stirred at room temperature for 12 hours to allow the formation of sodium cyclohexanoxide. After that, the sodium salt was added to 50 mL of a dioxane solution of hexachlorocyclotriphosphazene (1.00 g, 2.88 mmol). The chlorine replacement was allowed to progress for 72 hours under reflux to complete the reaction. The reaction medium was centrifuged to remove sodium chloride and unreacted sodium cyclohexanoxide. The solution layer was collected and dried under reduced pressure. The residue was redissolved in ethyl acetate (100 mL) and extracted with water (100 mL) twice. The organic layer was collected and the solvent was removed. The crude product was further purified by column chromatography using hexanes as an eluting solvent. The trimer after purification was dried under vacuum as a light yellow powder (3).

7.2.3 Preparation of Poly(dichlorophosphazene) ([NPCl₂]ₙ).

Poly(dichlorophosphazene) was prepared by the BCl₃-catalyzed thermal ring-opening polymerization of recrystallized and sublimed hexachlorocyclotriphosphazene (Fushimi Chemical Co., Japan) in evacuated Pyrex tubes at 220 °C (yield: 60%).22 3¹P NMR (acetone-d₆) δ (ppm): -17.26 (s).

The synthesis of poly[bis(cyclopentoxy)phosphazene] (10) is described as an example. Sodium cyclopentoxide was prepared by treatment of a suspension of NaH (4.14 g, 103.56 mmol, 60% mineral oil, pre-extracted by 200 mL ether to remove the mineral oil) in 200 mL dioxane with cyclopentanol (8.92 g, 103.56 mmol). The mixture was stirred for 12 hours at room temperature. The resultant cyclopentoxide suspension was added to a solution of poly(dichlorophosphazene) (1.50 g, 12.95 mmol) in 200 mL dioxane. The reaction mixture was stirred under reflux conditions (100 °C) for four days to complete the chlorine replacement. Solvent was removed under reduced pressure. The residue was redissolved in 40 mL THF, and the mixture was precipitated into deionized water twice. Then, the crude product was dissolved in 50 mL THF and purified by dialysis against a THF: methanol (80: 20) solvent system for 4 days (solvent changed once per day, Spectra/Por dialysis membrane MWCO: 12–14 KDa). The product was isolated by evaporation of the dialysis solvent and dried under vacuum at 50 °C to yield the polymer as a leathery material (10).

For the synthesis of 11 and 12, the reactions were carried out in Buchii pressure reactor using 1.5 L THF in total at 130 °C.

7.2.5 Synthesis of Poly[bis(trifluoroethoxy)phosphazene] 16.

The synthesis of 16 was followed a previously published procedure.2

7.2.6 Synthesis of Mixed-Substituent Poly(organophosphazenes) 17–24.

The synthesis of 15 is described in detail as an example. Sodium trifluoroethoxide was prepared from 2,2,2-trifluoroethanol (2.59 g, 25.90 mmol) and sodium hydride (1.04 g, 25.90
mmol, 60% in mineral oil, pre-extracted by 200 mL ether to remove the mineral oil) in 100 mL THF. The resultant alkoxide solution was added slowly to a solution of poly(dichlorophosphazene) (3.00 g, 25.89 mmol) in 300 mL THF and the mixture was stirred at room temperature for 24 hours. A cyclopentoxide solution prepared from NaH (1.56 g, 39.00 mmol, 60% in mineral oil, pre-extracted by 200 mL ether to remove the mineral oil) and cyclopentanol (3.34 g, 39.00 mmol) in 100 mL THF was added to the polymer solution and the mixture was stirred at reflux for 36 hours. The mixture was then concentrated to one quarter of its original volume, and was purified by re-precipitation from THF into deionized water (2 L) three times. The crude product was then re-dissolved in THF, and was further purified by dialysis versus acetone: hexanes: methanol (40: 40: 20) solvent system for 4 days (solvent changed once per day, Spectra/Por dialysis membrane MWCO: 12–14 KDa). The rubbery product (15) was isolated and dried under vacuum at 50 °C.

7.3 Results and Discussion

7.3.1 Small Molecule Cyclic Model Reactions.

The introduction of cycloalkanoxy side groups was first attempted with the cyclic chlorophosphazene trimer (NPCl₂)₃ as a model system (Figure 7-1) to set the stage for the more challenging macromolecular substitution reactions. The feasibility of this type of reaction had been reported earlier at the phosphazene small molecule level with respect to the linkage of cyclohexyloxy groups to the phosphazene cyclic trimer.¹³

It is well known that the size of side groups can significantly influence the rate of chlorine replacement in chlorocyclophosphazenes and it was important to monitor this effect before embarking on a polymer synthesis program. In this work the linkage of the small cyclobutoxide
units to the small-molecule ring system reached completion within a day at 66 °C in refluxing THF. The corresponding reactions for cyclopentoxide, cyclohexanoxide and cycloheptanoxide initially proceeded rapidly during the first few hours, but the reaction rates decreased drastically after three of the six chlorine atoms had been replaced. Complete substitutions for side groups 2, 3 and 4 required several days using a refluxing high boiling solvent. However, the synthesis of the phosphazene ring system with side group 5 ceased completely when a maximum of five cyclooctanoxo groups were attached to the phosphazene ring. The reaction conditions and characterizations of the cyclic trimer models are summarized in Table 7-1, and selected NMR characterizations are reported in Figure 7-2. These small molecule studies were then used as a starting point to design reaction conditions for the high polymers.

\[ \text{Cycloalkanoxide groups:} \]

1. \( R = \text{O} \)  2. \( R = \text{O} \)  3. \( R = \text{O} \)  4. \( R = \text{O} \)  5. \( R = \text{Octyl} \)

\( \text{Incomplete Substitution} \)

\[ \text{Cycloalkanoxide groups with methylene or ethylene spacers:} \]

6. \( R = \text{O} \)  7. \( R = \text{O} \)  8. \( R = \text{O} \)

Figure 7-1: Synthesis of phosphazene cyclic trimers.
7.3.2 Monitoring the Introduction of Cycloalkanoxy Side Groups at the High Polymer Level.

The chlorine replacement reactions at the phosphazene high polymer level became more challenging as the dimensions of the cycloalkanoxy nucleophiles become larger. Side groups with high steric hindrance characteristics can restrict the molecular mobility and solvation of the partly substituted macromolecules, leading to slow reactions or precipitation of the polymeric...
products from solution in the reaction solvents. Also, the lower reactivity of the larger side groups requires longer times to complete the chlorine replacement process. However, these slower reactions afford unique opportunities to monitor the substitution behavior and to study the regioselectivity of these large side groups for different sites along the backbone.\textsuperscript{23} The reaction of cyclohexanoxide with poly(dichlorophosphazene) (II) was even slower than with the cyclic trimer model system, as monitored by $^{31}$P NMR spectroscopy (Figure 7-3). Initially, a ratio of four molar equivalents of the cyclohexanoxide (4 eq. per repeating unit, 2 eq. per chlorine) was used for the synthesis while the reaction mixture was maintained at reflux in THF. The reaction ceased after four days. The original sharp singlet peak at around -17.3 ppm (peak A, PCl$_2$) shifted to -15.3 ppm (peak B) and -10 to -11 ppm (peak C and D). Peak B represents phosphorus centers with a single chlorine remaining [P(OCH$_6$H$_{11}$)Cl] structure. Peaks C and D both represent double cyclohexanoxy substituted phosphorus atoms [P(OCH$_6$H$_{11}$)$_2$], while peak C is from the P(OCH$_6$H$_{11}$)$_2$ with two adjacent single substituted phosphorus atoms [P(OCH$_6$H$_{11}$)Cl], and peak D represents the P(OCH$_6$H$_{11}$)$_2$ phosphorus centers flanked by one singly substituted phosphorus atom [P(OCH$_6$H$_{11}$)Cl] and one doubly substituted phosphorus atom [P(OCH$_6$H$_{11}$)$_2$]. The chlorine replacement was driven further by the addition of 4 more molar equivalents of sodium cyclohexanoxide (8 eq. per repeating unit, 4 eq. per chlorine). From the $^{31}$P NMR spectrum, a new peak, E, then appeared which represents di-substituted phosphorus centers flanked by two other di-substituted phosphorus units [P(OCH$_6$H$_{11}$)$_2$]. As these new peaks appeared and increased in intensity, the integration of the single substituted peak at -15.3 ppm decreased. Four additional molar equivalents of the nucleophile (12 eq. per repeating unit, 6 eq. per chlorine) were added after the reaction again ceased after 6 additional days. In the $^{31}$P NMR spectrum, peak E dominated while peaks B, C, and D decreased in integration. After the mixture had been stirred for a total of 20 days at reflux in THF, the chlorine replacement reaction became extremely slow to the point that no change could be detected by $^{31}$P NMR analysis. In order to obtain additional substitution, the
reaction mixture was transferred to a Buchii pressure reactor and heated to 130 °C with the addition of yet another 4 molar equivalents of the side group (16 eq. per repeating unit, 8 eq. per chlorine). The chlorine replacement reaction was then complete after 16 additional days under the forcing conditions of the pressure reactor. All the $^{31}$P NMR signals shifted toward the position of peak E until only peak E remained, thus indicating that all the phosphorus atoms now bore two cyclohexanoxy groups.

Figure 7-3: $^{31}$P NMR spectra showing the progress of the reaction of sodium cyclohexanoxide with poly(dichlorophosphazene).

Thus, initially the replacement of chlorine proceeds rapidly until approximately 80% substitution is achieved. Then the reaction becomes extremely slow afterwards. There are two reasons for the extremely long reaction time and forcing conditions needed to achieve complete chlorine replacement by cyclohexanoxide. First, sodium cyclohexanoxide is almost insoluble in
THF. Second, although the polymer remained soluble in THF after the attachment of cyclohexanoxy side groups, the bi-phasic reaction rate is limited by the surface area of the suspended particles of the nucleophile and is thus much slower than would be expected for a homogeneous reaction. Third and more important, the significant steric bulk of cyclohexanoxy side groups hinders the reaction by shielding nearby phosphorus centers from attack by incoming nucleophiles. At this point the rate of chlorine replacement declines rapidly and the aforementioned forcing conditions are necessary in order to bring about any increase in the percentage of substitution. Initially this seems paradoxical since the introduction of phenoxide into a polyphosphazene proceeds without difficulty. However, the non-planar structure of the cyclohexyl group causes it to occupy a much larger steric volume than phenoxide. Furthermore, the equilibration of the cyclohexyl group between the two chair conformations and the boat conformation means that it is capable of occupying an even larger hydrodynamic volume, thus further contributing to the shielding of neighboring phosphorus-chlorine centers. Figure 7-4 demonstrates how the adjacent substituted cyclohexanoxy groups shield the remaining chlorine atoms in the final stages of the macromolecular substitution. These solvent-accessible surface simulations represent the parts of molecules that can be accessed by reagents in solution, and beneath which the remaining chlorine atoms are buried. Therefore, these last chlorine atoms on the polymer are extremely difficult to replace unless a higher reaction temperature is used in a pressure reactor.
The introduction of cyclohexanoxy groups is one of the slowest macromolecular substitution reactions found in the study of several hundred poly(organophosphazenes). Monitoring the substitution patterns of such side groups is important because macromolecular substitution reactions are unusual in general polymer chemistry, and unique principles need to be taken into account. Bulky side groups tend to attack the least sterically hindered sites along the polymer chain. Single substitution at each phosphorus atom tends to occur first and quickly. A subsequent incoming nucleophile is likely to attack a center perhaps several repeating units down the chain from the first reaction site due to the steric shielding of the neighboring sites by the side groups. The very slow disappearance of peak B in Figure 7-3 indicates that cyclohexanoxide prefers to
attack non-geminally. Thus the incoming nucleophiles attack P–Cl bonds between already singly-substituted centers until they are forced to substitute geminally. The next most likely sites for nucleophilic attack are phosphorus centers with neighbors that have only one side group. Eventually the only remaining chlorine atoms are the ones attached to phosphorus centers with neighbors that are already linked to two organic side groups. These sites are the most sterically hindered of all and are therefore the last sites to succumb to nucleophilic chlorine replacement (Figure 7-4).

7.3.3 Synthesis of Other Poly[bis(cycloalkanoxy)phosphazenes] and Derivatives 9–15.

A series of high polymers with other cycloalkanoxy, or cycloalkylmethoxy or cycloalkylethoxy groups was then synthesized and the products are listed in Figure 7-5. The synthetic conditions and structural characterizations are summarized in Table 7-2. Complete replacement of chlorine in poly(dichlorophosphazene) by cyclopentoxide (10) proceeded with relative ease compared to that by cyclohexanoxide (NMR spectra, Figure 7-6). This is because of the smaller ring size and perhaps also because of the locked planarity of the five membered ring which occupies a much smaller steric volume. The substitution reaction for the smaller and also planar cyclobutoxide proceeded even faster with completion within a day.
Figure 7-5: Synthesis of poly[bis(cycloalkanoxy)phosphazenes] and derivatives.

\[
\begin{align*}
\text{Cycloalkanoxy groups:} \\
9. \quad & R = \text{O} \\
10. \quad & R = \text{O} \\
11. \quad & R = \text{O} \\
12. \quad & R = \text{O} \\
\text{(maximum 93%)}
\end{align*}
\]

\[
\begin{align*}
\text{Cycloalkanoxy groups with methylene or ethylene spacers:} \\
13. \quad & R = \text{O} \\
14. \quad & R = \text{O} \\
15. \quad & R = \text{O}
\end{align*}
\]

Figure 7-6: NMR spectra of polymer 10
An attempt was also made to achieve full chlorine replacement along the phosphazene chain by cycloheptoxide (12). The reaction conditions were similar to those for 11, with 10 molar equivalents of cycloheptoxide used per chlorine and immediate transfer into the Buchii pressure reactor at 130 °C. Even after 20 days under these forcing reaction conditions, 31P NMR analysis showed that approximately 7.5 mol% chlorine remained. Surprisingly, this polymer could be purified by standard precipitation procedures from solution in THF into deionized water followed by dialysis with no change in the 31P NMR spectrum after work-up. It might be expected that a polyphosphazene with any remaining P–Cl bonds would react with water either to form P–OH units or P–O–P crosslinks with the concurrent release of HCl, or to undergo extensive chain cleavage through the formation of unstable phosphazane intermediate species. However, polymer 12 did not crosslink even after extensive exposure to water, and the molecular weight remained in the same range as that of 11. This suggests that the cycloheptoxyl group is capable of shielding the remaining phosphorus–chlorine centers sufficiently to protect them from attack by water. It also explains why the remaining chlorine atoms are extremely difficult to be replaced by

### Table 7-2: Replace this with table caption above the table.

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<th>polymer</th>
<th>feed equivalents a</th>
<th>solvent, temperature (°C)</th>
<th>reaction time (h)</th>
<th>yield (%)</th>
<th>31P NMR (ppm)</th>
<th>1H NMR (ppm)</th>
<th>Mw (PDI) b</th>
<th>ru c</th>
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<td>2</td>
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<td>30</td>
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<td>10</td>
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<td>2</td>
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<td>60</td>
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a. Equivalents to Chlorine atoms; b. Molecular weight was measured using a GPC calibrated by polystyrene; c. Number of repeat units, calculated by GPC results; d. Reaction was carried out using a high pressure reactor.
a large excess of nucleophile during the reaction. Polymers 11 and 12 had significantly lower molecular weights compared with polymers 9 and 10 presumably due to depolymerization at the higher reaction temperature and a longer reaction time.

By comparison, the substitutions by cyclopentylmethoxide and cyclohexylmethoxide were substantially faster. Polymers 13 and 14 can be synthesized within a day in refluxing THF. A second methylene spacer group in the nucleophile would be expected to further increase access by the anionic site to the backbone phosphorus atoms and have only a limited shielding effect on the remaining P-Cl units. However, surprisingly, the reactivity of cyclohexylethoxide during the synthesis of 15 was slower than with cyclohexylmethoxide as shown in Table 7-2. However, this may not be mechanistically significant because the lower solubility of sodium cyclohexylethoxide in THF may be responsible for this decreased reactivity. The NMR spectra of 15 are shown in Figures 7-7.

Figure 7-7: NMR spectra of polymer 15.
7.3.4 Synthesis of Mixed-Substituent Polyphosphazenes 17–24.

The properties of phosphazene polymers can be tuned by introducing more than one type of side group. Poly[bis(trifluoroethoxy)phosphazene] (16) is one of the best studied poly(organophosphazenes). Moreover, the strong electron-withdrawal from phosphorus by the trifluoroethoxy group assists the replacement of nearby chlorine atoms in a very fast reaction. For this reason the synthesis of polymers with both trifluoroethoxy and cycloalkanoyl side groups encounter reduced synthetic challenges and also give rise to changed properties. A target ratio of 1:1 substitution of two side groups was pursued for all the mixed-substituent polymers 17–24 (Figure 7-8).

![Chemical Structure Diagram]

**Figure 7-8:** Synthesis of mixed-substituent polyphosphazenes: $x$ and $y$ represent the final ratios.
The addition sequence for the introduction of two different side groups plays a vital role in the structure and properties of mixed-substituent polyphosphazenes. All the mixed-substituent polymers discussed here were produced by the introduction of the trifluoroethoxy units first, followed by completion of the chlorine replacement with the cycloalkanoxide. This technique minimized substituent-exchange reactions that are possible when smaller and more reactive side groups replace bulkier groups. The sequence utilized in this work yielded a side group ratio close to the targeted value and also gave higher molecular weight polymers.26 Another important aspect of this synthetic technique is that, at the solution concentrations chosen for substitution reactions, gelation of the reaction mixture can occur during the initial stage of a reaction. This is due to the highly polar nature of the sodium trifluoroethoxide reagent, which raises the overall polarity of the reaction medium to the point that less polar polymers begin to precipitate at an early stage of the process. As the reaction continues, sodium chloride precipitates from the reaction mixture, and this removes the charged species from solution. Thus, as the overall polarity of the solution is lowered the polymer returns to a fully solvated state. In order to avoid this phenomenon, it is important that the trifluoroethoxide is added first to the solution of poly(dichlorophosphazene) and sufficiently slowly that the medium does not become so polar that the polymer begins to desolvate.

The syntheses of these mixed-substituent polymers are significantly easier than the preparation of the single substituent polymers due to the low steric hindrance and strong electron-withdrawal generated by the trifluoroethoxy groups. For example, complete replacement of chlorine by the cycloalkanoxides in the second step of the synthesis was achieved within two days in refluxing THF. However, the side group introduction sequence for polymer 24 had little influence on the final side group ratio. If the cyclohexylethoxy groups are linked to the polymer first, complete chlorine replacement in the second step by trifluoroethoxide can be accomplished at room temperature in 16 hours. The final polymer compositions were calculated based on the peak
integrations of two side groups in $^1$H NMR (Figure 7-9), and the synthesis of 17–24 are summarized in Table 7-3.

![Figure 7-9: NMR spectra of polymer 19.]

Table 7-3: Replace this with table caption above the table.

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<th>polymer</th>
<th>cycloalkanoxide feed equivalents</th>
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<th>$^{31}$P NMR (ppm)</th>
<th>$^1$H NMR (ppm)</th>
<th>$M_w$(PDI)</th>
<th>Repeating units</th>
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<td>1.5</td>
<td>24</td>
<td>56</td>
<td>-9.50 (s)</td>
<td>4.79–4.51 (br, 3H), 2.35–1.40 (m, 6H)</td>
<td>471 (2.1)</td>
<td>2190</td>
</tr>
<tr>
<td>18</td>
<td>1.5</td>
<td>24</td>
<td>67</td>
<td>-8.45 (s)</td>
<td>5.01–4.51 (br, 3H), 1.90–1.62 (m, 8H)</td>
<td>1785 (3.1)</td>
<td>7790</td>
</tr>
<tr>
<td>19</td>
<td>1.5</td>
<td>24</td>
<td>44</td>
<td>-7.63 (s)</td>
<td>4.58–4.40 (br, 3H), 1.98–1.28 (m, 10H)</td>
<td>422 (2.1)</td>
<td>1737</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>36</td>
<td>70</td>
<td>-8.38 (s)</td>
<td>4.48–4.24 (br, 3H), 1.93–1.36 (m, 12H)</td>
<td>1089 (2.3)</td>
<td>4240</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td>48</td>
<td>38</td>
<td>-8.76 (s)</td>
<td>4.48–4.25 (br, 3H), 1.85–1.25 (m, 14H)</td>
<td>197 (1.4)</td>
<td>730</td>
</tr>
<tr>
<td>22</td>
<td>1.5</td>
<td>16</td>
<td>56</td>
<td>-7.27 (s)</td>
<td>4.41 (s, 2H), 3.70 (s, 2H), 2.23 (br, 1H), 1.71–1.14 (m, 8H)</td>
<td>994 (2.2)</td>
<td>4090</td>
</tr>
<tr>
<td>23</td>
<td>1.5</td>
<td>16</td>
<td>60</td>
<td>-7.31 (s)</td>
<td>4.48 (s, 2H), 3.74 (s, 2H), 1.87–1.04 (m, 11H)</td>
<td>1002 (2.2)</td>
<td>3900</td>
</tr>
<tr>
<td>24</td>
<td>1.5</td>
<td>24</td>
<td>37</td>
<td>-7.60 (s)</td>
<td>4.45 (s, 2H), 4.10 (s, 2H), 1.78–0.85 (m, 13H)</td>
<td>324 (1.7)</td>
<td>1190</td>
</tr>
</tbody>
</table>

a. Equivalents to chlorine atoms; 1 eq. of the trifluoroethoxide was added in the first step for all the syntheses; b. Refluxing THF was used as the solvent; the substitution reaction with trifluoroethoxide in the first step was accompanied by stirring at room temperature for 24 hours for all the syntheses; c. Molecular weights were measured using a GPC unit calibrated by polystyrene; d. Number of repeat units, calculated from the GPC results.
7.3.5 Solubility.

The physicochemical properties of polymers reflect some combination of the nature of the backbone and the structure of the side groups. Thus, due to their synthetic versatility, poly(organophosphazenes) provide an excellent means for exploring the effects of changes in the shape, size, and flexibility of organic side groups.27

The solubility of polyphosphazenes is strongly controlled by the side groups. Cyclic aliphatic groups are among the most non-polar groups that have been linked to a phosphazene backbone. As shown in Table 7-4, the cyclic trimers with these side units are soluble in nearly all common solvents except methanol. The single-substituent polymers dissolve in less polar solvents such as chloroform, while the mixed-substituent polymers have higher solubility in more polar solvents such as acetone. THF is the universal solvent for all the polymers examined.

Table 7-4: Solubility of trimers and polymers in common organic solvents.

<table>
<thead>
<tr>
<th>compound</th>
<th>tetrahydrofuran</th>
<th>hexanes</th>
<th>chloroform *</th>
<th>dichloromethane</th>
<th>methanol</th>
<th>acetone</th>
<th>ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–8</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9–15</td>
<td>+</td>
<td>swelled</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17–24</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a. Chloroform is the best solvent for cycloalkyloxy-containing polymers
7.3.6 Glass Transition Temperatures.

The polyphosphazene backbone is inherently flexible. However, the side groups play a critical role in controlling glass transition temperatures.\textsuperscript{1} The glass transition temperatures of the polymers synthesized in this work and several other related polymers are listed in Figure 7-10. The glass transition temperatures of the cycloalkanoxy derivatized polymers are higher than those of linear alkylxoy containing polymers (which are close to -100 °C for some examples).\textsuperscript{10} The low glass transition temperatures of polyphosphazenes with linear alkoxy side groups are due to the torsional freedom of the backbone coupled with the torsional flexibility of the side groups. This low side group torsional barrier is partly related to the presence of an etheric oxygen at the point of attachment to the phosphazene backbone and to the ability of the side groups to undergo low-energy avoidance motions during backbone torsion. Conversely, cycloalkanoxy groups without a spacer unit are sterically bulky near the point of connection to the backbone and have fewer degrees of conformational freedom. This results in higher energy barriers to torsional motions of both the backbone and the side units, and consequently to higher glass transition temperatures. The striking difference in glass transition temperatures between polymers 9, 10 and 11 is due to the cyclobutyl and cyclopentyl groups being locked in a planar conformation, which lowers the steric profile compared to the cyclohexyl group. Meanwhile, the possibility of chair-chair stacking in the cyclohexyl system may also contribute to the higher T\textsubscript{g}. Similarly, polymer 11 has a higher glass transition temperature than a phenoxy-substituted polyphosphazene presumably due to the planar conformation of the benzene ring in the phenoxy derivative. Polymer 12, is an anomaly because this is not a 100\% substituted polymer. The 7.5\% unreacted chlorine clearly affects the glass transition temperature. Polymers 13 and 14 with one methylene group between each cycloalkyl group and the phosphazene backbone have significantly lower glass transition temperatures than their counterpart polymers 10 and 11 without the methylene
spacer. Thus, the increased distance between the bulky cycloalkyl unit and the polymer backbone reduces the effect of steric hindrance. Polymer 15 with an ethoxy spacer group has an even lower glass transition temperature than 14. None of the single-substituent polymers have detectable melting transitions before reaching the temperature of thermal decomposition.

The $T_g$ values of the mixed-substituent polymers can be compared to the value of -62 °C for poly[bis(trifluoroethoxy)phosphazene] (16). This is a semi-crystalline polymer with a mesophase transition at 80°C. The mixed-substituent species 17–24 have $T_g$ values that lie between that of 16 and the values for the single substituent polymers that lack trifluoroethoxy co-substituents. Moreover, the original sharp crystalline-to-mesophase transition at 80 °C in 16 was absent for all the mixed-substituent polymers due to the decrease in macromolecular symmetry caused by the random disposition of different side groups. Such behavior is consistent with those of other reported mixed substituent poly(organophosphazenes).
7.3.7 Thermal Decomposition Temperatures.

Thermo-gravimetric analysis data are unreliable indicators of the mechanism of thermal breakdown of most polymers. Different polyphosphazenes undergo cyclo-depolymerization, random fragmentation, or even pre-ceramic cross-linking at high temperatures depending on the side groups. Thermo-gravimetric analysis results obtained in this study are summarized in the Table 7-5. All the single- and mixed- substituted polymers containing cycloalkanoxy groups directly linked to the backbone start to lose weight below 300 °C. However, the resistance to
thermal breakdown improves significantly when methylene or ethylene spacer groups are present between the cycloalkane units and the skeleton.

Table 7-5: Summary of the TGA results.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;d&lt;/sub&gt; (°C)</td>
<td>233</td>
<td>262</td>
<td>266</td>
<td>275</td>
<td>316</td>
<td>258</td>
<td>337</td>
<td>352</td>
</tr>
<tr>
<td>Polymers</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>T&lt;sub&gt;d&lt;/sub&gt; (°C)</td>
<td>236</td>
<td>270</td>
<td>265</td>
<td>226</td>
<td>212</td>
<td>331</td>
<td>365</td>
<td>360</td>
</tr>
</tbody>
</table>

Note: T<sub>d</sub>s were calculated based on the Tangent method; TGA indicates when a polymer begins to lose mass by expelling smaller and volatile decomposition products instead of chain scissions or molecular weight decrease.

7.3.8 Hydrophobicity.

The side groups in most polyphosphazenes control the surface properties. As shown in Figure 7-11, all the single-substituent polymers 9–15 generate an advancing water contact angle near 90°. Polymer 16 is the most hydrophobic derivative due to the abundance of highly hydrophobic trifluoroethoxy side-groups. The advancing water contact angles of the mixed-substituent polymers are intermediate between each of their single-substituent counterparts. No hexadecane contact angle results were obtained since all the polymers absorb hexadecane.
7.3.9 Mechanical Properties.

The mechanical properties of these polymers are related to their glass transition temperatures or to the presence or absence of microcrystallinity. Selected polymers were examined for their tensile behavior. Species 10 and 16 are representative of the single-substituent polymers, with either cyclopent oxy or trifluoroethoxy side groups. Polymers 18 and 19 demonstrate the behavior of polymers with both trifluoroethoxy side groups and different cycloalkanoxy groups.

Compound 16, as shown in Figure 7-11, is a tough material. The polymer begins to orient and crystallize readily under strain. These crystalline domains act as physical “crosslinks”, which are responsible for the high Young’s modulus, high tensile strength, and a medium elongation to the

Figure 7-11: Summary of advancing water contact angles.
break point. Films from polymer 10 are rigid with even higher Young’s modulus. But the average break elongation is within 100% of strain, with a significant necking effect after the yield point. There are three reasons for such an early break behavior. First, the non-polar cycloalkanoxy side groups generate fewer dipolar associations (lower degree of crystallinity). Second, 10 has a higher glass transition temperature which suggests that the polymer chains are less flexible. Finally, the relatively lower molecular weight of 10 provides a reduced level of macromolecular chain entanglement. Co-substitution significantly alters the mechanical properties of all the polymers, especially since the absence of crystallinity greatly increases the film flexibility. The mechanical properties of the mixed-substituent polymers can also be altered by the size of cycloalkanoxy groups. Species 18 generates a longer maximum elongation and lower tensile strength than 19 due to the lower glass transition temperature, which in turn, results from the presence of the smaller cyclopentoxy groups.

Figure 7-11: Images of self-standing transparent membranes from 10 and 18 and a direct comparison of mechanical properties of 10, 16, 18 and 19.
The wide-angle X-ray diffraction study illustrated in Figure 7-12 supports the conclusions obtained from the mechanical analyses. Semi-crystalline thermoplastic 16, an opalescent film-former, has a sharp reflection signal at $2\theta = 21^\circ$ which indicates a high degree of crystallinity and significant macromolecular order.\textsuperscript{31,32} The single-substituent species 10 yields transparent films. These films show a slightly broadened, but still discernible X-ray signal at $2\theta = 21^\circ$, which suggests that the material still retains a low degree of crystallinity and certain spacing regularities.\textsuperscript{33} All the mixed-substituent polymers are transparent films, in which the signal at $2\theta = 21^\circ$ is significantly decreased, indicating either little or inconspicuous crystallinity. Thus, we conclude that the cycloalkanoxy groups serve as molecular irregularities to reduce the microcystallinity of the highly symmetric 16.

Data for the mechanical properties are summarized in Table 7-6. Most of the polymer films are robust and non-adhesive. They can be peeled intact from common surfaces, such as Teflon or polypropylene (Figure 7-13). Depending on the different applications, further changes to the ratios of cycloalkanoxy and trifluoroethoxy groups should allow the physical properties to be tuned within fine limits.\textsuperscript{34}
Figure 7-12: Wide angle X-ray diffraction traces.

Figure 7-13: Transparent films and membranes of mixed substituent polymers (8 cm × 8 cm).
Conclusions

A number of new poly(organo)phosphazenes with cycloalkanoxy or cycloalkane-alkyleneoxy side groups plus mixed-substituent species with trifluoroethoxy cosubstituents were synthesized, together with small molecule model molecules used to probe synthesis conditions. The syntheses of cyclobutoxy or cyclopentoxy single-substituent polymer (9 or 10) proceed more rapidly and under milder conditions than do the corresponding reactions leading to the synthesis of the cyclohexanoxy single-substituent polymer (11). Co-substitution to yield polymers with both cycloalkyloxy and trifluoroethoxy groups provides a means to reduce the synthetic challenges and tune the polymer properties. Structure–property relationships of the polymers were evaluated in terms of glass transition temperatures, thermo-gravimetric behavior, hydrophobicity, and mechanical behavior, with insights obtained into the influence of different cycloalkanoxy side units. Films prepared from these polymers are tough and non-adhesive. They may be appropriate for applications where film-forming ability, flexibility, and transparency are important. Overall, several of these polymers, such as the cyclohexanoxy-containing species, appear to have properties that are appropriate for gas transport membranes.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Break elongation (%)</th>
<th>Strength-at-break (MPa)</th>
<th>Yield elongation (%)</th>
<th>Yield strength (MPa)</th>
<th>Tensile strength (MPa)</th>
<th>Young's modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>78.75 ± 27.38</td>
<td>4.51 ± 0.44</td>
<td>16.42 ± 2.78</td>
<td>5.32 ± 0.60</td>
<td>5.32 ± 0.60</td>
<td>66.86 ± 13.26</td>
</tr>
<tr>
<td>16</td>
<td>406.12 ± 39.08</td>
<td>10.04 ± 2.89</td>
<td>26.72 ± 2.77</td>
<td>4.82 ± 0.49</td>
<td>10.04 ± 2.89</td>
<td>42.34 ± 5.74</td>
</tr>
<tr>
<td>18</td>
<td>602.15 ± 57.17</td>
<td>1.72 ± 0.49</td>
<td>14.84 ± 1.60</td>
<td>0.91 ± 0.32</td>
<td>1.92 ± 0.49</td>
<td>17.66 ± 3.01</td>
</tr>
<tr>
<td>19</td>
<td>342.08 ± 29.00</td>
<td>1.93 ± 0.31</td>
<td>14.79 ± 1.56</td>
<td>1.60 ± 0.19</td>
<td>2.08 ± 0.44</td>
<td>23.18 ± 2.99</td>
</tr>
</tbody>
</table>

7.4 Conclusions

Table 7-6: Summary of mechanical properties.
7.5 Acknowledgements

The authors thank Dr. Hemant P. Yennawar for assistance with the wide-angle X-ray diffraction experiments. This work was made with support under Contract No. RES1000026 awarded by National Energy Technology Laboratory (NETL)/ Department of Energy (DOE).

7.6 References

VITA

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