Synthesis and Functionalization of Poly(ethylene oxide-*b*-ethyloxazoline) Diblock Copolymers with Phosphonate Ions

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Abstract

Poly(ethylene oxide) (PEO) and poly(2-ethyl-2-oxazoline) (PEOX) are biocompatible polymers that act as hydrophilic "stealth" drug carriers. As block copolymers, the PEOX group offers a wider variety of functionalization. The goal of this project was to synthesize a poly(ethylene oxide)-*b*-poly(2-ethyl-2-oxazoline) (PEO-*b*-PEOX) block copolymer and functionalize pendent groups of PEOX with phosphonic acid. This was achieved through cationic ring opening polymerization (CROP) of 2-ethyl-2-oxazoline monomer onto PEO. These polymerizations used tosylsulfonyl chloride as initiator. Size-exclusion chromatography (SEC) was used to determine the molecular weights of the block copolymers. Two samples of 1:2 and one sample of 1:3 of PEO-to-PEOX block copolymers were made. These samples underwent partial hydrolysis of the PEOX pendent groups to form the random block copolymer, poly(ethylene oxide)-*b*-poly(2-ethyl-2-oxazoline)-*co*-poly(ethyleneimine) (PEO-*b*-PEOX-*co*-PEI). These reactions showed that there was a degree of control based on the moles of acid. Diethyl vinyl phosphonate was attached to the nitrogen of PEI units via Michael addition where the phosphorylation left <1% of PEI units unattached. The ethyl groups on the phosphonates were further hydrolyzed off phosphonate with HCl acid leaving phosphonic acid. After each step of synthesis, structures and composition were confirmed using ^{1}H NMR. Due to the nature of the phosphonic acid, the polymer can be utilized in the incorporation and release of cationic drugs.

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Table of Contents

List of Figures

List of Tables

CHAPTER 1: Introduction

In the field of drug delivery, it is imperative that nanoparticle design can adapt to various biological media and incorporate different facets of drugs such as anionic or cationic properties. These biocompatible polymeric carriers are often composed of amphiphilic block copolymers due to their ubiquitous nature and often controllable properties during and after synthesis. Poly(ethylene oxide) (PEO) is a biocompatible polymer component known for its hydrophilicity, low cytotoxicity, and low immunogenicity. By incorporating PEO in nanoparticle designs, size can be increased allowing for drug-carrying nanoparticles to avoid filtration via the kidneys while keeping consistent biodistribution even while circulating the blood stream. Poly(2-ethyl-2-oxazoline) (PEOX) is also another biocompatible polymer that exhibits similar "stealth" behaviors but can also be functionalized with biocompatible pendent substituents. In this thesis, phosphonic acid was used in replacing the pendent ethyl groups of PEOX. The anionic quality of the phosphonic acid has the potential to be pH controllable and provide an environment where cationic drugs and contrast agents can be held.

The second chapter reviews the controlled radical ring-opening polymerization and medicinal benefits of polyoxazolines and the biomedical implications of phosphonates. The Third chapter will describe the synthesis and characterization of PEO-*b*-PEOX block copolymers and the steps taken to functionalize the copolymers. This includes pendent group hydrolysis, Michael addition, and ethyl hydrolysis of phosphonate as well as ^{1}H NMR characterizations.

CHAPTER 2: Literature Review

2.1 Overview

The topics discussed in this literature review are separated into two sections. The first will describe the synthesis, properties and applications of polyoxazolines and its diverse usage in biomedicine. The second section will discuss phosphonates and the properties and chemistry are beneficial in biological systems.

2.2 Poly(2-oxazoline)s

2.2.1 Introduction

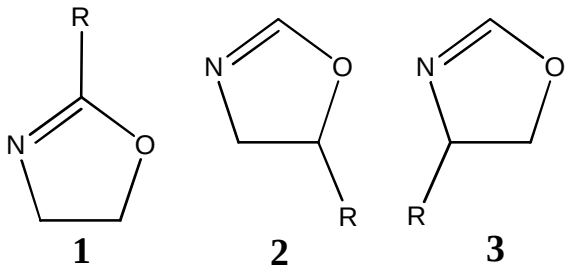
In recent years, the demand for more versatile biocompatible nanotechnology has directed attention to the growing research focused on the design of “smart” polymers. Before the 1990’s, polyoxazolines had relatively limited uses in industry, mostly in coating and paint dispersants [1]. These materials can be prepared by cationic polymerization in solution with conventional thermal heating or under microwave conditions [2-4]. Today, polyoxazolines have been shown to have a wide variety of uses, especially in the biomedical field due to their low cytotoxicity, tunable solution properties, resilience to degradation and uptake in the body, and the ability to be incorporated in a multitude of nanostructures [1, 2, 5, 6].

When developing materials for drug delivery, there are a variety of problems that stem from the complexity of biological systems. Some of these involve administration methodology, properties of the drug and how it will interact in biological media, target sites, and desired drug release rates [7, 8]. Even afterwards, drug designs must take into account inter- and intramolecular interactions that can cause harm to the biological system if left unchecked such as

waste removal functions, immunogenicity and organ accumulation [8, 9]. As a result, the designs of vehicles for drug delivery are complex while conversely, and ironically, such pharmaceutics need to be as simple as possible to avoid unnecessary side reactions. Biocompatible polymers such as poly(ethylene oxide) and poly(2-oxazoline)s are ideal since they provide versatile synthetic polymer platforms whereby complex structures can be attained while allowing for incorporating more complex groups [5, 9].

2.2.2 Structure, Synthesis and Properties

The monomer structure of polyoxazoline is the oxazoline ring which is comprised of nitrogen, oxygen, and a double bonded carbon that is arranged as a 5-membered ring. This cyclic imino ether comes in three distinct arrangements that differ from one another by the carbon-carbon bond, though 2-oxazoline has been the most widely used monomer in polymerizations (**2**) (**Figure 2.1**) [10].



R = alkyl or phenyl group

Figure 2.1 Arrangements of cyclic imino ether

While substituents can be present in the 4- and 5- positions, experiments, conducted by Saegusa and Kobayashi showed that this impeded polymerizations through steric crowding, often requiring specific catalysts or spatial arrangements to perform successful polymerizations [1].

Polyoxazolines are polymerized by cationic ring opening polymerization (CROP). First developed in 1966 by four independent groups, polyoxazoline synthesis follows a living cationic ring opening polymerization [9, 11-14]. It is initiated when the nitrogen of the ring, a weak nucleophile, substitutes onto the initiator thereby releasing a weak base. This leaves the positive charge distributed over the nitrogen, carbon and oxygen of the ring. Propagation occurs by subsequent repeated attack on the carbon in the 5-position [4, 13]. Termination is induced after the polymerization by adding another nucleophile such as hydroxide ion or an amine.

Well-defined polyoxazoline copolymers can be synthesized with narrow molecular weight distributions [15]. A crucial factor determining the polyoxazoline properties is the length of the 2-substituted chain. Hoogenboom *et al.* has reported experiments with libraries of various polyoxazoline monomers differentiated by the chain length of the 2- group that ranged from 1 to 9 carbons [2]. Three key points can be drawn from their results: (1) Side-chain length does not significantly affect the rate of polymerization of homopolymers except for when the substituent is methyl. Methyloxazoline polymerizes somewhat faster due to a higher nucleophilicity. (2) Dynamic Scanning Calorimetry (DSC) analysis showed that poly(2-alkyl-2-oxazoline)s that had side chains longer than butyl are semicrystalline, whereas polymers with ethyl and butyl side chains were completely amorphous. The glass transition temperatures (T_g) of polymers with pentyl and longer chains were not identified and this was attributed to the small changes in heat capacities at the glass transitions. (3) Surface energies, calculated by measuring contact angles of diiodomethane and ethylene glycol as test liquids, were influenced by chain length. Polymers with methyl to propyl chains had high surface energies of around 45 mN/m and those with longer chain lengths had low surface energies of around 22 mN/m [2].

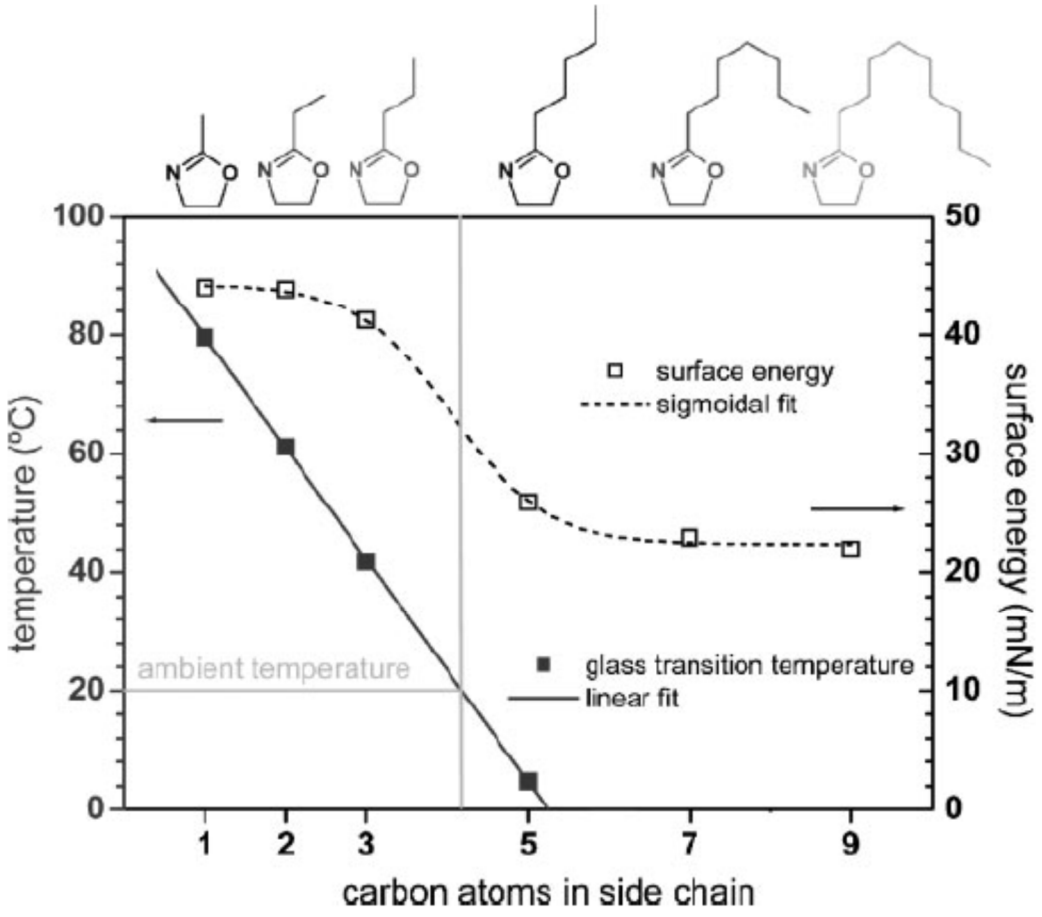


Figure 2.2 Monomer Structures, Tg, and Surface Energies of poly(2-n-alkyl 2-oxazoline) vs. Carbon Number. *Image reproduced from Poly(2-oxazoline)s: Alive and Kicking* [2] Used under Fair Use, 2013

Figure 2.2 details the surface energy of the polymers with regards to the length of the side chain and their glass transition temperatures. A sharp transition in surface energy correlates with a shift from the propyl to pentyl groups along the ambient temperature line (20 ^{O}C) suggesting that chain mobility may influence the capability for the side chain to populate the surface [16].

Another class of polyoxazolines is random copolymers from monomers with different side chain lengths. Park and Kataoka showed that different polyoxazoline compositions can influence properties [17]. For example, the melting point of poly(nonyloxazoline) at 150 ^{o}C

decreases linearly upon incorporation of ethyloxazoline. This indicates disturbance of the crystalline phase and results in the absence of crystallinity when more than 20 wt% of ethyloxazoline is incorporated into the chain. Likewise, T_g's for poly(methyloxazoline) and poly(ethyloxazoline) decrease with incorporation of nonyloxazoline. Surface energies for the copolymers were ~42 mN/m until the copolymers were comprised of ~70% wt% of nonyloxazoline. Interestingly, with high nonyloxazoline content, the T_g was near ambient temperature. At this stage, the surface energy decreased as the T_g dropped below ambient temperature due to the surface coverage by the nonyl side chains [2].

2.2.3 Applications in Biomedicine

In spite of their structural similarities there are certain core features that make polyoxazolines in general attractive for biomedicine. The potential for polymers to be effective vehicles for drugs has been a major driving force in the development of novel polyoxazolines. Synthetic polymers are attractive for drug and gene delivery due to the capability to tailor structure and properties. Polymers can improve the efficacy and bioavailability of drugs by protecting them from degradation and immune response, and improving solubility. Containment in the polymer vehicles can also be used to tailor and sustain drug release rates. Polyoxazolines have been incorporated and assembled into nanoscale structures such as micelles, liposomes, and hydrogels. It has also been shown that these polymers can avoid rapid immune response, similar to that observed polyethylene oxide [18].

Saccharide initiators have been used to polymerize 2-oxazolines [19]. This was achieved by first preparing a saccharide-substituted oxazoline ring, then forming a cationic oxazolinium initiator through the reaction of the saccharide-substituted ring with methyl triflate. The

saccharide-oxazoline cation was then reacted with a variety of 2-oxazoline monomers to produce polymers with a saccharide end group at the initiator end.

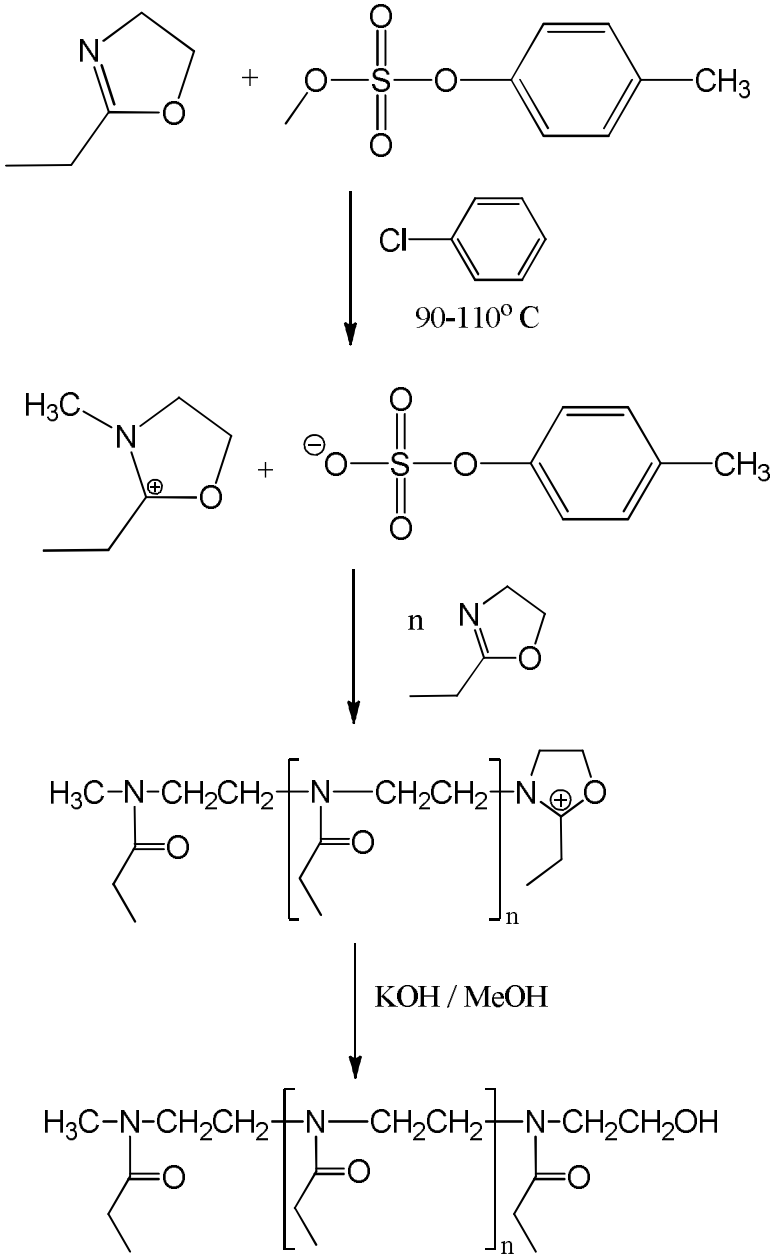


Figure 2.3 Polymerization of 2-ethyl-2-oxazoline with a tosylate initiator and termination with potassium hydroxide

Poly(2-methyl-2-oxazoline)s were demonstrated to be biocompatible by Goddard *et al* [6, 20]. A series of poly(2-methyl-2-oxazoline)-polyethylene glycol copolymers labeled with ^{125}I

were injected intraveneuosly into mice. It was observed that after 24 hours, 7% of 15 kDa copolymers remained in the blood while 28% of 29 kDa copolymers remained after the same time period. *Gaertner et al.*, discovered that poly(methyloxazoline) and poly(ethyloxazoline) with ^{111}In labels had negligible accumulation in any organs, except for the kidneys, but were quickly excreted [21]. Intravenously injected doses had only 0.8% (polymethyloxazoline) and 1% (polyethyloxazoline) left in the blood after 3 hours. Total accumulation activity per kidney was 0.4% and 0.6% for polymethyloxazoline and polyethyloxazoline, respectively, suggesting that excretion of the polymers is mostly done through the kidneys. In spite of this, investigations were conducted comparing with the clearance rates with two different nuclear tracers of kidney functions, Tc-diethylenetriaminepentaacetate (DTPA) and Tc-mercaptoacetyltriglycine (MAG3). It was shown that DPTA is filtered exclusively through renal glomerular filtration making the rate equal to glomerular filtration. MAG3 excretion is affected by glomerular filtration and tubuli secretion making it equivalent to the renal plasma flow rate. When comparing to the 5 kDa polyoxazolines, the clearance rates were much slower than the nuclear tracers suggesting that there were specific interactions between the polymers and proteins in the plasma. Overall, polyethyloxazoline was retained longer than polymethyloxazoline, and this was attributed to its more hydrophobic nature.

2.2.4 Stimuli Responsive Polyoxazolines

Stimuli responsive polymers ideally change properties drastically in response to a small change in environmental conditions. Most biological stimuli consist of changes in pH and/or salt and biochemical concentrations. It is well documented that polyoxazoline copolymers can respond in this manner [22-24]. The self-assembling nature of amphiphilic poly(ethyloxazoline)

copolymers is controlled by their structure and chemical environment. An example of this is with a series of poly(2-ethyl-2-oxazoline)-poly(ε-caprolactone) alternating multiblock copolymers prepared from short preformed blocks (approximately 1000-2500 g/mol). These act as hydrogels even though there are no covalent crosslinks. The copolymers were hydrophilic but water insoluble and exhibited reversible swelling behavior upon heating and cooling. Swelling ratios were dependent on the temperature, volume fractions of the blocks, and the length of the polyethyloxazoline block. At low temperatures (~15 °C), hydration of the hydrophilic polyethyloxazoline primarily dictated the swelling ratio. As temperature was increased (approaching 35 °C), maintaining the hydrophobic interactions from polycaprolactone within the gel became more important [1, 25]. Polyethyloxazoline has been shown to exhibit LCST behavior in water, but the short blocks in this study had LCST transitions outside of the range investigated.

Poly-2-ethyl-2-oxazoline-b-ε-caprolactone diblock copolymers have also been investigated. These copolymers form micelles in water with critical micelle concentrations of 1.0 to 8.1 mg/L. The outer shell is comprised of the hydrophilic poly(2-ethyl-2-oxazoline) block, and the shell is known to form complexes with polymethacrylic acid through hydrogen bonds. The carbonyl oxygen and nitrogen on the polyoxazoline are attracted to the carboxylic acids on the polymethacrylic acid due to hydrogen bonding [15, 26]. When pH drops below 3.5, the micelles precipitate, and upon increase of pH to 3.8 they can be redispersed.

The thermoresponsive nature of polyoxazolines has been investigated [9, 23, 27-29]. They become insoluble in water as temperature is raised through the lower critical solution temperature (LCST) and cloud points are observed. At low temperatures, the polymer dissolves in water due to hydrogen bonds. As temperature is increased, the hydrogen bonds are weakened

causing the polymers to aggregate due to the entropy gain associated with water dissociation [28]. The alkyl chain length of the polyoxazoline influences the LCST. Kwei et al. studied polyethyloxazoline thermosensitvity where cloud points were found to depend on polymer concentration and molecular weight (20-500 kDa). These factors correlated with the lower critical solution temperature (LCST) range of 61 - 64 °C. It was also found that addition of salts to these solutions influenced the LCST. Sodium chloride addition was found to decrease the LCST and cause precipitation, but conversely, LCST was increased with addition of tetrabutylammonium bromide. However, it must be noted that these tests were performed with a very high molecular weight sample of 500k [30].

Hoogenboom *et al*. measured the LCST transition of polyoxazolines with varying hydrophilicity, with and without the presence of added Hofmeister salts. As expected, the LCST increased with increasing hydrophilicity in the absence of the salts. These salts included NaSCN, $NaClO_4$, $LiClO_4$, NaI, LiI, NaCl, NaOAc, LiOAc, Na_2SO_4, and Li_2SO_4. It was demonstrated that addition of ^-SCN, ClO_4^-, and I^- increased cloud points. By contrast, Cl^-, ^-OAc, and SO_4^{2-} resulted in lower cloud points. The anions were found to have a greater effect than the cations. The cloud points of the most hydrophilic polymer studied, polyethyloxazoline, could be varied over a very broad range in temperature by adding these salts [31].

2.2.5 Polyoxazoline in Lipopolymers

Micelles and liposomes are in high demand due in part to the convenience of being able to self-assemble in water around a desired drug through the use of amphiphilic copolymers. The most commonly studied vehicles have involved poly(ethylene glycol) (PEG). There have been a number of cases where PEG-antibody immune responses have occurred [32]. Thus, Woodle *et*

al., prepared poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) lipo-polyoxazoline conjugates in combination with distearoylphosphatidyl ethanolamine (DSPE) as shown in **Figure 2.4** [33].

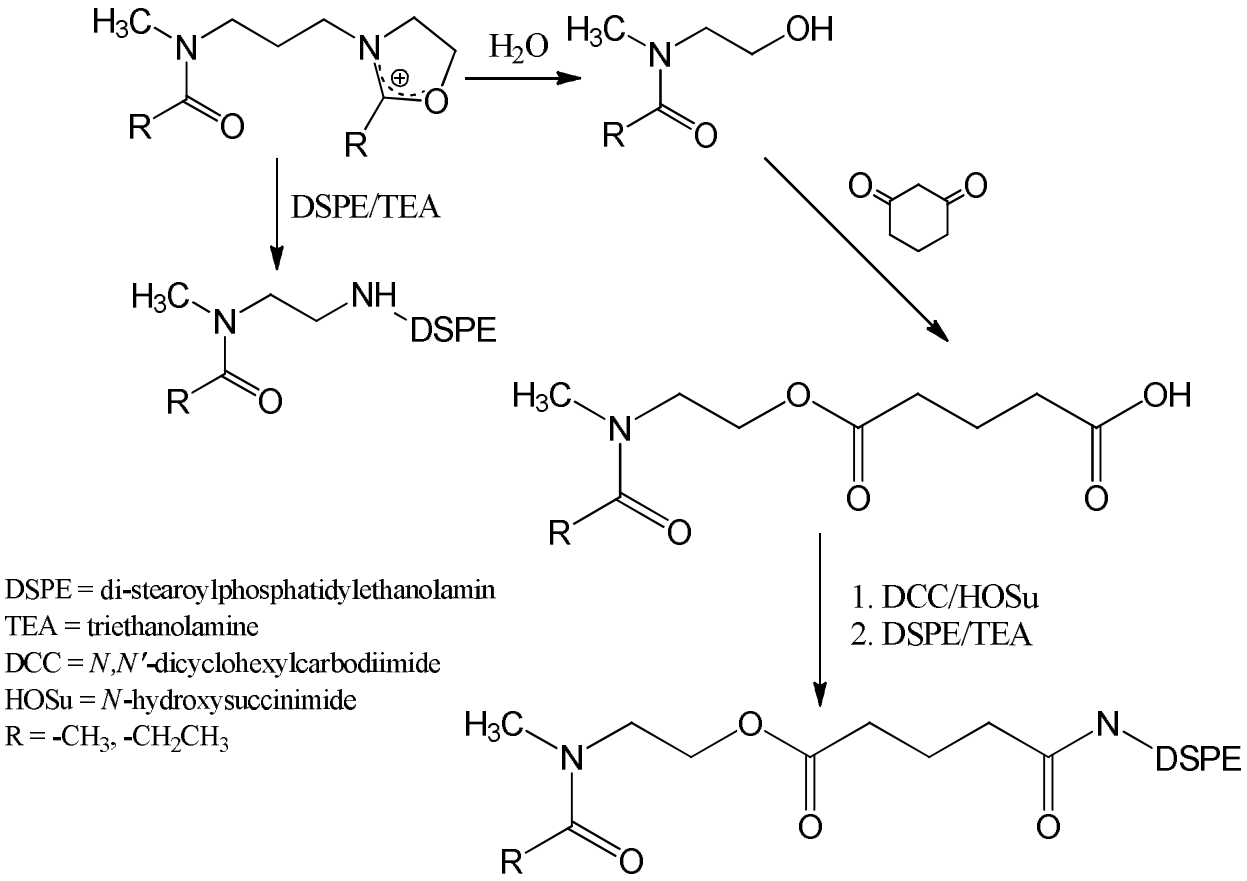


Figure 2.4 Representation synthesis of poly(2-oxazoline)/DSPE conjugates

These lipophilic polyoxazolines with ^{67}Ga labels were injected into the bloodstream of rats. They showed equivalent retention in the bloodstream as well as exhibiting steric colloidal stabilization due to high chain mobility and hydrophilicity. The hydrophilic oxazoline components of these materials suppressed their interaction with compounds in the blood that would cause immune response.

2.2.6 Polyoxazoline Vesicles

Polyoxazoline-based amphiphilic block copolymers are particularly attractive due to their self-assembly into micelles and vesicles. These nanocontainers have been used for a number of different applications. One such application is the encapsulation of calcium ions [34]. Poly(2-methyl-2-oxazoline)-*b*-polydimethylsiloxane-*b*-poly(2-methyl-2-oxazoline) (PMOXA-PDMS-PMOXA) was prepared and bound to platinum and glass electrodes in phosphate buffer [1]. This resulted in phosphate ions becoming incorporated into the micelle or vesicle. These membranes containing phosphate ions were incubated with calcium chloride in the presence of ionophores, and this lead to precipitation of calcium phosphates in the vesicle [1].

2.2.7 Gene Transfer Chemistry

Polyoxazolines can be converted to polyethyleneimine through hydrolysis of the pendent amides. The polyethyleneimine exists as a polycation at low pH. These polycations have been investigated as components of complexes with anionic polynucleic acids. Gene delivery has been investigated for over 25 years using both viruses and polycations to carry the DNA. [1, 35, 36]. An example of this is with a poly(ethylene glycol)-b-poly(ethylene imine). Synthesis of the PEG used a heterotelechelic poly(ethylene oxide) with an acetal group on one end and a mesylate on the other. This polymer was used as a macroinitiator for the cationic ring-opening polymerisation of 2-methyl-2-oxazoline to nominally make an acetal-PEG-PMOXA polymer. This was hydrolyzed using strong aqueous base to give the reported acetal-PEG-PEI. ^{1}H NMR confirmed the expected composition of the polyethyleneoxide-polyoxazoline block copolymer. However, it is not clear in light of the work described in this thesis that efficient initiation of the macroinitiator to form the block copolymer would have occurred under the conditions specified.

The NMR showed that the hydrolysis reaction to form the polyethyleneimine block was quantitative. Park *et al*. described the synthesis of poly(2-ethyl-2-oxazoline)-co-poly(ethyleneimine) random copolymers through partial acid hydrolysis of poly(2-ethyl-2-oxazoline) [37]. Increasing the acid concentration afforded polymers with increasing degrees of hydrolysis and therefore increasing charge densities on the backbone. These materials formed tightly compacted complexes with DNA. A 50k g/mol polyethyloxazoline that had been 88.0% hydrolyzed was found to have the most efficient complexation capacity. However, the high degree of hydrolysis also increased cytotoxicity. The higher the charge and molecular weight, the more cytotoxic the polymer became [38].

PEI has also been shown to have potential as a component of carriers for anticancer agents [39]. Folate receptors are proteins that are overexpressed on tumor cells. A folate-poly(ethyleneimine)-b-poly(L-lactide) copolymer was synthesized by preforming a partially hydrolyzed polyethyloxazoline oligomer terminated with amine groups. This was reacted with a carboxyfunctional poly-L-lactide oligomer, then further reacted with folate. A polymer/DNA complex was prepared with the folate-poly(ethyleneimine)-b-poly(L-lactide). It was found that the folate-poly(ethyleneimine)-b-poly(L-lactide) reduced toxicity relative to PEI but maintained acceptable transfection efficiency.

Triblock copolymers that integrated PEG to improve colloidal stability and decrease cytotoxicity have been reported. However, these authors did not provide any evidence that the molecular weights could be controlled. Nevertheless, the polymethyloxazoline componenet was hydrolyzed to yield PEI blocks, and the resultant materials were complexed with DNA. When screened for transfection efficiency, these copolymers resulted in improved transfection efficiency over PEI with less cytotoxicity [40].

2.2.8 Antimicrobial Polyoxazoline

Antimicrobial polymers may have advantages relative to traditional low-molecular-weight antimicrobial agents due to lower toxicity and less development of microbial resistance [41]. In a Minimum Inhibitory Concentration study done by Waschinski and Tiller, polyethyloxazoline and polymethyloxazoline were end-functionalized with various alkyl groups at the initiator end and various quaternary ammonium salts at their terminal end, and these were investigated as antimicrobial agents. Only the polymethyloxazolines exhibited antimicrobial activity. It was found that the potency of the materials depended on the end-group structures at both ends and also depended on the aggregate characteristics. The materials that were below their critical micelle concentrations (CMC) were more active than those above the CMC. It was hypothesized that the both of the endgroups interacted with the cell membranes, thereby destabilizing the membranes [1, 42, 43].

Tiller et al. have investigated polymethyloxazoline as an additive for a contact-active acrylate-based antimicrobial material without the release of low molecular weight antimicrobial agents into the environment [42, 44]. The polymer was initiated with a biocidal benzyl bromide functional initiator, and terminated with a acrylamide functional tertiary amine. These macromers were copolymerized with hydroxyethylmethacrylate and 1,3-glyceroldimethacrylate. The polymethyloxazoline chain was utilized as a spacer for the biocidal copolymers. **Figure 2.5** shows the structure of the polymer macromonomers used. The study demonstrated that by weight, the copolymer containing as little as 0.4% of the 2400 g/mol M_n macromer was highly antimicrobial [44].

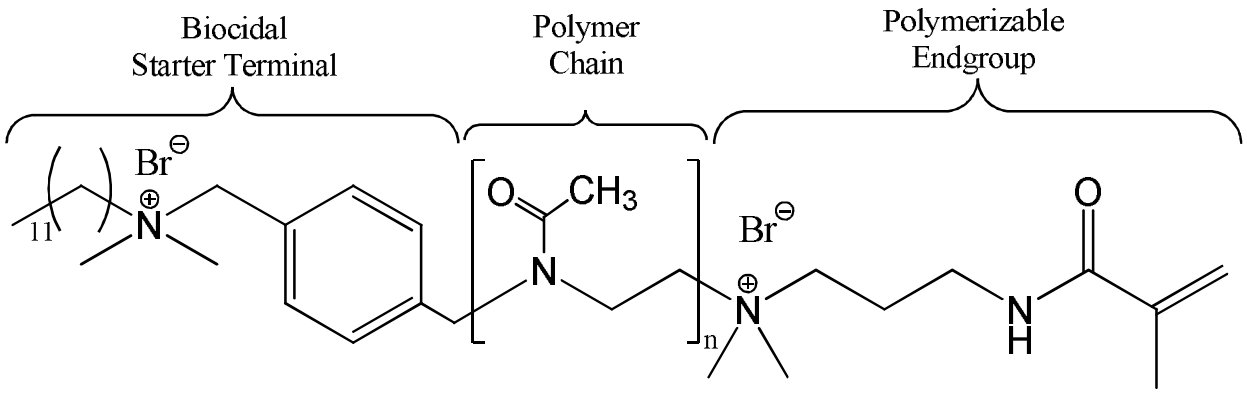


Figure 2.5 Structures of antimicrobial macromers

2.3 Phosphonates

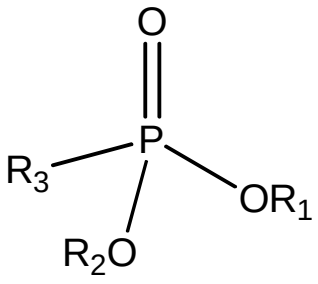
2.3.1 Introduction

Two industrial and biologically significant classes of organic compounds are phosphonates and phosphonic acids. They are known to have a wide variety of applications especially in medicine, finishes for paper and textiles, and in agriculture. However, the exact biological role of phosphonates is not yet fully understood. The first documented experiment with phosphonates was in 1865 with the synthesis of bisphosphonates used in textiles and fertilizers [45]. Naturally occurring phosphonates were discovered in rumen protozoa in 1959 leading to further investigations of their presence in fungi, bacteria, and higher order organisms such as the snail schistosome vector, *Biomphalaria* [46, 47]. Furthermore, because of the affinity that phosphonates have with metal ions, extensive research has been focused on bone regeneration utilizing chelating properties. As a result, phosphonates are used as osteoporosis drugs [48]. The biocompatible nature also has provided a bridging aspect between inorganic and organic media. One long-term impact of the research described in this thesis may be to utilize the

unique properties of polymers containing phosphonates to complex with cations. These may be applicable for bioimaging and drug delivery.

2.3.2 Chemistry of Phosphonate and Phosphonic acid

The structure of phosphonate is a phosphorus atom covalently bonded to two hydroxide or ester variants, double bonded to oxygen, and with a bond to an alkyl or aryl group as shown in **Figure 2.6.**



R_1, R_2, R_3 = H, Alkyl, or Aryl Groups
Figure 2.6 Basic structure of phosphonate compound

The oxygen substituents make the molecule soluble in water and provide a negative electronic environment to function as chelating agents. Phosphonates are often soluble in both water and organic solvents, while phosphonic acids are typically water-soluble but poorly soluble in organic solvents. Organophosphonates have a highly stable carbon-phosphorus bond strong enough to resist hydrolysis, thermal decomposition, and photolytic degradation. This is due to high activation energies despite similarities of the bond energies between a C-P and C-C bond which were determined to be 64 kcal and 62 kcal, respectively.

Phosphonic acid is a moderately strong acid and dibasic. **Table 2.1** presents the pK_a values of alkylphosphonic acids and **Table 2.2** shows the pK_a values of aliphatic bisphosphonic acids. According to **Table 2.1**, the pK_a values increase with growing numbers of carbon atoms

and branching degree. The pK_a's of aliphatic bisphosphonic acids, shown in **Table 2.2**, are affected by the length of the connecting carbon chains [49].

Table 2.1 pK_a Values of Unsubstituted Alkylphosphonic Acids in Water

Alkyl Group	pK_1	pK_2	pK_2 - pK_1
Methyl	2.33	7.76	5.43
	2.35	7.1	4.75
	2.38	7.74	5.36
	2.48	7.34	4.86
Ethyl	2.39	7.98	5.59
	2.45	7.85	5.4
	2.43	8.05	5.62
n-Propyl	2.45	8.06	5.61
	2.49	8.18	5.69
Isopropyl	2.55	7.75	5.2
	2.66	8.44	5.78
n-Butyl	2.59	8.19	5.6
Isobutyl	2.7	8.43	5.73
sec-Butyl	2.74	8.48	5.74
t-Butyl	2.79	8.88	6.09
Neopentyl	2.84	8.65	5.81
1,1-Dimethylpropyl	2.88	8.98	6.08
n-Hexyl	2.6	7.9	5.3
	2.4	8.25	5.85
n-Dodecyl	*	8.25	---
	1.22†	0.81†	5.59

* Low solubility of this compound in acid solutions prevented the determination of pK_a
† Values represented are on the molal scale and were obtained in 50 % wt ethanol

Table 2.2 pK_a Values of Aliphatic Bisphosphonic Acids in Water

Compound	pK_1	pK_2	pK_3	pK_4
$H_2O_3P(CH_2)_4PO_3H_2$	<2	2.75	7.54	8.38
$H_2O_3P(CH_2)_3PO_3H_2$	<2	2.65	7.34	8.35
$H_2O_3PCH_2CH(CH_3)PO_3H_2$	<2	2.6	7.00	9.27
$H_2O_3PCH_2PO_3H_2$	<2	2.57	6.87	10.33

2.3.3 Biomedical Applications of Phosphonate

The vast majority of natural and synthetic products involving phosphorus are phosphate esters. Phosphonate derivatives are also significant in industry and biologically relevant products [50]. Horiguchi and Kandatsu in 1959 were able to isolate 2-aminoethylphosphonate in rumen protozoa [51]. It was later discovered that the phosphonate was an integral component of the phosphonolipid structure in several varieties of sea organisms and in the human brain [50, 52]. Further studies by Seidel and Bowman discovered the enzyme phosphoenolpyruvate phosphonomutase (PEP mutase) which converts phosphates into phosphonates (**Figure 2.7**) [47, 53].

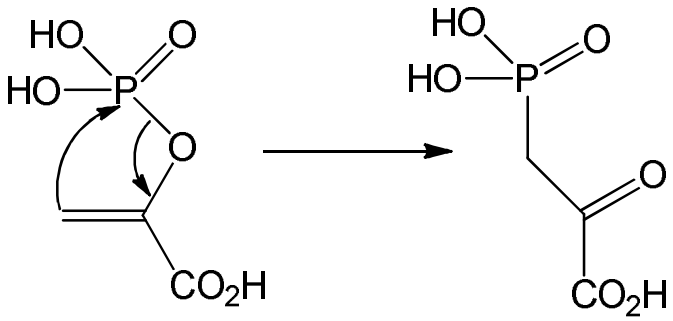


Figure 2.7 Reaction catalyzed by PEP mutase

The stereochemistry of the oxygen atoms allow for effective chelation of many harmful heavy metal cations in ground water such as lead, copper, and arsenic [54-56]. This affinity has been exploited as an oxidation stabilizer which has benefited the paper, textiles, and wood varnishing industries. The C-P bond coupled with strong cationic attraction of oxygen atoms has resulted in oligomeric phosphonates that, when coated onto wood or plastic casings, are resilient to combustion [57].

Phosphonates have also influenced the development of pesticides. Pesticide brand names such as Round Up^{TM} (glyphosate) utilize an aminoalkyl phosphonate as part of the active group. Pesticide development has been integral to phosphonate research. Butonate, trichlorfon, and *o,o*-

bisphenyl methylphosphonate are a few examples of phosphonate insectides and fungicides [58]. (**Figure 2.8**)

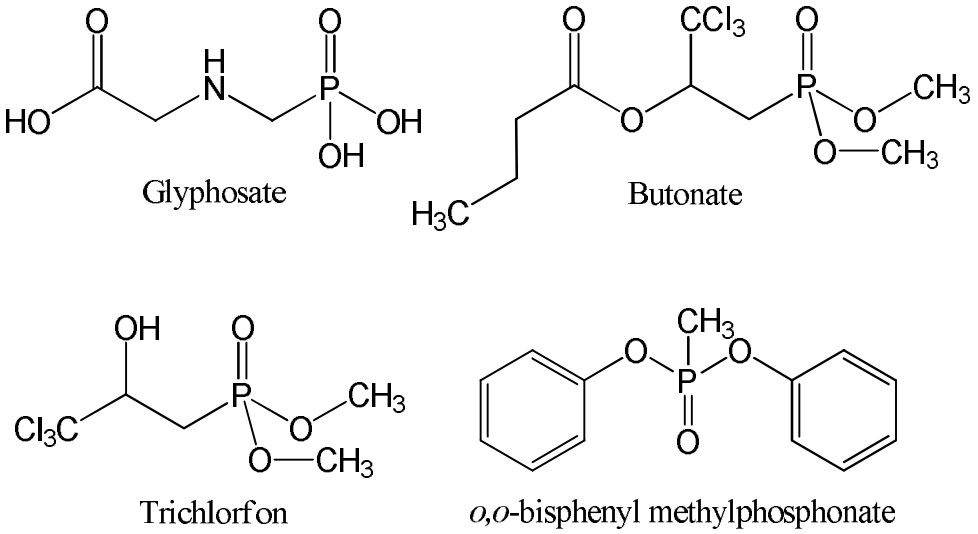


Figure 2.8 Examples of commercially used pesticides

The toxic nature of such pesticides, however, garnered interest from the military as biochemical weaponry. Gerhard Schrader introduced the deadly potential in 1936 of phosphorus-containing agents in the form of the first nerve agent, Tabun. Various dialkyl methylphosphonates were precursors to various nerve agents including Sarin, and Soman [59]. (**Figure 2.9**)

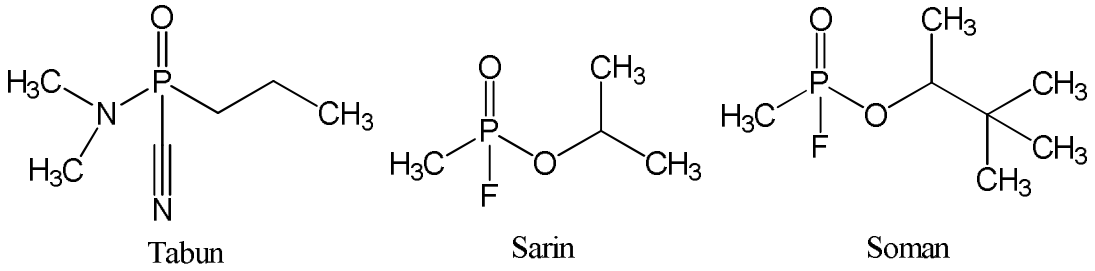


Figure 2.9 Structures of phosphorus–based chemical weaponry based on methylphosphonates

The field of medicine has greatly benefited from the functional potential of phosphonates. Therapeutic agents derived from acyclic nucleoside phosphonates were shown to be effective against certain invasive pathogenic species and microorganisms [60-62]. Several examples include Tenofovir disoproxil fumarate, an HIV treatment agent that is also effective against hepatitis B, Adefovir, a nucleotide reverse transcriptase inhibitor used against the Herpes virus, and Cidofovir, which treats the Human papilloma virus. Specifically, HIV and hepatitis C reproduction were inhibited through phosphonate interactions with proteases [59, 63, 64]. Alafosfalin is an antibiotic known to inhibit cell wall biosynthesis and it is potentially useful in the gastroenteritis treatment and bacterial urinary tract infections [59, 65, 66].

One subset of phosphonates that has been particularly useful in the clinic is bisphosphonates where two phosphonate groups are attached to a single carbon. These are used as treatments for bone related disease including osteoporosis and even bone and breast cancers [67-70]. In addition, bisphosphonates have shown promise in their ability to chelate radionucleotides and iron oxide contrast agents in an effort to gain further insight into cancers and their related biological processes [71, 72].

2.3.4 Phosphonates as Biocompatible Surface Media

Many implantable materials and devices need to be interfaced with biological components. This is particularly significant with metallic prosthetics. Exposure of the implants to the body can lead to adsorption of proteins, cell-surface interactions, or tissue development or regrowth. This makes the physical and biochemical properties of the implant surface a high priority for design. Phosphonic acids and phosphonates can produce a biocompatible interphase region to bridge metal and tissue. Titanium metal, used in orthopedics and dental implants, has

excellent mechanical properties. Titanium oxide is an inert compound that is easily integrated in bone regeneration [73, 74]. This is a key reason why it is used in dentistry. However, complete incorporation with the bone lattice via chemical bonding has not been observed. Titanium oxide and siloxane surfaces can be functionalized with phosphonates to produce self-assembled monolayers [73, 75]. These surfaces can then be further functionalized with cell-binding peptides to allow for successful surface interactions with bone tissue [76]. Phosphonic acid has only recently been utilized to form bioactive titanium surfaces and these show promise as they are biocompatible, are more hydrolytically stable than silane surfaces, and can be bound to various material surfaces [75, 76].

Gawalt et al. first reported the self-assembly of alkanephosphonates onto titanium oxide surfaces. In general, the surface of titanium oxidizes easily forming an oxide surface that is resistant to surface chemical modifications [73]. However, after aerosol application and solvent removal of alkanephosphonate in THF, heating the surface to 120 °C for 18 hours formed an alkanephosphonate film. This film was shown to be highly resistant to solvent removal and mechanical peeling.

However, investigations of cell-adhesion proteins interacting with phosphonic acid-modified titanium surfaces showed a substantial increase in immune responses. It was hypothesized that phosphonic acids were indiscriminately binding to many surface proteins of cells causing distortion and irritation at the interface [77]. Research conducted by *Adden et al.* sought to better integrate bone tissue with various phosphonic acid compounds in order to prevent aseptic loosening. This is an immune response where tiny particles between the joint and tissue cause inflammatory responses that can lead to bone degradation and loosening of the implant. (11-Hydroxyundecyl) phosphonic acid and (12-carboxydodecyl) phosphonic acid were

deposited onto silicon or titanium substrates by submersing these substrates in solution at a concentration below the critical micelle concentration. As the solvent evaporated, a phosphonate film became firmly attached on the substrate due to the partially organized aggregation at the surface. **Figure 2.10** presents a diagram of the procedure.

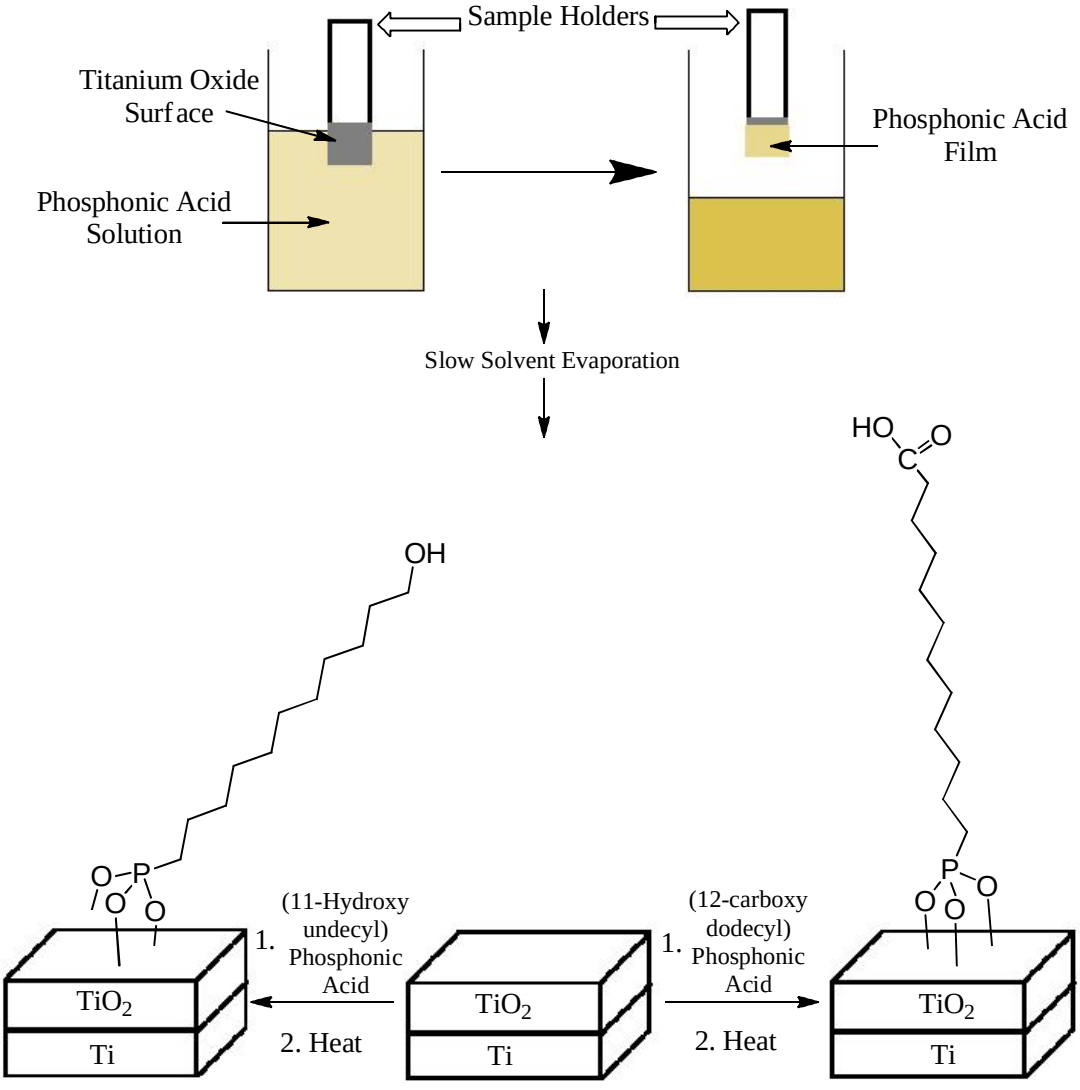


Figure 2.10 Diagram of "Tethering by Aggregation and Growth" (T-BAG) using Phosphonic Acid Solutions to Coat Titanium Oxide surfaces

A similar procedure has been used to immobilize cell-adhesive RGD peptides and BMP-2 proteins onto phosphonate-modified titanium oxide surfaces. BMP-2 protein enhances bone growth by differentiating mesenchymal cells into osteoblasts. Two methods were devised [76].

First, (11-hydroxyundecyl) phosphonic acid was immobilized then activated for protein binding with carbonyldiimidazole. Second, (12-carboxydodecyl) phosphonic acid was bound in a similar manner except with the use of *N*-hydroxysuccinimide to activate binding at the surface. Human BMP-2 proteins were bound to the carboxy group through their primary amine, forming a biocompatible cell adhesion layer that was specific for the osteoclast cells and promoted bone growth. Phosphonic acid was shown to be chemically and physically more resilient to degradation and aseptic loosening than the previously used silanes.

CHAPTER 3: Synthesis and Functionalization of Poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) Diblock Copolymers with Phosphonate Ions

3.1 Synopsis

This chapter discusses the synthesis, functionalization, and characterization of PEO-b-PEOX as it is converted into the phosphonic acid copolymer form. In recent years, amphiphilic block copolymers have been extensively investigated as potential drug carriers due to their self-assembly into various kinds of morphological structures in aqueous solutions. However, PEOX blocks can be additionally functionalized with other biocompatible molecules such as proteins. The phosphonic acid substituents provide an electronegative environment for cationic drugs, proteins, and metal ions to be held in. Such complexes can induce micelle or vesicle formation depending on the structure of the block ionomer, changes in pH, temperature, and ionic strength. In this study, we demonstrate a novel approach to prepare poly(ammonium phosphonate) block copolymers with PEO. This builds upon previous work that describes converting poly(2-oxazolines) to linear PEI or poly(2-oxazoline-co-PEI) [37, 78, 79]. PEO-*b*-PEOX block copolymers were synthesized using cationic ring-opening polymerization with a tosylate initiator. Of the three samples, two weight ratios were made: 1:2 and 1:3 PEO to PEOX. SEC, however, revealed slow initiation relative to propagation though further functionalizations were not affected by this. The samples were hydrolyzed with HCl to form the random copolymer PEO-*b*-PEOX-*co*-PEI. These copolymers were dialyzed in pH ~9 to free the nitrogen of the PEI group which would allow for Michael addition onto diethyl vinyl phosphonate forming PEO-*b*-PEOX-*co*-PEI-*co*-phosphonate copolymers. Further acid hydrolysis of the phosphonate forms the

final product, PEO-*b*-PEOX-*co*-PEI-*co*-phosphonic acid. Due to the ionic nature of the phosphonic acid these novel materials may bind strongly to opposite charged molecules and metal ions.

3.2 Experimental

3.2.1 Materials

Poly(ethylene oxide) methyl ether (M_n = 5800 g/mol), *p*-toluenesulfonyl chloride (TsCl, >98%), anhydrous methanol (>99.8%), calcium hydride (>90%), and manganese (II) chloride tetrahydrate (99.9%) were purchased from Aldrich and used as received. 2-Ethyl-2-oxazoline and chlorobenzene, both from Aldrich, were dried over CaH_2. 2-Ethyl-2-oxazoline was vacuum distilled into a dry flask under nitrogen. Chlorobenzene was distilled under nitrogen. Triethylamine (TEA, 99.5%) was obtained from Fluka. Bromotrimethylsilane (TMS-Bromide, 97%) and aqueous potassium hydroxide (1 M) were purchased from Alfa Aesar and used as received. Dialysis tubing (3500 g/mol MWCO) was obtained from Spectra/Por. Aqueous hydrochloric acid (2 M, LabChem) was used as received. Diethyl vinyl phosphonate (98%) was obtained from Epsilon Chiminie. Diethyl ether, methanol and dichloromethane (Fisher Scientific) were used as received. Anhydrous dichloromethane (>99.8%) was obtained from EMD Chemicals.

3.2.2 Synthesis of Tosylated PEO Macroinitiator

The synthesis of PEO methyl ether tosylate macroinitiator is provided. A 5800 g/mol M_n PEO oligomer (30.2 g, 5.21 mmol) was added to a 250-mL flask equipped with a magnetic stir bar and enclosed with a rubber septum bound with steel wire. The PEO oligomer was vacuum dried at 70°C for 24 h and dissolved in dichloromethane (70 mL). Et_3N (3.67 mL, 26.3 mmol) was added to serve as an acid scavenger. In a separate flask, TsCl (2.04 g, 10.7 mmol) was

dissolved in 50 mL of CH_2Cl_2. The tosyl chloride solution was syringed into the reaction flask. The reaction was stirred for 24 h at 25 °C. The reaction mixture was passed through a 0.2-µm Teflon® filter, then transferred to a separatory funnel, and washed with DI water 4X to remove salts. The organic phase was concentrated via rotary evaporation, and the resulting macroinitiator was collected via precipitation into diethyl ether, and then was dried overnight under vacuum at 50 °C.

3.2.3 Synthesis of Poly(ethylene oxide-*b*-2-ethyl-2-oxazoline)

Double hydrophilic poly(ethylene oxide-b-2-ethyl-2-oxazoline) (PEO-*b*-PEOX) block copolymers were synthesized via cationic polymerization of 2-ethyl-2-oxazoline from the end of the tosylated PEO macroinitiator. An representative procedure for preparing a targeted composition of ~2.5:1 wt:wt PEO:PEOX is as follows. A tosylated PEO macroinitiator (3.3 g, 0.57 mmol) was dried overnight at 80 °C in a 100-mL flask equipped with a magnetic stir bar and enclosed with a rubber septum. Chlorobenzene (20 mL) was added to dissolve the PEO macroinitiator, and then 2-ethyl-2-oxazoline (5.51 mL, 5.41 g, 55 mmol) was syringed into the macroinitiator solution. The reaction was performed at 110 °C for 24 h. The reaction mixture was cooled to room temperature and the polymer chains were terminated with 1 M KOH (1 mL, 1 mmol) in methanol. The diblock copolymer was isolated by precipitation into diethyl ether, and collected by filtration and dried at 50 °C *in vacuo* overnight.

3.2.4 Acid Hydrolysis of Poly(ethylene oxide-b-2-ethyl-2-oxazoline)

An acid hydrolysis procedure for removing a portion of the pendent amide groups from the PEOX blocks is provided. A PEO-*b* -PEOX diblock copolymer with 5800 g/mol PEO:14,300 g/ mol PEOX (5 g, 0.25 mmol, 36 meq of amides) was charged to a 100-mL flask containing a

magnetic stir bar and enclosed with a septum. $HCl_{(aq)}$ (2 M, 10.5 mL, 21 mmol) and 4.5 mL of DI water were syringed into the flask. The reaction was conducted at 90 °C and maintained for 24 h, then cooled to room temperature. The reaction mixture was diluted to 50 mL with DI water and placed in a 3500 g/mol MWCO cellulose acetate dialysis membrane and dialyzed against 4 L of DI water for 48 h. The pH of the receptor medium was adjusted to ~ 9 with KOH to remove acetates and other salts. The contents of the dialysis membrane were transferred to a 250-mL flask and freeze-dried. ^{1}H NMR was used to confirm the extent of hydrolysis.

3.2.5 Synthesis of Phosphonic Acid Functionalized PEO-b-PEOX-co-PEI

The ethyleneimine groups of the partially hydrolyzed copolymers were reacted via Michael addition with diethyl vinyl phosphonate. A representative procedure for addition of phosphonate groups to the PEO-*b*-PEOX-*co*-PEI copolymer is as follows. A PEO-*b*-PEOX-*co*-PEI (4.07 g, 0.25 mmol, 19.3 meq of amides, 16.7 meq of imines) was charged to a 100-mL flask equipped with a stir bar, and dissolved in DI water (10 mL). Diethyl vinyl phosphonate (7.75 mL, 8.22 g, 50 mmol) was added to the polymer solution. The reaction was performed at 80 °C for 24 h. The reaction mixture was diluted with DI water (40 mL) and placed in a 3500 g/mol MWCO cellulose acetate dialysis bag and dialyzed against 4 L of DI water for 48 h to remove excess diethyl vinyl phosphonate. The contents of the dialysis membrane were transferred to a 250-mL flask and freeze-dried.

PEO-*b*-PEOX-*co*-PEI-phosphonic acid was prepared by removal of the ethyl ester groups from PEO-*b*-PEOX-*co*-PEI-phosphonate [80, 81]. In a representative procedure, PEO-*b*-PEOX-*co*-PEI-phosphonate (0.50 g, 0.031 mmol, 0.625 meq of phosphonate groups, 1.25 meq of ethyl groups) was dissolved in anhydrous dichloromethane (5 mL) in a flame-dried 100-mL flask equipped with a stir bar, and enclosed with a rubber septum. TMS-Br (0.25 mL, 0.29 g, 1.88

mmol) was syringed into the reaction flask and stirred at 25 °C for 24 h, then the solvent and excess TMS-Br were removed via rotary evaporation. The product was further processed under vacuum at 60 °C for 4 h utilizing a KOH trap system. The resultant polymer was dissolved and reacted in methanol (3 mL) for 6 h to cleave the trimethylsilyl groups. PEO-*b*-PEOX-co-PEI-phosphonic acid was recovered by precipitation into diethyl ether and vacuum-dried at 25 °C for 24 h.

3.2.6 Characterization

^{1}H NMR spectral analyses of polymers were performed using a Varian Inova 400 NMR spectrometer operating at 400 MHz. The NMR parameters included a pulse width of 30° and a relaxation delay of 1 s at room temperature with 32 scans. All spectra of the polymers were obtained in D_2O at a concentration of 0.05 g/mL.

Size exclusion chromatography was performed using an Agilent Technologies 1260 Infinity series HPLC pump equipped with a degasser, autosampler, and temperature controlled column compartment. The detectors were a Dawn Heleos-II multi-angle laser light scattering detector and Optilab T-rEX refractive index detector both by Wyatt Technologies. The mobile phase was N-methylpyrrolidone containing 0.05 M LiBr and the stationary phase consisted of two Alpha M mixed bed columns from Tosoh Bioscience. The column compartment was maintained at 80 °C and the detectors at 50 °C. Samples were dissolved at approximately 1 mg/mL and filtered with a 0.2 µm Teflon® filter prior to sample loading.

The thermal decomposition behavior of polymer samples was determined using a thermogravimetric analyzer (TGA, TA Instruments, TGA Q5000) with a heating rate of 10°C/min under nitrogen. TGA measurements were conducted from 50 to 600°C. Prior to each

measurement, all samples were held at 100 °C for 15 min in the TGA instrument to remove any moisture.

3.3 Results and Discussion

3.3.1 Synthesis of Poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) and Modifications to Form Copolymers Containing Ammonium Phosphonate Zwitterions

Syntheses of the PEO-*b*-PEOX diblock copolymers were performed using cationic ring-opening polymerization of 2-ethyl-2-oxazoline from one end of PEO-tosylate macroinitiators as depicted in **Figure 3.1**.

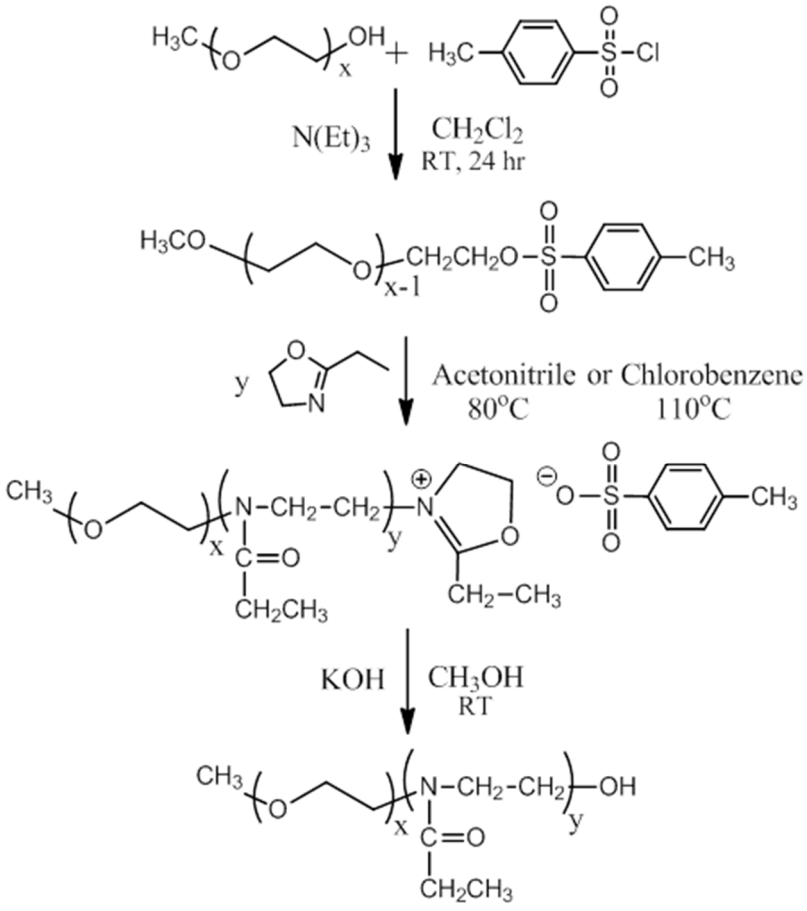


Figure 3.1 Synthesis of poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) copolymers

After polymerization was complete, the cationic chain ends were neutralized with KOH in methanol at 25 °C to form a hydroxyl endgroup. A portion of the pendent amides were then hydrolyzed to obtain a series of PEO-*b*-(PEOX-co-PEI) copolymers (**Figure 3.2**). It has been reported that the degree of deacylation on PEOX could be controlled by the stoichiometric molar ratio of HCl to amide up to a degree of deacylation of 0.4 [78]. Thus, various concentrations of HCl were used to control the extent of hydrolysis so that copolymers with different relative amounts of amide versus ethyleneimine units could be investigated. Ethyleneimine units on PEO-*b*-(PEOX-co-PEI) were post-functionalized with diethylvinylphosphonate (DEVP) via Michael addition in water to introduce pendent ammonium phosphonate groups as precursors for the corresponding phosphonic acids as shown in **Figure 3.2**.

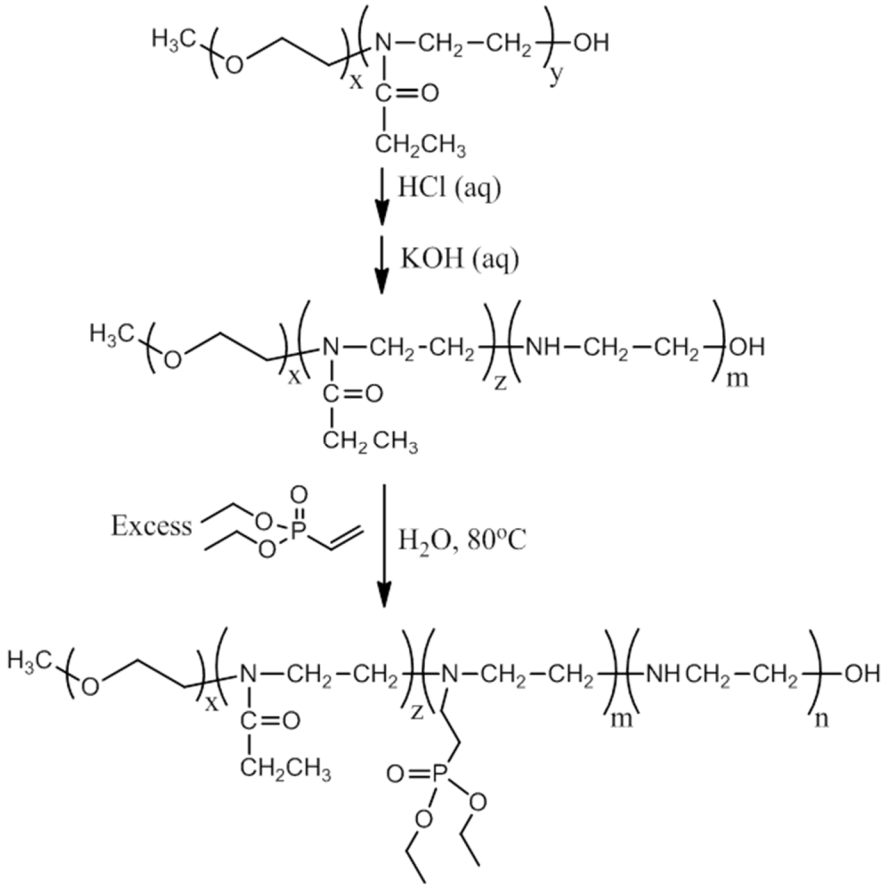


Figure 3.2 Synthesis of diethyl phosphonate derivatives of poly(ethylene oxide)-poly(2-ethyl-2-oxazoline) diblock copolymers through acid hydrolysis and Michael addition

Water likely accelerated the reactions based on literature reports [82]. The reactions were conducted with 3 equivalents of diethylvinylphosphonate per equivalent of ethyleneimine and resulted in 92-96% addition. The ethyl esters on the phosphonate pendent groups were removed by reacting the polymers with an excess of TMS-Br in anhydrous dichloromethane to form trimethylsilyl-phosphonates (**Figure 3.3**), then the silylated intermediates were converted to the corresponding phosphonic acid derivatives by methanolysis. These reactions were conducted under strict anhydrous conditions to avoid TMS-Br hydrolysis.

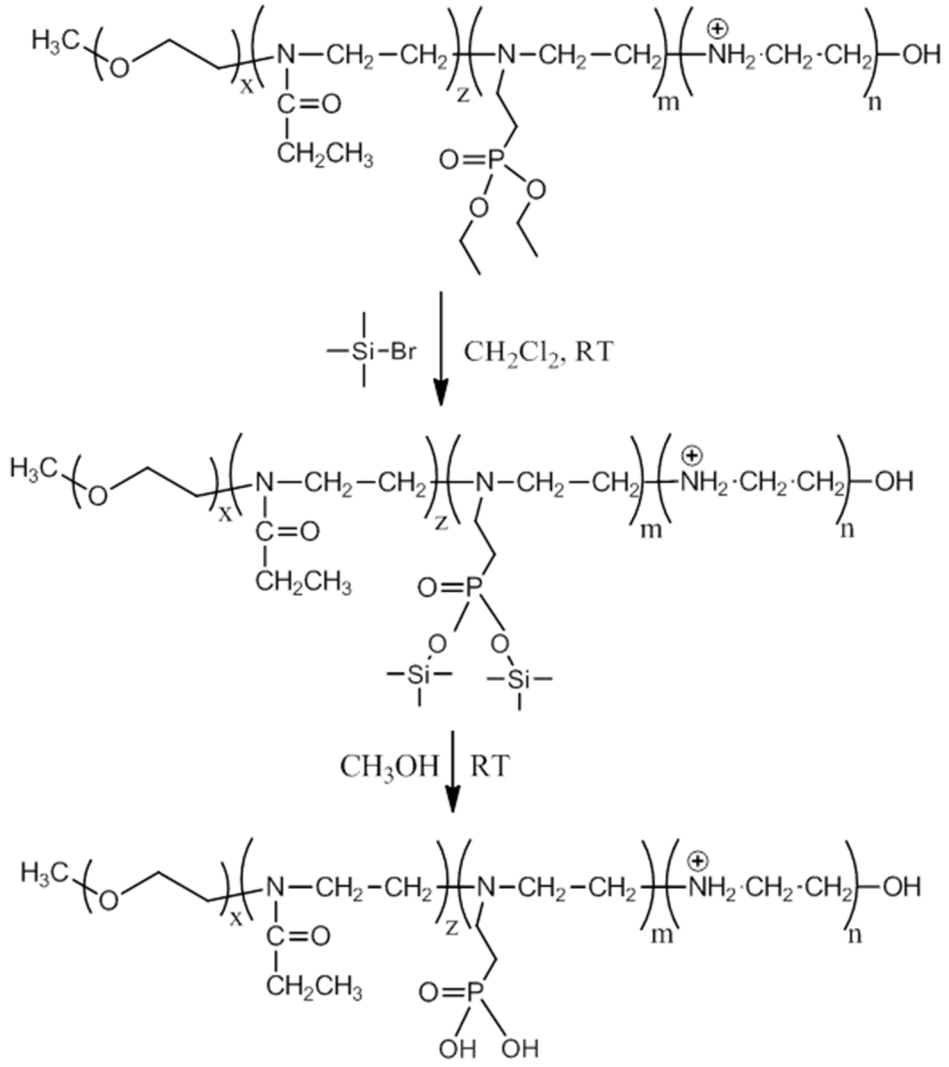


Figure 3.3 Conversion of diethyl phosphonate group to phosphonic acid

Size Exclusion Chromatography (SEC) was used to analyze the molecular weight distributions of the copolymers in NMP containing 0.05 M LiBr with multiple detectors (differential refractive index, viscosity, and multi-angle laser light scattering). The viscosity curves of these PEO-*b*-PEOX copolymers were bimodal as represented in **Figure 3.4**.

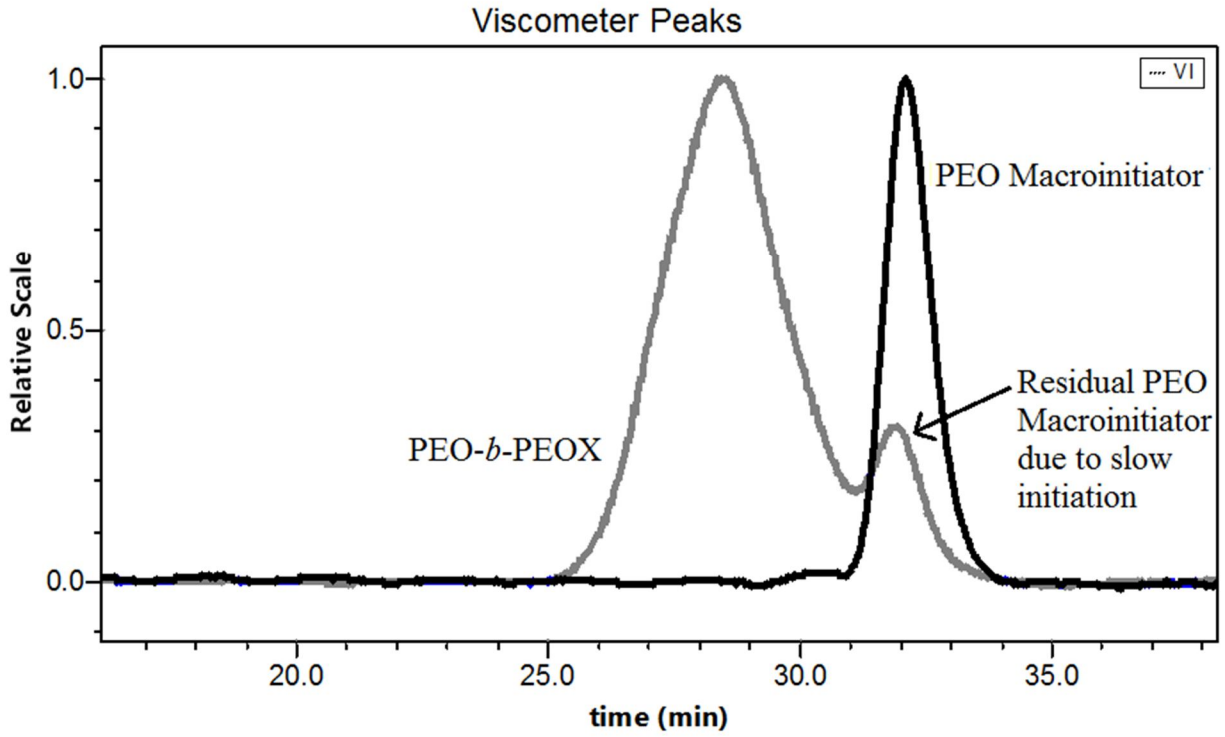


Figure 3.4 SEC Viscosity measurement of PEO-*b*-PEOX in comparison to PEO

The low molecular weight peak coincided with the SEC curve of the PEO-macroinitiator, and this signified that there was a substantial amount of PEO-macroinitiator remaining. The refractive index curves had a negligible peak in the macroinitiator region since the refractive index of the solvent and PEO were very similar. This allowed clear identification of residual macroinitiator as the source for the low molecular weight peak. It was reasoned that the rate of

initiation relative to propagation was too slow to yield well-defined block copolymers. Thus the materials were blends containing a major amount of the desired diblock copolymer combined with the residual PEO macroinitiator.

3.3.2 Copolymer Compositions Before and After Hydrolysis and Post-phosphorylation

^{1}H NMR spectra of the PEO-*b*-PEOX diblock copolymer-PEO blends provided the PEO to PEOX compositions as reported in **Table 3.1**. A representative spectrum is shown in **Figure 3.5**. Formation of the PEOX end blocks resulted in the appearance of broad peaks in the region around 3.3-3.5 ppm assigned to the methylene backbone protons of PEOX and pendent group protons at 2.2 ppm (-CH_2-) and 0.9 ppm (-CH_3). The experimental compositions determined via ^{1}H NMR were in good agreement with the targeted compositions.

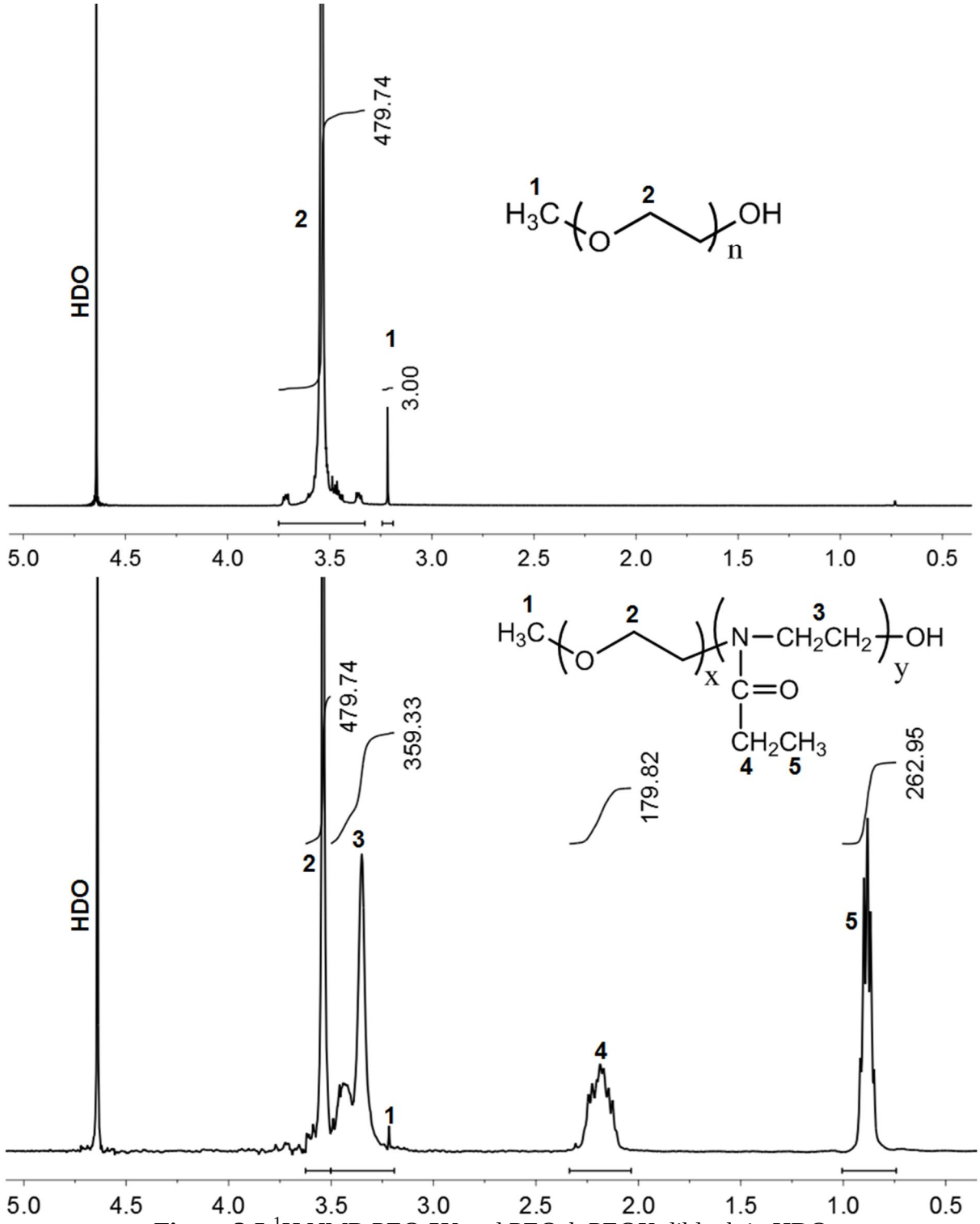


Figure 3.5 ^{1}H NMR PEO 5K and PEO-*b*-PEOX diblock in HDO

Table 3.1 Compositions of PEO to PEOX in copolymers

PEO-*b*-PEOX Copolymer	Targeted Composition (wt:wt)	Experimental PEO to PEOX Composition wt:wt
1	0.37:0.63	0.39:0.61
2	0.37:0.63	0.37:0.63
3	0.28:0.72	0.29:0.71

As the concentration of HCl in the amide hydrolysis modification was increased, the extent of hydrolysis also increased as listed in **Table 3.2**, but the exact degrees of hydrolysis that were achieved were always somewhat smaller than targeted. As discussed by Kem, this may be related to a polyelectrolyte effect where hydrolysis becomes slower as polymer units become protonated, especially at higher degrees of hydrolysis [78]. This does, however, provide a means to prepare copolymers with different degrees of hydrolysis.

Table 3.2 PEO-*b*-PEOX hydrolysis to form PEO-*b*-PEOX-*co*-PEI

Copolymer*	Targeted Hydrolysis	Experimental Hydrolysis
2A	60.0%	51.4%
3	58.8%	46.0%
2B	40.0%	27.4%

* Copolymer 2 described in Table 1 was hydrolyzed with 2 different concentrations of HCl. The third entry was derived from copolymer 3.

^{1}H NMR spectra reflected the pH sensitive character of the PEO-*b*-(PEOX-co-PEI) copolymers as shown in **Figure 3.6**.

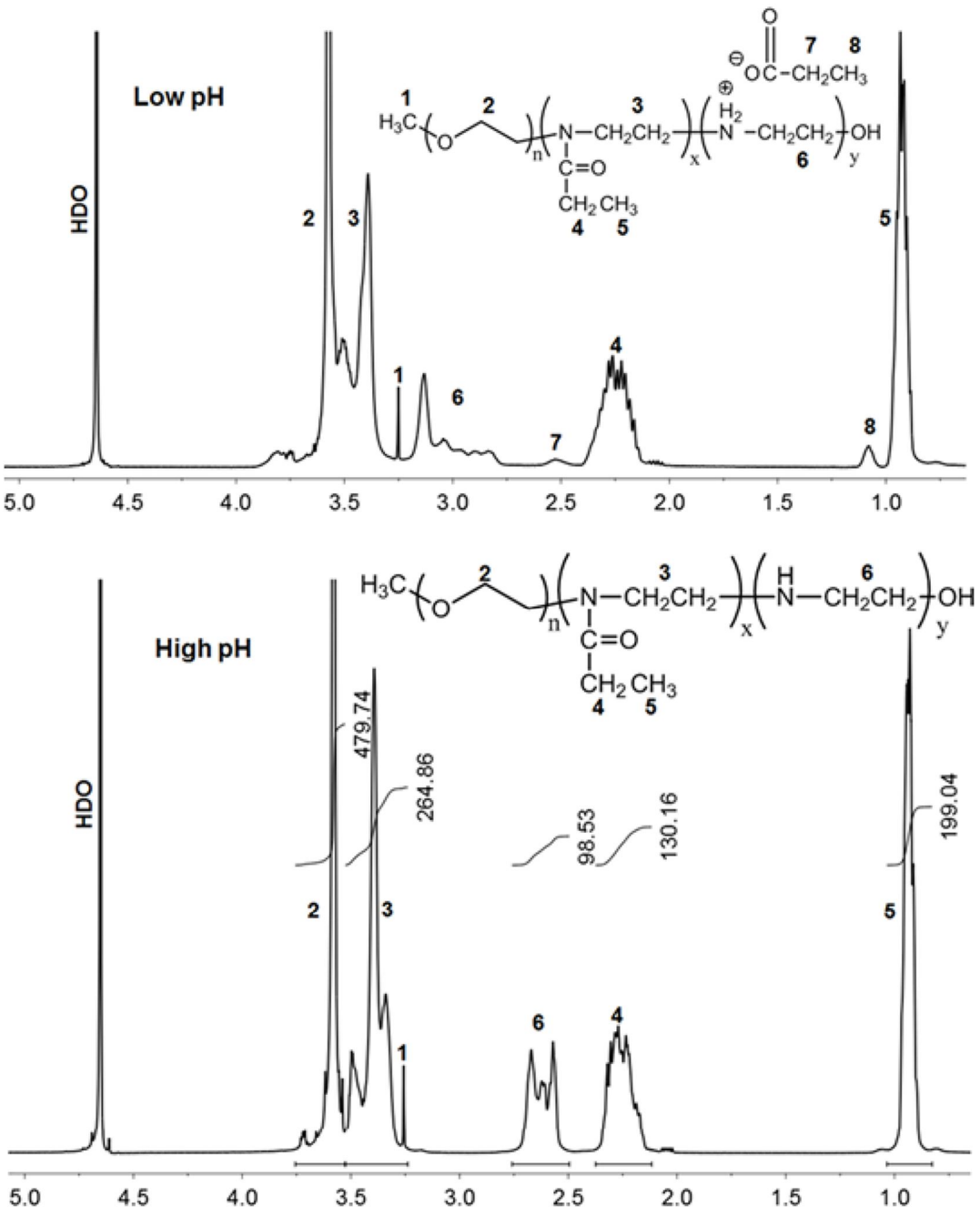


Figure 3.6 ^{1}H NMR Comparison illustrating pH influence on polymer mixture

The hydrolysis by-product after neutralization with KOH, propionate ion, was ionically bound to secondary ammonium groups on the ethyleneimine units of the backbone. The alkyl group proton resonances at 1.1 and 2.5 ppm on the associated propionate ions appeared on the ^{1}H

NMR spectra (**Figure 3.6**) when the polymers were dialyzed against deionized water. The backbone methylene protons on the secondary ammonium and 2-ethyl-2-oxazoline units were shifted downfield relative to those on the uncharged polymer. When a hydrolyzed copolymer solution was dialyzed against high pH (~9) water, resonances from the propionate ions completely disappeared (since they had been removed by dialysis) and resonances due to uncharged ethyleneimine and 2-ethyl-2-oxazoline units were shifted upfield. This demonstrated the complete removal of propionate and neutralization of ethyleneimine units to form free amines. The hydrolysis degrees of the PEOX component were determined by calculating the relative areas of proton resonances in the ethylenimine units relative to the pendent group protons of PEOX [83, 84].

A representative spectrum, as shown in **Figure 3.7**, denotes the difference between the bare PEI units with phosphorylated. The appearance of the ethyl peaks at 1.2 and 4.0 shows that the Michael addition was successful.

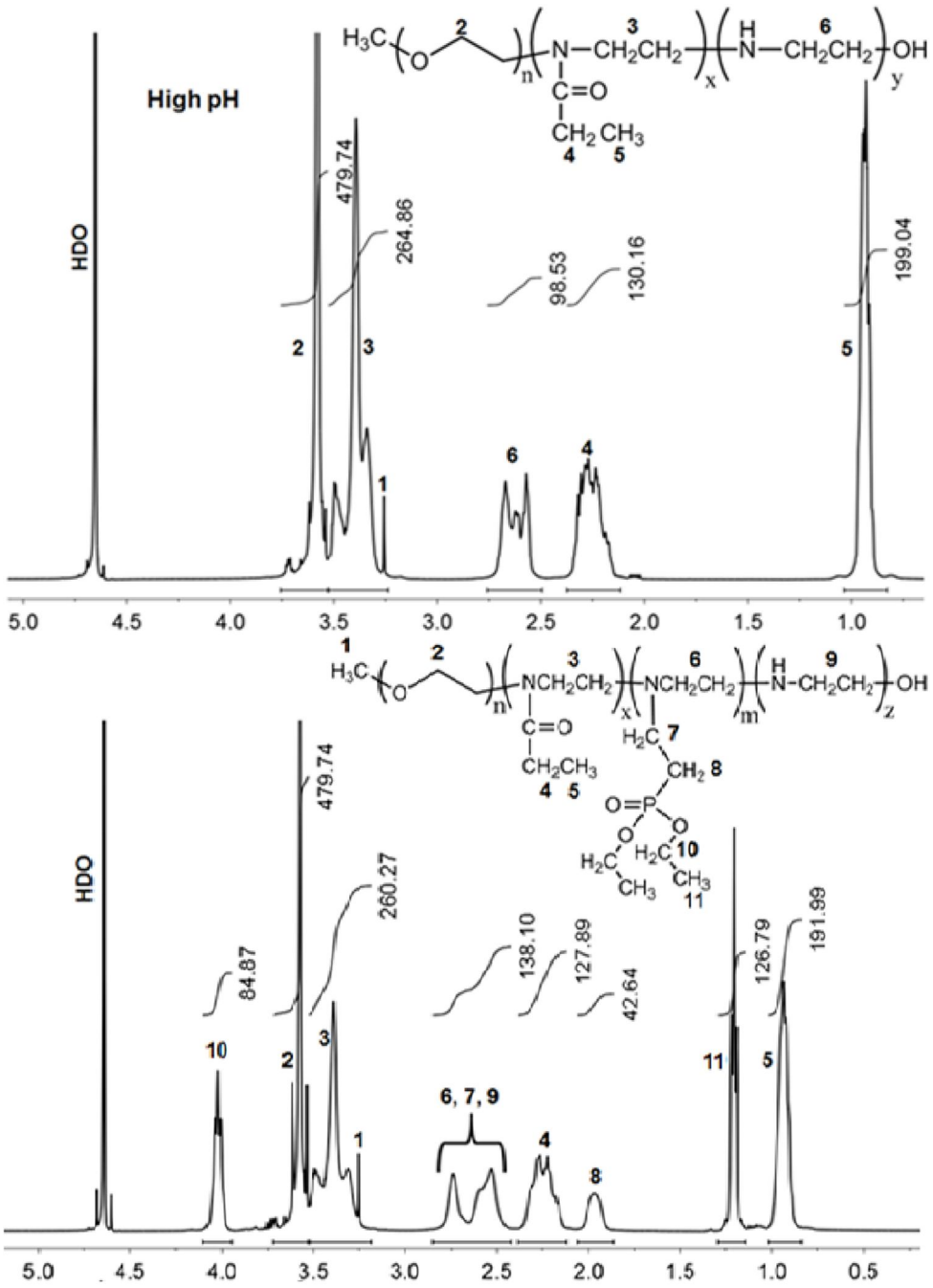


Figure 3.7 ^{1}H NMR **of** PEO-*b*-(PEOX-*co*-PEI) phosphorylation

The ^{1}H NMR spectra of the PEO-*b*-(PEOX-*co*-PEI-*co*-phosphonate) copolymers were used to analyze the relative amounts of oxyethylene, amide, ethyleneimine, and diethylphosphonatoethyl pendent groups (**Table 3.3**). **Figure 3.8** (top) shows three different

characteristic signals for the diethylphosphonatoethyl groups: (1) methylene protons adjacent to the phosphorus at 1.9 ppm, and (2) ethyl ester group resonances at 4.0 for -CH_2- and (3) 1.2 ppm for -CH_3 groups.

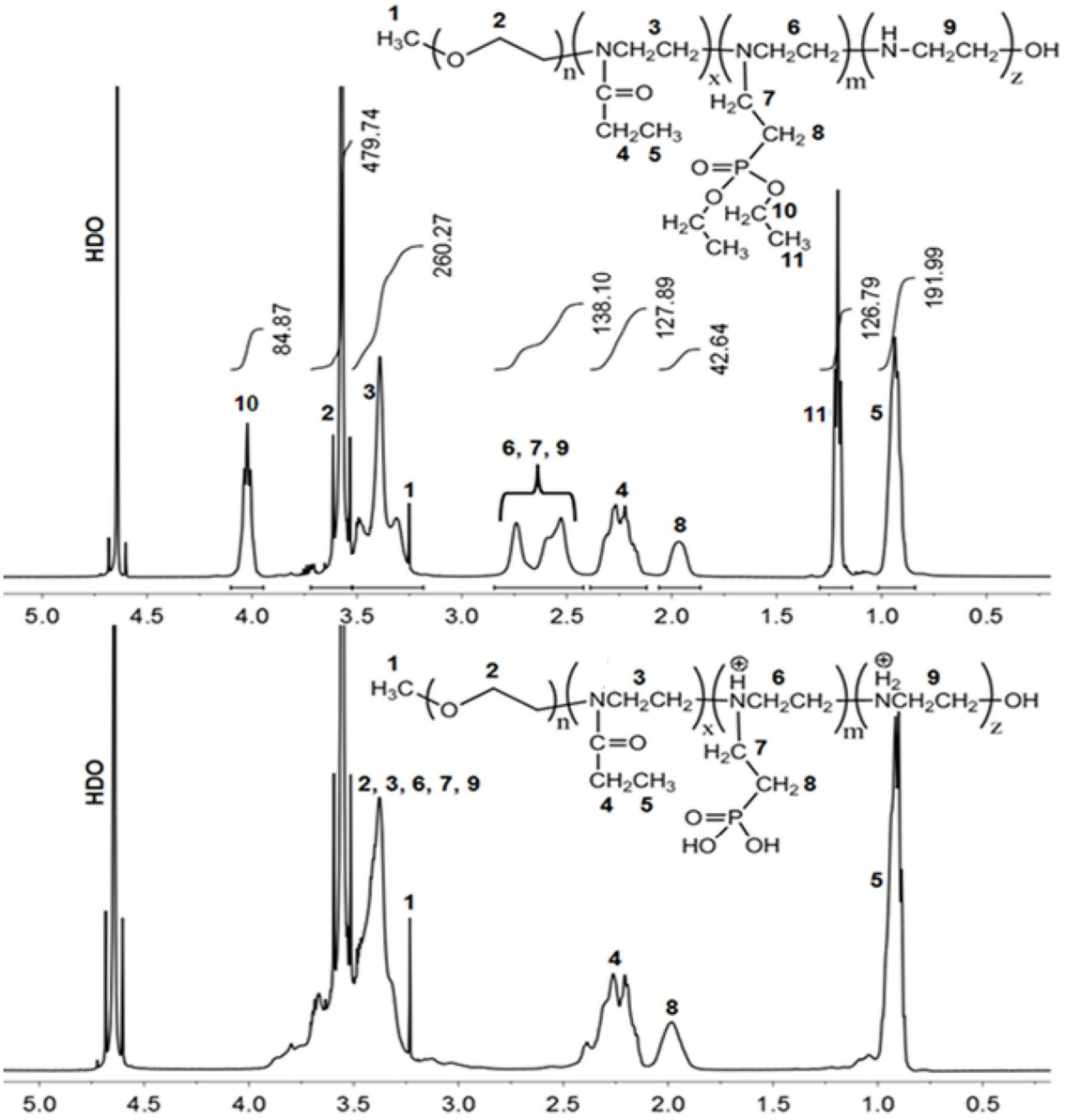


Figure 3.8 ^{1}H NMR of diethyl phosphonate conversion to phosphonic acid

The resonances at 2.4-2.8 ppm represent a combination of the methylene adjacent to nitrogen and the backbone methylenes in the phosphorus-containing units (total of 6 protons per

unit), and also the backbone methylenes in residual ethyleneimine units (4 protons per unit). The relative amounts of the phosphorus-containing units and ethyleneimine were calculated by subtracting the resonance integral at 1.2 ppm from the total integral at 2.4-2.8 ppm. The remaining part of the integral at 2.4-2.8 ppm was then representative of the small residual amount of ethyleneimine units. The peak integral at 0.9 ppm due to the methyl groups on the amides was utilized to calculate the relative amounts of those units. The resonances due to the ethyleneoxy and backbone amide units overlap, so the relative number of ethyleneoxy units could only be estimated by subtracting the appropriate component due to the amides from the total resonance at ~3.2-3.7 ppm.

Table 3.3 Compositions of PEO-*b*-PEOX-*co*-PEI-*co*-phosphonate

Copolymer	Ethyleneoxy	Amides	Phosphorus-containing units	Ethyleneimine
2A	120	42	42	2
2B	129	71	23	2
3	119	64	53	2

The conversion to the corresponding phosphonic acid was demonstrated by the disappearance of the diethylphosphonatoethyl signals at 1.2 ($-CH_3$) and 4.0 ($-CH_2$) ppm as shown in **Figure 3.8** (bottom). In addition, the methylene group proton resonances on the PEOX backbone as well as the methylene protons that have similar chemical environments (labeled **6**, **7** and **9**) showed a significant downfield shift that can be explained by the positive charge on the ammonium ions formed after phosphonic acid modification. The efficiencies of the Michael addition were high ranging between 92-96% phosphorylation of ethyleneimine units.

3.3.3 Influence of Modification on Thermal Stability

Thermogravimetric analyses (TGA) of PEO homopolymers, PEO-*b*-PEOX copolymers and their derivatives were conducted under a nitrogen atmosphere (**Figure 3.9**).

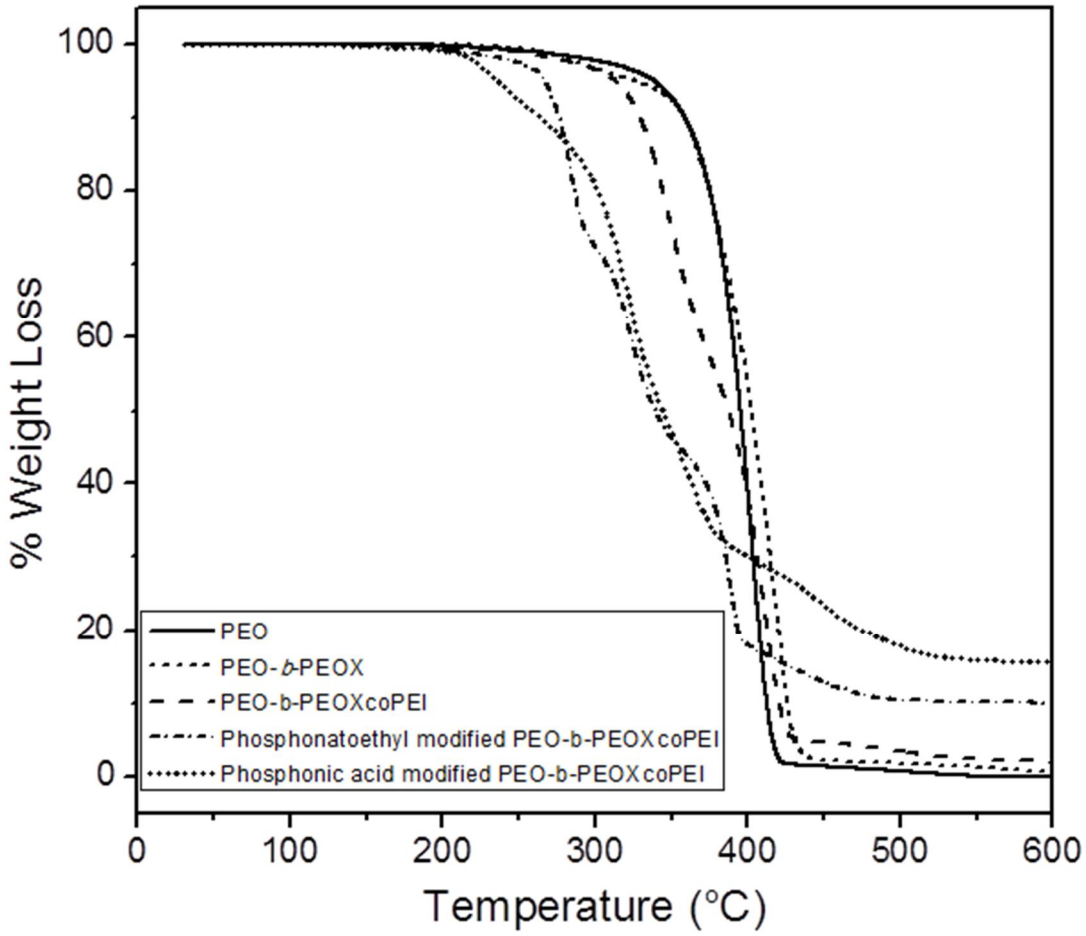


Figure 3.9 TGA graph depicting diblock series from PEO-b-PEOX to phosphonic acid modified final form

The thermograms show the weight loss profiles of each polymer as they were converted from the PEO to the PEO-*b*-(PEOX-*co*-PEI-*co*-phosphonic acid). TGA temperature scans revealed that both PEO and PEO-*b*-PEOX and the PEO-*b*-(PEOX-*co*-PEI) showed similar degradation behavior and did not lose a significant amount of weight up to 300-330 °C, and then they completely decomposed without the formation of char. By contrast, the phosphorus-

containing copolymers began weight-loss somewhat earlier (220-260 °C) but formed significant levels of char (10-17%). It has been reported that at elevated temperatures, dialkyl phosphonates pyrolyze to monoalkyl derivatives, and then condense to form P-O-P crosslinks that result in char [85-87]. Consistent with this premise, the amount of char after these tests corresponds to the weight percentages of the pendent phosphonates in these materials.

CHAPTER 4: Conclusions and Future Works

The synthetic strategies that have been developed led to PEO-*b*-PEOX diblock copolymers utilizing cationic ring-opening polymerization. However, according to SEC data, using tosylate initiators result in the copolymer being blended with residual PEO-macroinitiator. It was surmised that the relatively slow initiation of PEOX relative to propagation is the cause. However, the post-modification reactions to form ammonium phosphonate zwitterions were facile and controllable. Based on ^{1}H NMR, it was concluded that novel phosphonic acid-functionalized random copolymers were successfully synthesized. These new materials with strongly adhesive phosphonate zwitterions may provide strong complexes with many metals and metal oxides. Manganese metals and magnetite are some of metal complexes that are currently the focus of such complexation reaction. The PEO component in the copolymers may also provide steric dispersion stability in water for micellar complexes with metal oxide nanoparticles in their cores.

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