SYNTHESIS OF AMPHIPHILIC BLOCK COPOLYMERS FOR USE IN BIOMEDICAL APPLICATIONS

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ABSTRACT

The research presented in this thesis focuses on the synthesis of three amphiphilic block copolymer systems containing poly(ethylene oxide) (PEO) blocks. The polymer systems were developed for use in biomedical applications. The first of these is a series of poly(ethylene oxide-*b*-oxazoline) (PEO-*b*-POX) diblock copolymers for use in the progress towards novel non-viral gene transfer vectors. Poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) (PEO-*b*-PEOX) and poly(ethylene oxide-*b*-2-methyl-2-oxazoline) (PEO-*b*-PMOX) were investigated. The PEOX block was hydrolyzed with acid to form linear polyethylenimine (L-PEI). The polycation L-PEI is well known for its DNA binding efficiency but the water solubility of the resulting DNA/polymer complex is limited. Addition of a PEO block is directed towards the formation of a water dispersible DNA/copolymer complex. Dynamic light scattering of the PEO-*b*-PEOX and PEO-*b*-PEI block copolymers indicated that both systems existed as single chains in aqueous solution at pH 7.

PEO copolymers also play a significant role in the formation of magnetic magnetite nanoparticles, which are dispersible in water at biological pH (pH =7). There is significant interest in the design of magnetic nanoparticle fluids for biomedical applications including magnetic field-directed drug delivery, magnetic cell separations, and blood purification. For use *in vivo*, the magnetite nanoparticles must be coated with biocompatible materials. Such polymers render the nanoparticles dispersible in water. Harris[1] *et al*. synthesized PEO based,

polyurethane triblocks with pendant carboxylic acid groups for use in formation of stable aqueous magnetic fluids.

Building from this work, two polyurethane and polyurethaneurea systems were synthesized with 1300 g/mol PEOX and 2500 g/mol and PEOX2070 g/mol poly(ethylene oxide-*co*-propylene oxide) tailblocks, respectively. The PEO/PPO random copolymer contained about 25 weight percent PPO, and this disrupted the capacity of the PEO to crystallize. The PEOX based urethane triblocks were synthesized through reacting the tailblocks with the monomers for the center block whereas the PEO/PPO based polyurethaneurea was synthesized through forming the central urethane block with pendant acid groups first and then terminating the copolymer with the monofunctional copolymer. Terminal amine groups on the PEO/PPO tailblock afforded a triblock linked with two urea groups. The new polyurethanes with the PEOX tailblocks and the new polyurethaneurea with the PEO/PPO tailblocks could be utilized to efficiently stabilize magnetite nanoparticles in water.

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INTRODUCTION

In recent decades, the demand for amphiphilic block copolymers for use in magnetic and nonmagnetic targeted drug delivery and gene therapy has increased tremendously. Biocompatible amphiphilic block copolymers have uses in encapsulation of drugs, controlled release of low molecular weight drugs, nanogel carriers, and as gene delivery vectors.[2, 3] As such polymers are comprised of hydrophilic and hydrophobic segments, they can both bind to a desired biological moiety or magnetic particle and afford water affinity to the formed complex.[4]

Poly(ethylene oxide) (PEO) is prevalently used as the hydrophilic portion of an amphiphilic block copolymer because of its high water affinity and biocompatibility.[5, 6] When incorporated into a system, PEO provides the system with its hydrophilicity, compatibility in blood, and resistance towards recognition by the mononuclear phagocyte system.[7] These properties allow a designed complex to be placed within the human body without unfavorable reactions or immune response and can lead to longer circulation times in the blood stream.[3, 6, 7]

Further advances in polymeric materials helps to sophisticate the design of controlled drug delivery systems. Controlled drug delivery aims to regulate the amount of drug a patient is exposed to as well as target a specific site. By doing so, this prevents overdosage, exposure to unaffected areas, and reduces time of drug dispensation, all of which can increase patient health and comfort. Polymeric systems valuable to controlled drug delivery include hydrogels, degradable microspheres, degradable capsules, and stabilized magnetic nanoparticles.

A subset within controlled drug delivery is the design of delivery vehicles for genetic material to treat genetic disorders acquired at birth. There has been recent impetus towards the movement away from viral vectors and towards polymeric vehicles because of their increased DNA binding efficacy.[2, 8] Cationic polymers are of great interest because they easily interact

with the negatively charged phosphate groups along the DNA backbone. In particular, linear polyethylenimine (L-PEI) has received increased attention due to its improved DNA transfection efficiency *in vivo* and *in vitro*.[4, 6] Unfortunately, the interpolyelectrolyte complex formed with DNA has limited water solubility as do many polycation/DNA complexes.

Another avenue of interest for amphiphilic block copolymers for use in drug delivery is colloidal stabilization of magnetic nanoparticles.[9, 10] Magnetic materials could have a profound impact upon modern medicine being utilized for field directed targeted drug delivery, magnetic cell separation, blood purification, and in magnetic hyperthermia as a form of cancer therapy. In order to be utilized in such applications, it is required that the nanoparticles be sheathed with a non-toxic material to avoid elicitation of autoimmune responses by the body. Furthermore, the coating should provide the magnetic material with the ability to sterically prevent aggregation as well as afford water dispersibility for navigation in the bloodstream.

In Chapter 4, the synthesis of a PEO based triblock copolymer with a center block containing carboxylic acids is detailed. This block copolymer builds upon the triblocks developed by Harris, et al.[1] The details for synthesis of both the polymer and the formation of a stable, aqueous magnetic fluid of polymer coated magnetite nanoparticles are presented along with characterization of the magnetite/polymer complex. These particles are of great interest for field directed drug delivery.

Chapter 5 discusses a summary of work and suggested future work.

CHAPTER 1 LITERATURE REVIEW

1.1 Controlled Drug Delivery

The administration of drugs to increase fitness and health has always intrigued humans. Early written records have many examples of drug dispensation in the early Greek, Roman, Chinese, Arabian, and South American societies.[3] From these times and well into the 20th century, drug discovery was regarded as coincidental. For instance, penicillin was discovered by an accidental contamination of a bacterial plate.[4] Primitive drug administration involved ingestion or topical application. Today, the approach to drug discovery and administration has become more sophisticated and is multidisciplinary.[6] Advancements in biotechnology, molecular biology, pharmaceutical technology, physics, and chemistry, along with the past accumulated knowledge have greatly improved the development of novel drugs and delivery methods.[3]

Conventional methods of drug administration use single, transient dosage applications which must be repeated to maintain a drug plasma level above the minimum effective concentration for the desired pharmacological response.[7] Inconsistencies in the drug plasma level can result in inappropriate or reduced drug response as well as have detrimental effects on the human body.[7] A novel and intriguing solution is the development of systems with the goal of controlled release of therapeutic agents, including both small organic molecules and macromolecules.[3] Controlled drug delivery focuses on optimizing treatments by delivering an exact therapeutic dose for a finite time.[8, 11-14] This method includes regulating drug exposure duration and localization of drug administration.[3, 7]

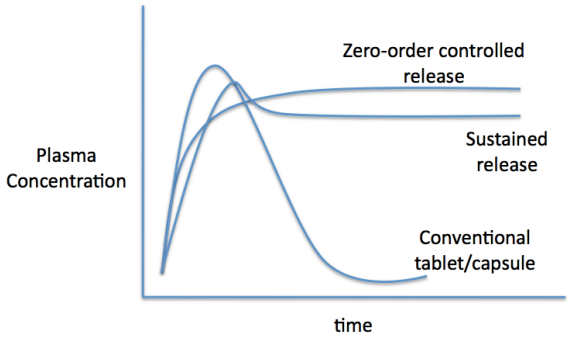


Figure 1.1: The concentration of a drug as a function of time varies with the administration method. Adapted from Li, *et al*, pg 15.[7]

Controlled drug delivery allows for consistency in the plasma drug concentration over time. Figure 2.1. provides exemplary comparisons of drug plasma concentrations and their maintenance. The time periods for dosage depend on the nature of the systems. The primary goal in drug administration is to provide the patient with the appropriate plasma drug concentration with reduced incidence of side effects. Side effects of drug administration are defined as undesired outcomes and include both hazardous overdosage of a drug as well as an inappropriate dosage amount resulting in an incomplete patient response. Conventional methodology of achieving a steady state for drug plasma levels involves a dosage regime determined by the pharmacokinetics of the drug.[15] Most often, in current administration systems, repeated short-duration dosages which often exceed the maximum desired level are used.[7] In addition to the risks of high dosages, broad fluctuations below the effective concentration can have undesirable side effects.[7] Thus, maintenance of a drug concentration between these two boundaries is of great importance to both drug efficacy and patient safety[4, 7] (fig. 2.2.).

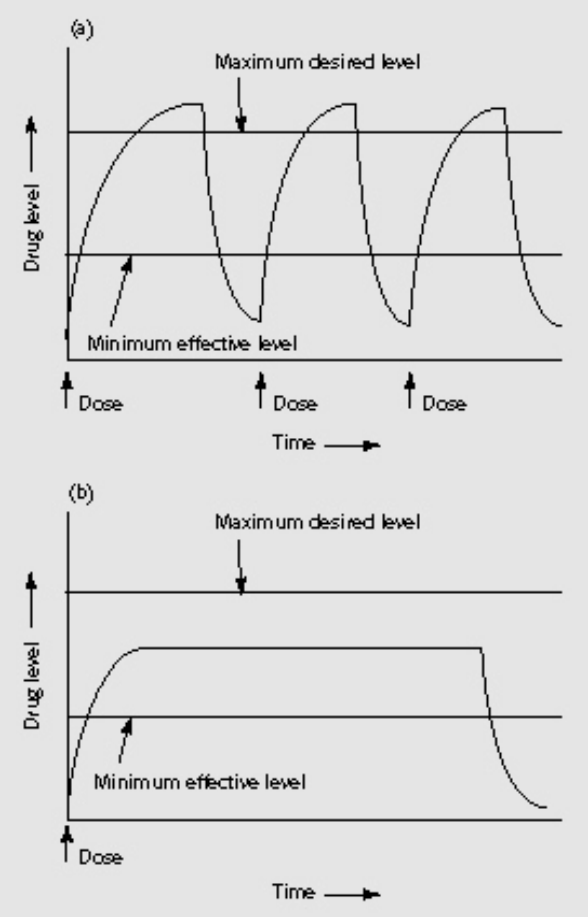


Figure 1.2: Demonstration of drug levels in a system depending on the administration method: (a) traditional delivery and (b) controlled drug delivery. Adapted from Brandon-Peppas, pg 3.[4]

With the advent of novel polymeric materials, the design of controlled drug delivery systems has become increasingly sophisticated. This includes modifications of systems to allow for targeting and specific site recognition.[6] With such amendments to delivery systems, the treatment of individual ailments without exposure to the rest of the body is possible. Advanced controlled drug delivery systems could be of great use in cancer chemotherapy.[16] The ability to target and locally treat only the tumor could potentially decrease administration time and the amount of chemicals used.[16] Effectively, this would reduce detrimental effects to the healthy tissues of a person with cancer and most likely increase the patient's comfort.

The past few decades in the drug delivery field have been marked by the successful development of a small number of polymeric controlled delivery systems, a selection of which are presented in Table 2.1.

Table 1.1 Examples of polymer-based controlled drug delivery systems. Adapted from Saltzman, pg 43.[3]

Drug/**Polymer**	Trade Name	Therapy
Estradiol/**poly[ethylene-*co*-(vinyl acetate)]**	Estraderm®	Estrogen replacement therapy
BCNU/**poly[carboxylphenoxypropane-*co*-(sebacic acid)]**	Gliadel®	Recurrent gliboblastoma multiforme
Leuprolide acetate/**poly(DL-lactide-*co*-glycolide)**	Lupron Depot®	Endometriosis
Levongestrel/**polysiloxane elastomer**	Norplant®	Implantable Contraceptive
Nitroglycerin/**poly[ethylene-*co*-(vinyl acetate)]**	Transderm-Nitro®	Prevention of angina
Pilocarpine/**poly[ethylene-*co*-(vinyl acetate)]**	Ocusert®	Glaucoma therapy
Progesterone/**poly[ethylene-*co*-(vinyl acetate)]**	Progestasert®	Contraceptive intra-uterine device (IUD)

The predominant approach to advancing the field of controlled drug delivery is to combine biologically inert polymers directly with the active agents (*i.e.* drugs, proteins, oligonucleotides).[3] The system should be tailored for its intended application (*i.e.* controlled rate of release, controlled duration of release, targeting capabilities).[3] Controlled drug delivery systems encompass many types of systems, including hydrogels, responsive systems, transdermal reservoirs, and degradable matrix delivery systems.[4, 6, 7]

Another aspect important to controlled drug delivery is the ability to target the therapeutic agent or its vehicle to a specific location in the body.[6] This drug targeting focuses on direct administration at the desired site without interaction with non-tissue materials.[6] There are three main approaches to drug targeting:[6]

(1) Drugs that only become active at the desired sites

(2) Tailoring drugs or drug-containing vehicles with functional groups that are specifically recognized by biological receptors at the destination

(3) Selective direction of the therapeutic agents to the target while preventing its recognition by other tissues and non-tissue

This research project has been focused on the synthesis of block copolymers that would be useful for the latter two methods.

Current designs of controlled and directed drug delivery include the use of microcapsules, liposomes, microspheres, and other microparticles.[3, 6] These systems must meet the requirements for an ideal macromolecular drug carrier:[6]

(1) Adequate capacity to carry the desired concentration of the drug

(2) Retention of water solubility when the drug is bound to the system.

(3) Hydrodynamic sizes large enough to avoid glomerular filtration but small enough to reach the desired cells

(4) The carrier should not be "captured" by adsorptive pinocytosis

(5) The carrier should have the desired rate of biodegradation in the desired extracellular or intracellular compartments

(6) The materials should be non-toxic and non-immunogenic

Another area of great intrigue is the use of magnetic materials to guide a drug to, or release a drug, at the targeted location.

Magnetic nanoparticles have great appeal as components of targeted, controlled drug delivery vehicles.[16] It may be desirable to attach a drug, gene, hormone, etc. to a polymer which is directly bound to the surface of the particle. The particle can then be directed to the desired site with a system of external magnetic fields and gradients. The therapeutic agent could also be

incorporated into a biodegradable polymer matrix which also contains a magnetic metal or metal oxide.[16, 17]

1.2 Polymers in Biotechnology for Drug Delivery

1.2.1 Biocompatible/Biodegradable Polymers

One important property of a polymer that is considered for medicinal use is biocompatibility. Biocompatibility has been defined to be "the ability of a material to perform with an appropriate host response in a specific situation."[18] Achieving biocompatibility in synthetic materials requires well understood orchestration of biology, chemistry, physiology, and physics in the material's design. The criteria for designing such polymers require them to be both non-toxic and immunologically compatible to host tissues[18] while maintaining processability. Moreover, they must be sterilizable, and their degradation rates should be controllable under the biological conditions specific to the desired application.[19]

The rate of biodegradability is of utmost importance for tailoring polymers for an individual application. For instance, Marra *et al.* synthesized a bone scaffold of a poly(caprolactone)-poly(D,L-lactic-*co*-glycolic acid) polymer blend which was designed with a degradation rate equivalent to the growth rate of bone tissue.[20] The polymer blend exhibited beneficial mechanical properties, such as good load-bearing properties and the material could be easily shaped into specific designs, while remaining bio-resorbable. Alternatively, a faster degrading polymer matrix would be desired for a controlled drug delivery vehicle which would release a drug rapidly upon reaching a specified destination.[20] For some applications, it is also important that the polymer perform other functions during the degradation process. In this respect, a

controlled drug delivery system could be designed to deliver drugs shortly after release as in a targeted vehicle or in a prolonged manner such as in a patch or implant.

Polymer biodegradation relies ultimately on chemical characteristics. These properties can also dictate their specific uses in drug delivery systems. The most influential property is the polymer's chemical structure, especially when the structure is tailored to have hydrolytically labile bonds.[6, 20] The relative stabilities of selected organic groups against hydrolysis is shown in Figure 2.3.

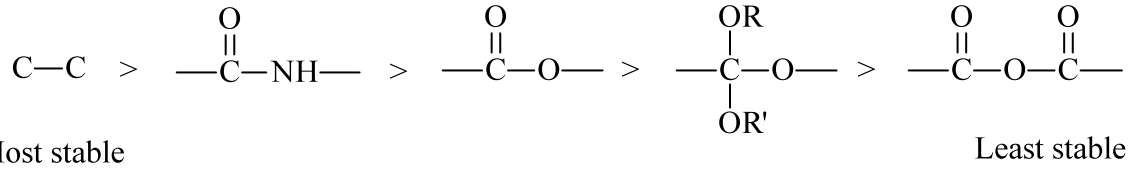


Figure 1.3: The hydrolytic stability of common organic groups.

Linear polymers without crosslinking typically exhibit the highest degree of biodegradability, as crosslinking often results in a reduction of degradation rate and matrix permeability.[6, 20]

1.2.2 Hydrophobic Biocompatible Polymers

Hydrophobic polymers typically do not readily degrade *in vivo* and account for the majority of synthetic materials used in clinical settings.[3] Examples include polysiloxanes, poly(methyl methacrylate), polyurethanes, and poly[ethylene-*co*-(vinyl acetate)] (EVAc).[6, 7] These systems are employed for applications requiring little change during the duration of use and limited induction of inflammatory response.[6, 7] Selected applications of these polymers include implants, dentures, artificial heart valves, and catheters.[6, 7]

1.2.2.1 Polysiloxanes

Polysiloxanes are partially inorganic polymers that are comprised of Si-O backbone units. These units create a physically unique chain that differs from an organic backbone by the larger Si-O-Si bond angle of 143° and the longer Si-O bond length of 1.63 Å. These distinctions result in exceptionally low glass transition temperatures and low melting points.[21] Polysiloxanes exhibit high oxygen permeability and high chain flexibility. They are hydrophobic and subsequently resistant to hydrolytic degradation.[3] The degree of hydrophobicity can be altered by changing the organic substituents on the silicon atom. Through modification, polysiloxane materials have found exemplary applications as sealants, lubricating oils, and biological implants. Polysiloxanes can also be customized by altering the molecular weight and degree of crosslinking. A generic polysiloxane repeat unit is provided in Figure 2.4.

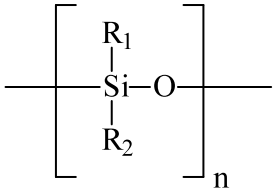


Figure 1.4: A repeat unit of a polysiloxane.

Polysiloxanes have great potential for use as nontoxic, hydrophobic drug delivery materials. Folkman and Long first made the observation that compounds of low molecular weight were able to diffuse, at a controlled rate, through walls of crosslinked polydimethylsiloxane (silicone) tubing.[3] Since this first development, numerous biological applications for polysiloxanes have been investigated. Stevenson *et al*. coated cobalt nanoparticles with polysiloxane triblock materials which afforded dispersibility in a polydimethylsiloxane (PDMS) carrier fluid. The protruding PDMS chains were not wetted by hydrophilic fluids, such as the vitreous humor of

the eye, and it was hypothesized that the magnetic materials would be of interest for internal treatment of retinal detachment.[22]

1.2.2.2 Polyurethanes

Polyurethanes are another class of polymers which can be designed to be either hydrophobic or hydrophilic, and these have been utilized in medical applications. Polyurethanes are normally synthesized by reacting isocyanates and alcohols to generate repeating carbamate (urethane) linkages along the chain (fig. 2.5.).[23] There is great variability in the properties of polyurethanes, depending on the choice of monomers. Additionally, the degree of biocompatibility of a polyurethane can depend on the polymer purity, and any low molecular weight reagents or catalyst residues can result in toxicity.[3]

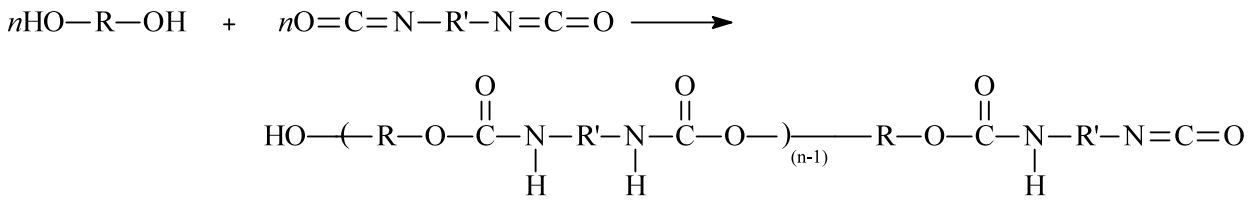


Figure 1.5: The synthesis of a generic polyurethane.

An intriguing aspect of polyurethanes is that they can be synthesized as elastomeric block copolymers with "soft" and "hard" segments.[3, 23] Formation of polyurethane elastomers is often carried out by a two step sequence. The first step involves reaction between an excess of diisocyanate with a hydroxyl terminated polyether or polyester. The resultant prepolymer is then reacted with a diol or diamine chain extender. The resulting copolymer has both urethane and urea linkages if it is chain extended with a diamine.[3, 23, 24] The morphologies of polyurethane

elastomers are often characterized by the association of hard segments into crystalline or glassy regions with the soft segments forming a continuous phase around the discrete regions. Biomer® was a commercial segmented polyurethaneurea marketed by Johnson and Johnson which was used in blood-contacting applications. It had excellent mechanical properties including little to no hysteresis after repeated stretching and was nonthrombogenic.[25] Similar polyurethaneureas are now marketed by the Polymer Technology Group.[26]

A similar process can be employed to form polyurethane block copolymers with hydrophilic and hydrophobic domains. Such amphiphilic polymers will be discussed in greater detail in the following chapters. Biomedical applications of polyurethanes include increasing the stability and biocompatibility of implants[27] and drug coatings that are less toxic and have cell-specific activity.[27]

1.2.2.3 Poly[ethylene-*co*-(vinyl acetate)]s

Poly[ethylene-*co*-(vinyl acetate)]s are remarkably biocompatible and have also been used in the design of biomaterials and drug delivery systems. EVAc statistical copolymers can be synthesized via free radical copolymerization. The materials employed for biomedical applications are usually predominately polyethylene (≈60% of total polymer).[6] The common structure of EVAc is presented in Figure 2.6. EVAc has a low degree of crystallinity which depends on composition (5-20%) and is extremely hydrophobic with less than 0.8% swelling in aqueous solutions.[6] EVAc copolymers are of interest for controlled drug delivery and comprise one of the principally studied classes of materials for such uses.[6] The FDA has approved EVAc for use in humans for implanted and topical devices.[6]

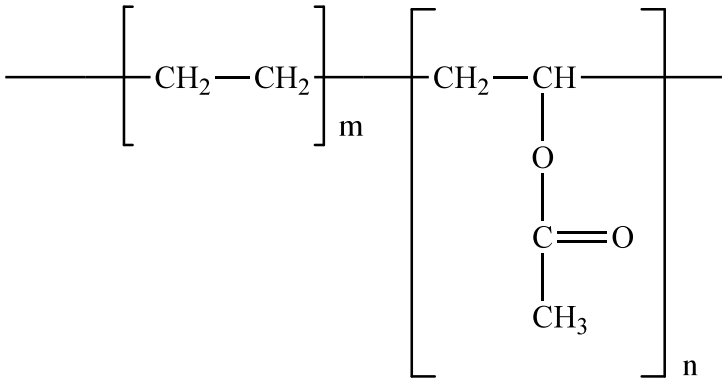


Figure 1.6: The general structure for poly[ethylene-*co*-(vinyl acetate)]

1.2.3 Biodegradable Biocompatible Polymers

1.2.3.1 Polyesters

The most prevalent biodegradable synthetic materials are polylactides (PLA), polyglycolides (PGA), and copolymers of lactic acid and glycolic acid (PLGA).[3] These polyesters are favored for biomaterials because of their desirable characteristics:[3] (1) they degrade into naturally occurring metabolites (glycolic acid and lactic acid), (2) their *in vivo* interactions have been well documented as a result of decades of use as sutures, (3) their degradation requires only water, and (4) a variety of useful materials with desirable properties can be developed from different compositions and sequences of the two monomers.[3]

PLA, PGA, and PLGA can be synthesized by the direct condensation of lactic acid and glycolic acid to yield low molecular weight oligomers. To generate high molecular weight products, the polyesters are synthesized by the ring-opening polymerization of the cyclic diesters glycolide and lactide (fig. 2.7.). Enantiomeric and diastereomeric stereochemical isomers for the

lactide monomer are important since these structures are retained in the polymers and greatly affect properties (fig. 2.8.). The choice of isomers influences the crystallinity of the final polymer. Stereoregular PLA or PDA with a high degree of crystallinity is synthesized from either the L,L or D,D enantiomers. By contrast, amorphous PDLA is produced from either the L,D stereoisomer or from stereoisomeric mixtures.

The degradation of PLA and PGA homopolymers is slow and can require up to a few months in the human body. However, their copolymers provide more rapid degradation. By copolymerizing lactic and glycolic acids (or the cyclic diesters), the crystallinity of the homopolymers is disrupted. The amount of crystallinity can be tailored by reacting different amounts of each monomer. Amorphous PLGAs are desirable for rapid drug delivery systems while the more crystalline polymers are appropriate for applications requiring increased physical strength.

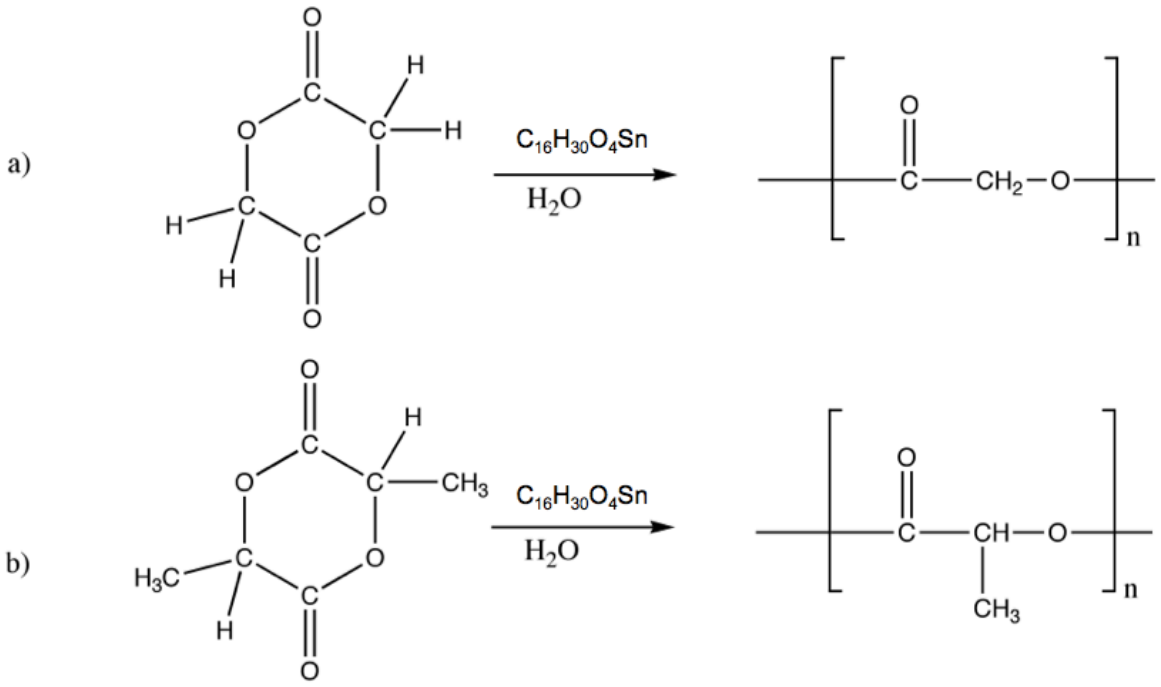


Figure 1.7: The synthesis of (a) polyglycolic acid and (b) polylactic acid via the ring opening polymerization of cyclic diesters.

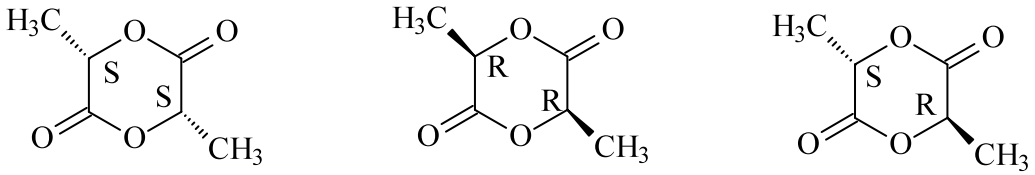


Figure 1.8: The stereochemistry of lactide monomers.

In an approach to design tissue specific drug vehicles, Maruyama *et al.* synthesized polymeric nanoparticles of poly(D,L-lactic acid) and poly(L-lysine-*graft*-polysaccharide) with narrow size distributions.[28] The grafted polysaccharide was a dextran, and it was found that the extent of grafting directly influenced the nanoparticle size and nanoparticle stability in

hydrophilic media. Additionally, the presence of dextran would theoretically facilitate the nucleic acid targeting capacity of the nanoparticle. The authors also demonstrated that incorporating the graft polysaccharide allowed for the PLA nanoparticles to encapsulate DNA.

Another biodegradable polyester of great interest for drug delivery is poly(ε-caprolactone) (fig. 2.9.). This polyester can be synthesized via numerous mechanisms, including cationic, anionic and coordination polymerization.[4, 6] The degradation rate of poly(ε-caprolactone) homopolymers is much slower than PGA and PLA homopolymers.[4] The degradation rate can be increased by copolymerization of ε-caprolactone with more rapidly degrading materials.[6]

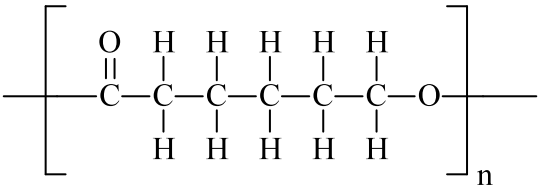


Figure 1.9: Poly(ε-caprolactone)

Drug release from a polymeric system is directly controlled by the rate of diffusion across the polymeric matrix.[29] The permeability of a polymer matrix is influenced by the state of the amorphous phase and materials with T_g's well below the use temperature tend to exhibit higher permeablities.[29] Poly(ε-caprolactone) offers a rapid initial delivery (at 37 °C) as a result of its glass transition temperature of ≈-60°C being well below the temperature of the body.[29] The combination of a slow degradation rate and high permeability make poly(ε-caprolactone) well suited for carriers of weak drugs requiring prolonged administration.[29]

Looss *et al.* investigated poly(ε-caprolactone) as a microparticle drug carrier for an injectable bone substitute (IBS).[29] The IBS was comprised of calcium phosphate granules combined with a hydroxypropyl,methylcellulose hydrogel. Looss and colleagues designed the IBS to release

therapeutic agents. Poly(ε-caprolactone) functioned as a resorbable encapsulant. It was found that poly(ε-caprolactone) was successful as a vancomycin carrier with a controlled release rate.

Another example of a poly(ε-caprolactone) biomaterial was demonstrated by Allen *et al.*[30] Poly(caprolactone-*b*-ethylene oxide) block copolymers were developed as drug delivery vehicles for neurotrophic agents. It was found that such copolymers formed small micelles with good biocompatibility, and that these had an extremely high loading capacity for hydrophobic drugs.

1.2.4 Hydrophilic Biocompatible Polymers

Hydrophilic polymers have potential for biomedical and drug delivery applications. They can be used to modify the surfaces of other materials or can be crosslinked to form gels. These gels, known as hydrogels, swell in water and can be used as drug delivery vehicles.

1.2.4.1 Poly(ethylene oxide) / Poly(ethylene glycol)

Poly(ethylene oxide) (PEO) is often employed for modifying surfaces (fig. 2.10).[5, 31] It has high solubility in water and occupies a larger volume in water than do other polymers of comparable molecular weights. PEO is also soluble in various organic solvents including dichloromethane, ethanol, and acetone. PEG/PEO materials below ~1000 g/mol are liquids at room temperature. As PEO is of great interest to this research, its synthesis, modification, and uses will be detailed in section 3. It is non-toxic and readily eliminated at average molecular weights below 50,000 g/mol. Exemplary applications of PEO include:

(1) Modification of a surface with PEO chains to reduce protein adhesion,

(2) Addition of PEO to aqueous solutions of nucleic acids and proteins to induce crystallization, and

(3) Induction of cell fusion by addition of high concentrations of PEO.

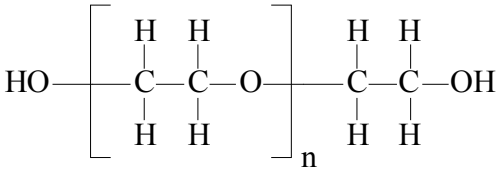


Figure 1.10: Structure of poly(ethylene oxide)

1.2.4.2 Naturally Occurring Polymers

There are numerous water-soluble naturally occurring polymers such as proteins, DNA and RNA, and polysaccharides. Many of these natural products, especially proteins and polysaccharides, are of interest for biomaterials. Collagen, the most abundant animal protein, is an extracellular matrix protein and offers biodegradability and weak antigenicity.[32] Maeda *et al.* used collagen in the design of a sustained drug delivery mini-pellet.[32] Human serum albumin, which has no affinity for collagen, was studied as a model to understand the release of drugs and proteins that do not interact with collagen. It was determined that the "mini-pellet" was effective as a drug delivery system and the rate of drug release could be controlled by changing the matrix density of the collagen.

Albumin is a widely studied serum protein often utilized in protein-based biomaterials.[6] For example, Widder *et al.* synthesized an albumin drug delivery microsphere which encased a drug, doxorubicin hydrochloride and a magnetic iron oxide, magnetite.[16] *In vitro* studies indicated drug release occurred after five minutes of exposure to water and steadily increased until

approximately 400 minutes had elapsed. The delay in release time was theorized to result from the method of administration in which the magnetite/drug/albumin microspheres were dispersed in oil and then introduced into an aqueous medium. It was hypothesized that the albumin molecules underwent conformational changes in the oil to expose the hydrophobic portions of the molecules. Subsequently, the temporary hydrophobic shield provided a window for transport to occur before drug release. *In vivo* tests suggested that the microspheres and the magnetite were non-toxic.

Polysaccharides, also known as carbohydrates, are polymers comprised of saccharide units, or sugars.[6, 33] In many cases, the units are glycosidic and are linked via condensation polymerization to form oligomeric and polymeric structures.[33] These macromolecules are a diverse class of polymers found in nature and serve animal and plant organisms as cellular structure elements, cell-cell recognition molecules, and as energy storage polymers.[33]

The variability of polysaccharides is attributed to significant chemical differences in the sugar monomers and the linkages between them. The basic chemical formula of sugars is $C_xH_yO_z$ with 5 and 6 membered heterocyclics (pentoses and hexoses, respectively) being the most common structures.[33, 34] The number, the sequences, the geometry of the linking group between the monomers, the stereochemistry of the hydroxyl substituents, and a range of functional groups varies between sugars.[33] The linkages between the saccharides can occur at different carbons and the linking bonds themselves can have different stereochemistry.[33, 34] Branching can also occur along a polysaccharide chain.[33, 34]

Dextran, a polysaccharide comprised exclusively of glucose, has been used in biomedical applications as plasma expanders (over the last 50 years), and more recently for the delivery of drug, protein/enzyme, and imaging agents.[35] In nature, dextran is utilized as a storage

macromolecule for yeast and bacteria.[34] In dextran, glucose is predominantly joined by α-(1,6) linkages, with infrequent -(1,2), -(1,3), and -(1,4) branches. The α-(1,6) linkages induce an open helix conformation of the macromolecule. Dextran has a narrow molecular weight distribution and has high water solubility.[35] Drugs and proteins can be readily conjugated to the numerous pendent hydroxyl groups of dextran either by direct attachment or via linking agents.[35]

Mehavar et al., investigated dextrans as controlled drug delivery agents and in other biological applications.[35] Dextran was also found to bind to the surface of iron oxides well. Molday and Mackenzie used dextran to sterically stabilize magnetite and produced particles with sizes of 30-40 nm in diameter.[36] Fifteen nanometers of the diameter was attributed to the iron oxide core. The dextran was further functionalized to contain additional hydroxyl groups for binding proteins to allow the nanoparticles to be utilized for immunospecific cell separations.

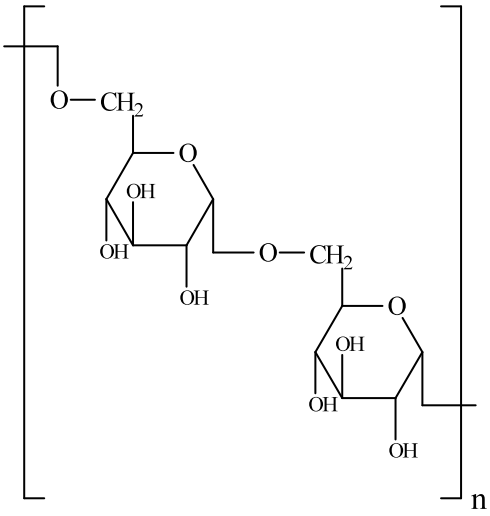


Figure 1.11: The repeat unit for dextran with α-(1,6) linkages. Adapted from Campbell, pg 58.[33]

1.2.4.3 Acrylates and Acrylamides

Polyacrylates, polymethacrylates and polyacrylamides are commonly employed in synthetic hyrdogels.[11, 37, 38] Synthetic hydrogels are three-dimensional hydrophilic networks which absorb large quantities of water.[11, 37, 38] In the hydrated state, the water content and mechanical properties of a hydrogel are reflective of soft tissue[11] and they are often biocompatible.[39] Hydrogels are often synthesized from acrylic acid, acrylamide, and/or 2-hydroxylethyl methacrylate (HEMA) (fig. 2.12). Lightly crosslinked pHEMA is prevalently used in biomedical hydrogels.[39]

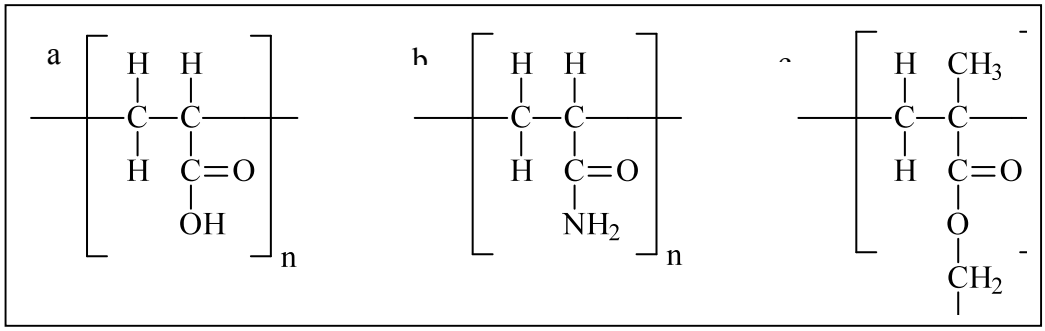


Figure 1.12: Exemplary polymers for synthetic hydrogels: (a) poly(acrylic acid), (b) polyacrylamide, and (c) poly(HEMA).

Poly(2-hydroxylethyl methacrylate) was first studied for biological applications by Wichterle and Lim[40, 41] and its first proposed uses were in the 1960's as a spongy breast augmentation material and as a nasal cartilage substitute.[11, 37, 38] Today, its applications range from hydrogels as soft tissue replacements,[42] to contact lenses.[40] Dzibula *et al.* studied pHEMA sponges as tissue implants which released drugs (e.g., insulin) over prolonged times.[11] Hsiue, *et al.* investigated pHEMA as a hydrogel carrier for the anti-cancer drug pilocarpine.[40] It was demonstrated that pilocarpine was not altered by the pHEMA and the rate of drug release could be controlled by the crosslink density of the hydrogel.[40]

When pHEMA is dry, it has physical properties comparable to bone tissue.[42] Macroporous gels of dry pHEMA have a tendency to calcify after lengthy implantation times.[42] Filmon *et al.*

investigated pHEMA based systems as alternatives to ceramic bone remodeling materials.[42] Unlike the majority of biological applications, it was desirable in this case for adhesion to occur between the polymer and cells, particularly between osteoblasts and adherents. The pendent alcohol functionality of pHEMA prevented such adhesion so these materials were modified by carboxymethylation (fig. 2.13). The modified pHEMA showed increased adhesion to the precursors to bone growth and later bone tissue growth.

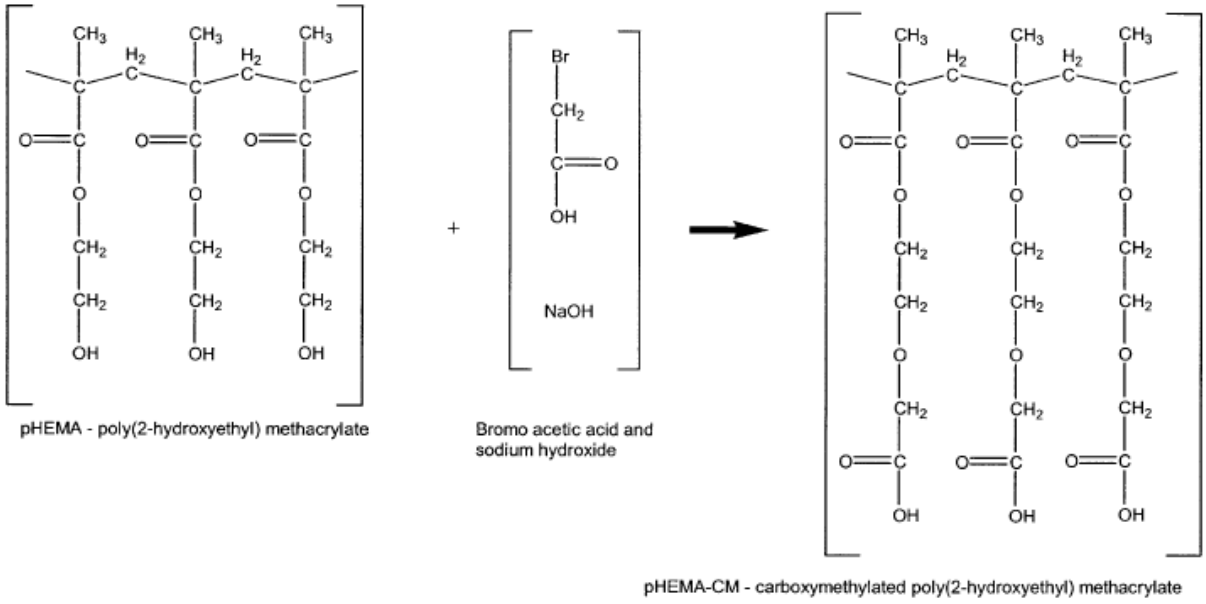


Figure 1.13: Carboxymethylation of the pendent alcohol functionality of pHEMA

An alternative example of pHEMA as a biomaterial was provided by Sefton *et al.*[43] Poly(HEMA-*co*-methyl methacrylate) coatings were utilized to microencapsulate insulin-producing cells with the hopes of implanting the protein-secreting cells in diabetic patients.[43] The spongy properties of the pHEMA based coating allowed for the appropriate glucose and oxygen supply via diffusion. Though the authors found that their exact design was not completely adequate for use *in vivo*, it was concluded that the use of pHEMA was promising for microencapsulating cells or proteins.[43]

1.3 Block copolymers

1.3.1 Introduction to block copolymers

Block copolymers are macromolecules of chemically unique segments. These polymers are distinguished by the arrangement of their well-defined sections, or blocks (fig. 2.14). Block copolymers with two discrete sequences of units, A and B, are known as A-B diblock copolymers. Also possible are polymers with three blocks A-B-A and multiblock $-(A\text{-}B)_n$- systems.[44] Nomenclature for block copolymers uses the suffix *block* for the sequence of units.

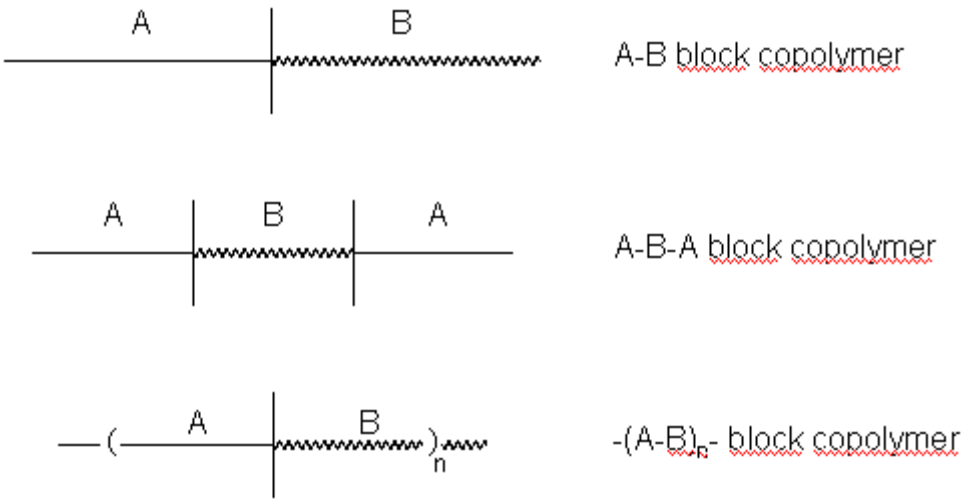


Figure 1.14: Three generic schemes of block copolymers.

Frequently, the phases of a block copolymer are immiscible and one block becomes dispersed in the other. One result of this microphase separation is the preservation of each segment's glass transition temperature and melting points.[45] In thermoplastic elastomers, a glassy polymer block is usually dispersed in a continuous matrix of the softer component.

There are various methodologies for the synthesis of block copolymers. The mechanisms that can be involved include free radical, anionic, cationic and step-growth polymerizations.[45] Two approaches for joining of the blocks, particularly utilizing step polymerizations, include *multi-prepolymer* and *single-prepolymer* strategies. The first approach involves synthesis of each respective block and then they are chemically joined. In the single-prepolymer route, one polymer segment is synthesized and the second block is often prepared in the presence of the first. When the first monomer is exhausted, the second monomer can be added to form the next block via chain extension. Polymers, such as those used in this process, with one or more functional endgroup(s) and the capacity to selectively react with a specific molecule are termed *telechelic polymers*.

Living polymerizations are a popular method for the synthesis of block copolymers by chain polymerizations. A living polymerization is characterized by specific characteristics:[23, 46, 47]

1. The rate of chain transfer and the rate of chain termination both equal zero.
2. Polymerization proceeds until full consumption of monomer occurs, and subsequent addition of a second monomer can result in continuation of polymerization.
3. The number of polymer chains and active centers is constant and independent of conversion.
4. The average molecular weight, M_n, is directly proportional to conversion.
5. Molecular weight control is provided by the stoichiometry of the reaction.

The first living polymerizations were accomplished by Szwarc and involved the anionic polymerization of styrene and 1,3-dienes.[23] Mechanistically, living polymerizations can be carried out by anionic or cationic methods, though anionic living polymerizations remain today as the most prevalent technique of the two.[23]

The ability to control and target the molecular weight of the polymer via the stoichiometry of the reagents is of great appeal for the formation of block copolymers. Living polymerizations provide for homopolymer segments of predetermined lengths. A segment can be designed to be monofunctional or difunctional, depending on the initiator used, with functional endgroups.[23, 47] The next segment can be subsequently added by sequential monomer addition via living techniques.[47]

A subset of block copolymers that is of great interest to the field of biomaterials are amphiphilic block copolymers. Macromolecules which are amphiphilic have discrete features that are either "water loving" or "water repelling" both occurring in one molecule. Amphiphilic block copolymers can have well defined hydrophobic and hydrophilic portions and have many diverse applications due to their ability to self-assemble into micelles and membranes.[48]

1.3.2 Poly(ethylene oxide)

Ethylene oxide, as are other cyclic ethers, is polymerized by ring-opening techniques. Ring-opening polymerizations can proceed by numerous mechanisms, depending on the monomer and catalyst. The thermodynamic polymerizability of a cyclic ether is a function of the change in free energy from the ring-opening. The reaction is usually driven by the thermodynamics favoring ring-opening which releases ring-strain. For most cyclic monomers polymerization to a linear structure is favored, with the thermodynamic feasibility being 3, 4 > 8 > 5, 7.[23] Predominantly, the initiating species for ring-opening polymerizations may be either cationic or anionic.

Anionic polymerization of ethylene oxide occurs by ring-opening, living techniques. Sodium or potassium hydroxide or alkoxide are commonly utilized to initiate polymerization. Through

S_N2 displacement, an alkoxide ion is formed at the chain end and propagation occurs via nucleophilic attack. The alkoxide ion is maintained and the chain end of the polymer remains active.

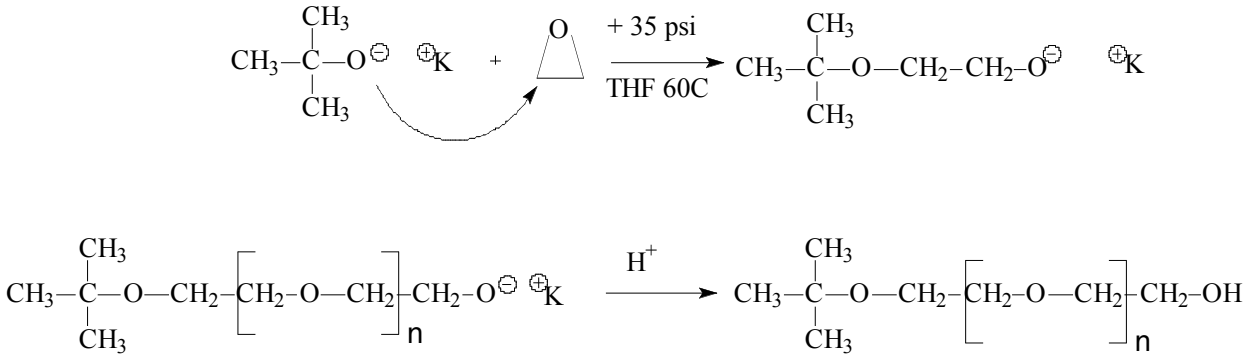


Figure 1.15: Anionic polymerization of monofunctional PEO

Poly(ethylene oxide)s, particularly the smaller hydroxylated poly(ethylene glycol)s, are one of the most prevalently used polymer classes for modifying surfaces of synthetic biomaterials.[49] When grafted onto a surface its hydrophilic nature, blood compatibility, and resistance to recognition by the mononuclear phagocyte system are transferred to the adapted material.[49] A PEO coating or layer surrounding another material may make the entire system "invisible" to the human body and consequently, many unfavorable interactions with the body can be avoided.

Gref *et al.* studied PEO 'stealth' grafts to establish the optimal coating thickness to minimize phagocyte interaction and protein adsorption.[49] Nanoparticles on the order of 160-270 nm were synthesized from diblock copolymers of PEO-poly(lactic acid) (PEO-PLA), PEO-poly(lactic-co-glycolic acid) (PEO-PLGA), and PEO-poly(caprolactone) (PEO-PCL) with PEO weights ranging from 2000 to 20,000 g/mole. An emulsion/solvent evaporation technique was used to produce the nanoparticles.

It was determined, via 2-D PAGE analysis, that the molecular weight of the PEO block greatly influenced the amount of protein adsorption on the surface of the nanoparticles.[49] A significant decrease in surface protein adsorption was recognized when the molecular weight of the PEO block was increased from 2000 to 5000 g/mole and negligible amounts of protein adsorption were observed at molecular weights of about 5000 g/mole. Assuming complete migration of the PEO chains to the surface of the nanoparticle, this would indicate an ~1.4 nm layer to be necessary for avoiding protein adsorption. Moreover, a reduction in phagocyte recognition and uptake corroborated this result.

1.3.3 Poly(2-oxazolines)

Oxazolines are classified as cylic imino ethers as they are compounds containing a nitrogen, oxygen, and double bonded carbon in a 5 membered ring.[50] These heterocycles can have one of three structures, depending on the location of the double bond (fig. 2.16). Of these structures, the 2-oxazolines and its derivatives have been predominantly used for polymer synthesis.[50] The 2, 4, and/or 5 positions on the 2-oxazoline ring can have substituents.[51]

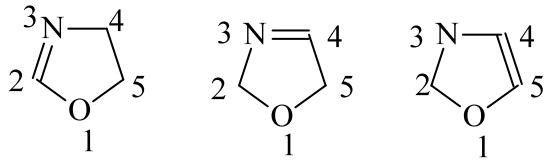


Figure 1.16: The three structures of oxazolines

The cationic ring-opening mechanism of polymerization for 2-oxazolines was established in the mid 1960's by at least four research groups (fig. 2.17). Such polymerizations are nucleophilic with the monomer being the nucleophile and the terminal positively charged species acting as the electrophile.[51] The cationic species is generated via reaction with a weak

nucleophile. Propagation of the chain occurs when the active cationic chain end reacts with another oxazoline molecule.

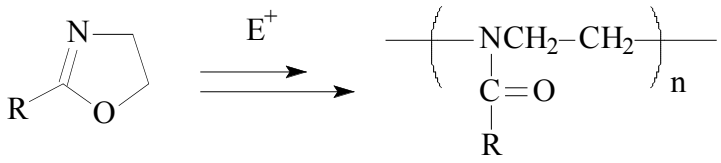


Figure 1.17: The generic scheme for the polymerization of 2-oxazoline.

The reaction mechanism can be categorized as either S_N2 or S_N1. In the S_N2 reaction the new bond is formed simultaneously with the bond that is being broken. The S_N1 mechanism is marked by the rate determining breaking of the onium bond in the positively charged species and the subsequent fast second step where the positively charged species and monomer react (fig. 2.18). Many heterocyles, such as 2-oxazolines and tetrahydrofuran, favor cationic mechanisms versus anionic mechanisms because the carbon-onium bond is a comparatively better leaving group that a carbon-heteroatom bond.[51]

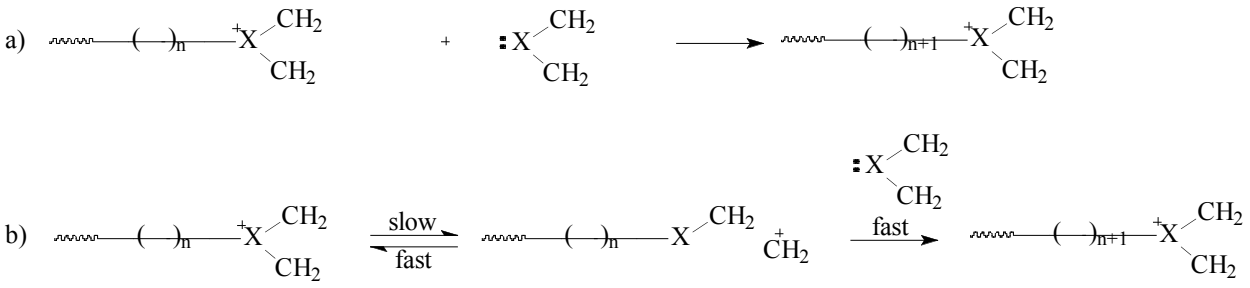


Figure 1.18: The general schemes of a cationic ring opening polymerization via (a) S_N2 and (b) S_N1 mechanisms.

Cationic initiators include lewis acids, stable cationic salts, esters of sulfuric, sulfonic and picric acids and acid anhydrides, alkyl halides, and strong protonic acids and their salts. The

cationic species is generated via reaction with the oxazoline weak nucleophile. Chain propagation occurs when the cationic chain end reacts with another oxazoline molecule. Figure 2.19 shows an example of the synthesis of a poly(2-oxazoline), poly(2-ethyl-2-oxazoline) (PEtOz).

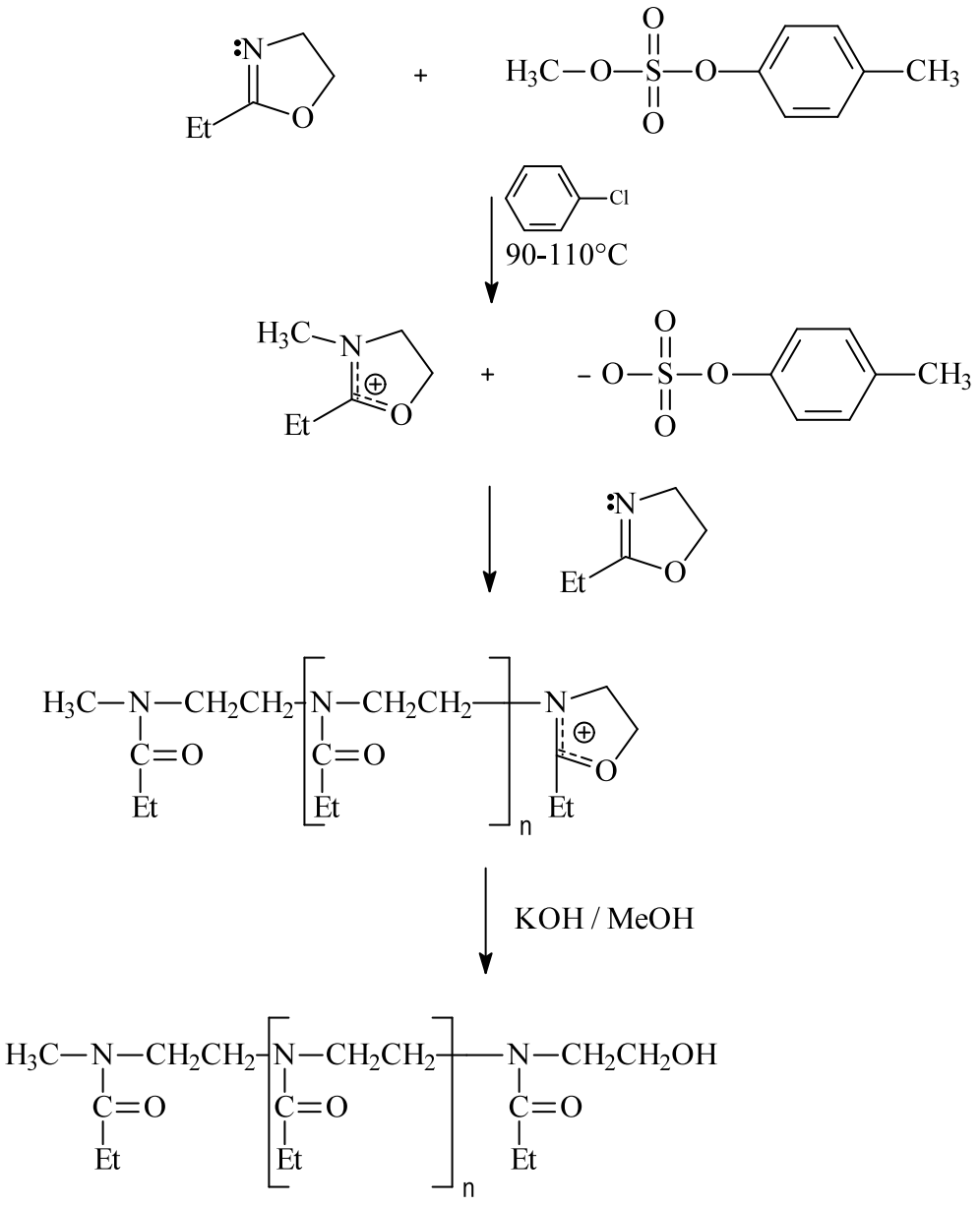


Figure 1.19: The polymerization of 2-ethyl-2-oxazoline with a tosylate initiator and termination with potassium hydroxide

Poly(2-oxazoline)s with either methyl or ethyl substituents are water soluble and can be readily converted to poly(ethylenimine) (PEI) via acid hydrolysis. As the PEI segments carry a positive charge, they can be utilized to coordinate with DNA. DNA is highly negatively charged, and thus it has an affinity for cationic polymers.[2] Gene therapy focuses on the production of therapeutic or essential proteins for which the required DNA sequence is introduced to the cell via non natural methods[42]. Viral vectors are often used for *in vivo* delivery of DNA to cells to provide for a sufficient pharmacological response.[47, 52] However, the use of viral vectors has drawbacks associated with both production of and the safety of the patient. Polymers can be used for non-toxic, non-viral vehicles which would not cause nonspecific inflammation and anti-vector immune reaction that are often induced by viral vectors. Moreover, synthetic DNA vectors are advantageous in terms of ease of reproduction and specific tailoring to the desired vehicle. Jeong *et al.* formed a random linear poly(2-ethyloxazoline) (PEOX) and poly(ethylenimine) (L-PEI) copolymer via the controlled partial acid hydrolysis of PEOX (fig. 2.20) and studied its transfection efficiency.[53] The PEOX-co-L-PEI was found to be less toxic than PEI homopolymers, yet retained an equivalently high DNA transfection efficiency.[53]

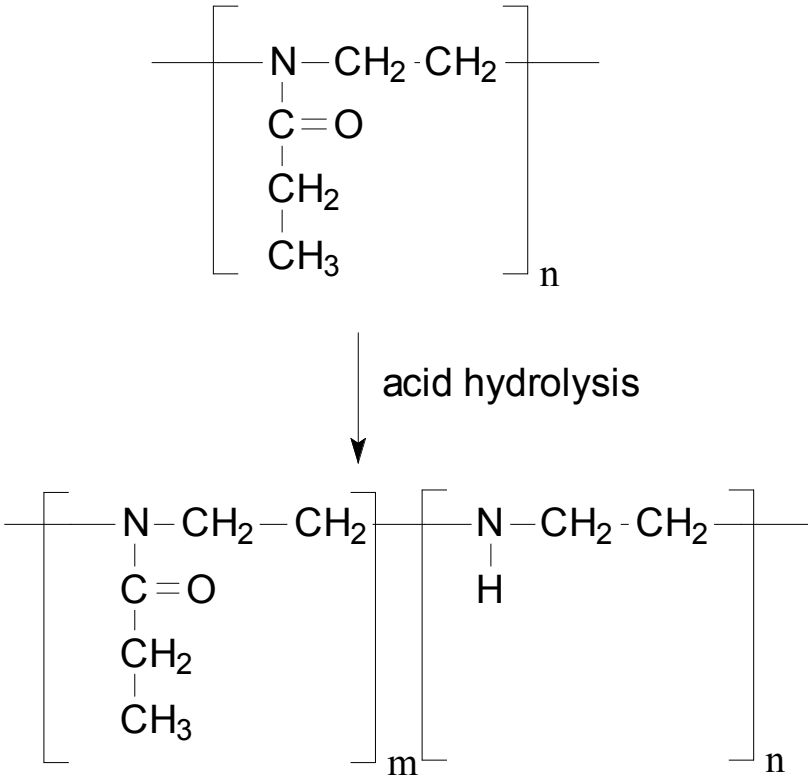


Figure 1.20: The partial acid hydrolysis of PEOX to form random PEtOz -*co*-L-PEI. Adapted from Jeong, *et al,* pg 395. [53]

A novel route for the synthesis of an amphiphilic block copolymer with α and ω functional groups was demonstrated by Akiyama *et* al.[54] An α-acetal PEO-*block*-PEI was synthesized by the use of a acetal-PEO-OSO_2CH_3 macroinitiator for a polyoxazoline block (fig. 2.21). The polyoxazoline was subsequently hydrolyzed to PEI with NaOH to form the PEG-PEI block copolymer (fig. 2.21.d). To form the acetal-PEO-OSO_2CH_3 macroinitiator, potassium 3,3-diethoxypropaneolate was used to initiate the anionic polymerization of ethylene oxide and a molar excess of methanesulfonyl chloride was used to provide a terminal sulfonate group. It was determined, via GPC and ^{13}C NMR, that the method was successful for producing the acetal-PEO-OSO_2CH_3 macroinitiator (fig. 2.21.a).

The PEO endgroup served as an initiator for the oxazoline polymerization without reaction with the opposite acetal endgroup.[54] Sulfonate derivates are known to have the capacity for initiation of various polyoxazolines.[54] The acetal-PEO-OSO_2CH_3 effectively initiated the polymerization of 2-methyl-2-oxazoline to produce polyoxazoline blocks with the targeted molecular weights (fig. 2.21.b-c). NaOH was found to efficiently hydrolyze the polyoxazoline block without reaction with the α-acetal group as acetal groups are relatively stable in alkaline conditions.

An earlier example of the use of a sulfonate ester endgroup acting as an initiator for 2-oxazoline polymerization is provided by Simionescu and Rabia.[55] Polyethylene glycol was reacted with tosyl chloride to form a p-toluenesulfonic acid ester of polyethylene oxide. Infrared spectroscopy was used confirm the tosylation of the PEO by monitoring the disappearance of the hydroxyl groups and the appearance of the tosyl aromatic group. The ester was then used to initiate the polymerization of a 2-oxazoline and the reaction progress was verified by ^{1}H NMR.

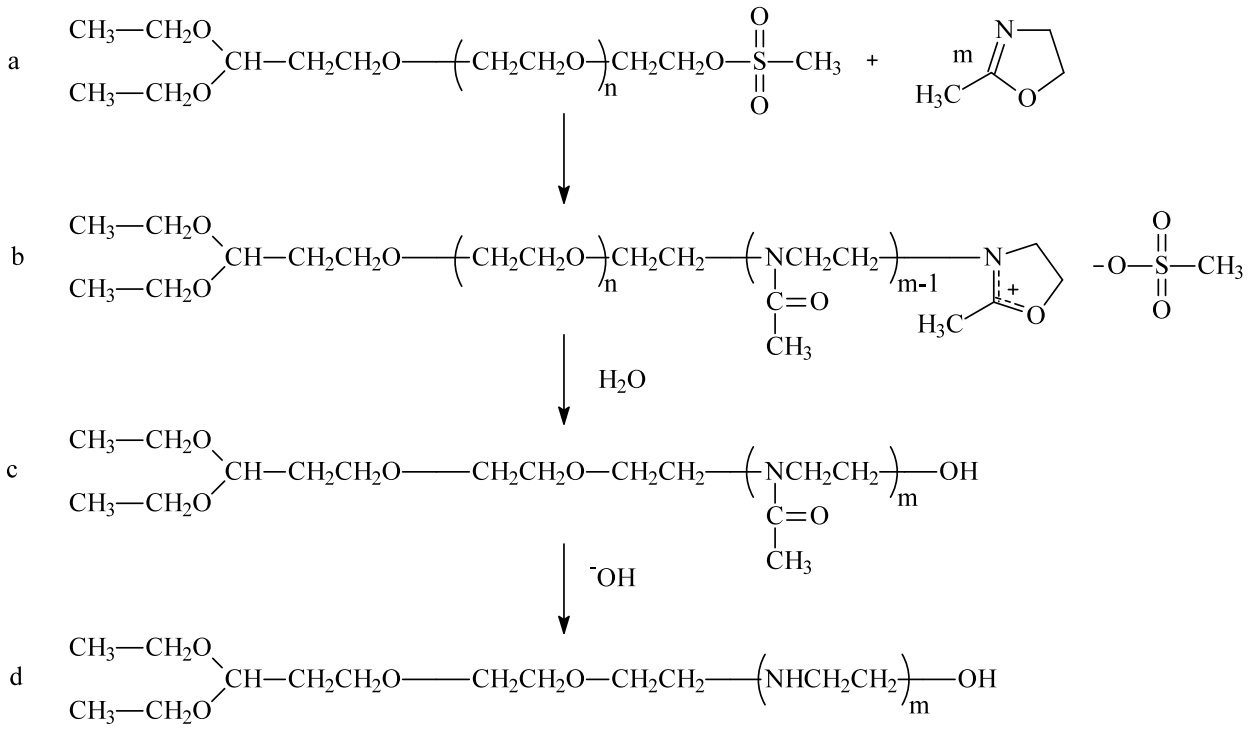


Figure 1.21: The synthetic route for acetal-PEO-PEI with acetal-PEO-OSO_2CH_3 as a macroinitiator. Adapted from Akiyama, *et al,* pg 5844.[54]

Lee *et al.* also utilized tosylate ester initiators for polymerization of a 2-oxazoline block to form amphiphilic block copolymers (fig. 2.22).[56] The polymerization of 2-ethyl-2-oxazoline was successfully initiated by a *p*-toluenesulfonate ester, and the resulting oxazolinium living endgroups were terminated with KOH to produce terminal hydroxyl groups. The hydroxyl groups were utilized to initiate either L-lactide or ε-caprolactone to synthesize the PEOX—PLA or PEOX—PCL amphiphilic block copolymers. For each case, ^{1}H NMR was used to confirm the completion of the reaction.

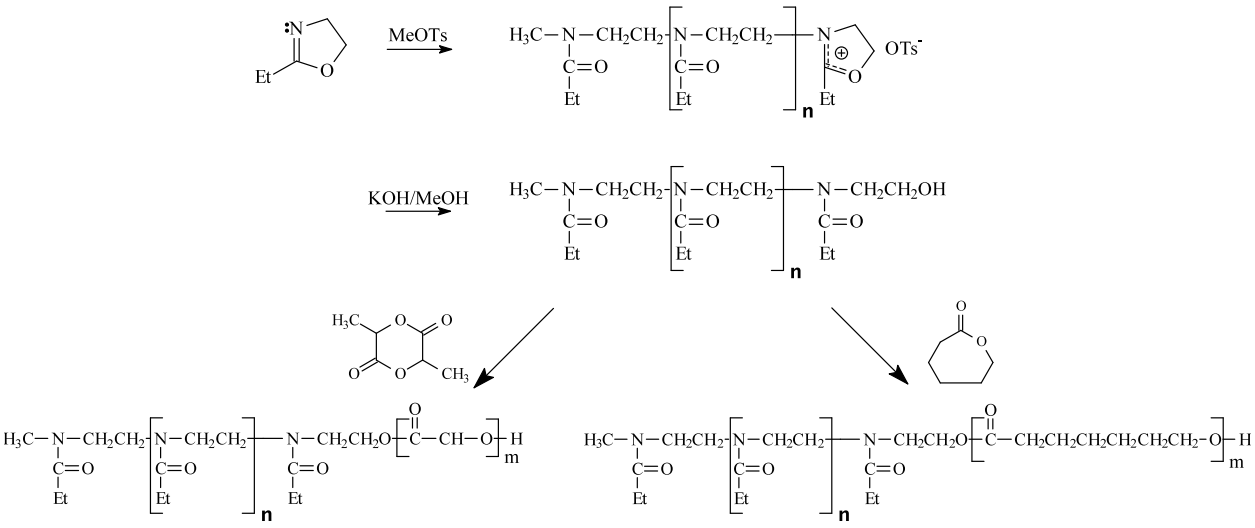


Figure 1.22: The polymerization of 2-ethyl-2-oxazoline with a tosylate initator and the following reaction with L-lactide or ε-caprolactone.

An alternative route of producing an amphiphilic block copolymer with a poly(ethylene oxide) central block and poly(2-oxazoline) tailblocks can be carried out with a methyltosylate initating species.[57] Miyamoto *et al.* established that a mesylated poly(ethylene oxide) endgroups could initiate either 2-ethyl-2-oxazoline or 2-methyl-2-oxazoline. The mesylated poly(ethylene oxide) was synthesized via the reaction of mesyl chloride and an α-,ω-hydroxy-difunctional poly(ethylene oxide).

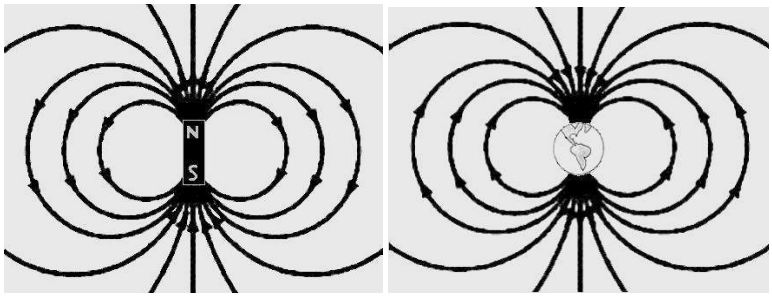
1.4 Magnetism

1.4.1 Introduction and Magnetic Terms

A simplistic and comprehensible method of approaching magnetism and magnetic fields is by understanding their relationship to electric charges. Yet while it is practical to study electric and magnetic interactions as separate entities, one must not overlook that in reality there is only one interaction between charged particles, the electromagnetic interaction.[58] Electricity involves the movement of charged particles, and when these particles have directional flow, a current is

established.[58] Magnetic interactions occur when one charged particle exerts a force upon another. This force is the magnetic force.[58] Magnetic charges have a paired behavior.[59] That is, each charge is always paired with another charge of opposite sign and equal magnitude. These charged pairs are called dipoles.[58-60]

Conveniently, the transmission of the magnetic force is denoted as the magnetic field.[58] Every moving charge creates a magnetic field which exerts a magnetic force upon another moving charge.[58] As magnetism is produced by electricity, it can also be viewed as an interaction between two currents.[58] Lines of magnetic force, field lines, can be used to depict the direction of the magnetic field.[50] These field lines also represent the magnetic dipole and are always drawn so that they are "north seeking" and never cross.[50] North and south are arbitrary designations used to represent opposite ends of the dipole.[50] Electric charges traveling in a current loop also can generate a magnetic field.[50] The flow of the magnetic field is depicted in the basic diagram of a bar magnet (fig. 2.23.a).[50] A more recognizable representation of a magnetic field involves the Earth (fig. 2.23.b).[58, 60]

a) 

b)

Figure 1.23: Illustration of the magnetic field lines of a bar magnet (a) and of the Earth (b). Adapted from Tipler, pg 9.[58]

1.4.1.1 Magnetic Equations

The definition of a magnetic dipole (μ) is:[58]

$$\mu = ml$$

where m is the measure, in meters, of the length vector, *l*, which runs from the South to the North pole. It is expressed in A·m.[250] The strength of the magnetic field (*H*) is calculated from the ratio of the current (I) to the area of the path of the magnetic dipole:[59]

$$\mathbf{H = I / (2 \pi r)}$$

The units for *H* are ampere per meter ($A \cdot m^{-1}$).

The intensity of magnetization (*M*) indicates the total magnetic dipole moment per unit volume (V) and has the units of $A \cdot m^{-1}$.[59, 60]

$$\mathbf{M = \mu / V}$$

A similar measurement is the magnetic induction (B), which is the magnetic flux per crossectional area of flow. Magnetic flux indicates the total number of field lines and is a measurement of the total amount of magnetism. Thus, the magnetic induction expresses the magnetism per unit area and is directly related to both ***H*** and ***M***:[59, 60]

$$\boldsymbol{B = \mu(H + M)}$$

The magnetic induction is expressed in Webbers per square meter or Tesla (T).[60]

The magnetic susceptibility (χ) measures the effectiveness of a magnetic dipole induction by an applied field. It value is useful for comparing the magnetic response of materials. Another method for magnetic material characterization is by determining the permeability (μ). Depending on the magnitude of the response, either susceptibility, permeability, or both can be used to characterize a material. The equations for χ and μ are written:[59, 60]

$$\chi = M/H \qquad \mu = 1 + \chi$$

1.4.2 Magnetism in Materials

Materials are initially segregated into two broad classes: those with a positive χ and those with a negative χ.[60] Generally, materials for which $\chi >> 1$ display magnetic properties that can be of potential use.[60] Another consideration is the temperature. The magnetic susceptibility is inversely related to temperature; that is, as the temperature increases, χ is observed to decrease in magnitude.[60] The relationship between χ and temperature:[59, 60]

$$\chi = C/(T)$$

C, the Curie constant, is a positive constant unique to the material of study. The curie temperature is the temperature above which a ferromagnetic material becomes paramagnetic.

The relationship between χ and temperature further classifies the material into one of five types of magnetic behavior: diamagnetism, paramagnetism, ferromagnetism, ferrimagnetism, and antiferromagnetism.[58-60] The simplest materials for which χ is equal to zero and χ is positive are classified as paramagnetic.[60] Another main grouping of materials is for those which are identified as having a χ which is negative. These materials are diamagnetic.[58-60] For all other materials this equation loses accuracy as the temperature decreases. These materials are marked by having a critical temperature at which the relation no longer holds.[60]

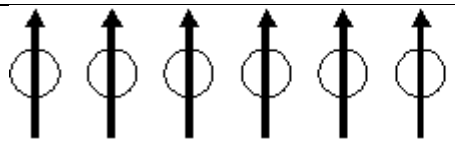
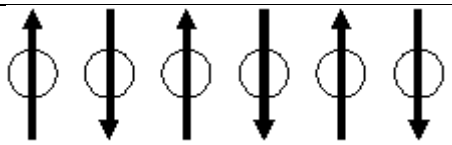
These classifications are based upon the material's response in an external magnetic field. Diamagnetism is inherent to all matter and is identified by the induction of a magnetic moment opposite to the applied field.[60] The result is a reduction of magnetic field strength.[60] This effect is not influenced by temperature fluctuations and is a relatively small contribution to a material's magnetism.[58] Diamagnetism is also characterized by possessing permanent magnetic dipoles.[58-60]

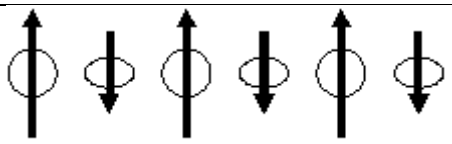
Paramagnetic materials have magnetic dipole moments with weak interaction and consequently a resulting random dipole orientation.[59, 60] When a magnetic field is applied, a partial alignment occurs between the dipoles and the field. Thermal motion disrupts much of the alignment, and subsequently, the net increase in field strength is relatively small.[58] However, the increase in magnetic field from paramagnetism is always greater than the decrease caused by diamagnetism.[59, 60] Also unlike diamagnetism, paramagnetism is affected by temperature.[60] At lower temperatures, thermal motion and the associated spin randomization are decreased allowing for a greater magnetic response.[59, 60]

Ferromagnetic materials possess permanent magnetic dipoles.[60] When a magnetic field is applied to a ferromagnetic material, there is an alignment of the magnetic dipoles with the magnetic field that increases the field strength. This occurrence is even observed in weak magnetic fields, and in cases with no magnetic field, an alignment of dipoles can still be observed. Antiferromagnetic materials also have atoms with permanent dipole moments, but the dipoles are antiparallel in alignment and subsequently such materials have small magnetic susceptibility and a zero overall magnetic moment. Ferrimagnetic materials also have an antiparallel arrangement, but the dipoles are of unequal magnitude. As a result, ferromagnetic materials exhibit a net magnetic moment. For ferrimagnetic and ferromagnetic materials,

temperatures above the Curie Temperature (T_C) cause the materials to lose their alignment and display paramagnetic behavior.

Table 1.2: Types of magnetism matter may exhibit. Adapted from Chen, pg 36.[60]

Magnetism	Electron Spin Alignment	Examples
Diamagnetic	No long range order; alignment against applied field	Inherent to all material
Paramagnetic	No long range order, alignment with applied field.	Metals such as Cr, Mn; diatomic gases O_2, NO; ions of transition metals and rare earth metals[51]; rare earth oxides
Ferromagnetic	 Individual atoms have permanent moments which align	Transition metals (*i.e.:* Ni, Fe, Co)
Antiferrimagnetic	 Atoms with permanent dipole moments; alternating moments from atom to atom resulting in no net magnetization	Compounds containing transition metals (*i.e.:* NiO, MnS, CuO)

Ferrimagnetic	 Atoms with alternating permanent dipole moments of unequal magnitude resulting in net magnetization	Iron Oxides (*i.e.:* Magnetite (Fe_3O_4), Maghemite (g-Fe_2O_3))

Bulk material that is ferromagnetic, antiferromagnetic, or ferrimagnetic is divided into regions of aligned spin arrangements.[60] Weis proposed that these uniformly magnetized domains minimize the field energy of a magnetized material. The width of the domain walls between the domains is a function of the lattice spacing of the crystal structure, the magnetocrystalline anisotropy, and the exchange energy (the preference for a constant equilibrium magnetic direction). The number of domains per particle of bulk material is directly related to the size of the particle, and decreases when the size of the particle decreases. Eventually, the material will reach a limit where a domain wall is no longer energetically feasible. This characterizes a single domain particle. .

Single domain particles respond and reach the saturation magnetization point at lower field strengths than those required for multidomain materials. The estimated maximum single-domain size for spherical particles is presented in Table 2.3. Single domain particles which reach rapid magnetization equilibrium relative to the experimental time are classified as superparamagnetic. Such materials differ from paramagnetic materials by having much larger susceptibilities and zero hysteresis, or residual magnetization after the removal of the applied field.[59]

Table 1.3: The maximum diameter of a single domained spherical magnetic particle

Material	D_s (nm)
Fe	14
Ni	55
Co	70
Fe_2O_3	166
Fe_3O_4	128

A hysteresis loop in which magnetization as a function of applied magnetic field demonstrates the response of a material to magnetization and demagnetization. Application of an effectively large magnetic field results in the progressive and then rapid increase in the magnetization of the material with the applied field until the maximum magnetization value, the saturation magnetization, is achieved[61]. The increase in magnetization with applied field is represented by points O-A-B in Figure 2.24. When the field is retracted, the magnetization of the sample decreases. Often, this decline in magnetization occurs at a slower rate and results in a non-zero magnetization value when the applied field is completely withdrawn. This remanence magnetization is indicated by point C (fig. 2.24.). To completely demagnetize the sample, a field is applied in the opposite direction until the saturation magnetization is reached in the opposite direction. This process is repeated and the hysteresis loop indicated by B-C-D-E-F-G-B is created. The distance O-D is the coercive field (H_c), which is the needed opposite field to return the sample's magnetism to zero.

Superparamagnetic materials do not exhibit a hysteresis loop with remanence magnetization. This is because when the magnetic field is removed, the material's magnetic domains

immediately lose their alignment and subsequently the material's net magnetization. For a superparamagnetic material, the curve would retrace its path and always intersect the origin.

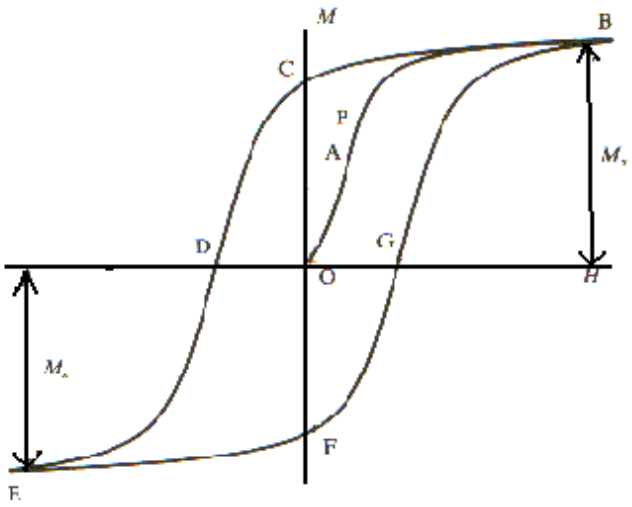


Figure 1.24: Hysteresis loop of a magnetic material demonstrating response to different magnetic fields

1.4.3 Ferrrofluids

The study of magnetic fluids, ferrofluids, was originated in 1965 by Papell and Rosensweig,[62-64] Rosensweig and Charles, Papirer and Martinet.[22] These stable magnetic fluids are comprised of the superparamagnetic nanoparticles, a surfactant, and the carrier liquid. The magnetic nanoparicles are small (3-15 nm), single domain, and have a molecular layer of surfactant for their dispersion.[64] These systems are entirely synthetic and do not occur in nature, but display the characteristic properties of fluids. When a magnetic field gradient is applied to a ferrofluid, it moves towards the region of higher field with the behavior of an intrinsic liquid magnet while still retaining its fluid properties.[63, 65]

The particles of a ferrofluid are single domain with nanometer dimensions and permanent magnetic poles. As the particles are superparamagnetic, their magnetic moments rapidly align

along the direction of an applied field and return to a random state when the field is removed. Superparamagnetic behavior results in a gain in magnetization with increased field strength.

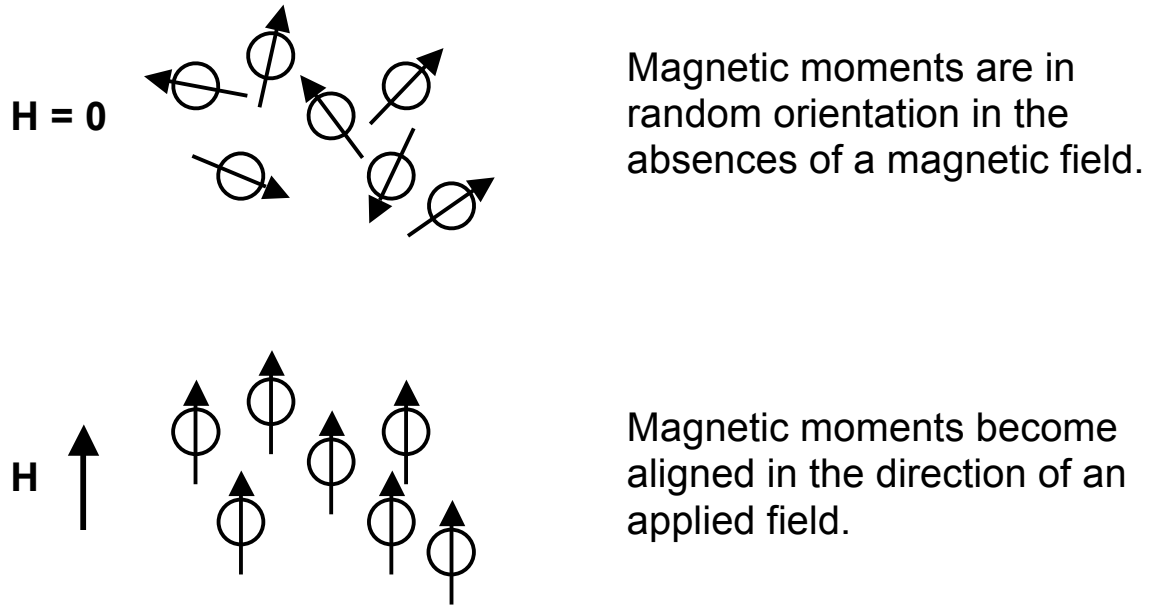


Figure 1.25: The effect of the application of a magnetic field upon a superparamagnetic material.

Transition metals and metal oxides with sizes ranging from 1-100 nm have the ability to form superparamagnetic dispersions in carrier fluids. These include Ni, Co, Fe, and iron oxides. The highest magnetic susceptibilities are demonstrated by the elemental metals. These pure transition metals have a high sensitivity to oxidation that is heightened by the large surface area of the nanoparticles. Ni, Co, and Fe oxidize under atmospheric conditions to antiferromagnetic alloys such as NiO, CoO, and FeO respectively. Since there is not an abundance of methodologies for prevention of nanoparticle oxidation, current research is focused on the use of iron oxides. Despite having a lower magnetization, these materials demonstrate oxidative stability and are more practical for applications in oxygen rich environments.

Magnetic data for transition metals and metal oxides is presented in Table 2.4.[66, 67] Magnetite, which is of great interest to this research, is highlighted.

Table 1.4: Magnetic data for select metals and metal oxides.

Metal/Metal Oxide	Saturation Magnetization (M_s) (emu/cm^{-3}) at 298 K	Cure Temperature (K)
Ni	485	631
Co (cubic)	1400-1422	1404
Fe (cubic	1700-1714	1043
γ-Fe_2O_3	394	820-986
$FeO\cdot Fe_2O_3$	**480-500**	**858**
$MnO\cdot Fe_2O_3$	410	573
$CoO\cdot Fe_2O_3$	400	793
$NiO\cdot Fe_2O_3$	270	858
$CuO\cdot Fe_2O_3$	135	728

1.4.4 Iron Oxides

1.4.4.1 Magnetite and Maghemite

The iron oxides magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are well-investigated iron oxides for use in drug delivery. Both have comparable physical properties, comparable crystalline structure and display ferrimagnetic behavior. Magnetite is made up of a 1:2 molar ratio ($FeO\cdot Fe_2O_3$) of Fe^{2+} which are tetrahedrally and octahedrally coordinated and Fe^{3+} ions which are solely octahedrally coordinated. Maghemite has a different sub-lattice arrangement with only Fe^{3+} ions that are tetrahedrally and octahedrally coordinated. These subtle differences

in structure and arrangement result in slightly lower magnetic properties in maghemite. The focus of this research is more concerned with magnetite.

Table 1.5: Comparison of the properties of magnetite and maghemite

	Crystal system	Cell dimensions (nm)	Density (g/cm^3)	Color	Magnetic susceptibility (emu/g)	Curie Temperature (K)
Magnetite	**Cubic**	**a_0 = 0.839**	**5.26**	**Black**	**90-98**	**850**
Maghemite	cubic or tetragonal	a_0 = 0.834	4.87	Reddish-brown	76-81	820-986

1.4.4.2 Magnetite

The chemical formula of magnetite is abbreviated Fe_3O_4, but it is more accurately written $FeO{\cdot}Fe_2O_3$. The inverse spinel structure of magnetite consists of ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions packed into an oxygen array.[68] The oxygen is arranged cubically along the [111] axis and the unit cell of magnetite consists of 56 atoms: 32 O^{2-} anions, 16 Fe^{3+} cations, and ? Fe^{2+} cations.

Several major synthetic pathways have been developed for the production of magnetite nanoparticles. These methodologies are classified into three main areas: size reduction from larger magnetite, aqueous precipitation of iron salts, and thermolysis-reduction of iron (III) organometallics. The nanoparticle morphology, structure, and dispersity are related to the synthetic conditions including the starting materials, solution concentrations, temperature, pH,

and the presence of surfactants or molecules that adsorb onto the surfaces of the particles as they form.[69] There are numerous aqueous precipitition techniques including oxidative hydrolysis of iron salts, water-in-oil microemulsions, and formation in magnetoferrin. The most prevalent method of forming magnetite in aqueous media is the chemical coprecipitation of ferrous and ferric salts by reaction with a strong base.[65]

The mechanism of magnetite formation via aqueous coprecipitation of Fe^{2+} and Fe^{3+} is not fully understood, but it is proposed that the Fe^{2+} reacts with base to form $Fe(OH)_2$ which subsequently reacts with hydrous oxides that are ubiquitous in the environment. Although the mechanism is vague, it is well known that polydisperse magnetite particles form with a cubic inverse spinel crystal structure and that the processes of nucleation and crystal growth are important. Nucleation occurs when the supersaturation of ion clusters in solution exceeds a critical value and subsequent growth is influenced by the solution conditions of the reaction. Qui. coprecipitated salts to form magnetite spheres with a diameter of 6.4 to 8.3 nm and saturation magnetizations of 63 to 71 emu/g (20 – 30 % less than bulk magnetite).[70] To determine these values, transmission electron microscopy and magnetrometry techniques were utilized.[70]

The stoichiometry of the formation of magnetite requires 1 mole of Fe^{2+} and 2 moles of Fe^{3+} that are commonly provided by the aqueous salt solutions $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 4H_2O$.[68, 71, 72] Fe^{2+} can oxidize to form alkagneite (β-FeOOH), and this emphasizes the need for precise laboratory techniquesThe salt solutions and materials to be used in the reaction should be deoxygenated and the dissolved salts should be utilized immediately to limit unwanted oxidation.[68] Gribanov *et al.* reported that the optimal pH for precipitation of magnetite from the salt solutions was 8.5 to 10.[69]

1.5 Magnetite Nanoparticle Stabilization

Magnetic nanoparticles can aggregate due to both Van der Waals attractive forces and their magnetic attractive forces.[73] Additionally, having a large surface-area to volume ratio predisposes magnetic nanoparticles to agglomerate to reduce their surface tension.[71] To counteract the strong self-attraction of magnetic nanoparticles, stabilization must be provided by a surfactant layer. Rosensweig and others have reported that a minimum steric coating thickness of 1-3 nm should be utilized.[64],[73] For biotechnological uses, the coating should enable dispersion in aqueous media, should be $\leq$ 15 nm to allow for renal clearance, and should limit interactions with biological macromolecules that result in opsonization and phagocytosis.

1.5.1 Magnetite Surface

Understanding the surface chemistry of magnetite is of great importance to optimize interactions candidate molecules to provide steric stabilization in dispersions. On the surface of magnetite, iron atoms can act as Lewis acids. In aqueous media, water interacts with the surface iron, thus resulting in functionalization of the surface with hydroxyl groups. The amphoteric nature of the hydroxyl groups allows for reaction with either acids or bases, depending on the environmental pH. The isoeletric point of magnetite occurs at a pH of ~6.8 and is the point at which the surface is equally positively and negatively charged (fig. 2.26.) At pHs above and below the isoelectric point, electrostatic repulsion stabilizes magnetite particles against aggregation caused by magnetic and Van der Waals attractive forces. Figure 2.26 illustrates the

electrostatic stabilization of magnetite. The failure to prevent aggregation around the isoelectric point for hydroxylated surfaces is indicated by the region labeled FLOC.

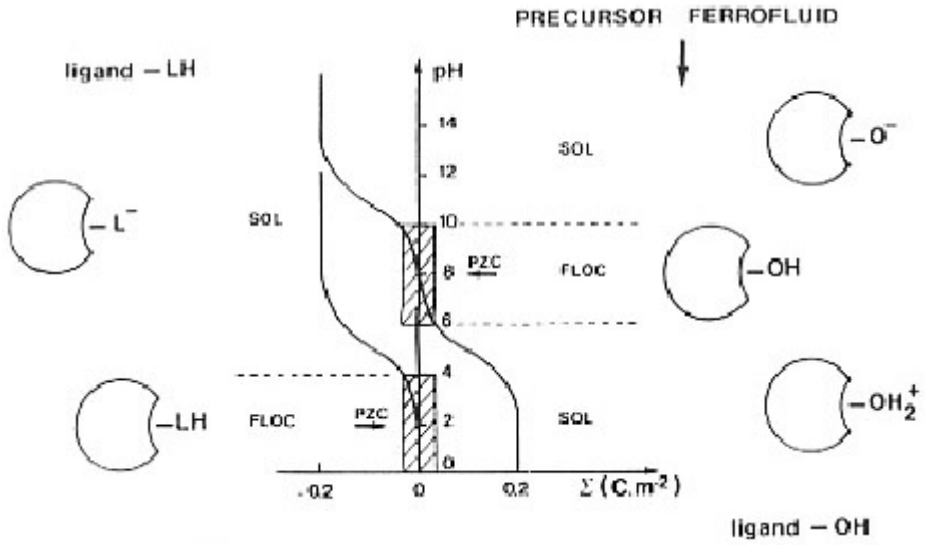


Figure 1.26: Illustration of the dependence of electrostatic stabilization on pH of solution. Aggregation (FLOC) occurs in the vicinity of the isoelectric point.

1.5.2 Steric Stablization of Magnetite Nanoparticles

It has been proposed that pendant carboxylate groups can exchange with the amphoteric hydroxyl groups on the surface of an iron oxide.[74] Shen *et al.* synthesized stable, hydrophilic Fe_3O_4 dispersions via a two step surfactant coating process. The primary surfactants were fatty acids, having carboxylic acid head groups which readily chemisorbed onto the surfaces of the Fe_3O_4. Another surfactant was then used to create a final, outward hydrophilic surface on the coated nanoparticle.[75] Fauconnier *et al.* demonstrated that gluconic acid, citric acid, and mixtures of both could be used to create stable maghemite dispersions at biological pHs.[74] Moreover, using HPLC, the authors were able to quantify the number of complexed carboxylates and it was suggested that they were comparable to the estimated number of surface hydroxyl groups.

An alternative to the approach provided by Shen *et al.* is the use of amphiphilic block copolymers. The utility of amphiphilic block copolymers is due to their chemical and structural versatility and their capacity to be tailored for a specific function.[75] In their design, the choice of copolymers, the molecular weights of the blocks, and the structures of blocks could be varied.[76]

CHAPTER 2 SYNTHESIS AND SOLUTION PROPERTIES OF POLY(ETHYLENE OXIDE-*b*-2-ETHYL-2-OXAZOLINE) AND POLY(ETHYLENE OXIDE-*b*-ETHYLENIMINE) DIBLOCK COPOLYMERS

2.1 Abstract

A series of poly(ethylene oxide-*b*-oxazoline) (PEO-*b*-POX) diblock copolymers were synthesized for potential use in the development of nanostructures to complex with anionic therapeutic molecules such as non-viral gene transfer vectors. The POX blocks were hydrolyzed with acid to afford linear poly(ethylenimine) (L-PEI), which is a polycation that has been explored in DNA complexes. Monohydroxyfunctional PEO with two molecular weights, 1923 and 4884 g mol^{-1}, were functionalized with a terminal tosylate group for use as macroinitiators for POX. The living cationic ring-opening polymerization of 2-ethyl-2-oxazoline was initiated with the tosylated PEO macroinitiator. The pendent amide groups of the poly(ethylene oxide-b-2-ethyl-2-oxazoline)s (PEO-*b*-PEOX) diblock copolymers were acidically hydrolyzed to form well-defined PEO-*b*-(PEOX-*co*-L-PEI). Dynamic light scattering established that both copolymers remained as single chains in aqueous solution at pH 7 with the PEO-*b*-PEI copolymers having smaller hydrodynamic radii (R_Hs) than the corresponding PEO-*b*-PEOX copolymers. The R_Hs for each copolymer series increased with increasing molecular weight of the second block, and had R_H values in water of 2-5.5 nm and 2-3 nm for the copolymers containing PEOX and PEI, respectively.

2.2 Introduction

There is considerable interest in gene delivery into eukaryotic cells to treat acquired and genetic diseases. Research over the past decade had been focused on the use of viral vectors because of their efficiencies.[53] Recently, gene delivery research has been expanded to exploring non-viral vectors. Several cationic polymers, for example, polyethylenimine (PEI), polyamidoamine, poly(*L*-lysine), and poly(2-(dimethylamino)ethyl methacrylate), have been investigated as potential DNA delivery agents in mammalian cells.[77] PEI has received much attention due to its DNA transfection efficiency *in vivo* and *in vitro*.[77, 78]

Linear and branched homopolymers have been investigated as gene delivery vehicles. Improved transfection efficiency has been achieved using linear PEI as compared to branched PEI.[78] Moreover, it has been postulated that greater flexibility of the linear chain promotes better DNA transfer.[79] It has also been reported that linear PEI has lower cell cytotoxicity than branched PEI.[53, 77]

PEI readily forms interpolyelectrolyte complexes with DNA through electrostatic interactions.[77] The cooperative interactions are formed between the negatively charged phosphate groups along the DNA backbone and the positively charged PEI backbone amino groups. Unfortunately, like other polycation/DNA complexes, the PEI/DNA complexes exhibit reduced water solubility.

Novel cationic carriers have been designed with hydrophilic block or graft copolymers to address the significant challenge of improving complex solubility. Poly(ethylene oxide) (PEO) has been employed to modify the surfaces of synthetic materials for *in vivo* applications.[53] The hydrophilic nature of the PEO imparts blood compatibility and immunogenicity to the copolymer materials.[53] Several hydrophilic block and graft copolymers containing PEO, such as PEO-*b*-polyspermine, PEO-*b*-polylysine, and PEO-*g*-PEI have been reported [77] . The copolymer

materials discussed in this thesis have been synthesized through the use of activated macroinitiators, and their aggregate characteristics as a function a molecular weight and composition have been extensively characterized within aqueous solutions.

This chapter focuses on the synthesis and solution properties of hydrophilic poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) (PEO-PEOX), poly(ethylene oxide-*b*-2-methyl-2-oxazoline) (PEO-PMOX), and PEO-PEI diblock copolymers (Figure 3.1). The PEO-PEOX diblock copolymers were synthesized by cationic ring-opening polymerization of 2-ethyl-2-oxazoline utilizing PEO-tosylate macroinitators. Acid hydrolysis of the amide groups in the PEOX block produced the linear PEO-PEI diblock materials. Aqueous solutions of the PEO-PEOX and PEO-PEI diblock copolymers were studied to measure their aggregate characteristics in water. A well-defined series of PEO-PEOX, PEO-PMOX, and PEO-PEI block copolymers, were developed with various block lengths.

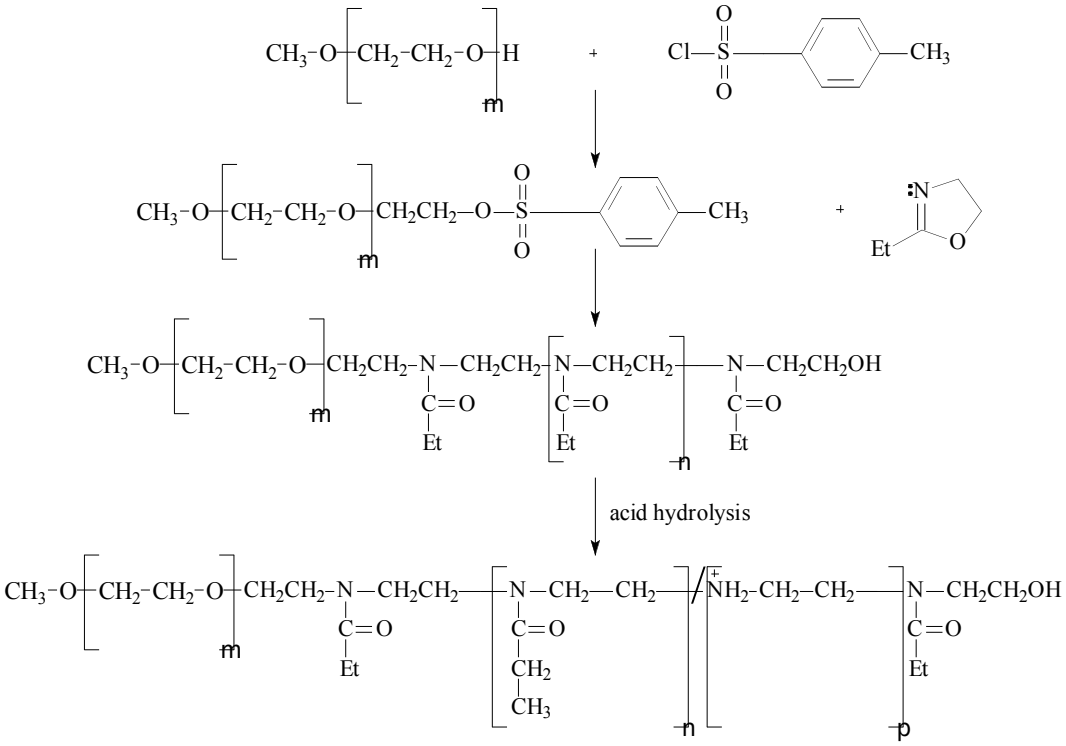


Figure 2.1: Synthesis of poly(ethylene oxide-*b*-2-ethyl-2-oxazoline) copolymers and their hydrolysis to produce copolymers containing polyethylenimine.

2.3 Experimental

2.3.1 Materials

Poly(ethylene oxide) monomethyl ether oligomers (PEO) were purchased from Aldrich Chemical Co. in two molecular weights: 1923 and 4884 g mol^{-1}. The 2-ethyl-2-oxazoline monomer (Aldrich) was dried over calcium hydride, distilled under a N_2 purge, and stored under N_2 at 10 °C prior to polymerization. Chlorobenzene (Aldrich, 99%) was dried over CaH_2, vacuum distilled into a clean, dry, round-bottom flask containing activated molecular sieves (4 Å) and stored under nitrogen at 25 °C. Acetonitrile (Aldrich 99.93%) was dried over CaH_2 and distilled prior to use. *p*-Toluenesulfonyl chloride (Acros, 99+%), triethylamine (Aldrich, 99.5%), KOH (Mallinckrodt), and NaOH (Fischer) were used as received.

2.3.2 Characterization

2.3.2.1 Size Exclusion Chromatography (SEC)

SEC was used to determine the molecular weights and molecular weight distributions of the PEO oligomers. The PEO samples were analyzed on a Waters 2690 GPC outfitted with four Waters Styragel HR columns, a Viscotek laser refractometer, and an online Viscotek 100 differential viscometric detector. Chloroform at 25 °C was used as the mobile phase with a 1.0 mL min^{-1} flow rate. Sample elution times were compared to those of a polystyrene calibration curve and the data was analyzed using a Universal calibration to obtain absolute molecular weights.

2.3.2.2 Proton NMR Spectroscopy

^{1}H NMR spectra were collected on a Varian Unity-400 spectrometer operated at a frequency of 399.952 MHz, a 22° pulse angle, an acquisition time of 3.7 s, and a 1 s recycle delay. Spectra were taken of the PEO oligomers in $CDCl_3$ to confirm number average molecular weights (M_n). ^{1}H NMR was used to monitor all reactions as well as to determine diblock compositions and extents of acid hydrolysis to produce the copolymers containing ethylenimine.

2.3.3 Synthesis of Tosylated PEO Macroinitiators

Two PEO tosylate macroinitiators were synthesized with different PEO molecular weights of 1923 and 4884 g mol^{-1}. The synthesis of the 4884 M_n PEO tosylate macroinitiator is provided. Fifty grams of PEO (0.010 mol) were dissolved in 150 mL of dichloromethane in a 500-mL,

round-bottom flask equipped with a magnetic stir bar. Next, 7.1 mL (0.051 mol) of Et_3N were added to serve as an acid scavenger. In a separate round-bottom flask, 2.31 g (0.015 mol) of *p*-toluenesulfonyl chloride were dissolved in 150 mL of CH_2Cl_2. The tosyl chloride solution was added to the first round-bottom flask via an addition funnel. The reaction proceeded for 12 h at 25 °C and was monitored by ^{1}H NMR. After the reaction was complete, the solution was filtered and washed 3-5 times with deionized water in a separatory funnel to remove salts. The tosylated PEO was collected via precipitation into cold anhydrous ethyl ether (<-10 °C). The macroinitiator was dried overnight under vacuum at 80 °C. ^{1}H NMR was used to determine the extent of tosylation, and it was found to be quantitative.

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

Hydrophilic PEO-PEOX diblock copolymers were synthesized with the tosylated PEO macroinitiator. A representative synthesis for the 1923 g mol^{-1} PEO:1200 g mol^{-1} PEOX is provided. Fifteen grams (0.007 mol) of the tosylated macroinitiator was dried overnight at 80 °C in a round-bottom flask equipped with a magnetic stir bar. Seventy mL of chlorobenzene were added to dissolve the PEO macroinitiator. With a syringe, 5.84 mL (0.083 mol) of 2-ethyl-2-oxazoline were added. The reaction was carried out at 90 °C for the first hour and then 110 °C until completion. A slow nitrogen purge was maintained and reaction progress was monitored by ^{1}H NMR. The polymer chains were terminated with an excess of KOH in methanol. Two layers formed upon termination, were separated, and 20 mL of chloroform were added to the organic layer. The organic layer was washed 3-5 times with deionized water in a separatory funnel to remove salts. The copolymer was collected via precipitation into cold anhydrous ethyl ether (<-10 °C) and dried overnight under vacuum at 80°C.

2.3.5 Synthesis of Poly(ethylene oxide-*b*-2-methyl-2-oxazoline)

A series of PEO-PMOX diblock copolymers were synthesized similarly to the PEO-PEOX copolymers. A representative synthesis for the 4884 g mol^{-1} PEO : 1400 g mol^{-1} PMOX is provided. Fifteen grams (0.007 mol) of the tosylated macroinitiator was dried overnight at 80 °C in a round-bottom flask equipped with a magnetic stir bar. Seventy mL of acetonitrile were added to dissolve the PEO macroinitiator. Ten mL (0.098 mol) of 2-methyl-2-oxazoline were added with a syringe. The reaction was carried out at 85 °C under a slow nitrogen purge. The reaction was monitored by ^{1}H NMR. The polymer chains were terminated with an excess of KOH in methanol. After termination, the solvents were removed by vacuum stripping and the polymer was dissolved in 30 mL of chloroform. The solution was washed 3-5 times with deionized water in a separatory funnel to remove salts. The copolymer was collected via precipitation into cold anhydrous ethyl ether (<-10 °C) and dried overnight under vacuum at 80°C.

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

The acid hydrolysis of the 4884 g mol^{-1} PEO : 540 g mol^{-1} PEOX diblock copolymer is provided. Ten grams of the diblock were dissolved in 100 mL of deionized water. HCl (0.18 moles) was added and the reaction was refluxed at 100 °C and hydrolysis was followed via ^{1}H NMR. After hydrolysis, the solution was neutralized with a 0.1 M NaOH solution. Dialysis

(2000 g mole^{-1} limit) of the solution was carried out over several days to ensure removal of all salts and reagents. The hydrolyzed copolymer was recovered by removal of water via vacuum stripping and dried overnight at 110 °C. ^{1}H NMR was used to confirm the extent of hydrolysis.

2.3.7 Solution Properties of Poly(ethylene oxide-*b*-2-ethyl-2-oxazoline)

Aqueous solution properties were studied by dynamic light scattering (DLS). Solutions of each polymer were prepared in deionized water from a NANOpure II ion exchanger (Barnstead) with a resistance above 17 MΩ·cm at concentrations of 0.0625 - 100 mg mL^{-1}. The pH was adjusted to 7 ± 0.05 using dilute NaOH. Measurements were made with a DynaPro-801 TC (Protein Solutions) at an angle of 90° and a wavelength of 836 nm. Solutions were filtered with 0.1 μm Anotope syringe filters (Whatman) during injection. A minimum of 15 data points were taken for each sample. Size distribution analysis was done using the Regularization algorithm. All experiments were performed in triplicate at 25 ± 0.2 °C.

2.4 Results and Discussion

2.4.1 Determination of PEO Tailblock M_n and Polydispersity

The M_n of the PEO oligomers determined via SEC were comparable to those established by ^{1}H NMR (Table 3.1). Reaction conditions were based upon 1923 and 4884 g mol^{-1} molecular weights. Additionally, the SEC results indicated that the molecular weight distributions of the PEO chains were narrow (the PDI of the 1923 and the 4884 g mol^{-1} oligomers were recorded as 1.06 and <1.03, respectively) (Figure 3.2).

Table 2.1: PEO M_n determined via ^{1}HMR and GPC.

M_n (g mole^{-1})	**M_n (g mole^{-1})**	**PDI**

^{1}H NMR	GPC	
1930	1770	1.06
4844	4940	< 1.03

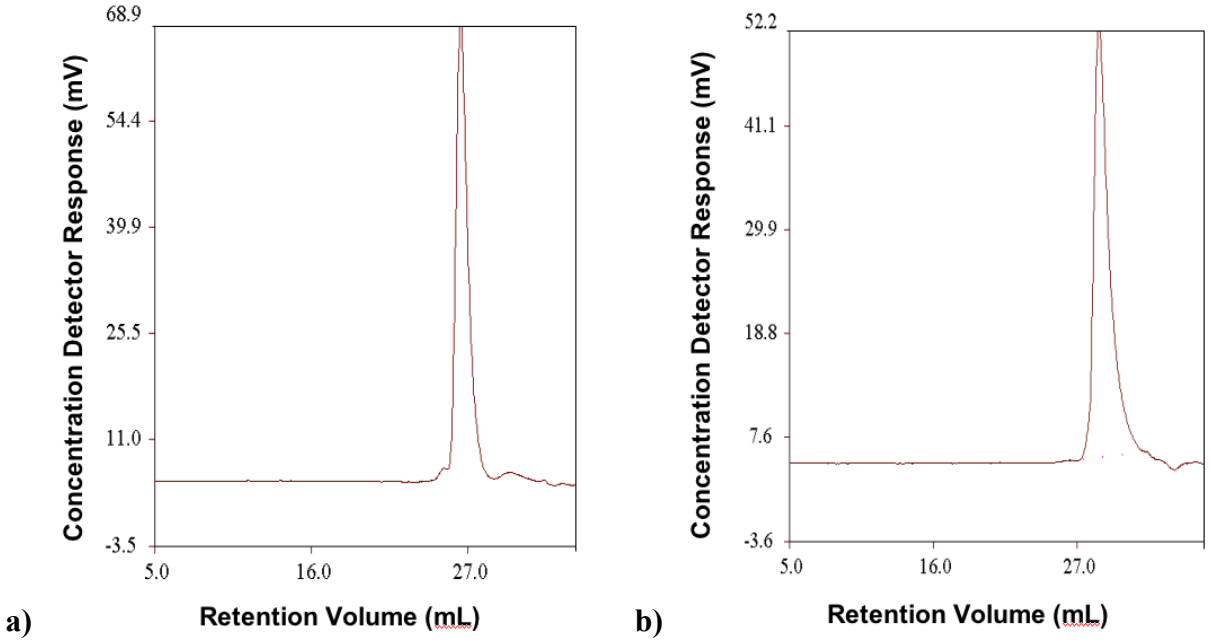


Figure 2.2: GPC Chromatogram for a) 4844 g mole^{-1} PEO and b) 1930 g mole^{-1} PEO

2.4.2 Synthesis of Tosylated PEO Macroinitiators

Synthesis of the tosylated PEO macroinitiator was monitored by ^{1}H NMR by following the disappearance of the PEO hydroxyl endgroup at 2.42 ppm and the appearance of the PEO methylene peak, which was bound to the tosyl endgroup. As the PEO was tosylated, these protons became nonequivalent to the protons of the repeat unit and the peak was observed at 4.19 ppm. ^{1}H NMR of the macroinitiator was performed to quantify the extent of tosylation (Figure

3.3). When the integration of the PEO terminal methoxy peak (e) at 3.41 ppm was set to 3, the terminal toluene peak of the tosyl group (f) was observed to also be 3. Moreover, integrating the peak of the PEO methylene protons bound to the tosyl group (c) gave a value close to 2. These results strongly suggested that these macroinitiators were quantitatively tosylated.

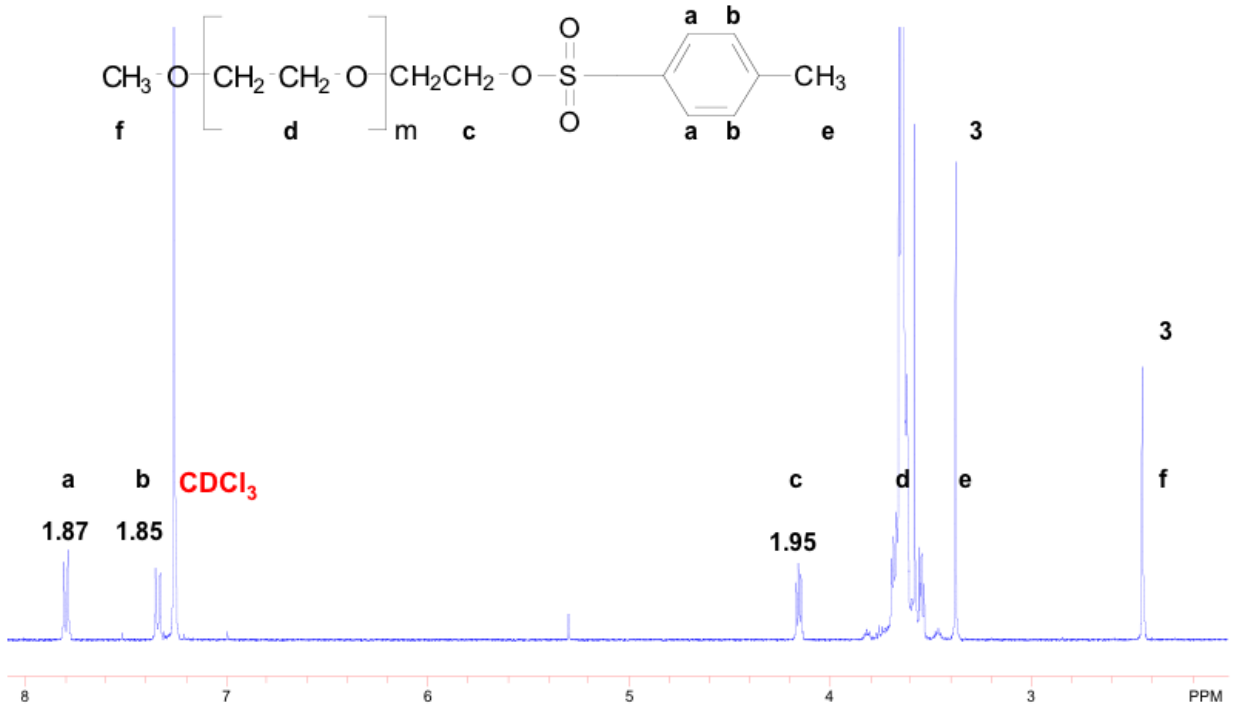


Figure 2.3: ^{1}H NMR of the1930 g mole^{-1} oligomer suggests quantitative tosylation of the PEO chains. The ratio of methoxy end group protons (e) to the protons of the tosyl end group (f) were equivalent.

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

Syntheses of the PEO-*b*-PEOX diblocks were carried out via cationic living ring-opening polymerization with the tosylate PEO macroinitiator serving as the initiating species for the synthesis of the PEOX block (fig. 3.1). The reaction was monitored via ^{1}H NMR to observe the disappearance of the nonequivalent methylene peaks on the monomer and the appearance of the single peak of repeat unit methylenes at 3.49 ppm. During the reaction, an orange color indicative of the living chain ends was observed. After termination with KOH/MeOH, two layers were formed with the polymer contained in a milky white chlorobenzene layer. The diblock copolymers were recovered by the same method that was employed for recovery of the macroinitator. ^{1}H NMR provided the PEOX block lengths and copolymer compositions as reported in Table 3.2**.** The integral of the terminal methoxy peak located at 3.4 ppm was set to 3 and the degree of polymerization was determined by the repeat unit backbone methylene protons and compared to the protons of the methylene (2.35 ppm) and methyl (1.11 ppm) group of the N-propionyl group of the repeat unit (fig. 3.4.). The experimentally determined PEOX M_n via ^{1}H NMR for the copolymers was in good agreement with the targeted block lengths.

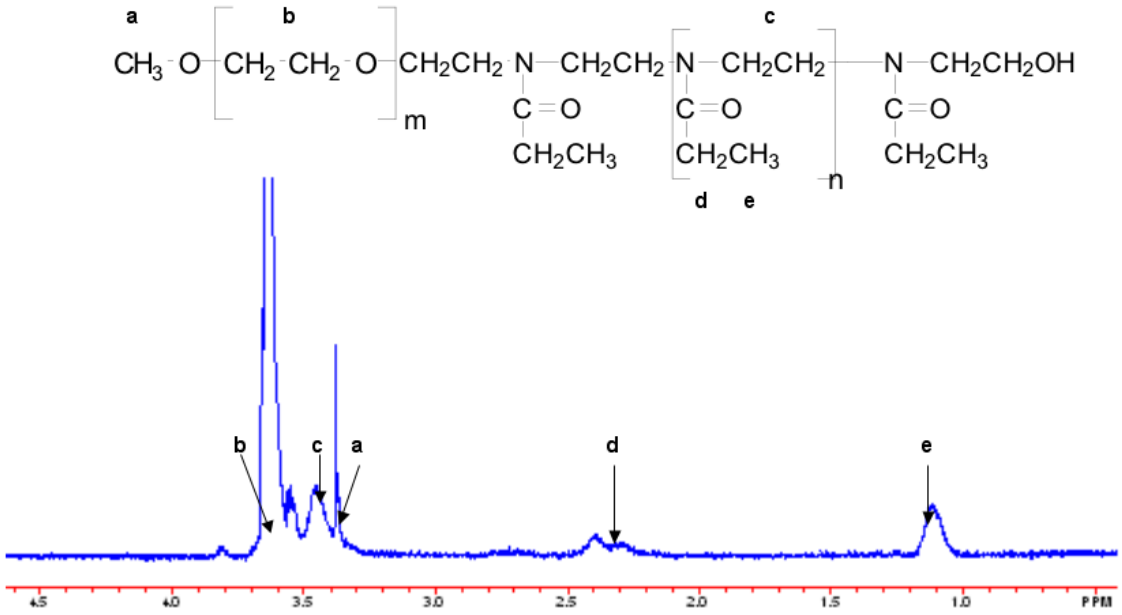


Figure 2.4: ^{1}H NMR spectra of PEO(5k)-*b*-PEOX(1.4k) with structural peak assignments.

Table 2.2: The composition of PEO-b-PEOX diblock copolymers.

Copolymer	**Targeted PEOX M_n (g mol^{-1})**	**Composition from ^{1}H NMR (PEO g mol^{-1} : PEOX g mol^{-1})**	**Average Repeat Units (PEO : PEOX)**
PEO(2k)-PEOX(0.6k)	550	1923 : 634	44 : 6
PEO(2k)-PEOX(1.2k)	1200	1923 : 1,250	44 : 13
PEO(5k)-PEOX(0.5k)	550	4884 : 540	111 : 5
PEO(5k)-PEOX(1.4k)	1,400	4884 : 1,470	111 : 15

PEO(5k)-PEOX(3k)	3,000	4884 : 2,978	111 : 30
PEO(5k)-PEOX(5k)	5,000	4884 : 5,040	111 : 51
PEO(5k)-PEOX(10k)	10,000	4884 : 8,910	111 : 90
PEO(5k)-PEOX(20k)	20,000	4884 : 23,405	111 : 236

2.4.4 Synthesis of Poly(ethylene oxide-*b*-2-methyl-2-oxazoline)

PEO-*b*-PMOX diblocks were synthesized via cationic living ring-opening polymerization in a manner comparable to the syntheses of the PEO-PEOX diblocks. The polymerizaiton of the PMOX block was followed via ^{1}H NMR. In likewise fashion for the PEO-*b*-PEOX diblocks, the disappearance of the nonequivalent methylene peaks on the monomer and the appearance of the single peak of repeat unit methylenes was observed. As the reaction progressed, a light gold color consistent with cationic end groups was observed. After recovery, ^{1}H NMR was employed to determine PMOX block lengths and copolymer composition (Table 3.3). There was good agreement with the experimentally determined PMOX M_n via ^{1}H NMR and the targeted block lengths.

Table 2.3: The composition of PEO-*b*-PMOX diblock copolymers.

Copolymer	Targeted PMOX M_n (g mol^{-1})	Composition from ^1H NMR (PEO g mol^{-1} : PMOX g mol^{-1})	Average Repeat Units (PEO : PMOX)
PEO(5k)-PMOX(1.4k)	1,400	4884 : 1,390	111 : 16
PEO(5k)-PMOX(5k)	5,000	4884 : 6,230	111 : 73
PEO(5k)-PMOX(10k)	10,000	4884 : 9,740	111 : 115
PEO(5k)-PMOX(20k)	20,000	4884 : 19,800	111 : 232

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

The procedure for conversion of PEOX to L-PEI via acid hydrolysis was adapted from the work of Jeong *et al*.[53] The PEO (5k)-*b*-PEOX (0.5k) was quantitatively hydrolyzed (99%). The remaining diblock copolymers were hydrolyzed to ~85% L-PEI conversion. Water solubility was preserved in the PEO-*b*-PEI copolymers for cases of longer L-PEI blocks by utilizing partial hydrolysis. ^1H NMR confirmed that the dialysis procedure utilized in recovering the copolymers was effective in removing the hydrolysis byproducts (fig. 3.5). The spectra of the polymers after dialysis were free of propionic acid, which was eliminated in hydrolysis of the PEOX blocks to form L-PEI.

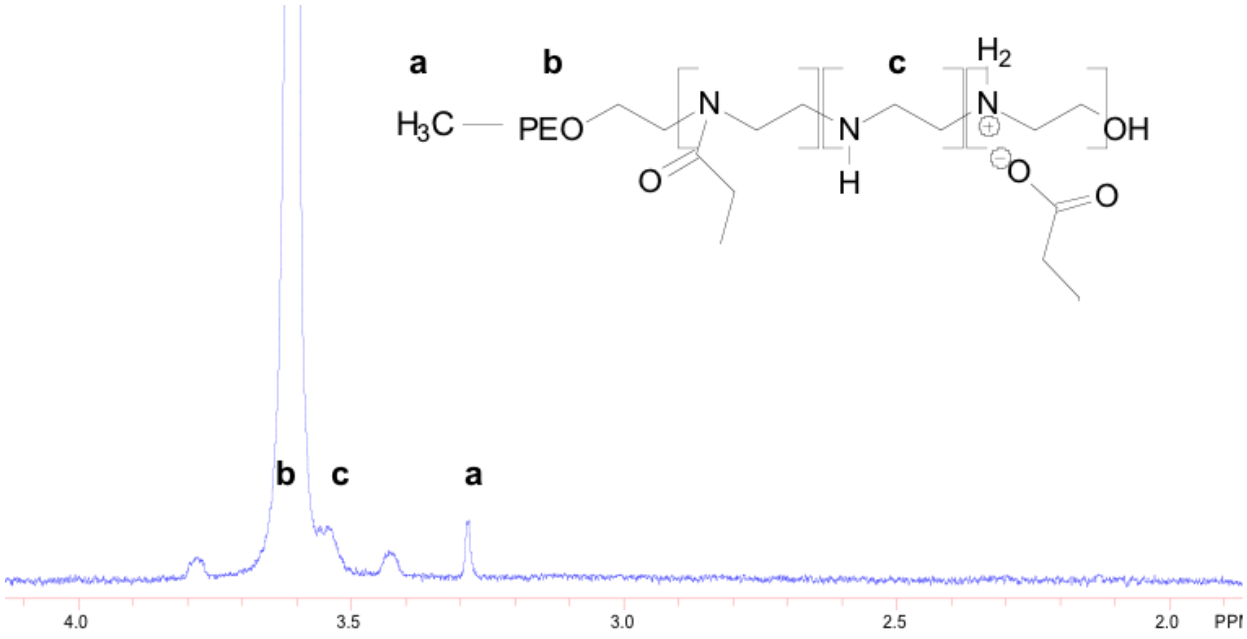


Figure 2.5: ^{1}H NMR of the purified 4884 g mole^{-1} PEO : 234 g mole^{-1} PEI (obtained via hydrolysis of the 4884 g mole^{-1} PEO : 540 g mole^{-1} PEOX) demonstrates the dialysis procedure was effective for removal of propionic acid. Since this polymer was quantitatively hydrolyzed, there also were no pendent methylene and methyl protons indicative of PEOX repeat units.

2.4.6 Solution Properties

DLS experiments indicated that the PEO-*b*-PEOX and the PEO-*b*-PEI diblock copolymers existed as single chains (unimers) when dissolved in water at pH 7 with <0.002M NaCl. At pH 4.8, PEI is approximately 50% protonated,[7] so the PEI block was only lightly charged. The hydrodynamic radii, R_H, of the PEO-*b*-PEOX copolymers were consistently larger than their PEO-*b*-PEI counterparts, which is reasonable in light of the larger PEOX pendant group. Values of R_H determined for the diblock copolymers are presented in Table 3.4, along with calculated size based on a random flight model:

$$\left\langle R_g^2 \right\rangle = \left(nl^2 C_\infty \right) / 6 \qquad (1)$$

In equation 1, R_g is the radius of gyration, n is the number of backbone bonds, l is the average bond length, and C_∞ is characteristic ratio for PEO. The radius of gyration is related to the hydrodynamic radius by the following, assuming non-draining spheres:[80]

$$R_H = 0.875 R_g \qquad (2)$$

Hydrodynamic radii were calculated for the copolymers using the characteristic ratio for PEO (4.05)[81] and PEOX (1.67)[82] for both series of copolymers with the assumption that the PEI block is not stiffer than the PEOX block.

Table 2.4: Solution properties of PEO-*b*-PEOX and PEO-*b*-PEI diblock copolymers.

Copolymer	PEO-PEOX Meas. R_H (nm)	PEOX Ave. Repeat Units	PEO-PEI Meas. R_H (nm)	R_H (Eq. 2) (nm)
PEO(2k)-PEOX(0.6k)	1.1 ± 0.5	7	-----	1.6
PEO(2k)-PEOX(1.2k)	2.0 ± 0.1	14	-----	1.7
PEO(5k)-PEOX(0.5k)	1.8 ± 0.3	6	1.5 ± 0.6	2.3
PEO(5k)-PEOX(1.4k)	2.0 ± 0.3	16	1.6 ± 0.6	2.5
PEO(5k)-PEOX(3k)	4.6 ± 0.3	34	1.6 ± 0.4	2.7
PEO(5k)-	5.9 ± 0.4	57	4.4 ± 0.3	2.9

PEOX(5k)				
PEO(5k)-PEOX(10k)	4.2 ± 0.6	115	2.9 ± 0.9	3.3
PEO(5k)-PEOX(20k)	5.1 ± 0.9	230	-----	3.8

2.5 Conclusions

Hydroxyl terminated monofunctional PEO oligomers were tosylated and utilized as macroinitiators for the cationic living polymerization of 2-ethyl-2-oxazoline to form PEO-*b*-PEOX diblock copolymers. The PEO-*b*-PEOX diblock copolymers were hydrolyzed to form well-defined PEO-*b*-(PEOX-*co*-L-PEI). Both copolymers remained as single chains in aqueous solution at pH 7 with the PEO-*b*-PEI copolymer having a smaller R_H than the corresponding PEO-*b*-PEOX copolymer.

CHAPTER 3 SYNTHESIS OF PEO-BASED POLYURETHANEUREA TRIBLOCK COPOLYMERS AND STABLIZATION OF MAGNETITE NANOPARTICLES

3.1 Abstract

There is considerable interest in the formation of magnetic magnetite nanoparticles, which are dispersible in water at biological pH (pH =7). Magnetic nanoparticles have potential utility in fluids for biomedical applications including magnetic field-directed drug delivery, magnetic cell separations, and blood purification.[62, 63] It is necessary to coat the magnetic particles with biocompatible materials for use *in vivo*. The reseach presented in this chapter continues the work of Harris[1] *et al*, who synthesized PEO based, polyurethane triblocks with pendant carboxylic acid groups for use in formation of stable aqueous magnetic fluids. A triblock polyurethaneurea was synthesized with 2070 g/mol poly(ethylene oxide-*b*-propylene oxide) tailblocks and a central carboxylate-functional polyurethaneurea block. The PPO content of the tailblock (~25%) was approximately the minimum amount of PPO required to disrupt the crystallinity of the PEO. The triblock was synthesized by forming the central polyurethane block with pendant acid groups first and then terminating the polymerization with the monohydroxyfunctional PEO/PPO polymer. Two urea linkages are present in each triblock due to the terminal amine groups on the PEO/PPO prepolymer. The PEO/PPO-polyurethaneurea formed complexes containing ~19% magnetite (81% polymer stabilizer) and ~48% magnetite (52% polymer stabilizer) that were dispersible in water.

3.2 Introduction

Magnetic nanoparticles have great utility in a variety of biomedical applications. These applications include magnetic field-directed drug delivery for chemotherapy, cancerous tumor

hyperthermia, magnetic cell separations, and blood detoxification.[16, 36, 83] The iron oxide magnetite (Fe_3O_4) is an ideal choice for use in biotechnology because it readily forms single domain, superparmagenetic nanoparticles. Additionally, magnetite has been shown to be safe for in vivo applications with a high LD_{50} in rats equal to 400 mg/kg.[84]

Magnetite nanoparticles share a significant challenge with other nanoparticles. The extremely large surface energies of the particles and the magnetic attractive forces drive the nanoparticles towards aggregation. Moreover, dispersions of bare magnetite particles at physiological pHs are hindered because it has an isoelectric point at pH = ~6.8. The implication is that uncoated magnetite nanoparticles cannot be stabilized against aggregation through electrostatic repulsive forces at the body's natural pH. The strong attractive forces of the nanoparticles and lack of electrostatic stabilization necessitate the use of steric stabilzation to prevent agglomeration. The selection of polymers which are biocompatible and water soluble aids the formation of magnetic complexes with low toxicity and high potential for use in the body.

This chapter reports the synthesis of an amphiphilic triblock polyureaurethane containing carboxylic acid pendant groups and poly(ethylene oxide) tails (fig. 4.1). The carboxylic acid groups readily bind to the surface of magnetite nanoparticles[85] and the PEO affords water dispersibility and reduced toxicity. The research builds upon the work of Harris[1] *et al*. and provides a new method for forming the amphiphilic triblocks.

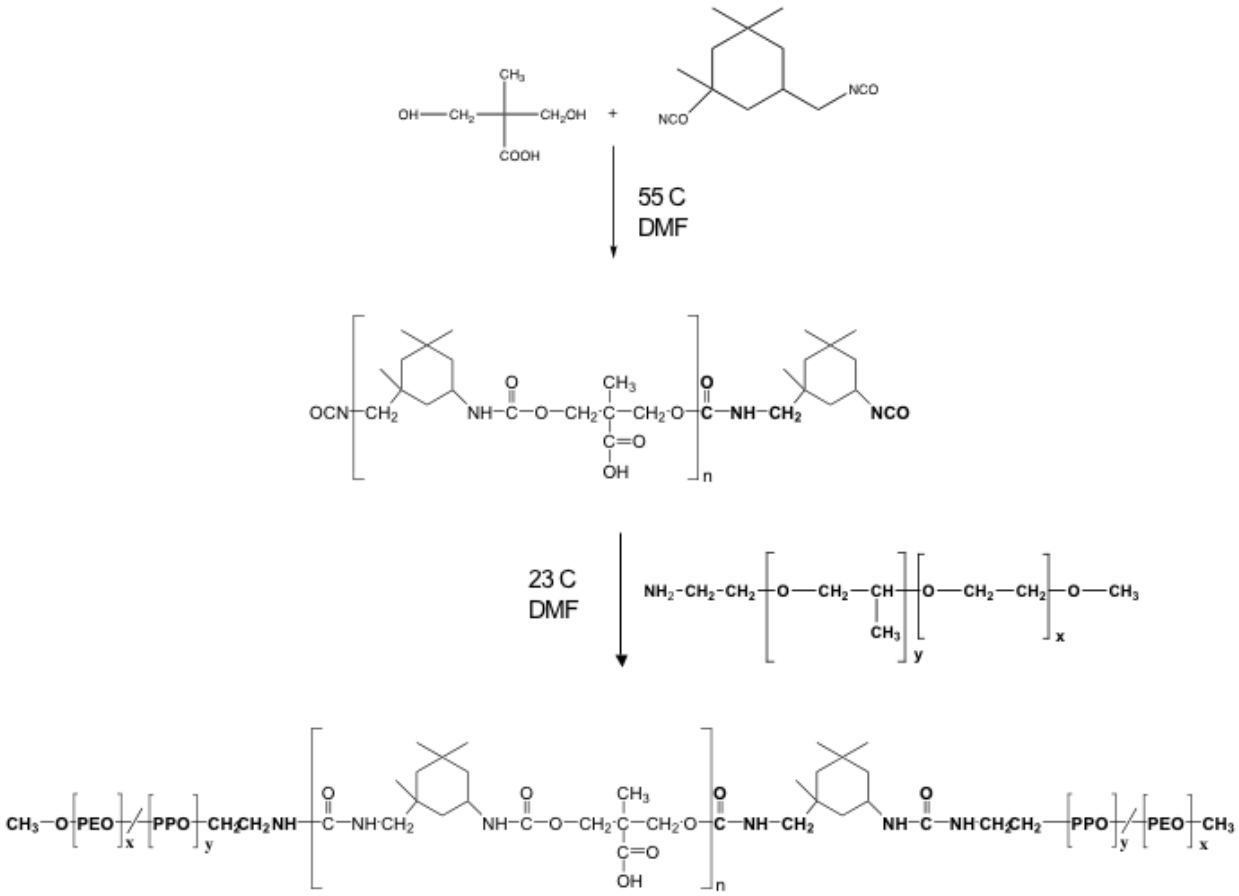


Figure 3.1: Synthetic scheme for the amphiphilic triblock stabilizer. The center block is a polyurethaneurea with pendant carboxylic acid groups for binding to the surface of the magnetite. The tailblocks of the copolymer are PEO.

3.3 Experimental

3.3.1 Materials

Amine terminated, monofunctional poly(ethylene oxide-*co*-propylene oxide) was donated from Huntsman Chemicals and had a molecular weight of 2070 g/mol. It was dried under vacuum at 60 ^{O}C for 24 h, then stored under nitrogen until used. Isophorone diisocyanate (Aldrich Chemical Co.) was dried over calcium hydride, distilled under vacuum, and stored under N_2 at 25 °C prior to polymerization. Bis(hydroxymethyl)propionic acid (Aldrich) was dried in a

vacuum oven at 60 °C for 2 days prior to use. $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ (Aldrich) were stored under N_2 in separate round bottom flasks that were wrapped in foil and then placed in a dessicator and used without further purification. All water used was filtered with Millipore gradient A10 (specific conductance ≈0.52 μS/cm) and deoxygenated for a minimum of 30 minutes with ultrahigh purity N_2 (99.9+%). Ammonium hydroxide (50% v/v aqueous, Alfa-Aesar) was deoxygenated prior to use for a minimum of 30 min with ultrahigh purity N_2. Dichloromethane (Burdick and Jackson) was used as received. Hydrochloric acid (Aldrich) was diluted to a 25 % v/v aqueous solution. Biotech grade DMF (99.9+%) was dried over calcium hydride and vacuum distilled before use.

3.3.2 Instrumentation

3.3.2.1 In situ Fourier Transmission Infrared Spectroscopy

A Bruker Tensor 27 infrared spectrometer outfitted with a Remspect high temperature *in situ* immersion probe was used to monitor the synthesis of the triblock polyurethaneurea stabilizers. After the reagents were added, time zero was noted and the instrument was programmed to acquire 1 spectrum every 15 minutes for 4 days. The disappearance of the isocyanate peak (2253 cm^{-1}) was followed to determine reaction progression.

3.3.2.2 Proton NMR spectroscopy

^1H NMR spectra were collected on a Varian Unity-400 spectrometer operated at a frequency of 399.952 MHz, with a 22° pulse angle, an acquisition time of 3.7 s, and a 1 s recycle delay. Spectra were taken of the triblocks in $CDCl_3$.

3.3.2.3 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed on a TGA Q1000 from TA Instruments, Inc. TGA samples were ramped from 25 °C to 600°C at 10 °C / min in a N2 environments.

3.3.2.4 Transmission electron microscopy (TEM)

A Philips 420T TEM run at 100 kV was used to obtain electron micrographs of the polymer/magnetite complexes. The polymer/magnetite complexes were diluted in water until a color resembling "weak tea" was achieved. Drops of the solution were cast onto a carbon-coated grid and the water was allowed to evaporate overnight.

3.3.2.5 Magnetometry

Analysis of magnetic properties was performed using a Quantum Design magnetic properties measurement system (MPMS-7) outfitted with a superconducting quantum interference device (SQUID). Magnetic measurements (σ) were taking at several applied fields (H) from -70,000—+70,000 Oe at 300 K and 5 K, with 100 Oe spacings between -1000 Oe and 1000 Oe. The samples were cooled in a zero applied filed and in an applied field (70,000 Oe) prior to conducting low-temperature measurements. The intention of these measurements was to establish the nanoparticles to be superparamagnetic. This was performed via analysis at behavior at low temperatures, the saturatization magnetization at 300 K, and the hysteretic behavior of the sample at 300 K. For each sample a Henkel plot was generated using methodology detailed by Allen *et al*.[86]

3.3.3 Synthesis of carboxylic acid containing triblocks

To synthesize the triblock, the center carboxylic acid containing block was formed first followed by termination with the PEO/PPO (Jeffamine) end blocks. The synthesis described

below is for a center block containing two carboxylic acids; however, the length of the central block can be easily changed to incorporate more or less acid groups as needed. A clean, dry flask was equipped with the *in situ* FTIR probe, a slow N_2 purge, and a magnetic stir bar. Isophorone diisocyanate (6.1 mL, 0.03 moles) was charged to the flask and heated to 55 °C. Bis(hydroxymethyl)propionic acid (2.9 g, 0.02 moles) was dissolved in a minimal amount of purified DMF (23.8 mL) and added to a the reaction vessel. At the addition of the bis(hydroxymethyl) propionic the first time point of the FTIR was taken.

The decrease of absorption from the isocyanate peak at 2253 cm^{-1} was monitored to follow its incorporation into the central oligomer. When 67% of the reactive isocyanate peak had been consumed, the reaction was cooled to room temperature. The formation of the central block required 43 hours. The reaction was then terminated via addition of the monofunctional PEO/PPO copolymer (Jeffamine) (15 g, 0.0145 mol dissolved in 15 mL of DMF). Termination occurred within 15 minutes after addition of the PEO/PPO oligomer and the reaction was allowed to stir at room temperature for two additional hours to ensure that all isocyanate end groups were reacted.

The first step in purification of the triblock was to remove the DMF at ≈53 °C and ≈500 mTorr. The polymer was dissolved in 200 mL chloroform, and precipitated into an excess of cold hexane.. After precipitation, the hexane was decanted, and the polymer was transferred to a round bottom flask, and dried overnight at 70 °C under vacuum.

3.3.4 Magnetite formation and steric stabilization

A procedure for preparing a stabilized magnetite composition using the triblock copolymer is provided. This composition targets 30 wt % magnetite in the polymer-magnetite complex. Prior to use, the water and NH_4OH were deoxygenated by bubbling Argon through the liquid for

30 min. The first reaction in the formation of the stable magnetite complexes is the formation of magnetite nanoparticles in anaerobic conditions at ambient temperature. Aqueous solutions of $FeCl_3 \cdot 6H_2O$ (0.389M, 2.0 g) and $FeCl_2 \cdot 4H_2O$ (0.195M, 0.736 g) were prepared separately under N_2 and combined into a 3-neck, 250-mL, round bottom flask equipped with a mechanical stirrer and pH electrode. After mixing the aqueous iron salts, NH_4OH (50% v/v aqueous) was rapidly added to the stirring solution until a pH of 9.5 was achieved (~10 mL). The solution quickly turned black, indicative of the formation of magnetite. The nucleation and growth of the particles was carried out for 30 minutes under an inert atmosphere.

Next, the N_2 purge was removed and a solution of the triblock copolymer dissolved in CH_2Cl_2 (2g triblock in 12 mL CH_2Cl_2) was added to the reaction and the heterogeneous mixture was stirred for 30 minutes at 25 °C to adsorb the polymer onto the magnetite surfaces. Then a N_2 purge was reintroduced to remove the CH_2Cl_2 for 2 h. At the end of the reaction, the complexes were neutralized to a biologically sustainable pH of 7.4 with dilute HCl (25% v/v aqueous). Particle aggregates were removed by centrifugation for 30 minute intervals which were repeated until there an absence of a pellet was observed. The particles were transferred to a dialysis membrane (Spectra pore 7, MWCO 1000) and were dialyzed against water for three days, refreshing the dialysis water twice/day. After dialysis, the water was removed from the magnetic complexes via rotovap and they were dried at 60 °C under vacuum overnight.

3.4 Results and Discussion

3.4.1 Synthesis of carboxylic acid containing triblock copolymers

Triblock copolymers comprised of a polyurethaneurea center block containing pendant carboxylic acid groups and PEO tail blocks were synthesized (fig. 4.1). The central block was bound to magnetite nanoparticles through interactions of the carboxylic acid groups with the

magnetite particle surfaces, and the tail blocks provided a hydrophilic brush layer around the particles that enabled their dispersion in water. A variation on the method based on the triblock synthesis recorded by Harris[1] *et al* was used and the center urethane block was formed prior to reaction with the PEO tailblocks. The impetus for this change was driven by a desire to form the polymer without the use of the dibutyltin dilaurate catalyst as used by the reported synthesis. It has been long desired to progress towards the incorporation of functional end groups on the PEO tailblocks for use in site specific targeting and fluorescence, yet it was found the catalyst coordinated unfavorably with certain biomoieties. To circumvent the use of the catalyst, the reverse procedure was developed to terminate the center block with an amino-functional PEO/PPO. The reaction between the isocyanate and the amine group to form a urea linkage can be done at room temperature without catalyst.

The reaction between the isophorone diisocynate and bis(hydroxymethyl) proprionic acid to form the urethane center block with carboxylic acid pendant groups was followed via *in situ* FTIR. To monitor the reaction, the decrease of the isocyanate peak (at 2270 cm^{-1}) as it was incorporated into the polymer was observed (fig. 4.2). When the peak exhibited a decrease by 75%, the reaction was cooled to room temperature for termination via addition of the jeffamine. After addition of the tailbocks, the complete disappearance of the isocyanate peak was observed (fig. 4.2). The complete synthesis of the triblock occurred in under 48 hours without the presence of catalyst.

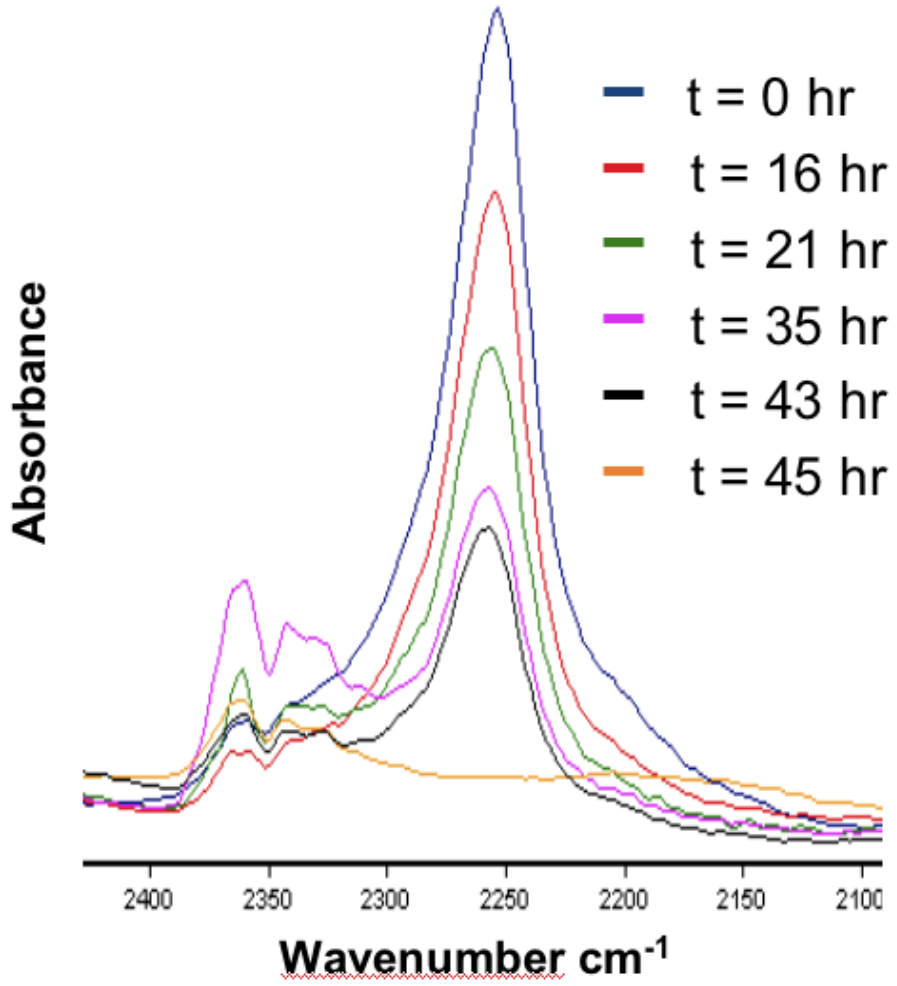


Figure 3.2: The reaction progress was followed via *in situ* FTIR by following the disappearance of the isocyanate absorption.

3.4.2 Magnetite formation and steric stabilization of nanoparticles

Two magnetite/triblock complexes were targeted, one containing 50 wt% magnetite and the other with 30 wt% magnetite. The triblock copolymer was adsorbed onto their surfaces and stable, aqueous dispersions of magnetite-polymer nanoparticles formed. TGA analysis of the two

complexes indicated they were 19% and 48% magnetite in composition, 81% and 52% polymer respectively. The average size of the magnetite nanoparticles was established with TEM to be approximately 10 nm in diameter and SQUID magnetrometry confirmed that the particles were superparamagnetic.

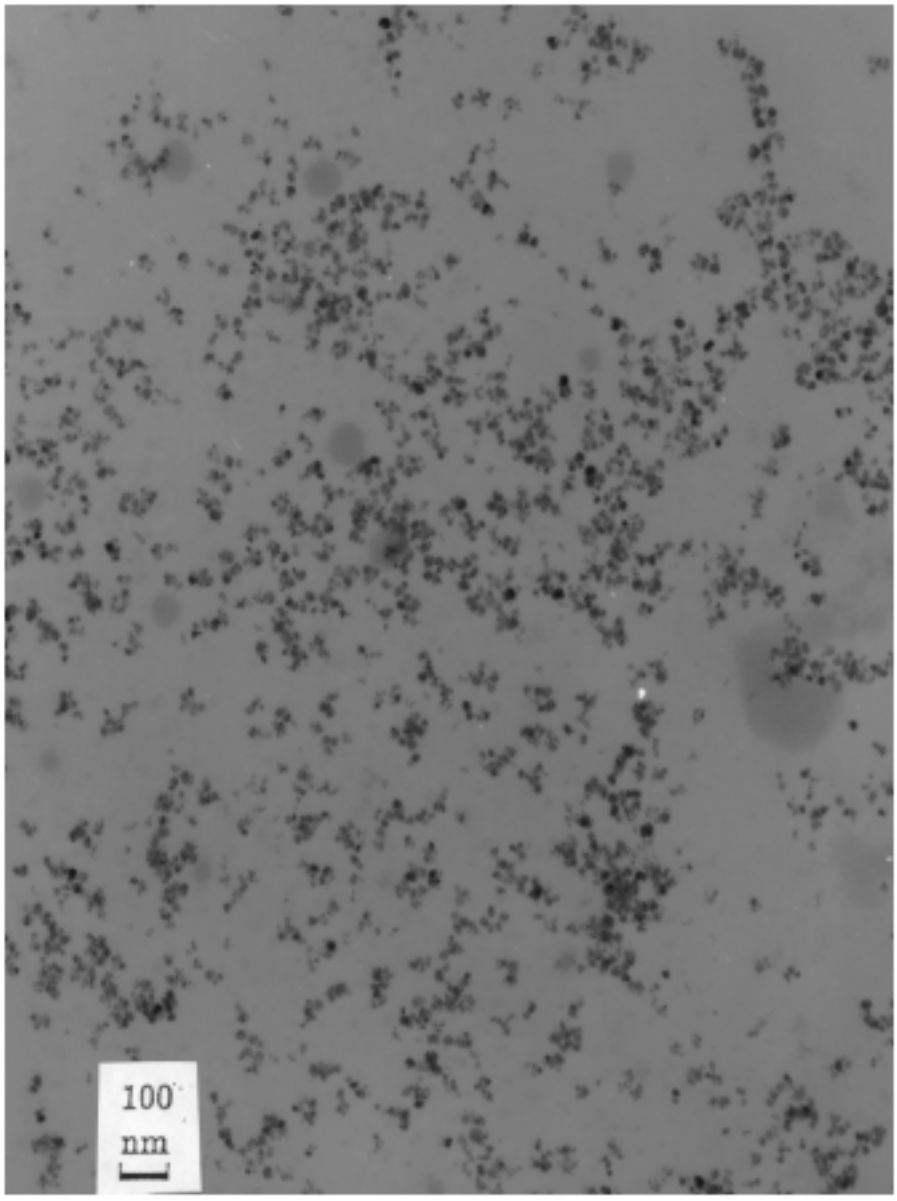


Figure 3.3: TEM showing magnetite-polymer complexes cast from water.

CHAPTER 4 SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Synthesis of Block Copolymers

Two series of well-defined poly(ethylene oxide-*b*-oxazoline) (PEO-*b*-POX) diblock copolymers were synthesized from tosylated-PEO homopolymers. The PEO homopolymer molecular weights were 1923 g/mol and 4884 g/mol and were used as macroinitiators for poly(2-ethyl-2-oxazoline) (PEOX) and poly(2-methyl-2-oxazoline) (PMOX) blocks. Table 5.1 outlines the block compositions of the PEO-POX diblocks as determined via ^{1}H NMR. The PEOX blocks of the PEO-PEOX diblocks were successfully acid hydrolyzed to yield PEO-PEI diblock copolymers.

Table 4.1: Block compositions of PEO-PEOX and PEO-PMOX diblock copolymers determined by ^{1}H NMR.

PEO : PEOX M_n (g mol^{-1})	PEO : PMOX M_n (g mol^{-1})
1923 : 634	4884 : 1,390
1923 : 1,250	4884 : 6,230
4884 : 540	4884 : 9,740
4884 : 1,470	4884 : 19,800
4884 : 2,978	-
4884 : 5,040	-
4884 : 8,910	-
4884 : 23,405	-

Dynamic light scattering was used to analyze the solution properties of the PEO-PEOX and the PEO-PEI diblock copolymers in aqueous solution. It was determined that both series of diblocks remained as single chains at pH 7. Each PEO-PEI diblock was found to have smaller R_H than the analogous PEO-PEOX diblock. Moreover, increasing the molecular weight of the PEOX and PEI blocks in both copolymers corresponded to an increase in R_H, with R_H values of 2-5.5 nm and 2-3 nm for the PEOX and PEI containing copolymers, respectively.

Future considerations for PEO-POX and PEO-PEI systems would include investigating their role as potential non-viral gene transfer vectors. It would be of interest to determine the binding efficacy of the PEI block to DNA and compare it to values determined for L-PEI homopolymer. Also of interest would be to study the solution properties of PEO-PEI/DNA complexes in aqueous solutions to determine the level of significance the PEO blocks play in developing a water soluble polycation/DNA complex. Cytotoxicity testing of the PEO-PEI would also be prudent to ensure the polymers are biocompatible for use as gene delivery vehicles.

A triblock comprised of a urethane center block with pendant carboxylic acid groups for binding to the surface of magnetite particles and PEO/PPO tailblocks was synthesized. The synthesis was carried out without a potentially toxic metal catalyst. The triblock stabilizer was used to prepare water dispersible, stable magnetite/polymer complexes. TGA analysis of these complexes indicate they were 19% and 48% magnetite in composition, which was close to the targeted amounts of 30% and 50%, respectively.

Continuing research towards developing magnetite particle dispersion stabilizers that have biological moieties and fluorescent tags is of interest. These specific groups could be bound to the ends of the PEO tailblocks and could impart cell or organ targeting capability for applications such as cell recognition, blood detoxification, and targeting of drugs.

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