Synthesis, characterization, and application of biodegradable polymeric prodrug micelles for long-term drug delivery

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Abstract

Synthesis, characterization, and application of biodegradable polymeric prodrug micelles for long-term delivery.

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Long-term drug delivery has several advantages such increasing bioavailability of drugs, reduction of pharmaceutical side effects, and increased patient compliance. Nanoparticles, such as block copolymer micelles, worm-like micelles, liposomes and polymersomes, have been proposed recently in the literature as promising drug delivery vehicles. Specifically, block copolymer micelles have advantages for drug delivery such as small size (< 100 nm) and hydrophilic outer shell that provides for increased \textit{in vivo} half-life imparting a “stealth” nature to the particles. Drugs covalently attached to a polymer chain, known as prodrugs, can significantly enhance drug incorporation efficiency. Additionally, the drug release could be further controlled through the conjugate chemistry.

In this work a biodegradable block copolymer prodrug was synthesized with the antipsychotic drug haloperidol. These polymeric prodrugs were formulated into nanoscale micelle-like structures, which were then characterized for size, morphology, stability, and drug loading capability. Micelles were found to be stable for use as a drug delivery vehicle, remaining intact upon dilution below the critical micelle concentration. \textit{In vitro} release was evaluated from various formulations of micelles, and ranged from 3 to 5 days. Release from polymeric prodrug micelles most closely approached a linear release profile. Furthermore, \textit{in vivo} behavioral studies were performed to assess haloperidol bioactivity from drug loaded micelles on ketamine induced hyperlocomotion. Results were consistent with \textit{in vitro} release data, showing that conjugate and combination micelles continued to release drug 4 days post injection, attenuating the effects of the ketamine induced hyperlocomotion. Additionally, results indicate that the sedative side effects of haloperidol were reduced with the micelle delivery systems as compared to the acute haloperidol injection.
CHAPTER 1: INTRODUCTION

The main objectives for a drug delivery system can include all or some of the following characteristics: increase a hydrophobic drug’s solubility in vivo, reduce drug toxicity, improve drug bioavailability, target drug delivery to a specific site, and sustain release systemically. Polymers offer an engineering strategy that can optimize the chemical and physical properties to achieve these aspects of a drug delivery system. The most widely used polymers for drug delivery have been poly ε-caprolactone (PCL), poly (alkylcyanoacrylate) (PACA), poly (lactic acid) (PLA), and the copolymer, poly (lactide-co-glycolide) (PLGA) [1, 2]. In addition, block copolymers of PLA and poly (ethylene glycol) (PEG) or poly (amino acids) have been used to make nanoparticles, polymersomes, worm-like micelles and micelle-like structures [3-5]. These polymers are known for both their biocompatibility and resorbability through natural pathways. Moreover, the use of PEG on particle surfaces can reduce opsonization by preventing protein adsorption on the particle surface and therefore slow clearance by the immune system.

During the 1980s and 1990s several microparticle drug delivery systems were developed to improve the efficiency of drugs and minimize toxic side effects [6, 7]. Initial promise for microparticles was dampened by the fact that there was a size limit for the particles to cross the intestinal lumen into the lymphatic system following oral delivery. Likewise, the therapeutic effect of drug-loaded particles was relatively poor due to rapid clearance of the particles by phagocytosis. In recent years some headway has been made in solving this problem by the addition of surface modifications and active targeting moieties to nanoparticles. Nanoparticles have a further advantage over larger
microparticles, because they are better suited for intravenous (IV) delivery. The smallest capillaries in the body are 5 to 6 \( \mu \text{m} \) in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 \( \mu \text{m} \), without forming aggregates, to ensure that the particles do not form an embolism [8].

Nanoparticles, including solid polymer nanoparticles, liposomes, polymersomes, worm-like micelles, and block copolymer micelles, have become an important area of research in the field of drug delivery because they have the ability to deliver a wide range of drugs to varying areas of the body for sustained periods of time. There is a medical need for long-term circulating carriers to maintain steady-state drug levels, for enhanced imaging capabilities, and as synthetic blood substitutes. The small size of nanoparticles is integral for systemic circulation.

This research focuses on the delivery of a model compound, haloperidol, via block copolymer micelles made from a PEG-PLA prodrug. To achieve the polymeric prodrug structure haloperidol was covalently linked via an ester bond to the end of the PLA polymer block. Block copolymer micelles have great potential as drug delivery vehicles because of their small size, ease of synthesis, and their hydrophilic outer shell. It is therefore the goal of this work to fully characterize the production of a polymeric prodrug and its subsequent synthesis into nanoscale micelle-like structures. Finally, these polymeric prodrug micelles are evaluated for their potential as a long-term drug delivery vehicle.
1.1 References


CHAPTER 2: BACKGROUND

2.1 Controlled drug release

Conventional oral drug delivery involves the periodic dosing of a compound which results in drug levels which oscillate around the desired steady state level, and between the side effect level and the minimum therapeutic level, within the ideal therapeutic window (Figure 2.1). For example haloperidol, an older antipsychotic medication used to treat schizophrenia, is given as 1 to 2 mg doses 3 to 4 times per day. A long-term delivery system, which would maintain a steady state level of drug in the plasma, eliminates the potential for missed doses, and therefore severe fluctuations outside the therapeutic window as seen in Figure 2.1. The half-life of a drug is the point at which half of the drug has been eliminated from the body. In Figure 2.1 the half-life is designated at 24 hours. After three doses at the half-life frequency, the drug concentration oscillates around the mean steady state drug level. The effects of partial compliance are illustrated when the patient misses dose 6, 7, and 8. For the chosen model, the patient is without desired drug level (dashed line) for 6 days after missing three doses. Also note, the amplitude of the oscillations is dependent on the frequency of the dosing, such that more frequent administration of smaller doses will result in tighter oscillations around the mean. These oscillations will approach a straight line as the frequency of dosing approaches constant infusion. One goal of long-term delivery platforms is to approach that ideal constant infusion profile. Many pharmaceutical compounds used with conventional dosing have drawbacks such as adverse side effects, severe fluctuations in plasma concentration, poor bioavailability, and poor patient adherence. Above the therapeutic window the patient is likely to experience side effects,
Figure 2.1 Conventional oral drug delivery showing the effects of noncompliance
and below the effective level the drug concentration is not sufficient to be effective in the body. Drugs with low oral bioavailability or a short systemic duration require frequent administrations, which adversely affect patient adherence. For example, high doses of antibiotics and antiparasitics are administered to treat gastrointestinal bacteria and parasites since only 10 to 15% of the drug administered is absorbed [1]. The increased mucoadhesivity of nanoparticles could be effective in treating these pathogens with lower doses of drugs.

Patients that require drugs that necessitate chronic administration, frequent administration, or exhibit extreme side effects above the intended level, would benefit from the ability to maintain constant plasma levels within the therapeutic window, i.e. a uniform release of the drug over time. Such a profile can potentially be achieved using polymeric drug delivery devices to release a compound in a controlled and predictable manner. Many drug delivery vehicles such as microparticles and implants improve the release profile over a longer period of time than conventional oral dosing. Furthermore, it has been proposed that these benefits will extend to additional technologies including nanoparticles, polymeric micelles, and polymersomes [2-4]. The polymer matrix can also serve to protect drugs with reduced stability, such as proteins, from harmful stomach acids prior to absorption [5].

2.1.1 Motivation and medical need

Recently, there has been increased interest in promoting improved medication adherence through new delivery systems across a broad array of medical specialties and diseases. The introduction of the transdermal contraceptive patch has allowed better adherence through the systemic delivery of hormones to prevent pregnancy [6].
Similarly, there have been advances in patch technology to yield novel transdermal therapeutic systems for the delivery of buprenorphine for pain relief [7]. Biodegradable microparticles and nanoparticles are also being developed to deliver a wide range of drugs from anti-tumor agents to vaccines [8-12]. These new methods of medication administration have provided patients with additional options to aid in medication adherence.

The main objectives for a drug delivery system can include all or some of the following characteristics: to increase a hydrophobic drug’s solubility in vivo, to reduce drug toxicity, improve drug bioavailability, target drug delivery to a specific site, and sustain release systemically. A drug’s bioavailability is the fraction of therapeutically active compound that reaches systemic circulation from any route of administration.

2.2 Biodegradable polymers

Biodegradable polymers retain their properties for a limited period of time in vivo and then gradually degrade into materials that can become soluble or are metabolized and excreted from the body. In order to be used for in vivo applications the polymers used for such systems must have favorable properties for biocompatibility, processability, sterilization capability, and shelf life.

The polymer chosen to formulate the nanoparticles will strongly affect the structure, properties and applications of the particles. For each application and drug, one must evaluate the properties of the system (drug and particle) and determine whether or not it is the optimal formulation for a given drug delivery application. In one example a carboxylic end group of PLGA was conjugated to a hydroxyl group of doxorubicin and formulated into nanoparticles [13]. This modification produced a sustained release of the
drug that was approximately six times longer than with unconjugated drug [14]. In another case, the anticancer protein drug cystatin was encapsulated in PLGA nanoparticles and it was found that a number of factors from the formulation parameters to the polymer structure had an impact on preservation of the protein activity [15]. For example, the presence of a carboxylic end group on PLGA may play an important role in the preservation of the protein drug’s activity throughout the release period [15]. However, the presence of the carboxylate end group can increase the initial rapid release of drug from these particles by increasing the hydrophilicity of the polymer matrix and thus the diffusion rate of water into the polymer matrix. Under differing circumstances a methyl capped polymer could be used to dampen the initial release rate. Conversely, Budhian et. al. have proposed that carboxylic acid end groups on PLGA can significantly increase drug loading and decrease the initial burst release from the particles [16]. Consequently, one can see that the choice of a single parameter, such as the polymer, can have a different impact on each polymer drug system.

2.2.1 Poly (lactic acid)/Poly (lactic acid-co-glycolic acid)

Many biodegradable systems rely on the random copolymers of PLGA, (Figure 2.2A) or the homopolymer PLA (Figure 2.2B). These classes of polymers are highly biocompatible and have good mechanical properties for drug delivery applications [17-21]. In addition, PLGA and PLA have been approved by the FDA for numerous clinical applications, such as sutures, bone plates, abdominal mesh, and extended-release pharmaceuticals [22-24]. The biomedical uses of PLA have been reported since the 1960s [25]. Numerous systems already utilize PLA to successfully achieve long term delivery, including several microparticle and nanoparticle systems, such as controlling
metabolism using a thyrotropin-releasing hormone [26], l-dopa to treat Parkinson’s Disease [27] and naltrexone in treating narcotic addiction [28]. These polymers degrade chemically by hydrolytic cleavage of the ester bonds in the polymer backbone. Its degradation products, lactic acid and glycolic acid, are water soluble, non-toxic products of normal metabolism that are either excreted or further metabolized to carbon dioxide and water in the Krebs cycle [26, 29]. PLA degradation is represented schematically in Figure 2.3. PLGA and PLA belong to a broad class of polymers known as polyesters, and can be synthesized by a polycondensation reaction, or ring-opening polymerization (Figure 2.4) [30-32]. Ring-opening polymerization is currently the preferred method for synthesis for PLGA and PLA due to shorter reaction times and higher monomer conversion rates. PLA occurs naturally as the pure enantiomeric poly (L-lactic acid) (LPLA) with a semicrystalline structure. However, most types of PLA used for biological applications exist in the racemic D, L form (DLPLA) and are amorphous polymers. DLPLA and PLGA have glass transition temperatures above body temperature. glycolic acid monomeric units, greatly influences the degradation rate [30]. A higher proportion of glycolic acid monomers incorporated into the copolymer will increase the degradation rate, and subsequent erosion, by increasing the hydrophilicity of the polymer and allow more biological fluids to penetrate the polymer matrix. In addition, the slower degradation by PLA is in part due to the steric effect of the alkyl group which hinders the hydrolytic attack by water [33].
Figure 2.2 Chemical structures of poly (lactic acid – co –glycolic acid) (PLGA) (A) and poly (lactic acid) (PLA) (B)
Figure 2.3 Schematic hydrolysis of poly(lactic acid) into monomer units
Figure 2.4 Schematic of lactide and glycolide monomers for ring-opening polymerization, and final products, PLGA and PLA.
2.2.2 Poly (ε-caprolactone)

PCL is a biodegradable and nontoxic polyester (Figure 2.5A). PCL is similarly polymerized to PLA and PLGA, by ring-opening polymerization [34, 35]. PCL is a semicrystalline polymer due to its regular structure. Its melting temperature is above body temperature (59-64°C), but its $T_g$ is $-60^\circ$C, so in the body the semicrystalline structure of PCL results in high toughness, because the amorphous domains are in the rubbery state [36, 37]. Hydrolysis of PCL yields 6-hydroxycaproic acid which enters the citric acid cycle and is metabolized. Since PCL degrades slowly it has been used in blends and copolymers with other biodegradable polymers [37, 38]. Combinations of polymers allow the user to tailor mechanical properties and degradation kinetics, among other characteristics, to suit the needs of a specific application. PCL has been used clinically as a degradable staple for wound closure and as a 1-year drug delivery system for contraceptives [37]. The amorphous regions of a semi-crystalline polymer degrade prior to the crystalline domains, leading to a change in the release profile [39]. Thus, polymers that have a higher percent crystallinity are more impervious to water and therefore degrade at a slower rate. The drug entrapped in the amorphous region is released first and at a faster rate than the drug entrapped by the crystalline domains. The percent crystallinity in a polymer depends on the type of polymer used in the application, the polymers composition, and the processing conditions for the polymer system. PCL typically has the highest percent crystallinity and the slowest degradation rate as compared to the most common biodegradable polymers typically used for drug delivery such as PLA or PLGA.
Figure 2.5 Poly (ε-caprolactone) (A), Poly (alkylcyanoacrylate) (B), Poly (ethylene glycol) (C).
2.2.3 Poly (alkylcyanoacrylate)

Biodegradable and biocompatible cyanoacrylate-based nanoparticles have been used for drug delivery as well (Figure 2.5B) [40-42]. They are considered to be promising drug delivery systems due to their mucoadhesive properties and ability to entrap a variety of biologically active compounds. PACA is not fully biodegradable, being comprised of an acrylate backbone with pendant ester groups [43]. The byproducts of degradation are alkyl alcohol and poly(cyanoacrylic acid), which at low molecular weights can be renally excreted. Cyanoacrylate based nanoparticles are synthesized by dispersing monomer in an aqueous solution at a low pH in the presence of an emulsifier [44]. While the nanoparticle formation differs from the more common polyester nanoparticle formation, some of the same issues for drug delivery must be confronted, such as burst release, particle size, and the effect of the emulsifier on these parameters [45]. Moreover, these polymers have a more rapid degradation rate than PLGA copolymers, which in some cases may be more desirable, for instance when multiple dosing of nanoparticles is required. However, one must consider that a rapid degradation rate may increase toxicity with high concentrations of breakdown products.

Many types of PACA nanoparticles with varied alkyl chains have been used for drug delivery. Poly (butylcyanoacrylate) (PBCA) nanoparticles have been successful in delivering drugs to the brain [46]. Sham et. al. were able to successfully deliver PACA nanoparticles encapsulated within carriers into the lung for potential drug administration application [47]. PACA nanoparticles have been polymerized around a magnetic core, thereby masking the electrical surface properties of the magnite, which has promising medical applications [48]. PECA nanoparticles have also been prepared using the
emulsion polymerization technique in the presence and absence of poly (ethylene glycol) (PEG), using Pluronic F68 as the stabilizer. As expected, PEG was shown to increase the release rate in vitro and impart some stealth capabilities to the particles [42]. In addition, PEGylating PACA particles may aid in the reduction of particle toxicity [49]. PEGylated PACA nanoparticles were also used to deliver paclitaxel to tumor sites using conjugated transferrin to actively target cancer cells [41].

2.3 Poly (ethylene glycol)

PEG is a hydrophilic, nonionic polyether that has been shown to exhibit excellent biocompatibility (Figure 2.5C). PEG is nontoxic and has been approved by the FDA for internal consumption [50]. Intravenously administered PEG is excreted by the kidneys [51]. In aqueous solutions PEG acts as a highly mobile molecule with a large exclusion volume [50]. PEG is a neutral polymer with hydroxyl end groups that are weak hydrogen bond acids, and weakly basic ether linkages in the backbone. PEG molecules can be added to drug delivery vehicles via a number of different routes including covalent bonding, blending during preparation, or surface adsorption [49, 52-56]. PEG can be modified at either end group for attachment to other molecules or polymers. When PEG is covalently attached to protein, most of the bioactivity is maintained, and when covalently linked, PEG can help solubilize other molecules. PEG is soluble in water and several organic solvents [50].

One of the greatest interests in PEG for drug delivery systems is its ability to extend residence time in the body. The mononuclear phagocytic system (MPS) (also known as the reticuloendothelial system) is encompassed by a range of cells capable of phagocytosis. The primary functions of the MPS are to remove senescent cells from
circulation and to provide phagocytic cells for both inflammatory and immune responses. Phagocytosis is a special form of endocytosis in which large particles are ingested by phagosomes. The immune system recognizes hydrophobic polymer particles as foreign bodies and thus they are rapidly cleared ending up in the liver or the spleen [57]. If sustained systemic circulation is required then the nanoparticle surface must be modified in order prevent this rapid clearance [53]. PEG-containing PLGA nanoparticles have been shown to extend the half life of the protein, bovine serum albumin, in rats from minutes to hours [54, 58]. PEG modification can extend the particle half lives in humans up to 45 hours [59]. Another study compared the pharmacokinetics of PLGA nanoparticles versus PEG-PLGA nanoparticles. The pharmacokinetics of PLGA nanoparticles were dose dependent, with decreased particle clearance at higher doses, indicating saturation of the MPS. However, the pharmacokinetics of mPEG-PLGA nanoparticles did not exhibit the same dose dependent clearance and as expected, they exhibited a longer residence time compared to the PLGA nanoparticles [60]. Because of this behavior, PEG functionalized nanoparticles are often referred to as “stealth nanoparticles” [55].

The presence of PEG on the surfaces of particles reduces protein adsorption by a combination of elastic and osmotic contributions [59]. As proteins approach the particles surface, the volume restriction due to the PEG chains results in a loss of their conformational entropy. As proteins approach the surface, the number of available PEG conformations is reduced due to compression of the polymer chains by the protein. In this region of interfacial mixing, a positive heat of solution can also result. The reduction in entropy and increase in enthalpy result in an increase of the free energy of mixing
between the particle and the protein, the outcome of which is protein separation from the particle surface. The osmotic contribution occurs when particles approach one another, there is an increase in polymer concentration in the area between the surfaces. This necessitates an influx of water, forcing separation [61]. While the presence of PEG on the particle surface extends particle half-life in vivo, a population of stealth particles still undergo rapid hepatic and splenic clearance after administration. It has been proposed that this may be due to heterogeneity of PEG density on particle surfaces [62]. This may also be caused by a limited pool of opsonic factors yet unidentified, which are unaffected by the volume restriction properties of PEG. These factors may be limited in concentration, especially under normal physiological conditions [59]. While PEGylation does not completely suppress complement activation, longer PEG chains were found to have better steric/osmotic suppression. Macrophages may also be able to recognize particles that sterically repel normal plasma opsonins, but this recognition seems to occur only during abnormal physiological conditions i.e. pathological sites such as tumors or infection sites [63].

The degree to which proteins adsorb onto particle surfaces can be minimized by increasing the PEG density on the particle surface and increasing the molecular weight of the PEG chains used [53]. For example, Leroux et al. [21] showed that an increase in PEG molecular weight in PLGA nanoparticles was associated with less interaction with the MPS, and longer systemic circulation. PEG has been shown to impart stability on PLA particles submerged in simulated gastric fluid (SGF). Tobio et. al. showed that after 4 hours in SGF, 9% of PLA nanoparticles converted to lactic acid versus 3% conversion for PEG-PLA particles [56]. PEG is also believed to facilitate mucoadhesion and
consequently transport through the Peyers patches of the GALT [64]. In addition, PEG may benefit nanoparticle interaction with blood constituents. PLGA nanoparticles have been shown to cause damage to red blood cells, while PEGylated nanoparticles cause less damage [65]. It should be noted that the red blood cell damage was also concentration dependent. The presence of proteins adsorbed on to PLGA particle surfaces can also retard degradation [56]. Consequently, the presence of PEG has a number of important functions for the use of polymeric particles for drug delivery.

2.4 Drug release

Polymeric drug release occurs in one of two ways: erosion or diffusion. Release from biodegradable polymers is frequently governed by a combination of both mechanisms, which depends on the relative rates of erosion and diffusion.

Erosion is defined as the physical dissolution of a polymer as a result of its degradation [66]. The erosion of water insoluble polymers begins with degradation by chain scission via hydrolysis. This progressive degradation changes the structure of the polymer matrix through the formation of pores. This allows the release of the degradation products, i.e. oligomers/monomers, which ultimately leads to mass loss, or the erosion of the polymer [33, 67]. Hydrolysis is a reaction between water molecules and bonds in the polymer backbone, typically ester bonds, that repeatedly cuts the polymer chain until it is returned to monomers. Other biodegradable and natural polymers are enzymatically degradable, which is also a type of chain scission. As water molecules break chemical bonds along the polymer chain, the physical integrity of the polymer degrades and allows drug to be released.
There are two possible mechanisms of erosion. When water is confined to the surface of the matrix, chain scission will occur only on the surface and drug will be released as the polymer matrix erodes. In this case the degradation rate is faster than the penetration of water into the polymer bulk, this is called surface erosion (Figure 2.6A). The matrix degrades and drug is released only from the surface, while the internal regions remain unchanged. When a polymer’s structure and composition are heterogeneous, surface erosion can occur unevenly (Figure 2.6B). The matrix degrades and the drug is released from the surface, but since the polymer matrix is not homogeneous the surface degradation is not evenly distributed. If the diffusion of water into the polymer matrix is faster than the rate of hydrolysis then erosion will occur throughout the entire material, which is also called bulk erosion (Figure 2.6C). The matrix is degraded and drug is released from the entire volume of the system. As the polymer matrix is eroded, drug molecules are free to be released via diffusion as well. In many cases, the erosion of a polymer matrix in vivo is some combination of these mechanisms. Degradation by surface erosion alone may be preferred in some cases, because the degradation rate can be controlled through the surface area of the matrix [68]. This generally occurs with rapidly degrading polymers such as polyanhydrides and poly (ortho esters) [67]. However, for certain types of structures, such as a sphere, as surface erosion occurs over time, the overall surface area becomes smaller which in turn decreases the release rate [69]. More complex shapes are available to manage the evolving surface area to create the desired release rate [70].
Figure 2.6 Homogeneous surface erosion of a polymer matrix (A), heterogeneous surface erosion (B), bulk erosion (C).
The overall kinetics of bulk erosion, which is seen with polyesters such as PLGA and PLA, are complicated. The degradation rate of the polymer backbone follows first-order kinetics [71, 72]. However, erosion, or the mass-loss from the polymer bulk, is more complex. After a significant period where no mass loss occurs, erosion can set in spontaneously where there is rapid dissolution of oligomers or monomers from the matrix in a short period of time [67]. The latter process is dependent on a number of factors, namely: water uptake, dissolution and diffusion of the degradation products, and morphological changes in the matrix. This has an obvious impact on release of pharmaceutical agents from polymer matrices. Lower molecular weight PLA has been shown to maintain its mass for approximately 2 days [73]. It should also be noted that as degradation within the polymer bulk occurs, the presence of degradation products can alter the local pH and create a pH gradient throughout the polymer matrix [67].

Polymer molecular weight, while being an important determinant of mechanical strength, is also a key factor in determining the rate of release of compounds from biodegradable polymers. As chain scission occurs over time, pores are introduced into the polymer matrix. Low molecular weight polymers lose their structural integrity more quickly than high molecular weight polymers and as a result they erode faster. Consequently, lower molecular weight polymers release drug molecules more quickly [74, 75]. This can be used to further engineer a system to control the release rate. A combination of molecular weights could be used to tailor a system to meet the demands of specific release profiles.
In the case of diffusion-controlled release, the drug’s concentration gradient in the polymer matrix is the driving force for the molecules to diffuse into the surrounding medium.

The diffusion of a drug molecule through the polymer matrix is dependent upon the solubility of the drug in the polymer matrix and the surrounding medium, the diffusion coefficient of the drug molecule, the molecular weight of the drug, its concentration throughout the polymer matrix, and the distance necessary for diffusion. Drug can be either distributed evenly throughout the matrix or encapsulated as a reservoir [76]. The release rate for the reservoir system also depends on the membrane thickness and area. Practically, reservoir systems often have a lag period after placement in vivo, as opposed to the burst release present for most other systems. However, these systems need to be carefully engineered to prevent premature membrane rupture that might release a toxic amount of drug into the body.

When a drug is dissolved in the matrix and the mechanism for delivery is diffusion, then the driving force for release is the concentration gradient. Therefore, the release predictions can be made based on Fick’s laws of diffusion [68]. Cumulative release from diffusion controlled devices is inversely proportional to the square root of time [70]. This presents an engineering challenge because surface area becomes smaller due to degradation, with a resulting decrement in the release rate.

The specific chemical and biological characteristics of the drug and the polymer are crucial in designing a polymeric delivery system. For example, drugs with greater hydrophilicity can increase the overall release rate by promoting water uptake and therefore increasing degradation, which in turn increases drug release [77]. The drug’s
molecular weight, solubility in biological fluids as well as its miscibility in the polymer matrix will influence the drug’s diffusivity from the system and the concentration profile of the drug throughout the matrix. Since polymeric delivery systems are rarely homogenous throughout the entire matrix, the drugs diffusivity, and therefore release rate, can change with the local polymer composition and structure. One example of this phenomenon showing the effect of the drug and polymer interaction is the release of the prodrug 3-methoxyxanthone, and its active form xanthone, from PLGA nanocapsules [78]. The differing release profiles of these prodrugs versus the active drug from identical carriers suggest a physical interaction of the drug with the polymer when given the nanocapsule structure [78].

Frequently, diffusion controlled release is important in the early stages of drug release. For many of the polymeric delivery systems there is some concentration of drug molecules entrapped near and adsorbed onto, the surface of the matrix. Upon immersion into a medium, the release of these drug molecules is controlled by the rate of diffusion of the drug into the surrounding environment. In some geometries, this can cause a problem referred to as the “burst effect,” that can potentially release a toxic amount of drug into the body within the first 24 hours [79]. This burst release is part of what is frequently referred to as a biphasic release profile [12]. During the first phase, the burst release, the structural integrity of the nanoparticles is maintained. The second phase, or the linear release, is characterized by pore formation, particle deformation and fusion [17]. In Figure 2.7A two possible concentration profiles of drug molecules throughout the matrix are shown. Figure 2.7B shows a schematic corresponding to the release profile of drug molecules where a disproportionate percentage of drug is located near the surface and is
released rapidly upon immersion in vivo after which uniform release is achieved. Figure 2.7C represents homogeneous distribution of drug molecules throughout the polymer matrix, and consequently a more uniform drug release.

One proposed method for eliminating the burst release is by tailoring the collection of the nanoparticles to remove the drug attached to the surface. PLA nanoparticles with incorporated anti-ischemic drug N\textsuperscript{6}-cyclopentyladenosine were collected using gel filtration. Drug molecules near the surface were removed, and while the overall encapsulation efficiency was diminished, the burst release was eliminated from the particles [80]. It is also worth noting that increasing the theoretical loading of drug into nanoparticles often increases the burst release. For example, PLA nanoparticles containing 16.7% savoxepine released 90% of their drug load in 24 hours, as opposed to particles containing 7.1% savoxepine, which released their content at a reduced rate over three weeks [57]. Finally, polymer choice may have a significant effect on the burst release phenomena. When using polymers which interact with a drug, like PLGA with a free COOH group and proteins, the burst release is lower and in some cases absent, and drug release is prolonged [16, 75, 81].
Figure 2.7 Schematic representation of burst release phenomena.
2.5 Nanoparticulate delivery systems

2.5.1 Solid polymer nanoparticles

A wide variety of drugs can be delivered via a number of routes using nanoparticulate carriers. Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, and biological macromolecules [15, 75, 82-87]. They can be modified for delivery to the lymphatic system, brain, arterial walls, lungs, or made for long-term systemic circulation [47, 59, 88-91]. Therefore, numerous protocols exist for synthesizing nanoparticles based on the type of drug used, required characteristics, and the desired delivery route. Four of the most important characteristics of nanoparticles are their size, encapsulation efficiency, zeta potential (surface charge), and release characteristics. For self-assembled nanocarriers, discussed later in this chapter, particle morphology, or shape, may also have an impact on their in vivo delivery characteristics.

There are several different methods for preparing solid polymer nanoparticles. Additionally, numerous methods exist for incorporating drugs into the particles. For example, drugs can be entrapped in the polymer matrix, encapsulated in a nanoparticle core, surrounded by a shell-like polymer membrane, chemically conjugated to the polymer, or bound to the particle’s surface by adsorption.

The most common method used for the preparation of solid, polymeric nanoparticles is the emulsification-solvent evaporation technique [11, 74, 92-94]. This technique has been successful for encapsulating hydrophobic drugs, but has had poor results incorporating hydrophilic compounds. Solvent evaporation is carried out by dissolving the polymer and the compound in an organic solvent. The emulsion is
prepared by adding water and a surfactant to the polymer solution. In order to minimize the particle size, the interface between the oil and water phase must be increased. To overcome this energy barrier and form the emulsion, energy must be added to the system, usually in the form of sonication or homogenization. The surfactant stabilizes the droplets formed during the energy input. The nanoparticles then “harden” as the solvent is removed, i.e. evaporation, extraction, or diffusion. The nanoparticles are usually collected by centrifugation and lyophilization.

A modification on this procedure has led to the protocol favored for encapsulating hydrophilic compounds and proteins, the double or multiple emulsion technique [54, 64, 74, 75, 83, 95]. First, a hydrophilic drug and a stabilizer are dissolved in water. The primary emulsion is prepared by dispersing the aqueous phase into an organic solvent containing a dissolved polymer. This is then reemulsified in an outer aqueous phase also containing stabilizer. The basic procedure for obtaining the nanoparticles is similar to the single emulsion technique for solvent removal.

All of the previously mentioned techniques use toxic solvents that could degrade certain drugs and denature proteins if they come into contact during the process. Consequently, an effort has been made to develop other techniques in order to increase drug stability during the synthesis. One such technique is the emulsification-diffusion method [96, 97]. This method uses a partially water-soluble solvent like acetone. Water is added to the emulsion, to allow for the diffusion of the solvent into the water. The solution is stirred leading to the nanoprecipitation of the particles. They can then be collected by centrifugation, or the solvent can be removed by dialysis. PLGA nanoparticles made using this technique can be seen in Figure 2.8.
Figure 2.8 Scanning electron micrograph of haloperidol loaded PLGA nanoparticles made using the emulsification solvent diffusion method.
Maintenance of protein activity during nanoparticle formation is a design challenge for emulsification procedures [98]. Protein activity can be affected by solvent selection and method of emulsification [99]. Dziubla et. al. found that loading efficiency could be increased eight fold by including a freeze thaw cycle into production, while maintaining ~60% activity of the enzyme catalase in PEG-PLGA nanoparticles [99]. In another study the encapsulation efficiency could be maximized when the pH of the internal and the external aqueous phases were brought to the isoelectric point of the peptide being encapsulated [81]. A synergistic effect between mechanical stirring and ultrasound was shown to produce nanoparticles of 300 nm in diameter while maintaining 85% of the starting activity of the cystatin protein [15]. The addition of protein protectants such as BSA or sugars was also found to aid in maintaining the biologically active, three-dimensional structure of cystatin [15]. These protectants may serve to shield proteins from interfaces during nanoparticle formation and lyophilization. The method of producing polymeric nanoparticles has several independent variables. Consequently, total drug loading, nanoparticle stability and release characteristics may vary with slight changes in processing parameters. First, one must consider the selection of the components used in the nanoparticle production, including the polymer, the polymer molecular weight, the surfactant, the drug, and the solvent [100]. Some other processing variables include the time of emulsification, the amount of energy input, and the volume of the sample being emulsified. As energy input into an emulsion increases the resulting particle size decreases [99]. In addition there are four separate concentrations that can be altered: the polymer, drug, surfactant, and solvent. Finally, the recovery of the particles can be changed depending on the method of lyophilization or centrifugation.
Different surfactants may produce particles of different sizes [101]. In a study conducted by Kwon et. al. [16], PLGA nanoparticles prepared using didodecyl dimethyl ammonium bromide (DMAB) were smaller than particles prepared with poly(vinyl alcohol (PVA) [96]. Another promising stabilizer for nanoparticles is the amphiphile d-\(\alpha\)-tocopheryl polyethylene glycol 1000 succinate vitamin E (TPGS). TPGS has high emulsification efficiency, can increase incorporation efficiency when used as a matrix component, and can be used as a cellular adhesion enhancer. TPGS can be used at as low a concentration as 0.015\% (w/v), in fact a lower concentration decreases particle size and polydispersity [102].

The amount of stabilizer used will also have an effect on the properties of the nanoparticles. Often a low concentration of surfactants will result in a high degree of polydispersity and aggregation [103]. Alternatively, if too much of the stabilizer is used, the drug incorporation could be reduced due to interaction between the drug and stabilizer. However, when the stabilizer concentration is between the “limits”, adjusting the concentration can be a means of controlling nanoparticle size. For example, increasing the PVA concentration has been shown to decrease particle size [74, 83]. However, when using the emulsification diffusion method, Kwon et. al. found that a PVA concentration from 2-4\% was ideal for creating smaller nanoparticles, ~100 nm in diameter [96].

When considering a particular polymeric nanoparticle for a given drug delivery application, particle size is one of the most important characteristics. It is necessary to first determine what the goal of the nanoparticle delivery system is before determining the size desired. For example, if the goal is delivery to extracellular targets then the ideal
size is between 30 and 100 nm [46, 104]. For systemic circulation particles should be in
the range from 50 to 500 nm [59]. Cells can internalize particles ranging from 30 to 300
nm [105]. It also appears that there is a lower size limit for ideal delivery, with ~75% of
particles with a diameter of ~ 30nm cleared by the liver 3 hours post injection. However,
70 nm particles had less than 50% taken by the liver [106]. Therefore, it appears that
particles below a certain size limit can get though the fenestrae of the liver endothelial
lining and accumulate.

There are trade offs when optimizing particle size by altering the molecular
weight of the polymer. Smaller diameter solid nanoparticles can be prepared with lower
molecular weight polymer, at the expense of reduced drug encapsulation efficiency.
Furthermore, an increase in polymer concentration during preparation increases both the
encapsulation efficiency and the size of the nanoparticles [74, 75, 96].

The synthesis method can also have a profound effect on the encapsulation
efficiency. In loading paclitaxel into PLGA nanoparticles using the nanoprecipitation
method, when the drug and polymer were mixed first and then solubilized in the organic
solvent prior to fabrication, encapsulation efficiency was 15% [12]. However, when a
solution of drug was used to dissolve the polymer prior to fabrication, nearly 100%
encapsulation efficiency was achieved.

Overall, solid polymer nanoparticles provide an interesting and versatile area for
drug delivery research. However, there are several other types of nanocarriers that may
offer a simpler fabrication mechanism, can better take advantage of stealth properties or
may have better biomimetic characteristics.
2.5.2 Liposomes

Liposomes were one of the first classes of nanoparticulate drug delivery vehicles investigated [107, 108]. Liposomes serve as excellent mimics of naturally occurring lipid bilayers [109]. Investigations with liposomes to mimic natural cell functions, especially those involving membrane transport, have led to their use as vehicles for drug delivery [107, 110-112]. Liposome membranes are organized into spheroid shells (Figure 2.9A), which can be uni- or multilamellar and can vary in diameter from less than 100 nm up to 10 microns [113]. There are several methods for liposome formation, but the most common are extrusion, sonication, and film rehydration [107, 114].

A primary advantage of liposomes is their high level of biocompatibility. Liposomes now constitute a mainstream technology for drug delivery; clinical approval has been given to liposomal formulations of anticancer drugs doxorubicin (Doxil®/Caelyx® and Myocet®) and daunorubicin (Daunosome®) [115, 116]. Another advantage of liposomes is their ability to transport a diverse array of drugs that can be hydrophilic, lipophilic, or amphiphilic. This relates to the amphiphilic nature of phospholipid molecules themselves, which self-assemble in water to form bilayers that enclose an aqueous interior. Hydrophilic drugs can therefore be entrapped within the aqueous core, whereas hydrophobic drugs partition into the hydrophobic region of the bilayer characterized by hydrocarbon chains. Loading techniques such as the ammonium sulfate method and the pH gradient method can be used to place amphiphilic drugs (e.g., doxorubicin and vincristine, respectively) at the inner-phospholipid-monolayer/water interface [116-118]. Generally, liposome drug loading is determined by equilibrium
Figure 2.9 Liposomes (A), polymersomes (B), and worm micelles (C). Gray areas represent the hydrophilic block and black areas represent hydrophobic areas.
partitioning of drug in the liposome. Consequently, liposomes are beneficial for incorporating small molecules and hydrophilic compounds.

A major disadvantage of liposomes as drug-delivery vehicles is their rapid clearance from blood via the MPS. This limitation has been overcome the addition of PEG to create ‘PEGylated Stealth®’ liposomes [117-119]. Incorporation of PEG into ordinary liposomes increases their circulation half-life from minutes to hours [116, 120]. PEG enhances the compatibility of lipid membranes by mimicking the glycocalyx, a carbohydrate-rich coating on cells [114]. In addition to conveying this stealth quality, PEG also increases the susceptibility of liposomes to ultrasound-induced leakage [121]. This quality might prove useful in development of a targeted, localized drug-delivery system using external ultrasound as a remote mechanical stimulus to trigger drug release. Liposomes can also be decorated with glycoproteins and sugar chains to target specific cells [122].

2.5.3 Polymersomes

Recently, interest has focused on polymeric vesicles, composed of hydrophobic-hydrophilic diblock copolymers or “super-amphiphiles”, as drug delivery vehicles (Figure 2.9B) [3, 123-126]. The term “super-amphiphile” comes from the exaggerated hydrophilic to hydrophobic domains which are much larger than naturally occurring surfactants [127]. The advantages of these polymersomes, as compared to liposomes, include enhanced mechanical stability, due to a thicker bilayer membrane, and greater flexibility to tailor bilayer characteristics, such as chemical composition [3, 114, 127-129]. Polymersomes have been found to be up to ten times less permeable than liposomes even though membrane thickness may be only a few nanometers. The
increased length of the amphiphile and its conformational freedom allows for greater tough-ness and reduced permeability as opposed to natural amphiphiles [3]. This reduced permeability may enhance polymersome’s benefit in drug delivery by decreasing the rate of release. Preparation of polymersomes is similar to that of liposomes [113, 114]. It has been speculated that protein interactions with polymersomes will greatly differ from their interactions with liposomes, thereby affecting drug delivery characteristics such as circulation time \textit{in vivo}. Indeed, Photos et al [130] have shown that the circulation time of polymersomes increases with the bilayer thickness. Polymersomes were shown to be inert to phagocytosis \textit{in vitro} when exposed to serum and they exhibited thermal stability [114]. Pata and Dan [131] have found that the characteristics of the polymeric bilayer, when compared to the lipid bilayer, are quite different and can be used for tailoring the carrier properties. Recently, Meng et al. have synthesized biodegradable polymersomes from block copolymers of PEG-PLA [20]. A model hydrophilic substance, carboxyfluorescein was incorporated into these polymersomes by adding the compound to the aqueous phase during preparation [132]. The release of this molecule was found to be controlled by first order kinetics, confirming release from a polymersome system to be a membrane controlled reservoir system. Discher et. al. were able to synthesize polymersomes from PEG-PLA and PEG-PCL block copolymers [133]. These vesicles showed comparable loading of doxorubicin to liposomes. In addition, increased release rate caused by a greater PEG content could be offset by the use of the more hydrophobic PCL as the lipophilic block. The release from these polymersomes seems to be dependent on the formation of pores in the membrane structure caused by degradation of the PLA or PCL chains [133].
Polymeric vesicles have also been synthesized from multiblock copolymers of Pluronic F27 with a PLA block on either end [134]. The vesicle structure is characterized by a hydrophilic core with hydrophobic layers, and can form several conformations, such as bilayer or onion-like vesicles. The PLA-F27-PLA copolymers exhibit a decreased $T_g$ and $T_m$ that indicate an increased permeability and chain mobility in aqueous solutions. This may explain the high burst release from these vesicles, but does not eliminate this class of particles as a drug delivery system [134].

2.5.4 Worm-like micelles

Cylindrical worm micelles are a promising new class of supermolecular drug carriers (Figure 2.9C) [135, 136]. The structure of these carriers is highly dependent on the hydrophilic block weight fractions that govern the morphological complexity from polymersomes to worm-like micelles to spherical micelles [137]. This will be discussed in greater detail later in this chapter. For worm micelles, even if they are microns long, while nanometers in diameter, they can "worm" through small pores and circulate for weeks. Additionally due to large surface area, targeted worm-like micelles can bind with high affinity to surfaces or cells that bear suitable receptors. After cellular internalization worm micelles can deliver of a relatively large amount of drug all at once [138].

Since polymeric worm micelles are a relatively new carrier, it is important that several system characteristics be elucidated, such as: polymer molecular weight versus worm diameter, worm stability, and flexibility [126]. Nanoscale worm micelles can be very stable; they appear similar to filamentous phages that have been used with great success *in vivo* for phage display of targeting ligands (including tumors) [139]. Unlike phages that carry nucleic acids, worm micelles may carry lipophilic drugs. Under flow
conditions worm micelles were shown to orient and stretch with scaling similar to DNA [140]. More importantly, under flow condition worm micelles were stable and did not fragment. Worm micelles have been shown to be stable in aqueous solutions for up to a month [138]. Worm micelle stability can be enhanced by the addition of electrolytes that add stiffness to the structure and allow them to grow to greater than 10 microns in length, without branching [141]. Worm micelles have also shown the capacity to be chemically crosslinked without disruption of the cylindrical structure [135]. This is done using a water based redox reaction that leads to chemical crosslinking at the core and can be used to improve the worm micelles viscoelastic properties.

2.6 Targeted drug delivery

2.6.1 Oral delivery

In recent years, significant research has been done using nanoparticles as oral drug delivery vehicles. Oral delivery using nanoparticles has been shown to be far superior to free drug in terms of bioavailability, residence time, and biodistribution [142]. Antifungal drug encapsulated in particles of less than 300 nm in diameter was detected in the lungs, liver, and spleen of mice seven days post oral administration, whereas oral-free formulations were cleared within 3 hours post administration [142]. For this application, the major interest is in lymphatic uptake of the nanoparticles by the Peyers Patches in the GALT (gut associated lymphoid tissue). There have been many reports as to the optimum size for Peyers Patch uptake ranging from less than 1 μm to 5 μm [83, 94]. However, it has been shown that microparticles remain in the Peyers Patches while nanoparticles are disseminated systemically [75, 143, 144]. Jani et. al. showed that 34% of particles delivered orally with a diameter of 50 nm were absorbed and 26% of 100 nm
particles were absorbed [145]. However, no radiolabelled particles over 300 nm were detectable in the blood after oral administration. This study was performed with polystyrene nanoparticles. Consequently, the small size combined with a polymer more suitable for interaction with the intestinal mucosa could lead to a more pronounced improvement in oral bioavailability.

Oral delivery of nanoparticles has focused on uptake via the Peyers Patches in the GALT. Peyers Patches are characterized by M cells that overlay the lymphoid tissue and are specialized for endocytosis and transport into intraepithelial spaces and adjacent lymphoid tissue. There have been several differing opinions as to the ease of nanoparticle transport through the M cells, and the method by which this occurs [146, 147]. One theory is that nanoparticles bind the apical membrane of the M cells, followed by a rapid internalization and a “shuttling” to the lymphocytes [104, 147]. The size and surface charge of the nanoparticles are crucial for their uptake. There have only been two published phase I clinical trials examining the oral uptake of PLGA nanoparticles encapsulating E. coli antigens, with no clear benefit determined [146]. There is some promise in identifying M cell receptors and targeting them on the surface of nanoparticles. The carbohydrate epitope, sialylated Lewis antigen A (SLAA) has been identified on human M cells [146]. This application of targeted nanoparticles in oral delivery holds tremendous promise for the development of oral vaccines and in cancer therapy. In addition, it has been found that the addition of a matrix material component, montmorillonite, can greatly enhance the cellular uptake of nanoparticles by Caco-2 and HT-29 cells without disturbing the physical or release characteristics of the nanoparticles [148].
Nanoparticles can be engineered not only for oral absorption themselves, but can be used to deliver a drug directly to the source for gastrointestinal uptake, thereby protecting the drug from low pH and enzymes in the stomach. pH sensitive nanoparticles made from a poly(methylacrylic acid and methacyrlate) copolymer can increase the oral bioavailability of drugs like cyclosporin A by releasing their load at a specific pH within the gastrointestinal tract. The pH sensitivity allows this to happen as close as possible to the drug’s absorption window through the Peyer’s patches [149].

2.6.2 Brain delivery

Kreuter et. al. [29] were able to deliver several drugs successfully through the blood brain barrier using polysorbate 80 coated PACA nanoparticles [46]. It is thought that after administration of the polysorbate 80-coated particles, apolipoprotein E (ApoE) adsors onto the surface. The ApoE protein mimics low density lipoprotein (LDL) causing the particles to be transported across the blood brain barrier via the LDL receptors. The effects of polysorbate-80 on transport through the blood brain barrier were confirmed by Sun et. al. with PLA nanoparticles [150]. Nanoparticles were also functionalized with a thiamine surface ligand [151]. These particles, with an average diameter of 67 nm, were able to associate with the blood brain barrier thiamine transporters and thereby increase the unidirectional transfer coefficient for the particles into the brain.

2.6.3 Cancer therapy

One of the most common targeting applications for nanoparticles is against cancerous cells and tumor sites with incorporated chemotherapeutic agents. Oral delivery
of chemotherapy is of high interest because chemotherapeutic agents are eliminated by the first pass effect with cytochrome p450 [101]. Another reason cancer therapy has attracted attention is due to the phenomena known as the enhanced permeation and retention effect (EPR). The vasculature around tumor sites is inherently leaky due to the rapid vascularization necessary to serve fast growing tumors [152]. In addition, poor lymphatic drainage at the tumor site prevents elimination of the particles from the tumor tissue. Another aspect of tumors that can be exploited by nanoparticles is the overexpression of specific antigens on cancer cells. Yamazaki et. al. used sugar chain remodeled glycoprotein-liposome conjugates for binding to E-selectin, and showed uptake of the particles by solid tumor tissue [122]. Cancer cells over express the transferring receptor, which is present in most cells and is normally used for iron uptake. Consequently, paclitaxel loaded PACA nanoparticles with a PEG linker chain conjugated to transferrin were able to increase the lifespan in tumor-bearing mice with a decreased weight loss compared to mice given conventional paclitaxel [41]. The decreased weight loss may point to a reduction in paclitaxel associated side effects.

One method for incorporating ligands for targeting that is attracting attention, is by exploiting the high affinity binding of biotin and avidin. Each avidin molecule binds four biotins. Using the multifunctionality of this technology, drugs and homing molecules for cancer cells can be combined [153]. The biotinylated block copolymer PEG-PCL can be associated with an avidin bound ligand, lectin. Using this system, the amount of nanoparticles associated with Caco-2 cells (a colon cancer cell line) is increased dramatically [154]. NeutrAvidin™, used as a model protein, was covalently attached to the surface of PLA nanoparticles via sulfhydryl groups [155]. Using this
technology, biotinylated antibodies or ligands can be attached to the particle surface. Biotinylated worm-like micelles have also been found to be stable in an aqueous solution for at least a month, and have the potential for the delivery of large quantities of hydrophobic drugs or dyes [138].

2.7 Block copolymer micelles

One promising class of nanoparticles for drug delivery is polymeric micelle-like structures. These micelle-like structures are formed using block copolymers. Block copolymers are defined as one type of homopolymer chain attached to a chain or chains of type of homopolymer. Block copolymers offer the ability to alter the properties of a homopolymer by the addition of a second repeating unit. This offers the potential to combine desirable properties of two different homopolymers into one single copolymer. Each block present in the copolymer shows the behavior ($T_g$, $T_m$, crystallinity) of that corresponding homopolymer, providing that the block lengths are not too short. This typically occurs due to the fact that the A blocks from different polymer molecules aggregate with each other, and B blocks will aggregate likewise [156]. Micelles can self-assemble from block copolymers composed of hydrophilic and hydrophobic segments (Figure 2.10). In Figure 2.10 the straight alignment in the core and the hole in the center is an artifact of the graphical representation. In actuality these hydrophobic segments become entangled and form a solid core. The hydrophobic segment creates the inner core of the micelle while the hydrophilic segment creates the outer shell, referred to as the
Figure 2.10 Schematic representation of block copolymer micelle. Gray area represents core forming hydrophobic block and black area represents the hydrophilic corona.
corona, in an aqueous media [157]. Polymeric micelles can be used as a drug delivery vehicle by either physically entrapping drug in the core (i.e. hydrophobic drugs can be trapped inside the micelle by hydrophobic interactions), or by chemically conjugating the drug to the hydrophobic block prior to micelle formation [158, 159].

### 2.7.1 Thermodynamics of micelle formation

Micelles can be referred to as a colloidal dispersion, with micelles being the dispersed phase and the aqueous media being the continuous phase [160]. Micellization in aqueous media is an entropically driven process, similar to surfactant micelles [161]. Micellization occurs in two stages. First, the block copolymer is dissolved in a good solvent for both blocks. A selective solvent for one of the blocks, typically water, is added in a larger volume [4]. In this case the hydrophobic block collapses to form a system of polydisperse particles. The presence of the hydrophobic block in water causes a decrease in water entropy that induces an increase in the degree of structuring of water molecules, leading to cavity formation [161]. Hydrophobic blocks aggregate and the structure of water is restored, thus increasing water entropy. As the collapsed unsolvated hydrophobic blocks relax into a more favorable confirmation, the core gets entangled. This overcomes the loss of entropy from the localization of hydrophobic blocks into the micelle core, leading to an overall negative Gibbs free energy, and confirming the spontaneity of the process. The major driving force for the formation of micelles is the decrease in free energy of the system due to removal of the hydrophobic blocks from the aqueous milieu, while the hydrophilic blocks stabilize the particles.

Micelles spontaneously form when the concentration of the amphiphile unimers is higher than a critical concentration called the critical micelle concentration (CMC).
Below the CMC amphiphilic molecules have a strong tendency to be absorbed at the air water interface [160]. At the CMC the amphiphile becomes saturated at this interface and in the bulk solvent, and it becomes entropically favorable for micelles to form to minimize the free energy of the system. At this point some quantity of solvent is still present, creating a “swollen” core. This is the point at which loose aggregates are formed, which exhibit a larger size than micelles formed at higher concentrations of polymer [4]. The CMC of polymeric micelles is significantly lower than that of small molecule surfactant micelles. This renders polymeric micelles more suitable for drug delivery applications because the lower CMC will be indicative of micelles remaining stable at larger dilutions. It has been shown that incorporation of small molecules into the micelle core does not significantly alter the CMC [4]. However, recent mathematical simulations of micelle formation and drug incorporation have indicated that the presence of drug molecules in the core pushes the hydrophobic blocks out and increases the overall diameter of the micelles [162]. As compatibility between the blocks increase the CMC increases as well [160].

Dissociation of micelles is also an important aspect of micelle stability. It is important to distinguish between thermodynamic stability, which is a function of the CMC, and kinetic stability, which is the actual rate at which micelle dissociate back into unimers below the CMC. Surfactant micelles have dissociation kinetics similar to their rates of association at their CMC. However, this process is more complicated with block copolymer micelles; the rates of formation and dissociation are both much slower and are distinctly different processes [163]. Micelle stability is enhanced by crystallization or rigidity in the polymer core, as well as entanglement of polymer chains in the micelle
core, which helps prevent rapid dissolution of block copolymer micelles \textit{in vivo}. Using nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC), the hydrophobic block has been shown to form a solid core [4, 164]. This is indicative that, once formed, these micelles no longer exhibit a dynamic nature between unimers and micelles, and below their CMC micelles may remain intact for longer periods of time.

One of the most important benefits of micelles for drug delivery is the ability to solubilize drugs in the core as a part of the spontaneous self-assembly process. The efficiency of micelles to solubilize compounds in their core is defined by the Flory-Huggins interaction parameter which is expressed in terms of the solubility parameters of the core forming block, $\delta_c$, and the drug, $\delta_d$ [165].

$$\chi_{c,d} = \frac{(\delta_d - \delta_c) v_d}{kT} \quad (2.1)$$

In this equation, $v_d$ is the molar volume of the drug, $k$ is the Boltzmann constant, and $T$ is the absolute temperature. Lower values for the Flory-Huggins parameter indicate compatibility between the core forming block and the drug. In addition, the solubilization is often expressed in terms of the partition coefficient, $K$, between the micelle core and the aqueous phase.

$$K = \frac{[\text{drug\_inside\_micelle}]}{[\text{drug\_dissolved\_aqueous\_phase}]} \quad (2.2)$$

With determination of the partition coefficient, the free energy of solubilization can be determined.

$$\Delta G_s^o = -RT \ln K \quad (2.3)$$
Despite the fact that the core of the micelle is hydrophobic, there is still an anisotropic distribution of water in the micelle core, with the highest concentration being near the surface (the interface with the hydrophilic block), and decreasing in concentration towards the center of the core [160]. As a result of this, the spacial positioning of drug molecules within the micelle core is dependent on its polarity [160]. An increase in the hydrophobic chain length will facilitate an increase in drug incorporation into the micelle core. It was shown experimentally that increasing the molecular weight of the hydrophobic block, PLA, correlated to increased drug incorporation [166]. However, the increase in the hydrophobic block molecular weight will increase the micelle diameter. This increase in diameter will decrease the Laplace pressure caused by the interfacial curvature [160]. Consequently, it is found that for some drug polymer micelle systems there is a trade off between micelle size and drug incorporation. Interestingly, this is also found with nanoparticles that do not self assemble, such as solid polymer nanoparticle discussed previously in this chapter.

2.7.2 Micelles for drug delivery

Micelles have several benefits as drug delivery vehicles. Their hydrophilic outer shell and small size (less than 100 nm) render these particles nearly invisible to the MPS, allowing for extended circulation in the bloodstream. Micelles can increase the bioavailability of poorly soluble drugs by sequestering them in the core. By increasing a drugs bioavailability, it is possible to deliver a lower dose of drug, thereby decreasing the resulting side effects. Their small size and increased residence time make them an excellent carrier to take advantage of the passive targeting benefit of the EPR effect. The extended hydrophilic block which forms the corona provides a convenient site for
covalent modification of moieties for active targeting. The spontaneous formation of micelles allows them to be prepared in large quantities, easily and reproducibly.

Multi-block copolymers, such as Poly (ethylene glycol)-block-Poly (propylene oxide)-block Poly (ethylene glycol) (PEG-b-PPO-b-PEG), commercially known as Poloxamers® and Pluronics®, can also self-assemble to create block copolymer micelles (Figure 2.11). Micellization behavior of these block copolymers varies with temperature, composition and block molecular weight. CMC of these polymers is often too high to be stable for drug delivery from micelles due to the weak hydrophobic interaction of the PPO block [167]. These micelles are extremely temperature dependent [168]. Their micellization process is believed to be an endothermic process driven by a decrease in polarity of the blocks as temperature increases, although the same entropy gain in water is seen when the hydrophobic blocks aggregate [168]. Pluronics® conjugated to brain-specific antibodies or insulin, have been used to solubilize haloperidol and other small compounds such as FITC [169]. Pluronics have also been used to effectively incorporate drugs such as diazepam and indomethacin [170, 171]. In addition, similar results were found with Pluronic incorporated doxorubicin on tumor regression and reduction in toxicity, as with diblock copolymer micelles discussed later in this chapter [172]. PEG-Poly(amino acid) (PEG-PAA) block copolymers (Figure 2.12) have received a great deal of attention for drug delivery because the biodegradable amino acid core has free functional groups for chemical modification [159]. Yokoyama et. al. have conducted numerous studies on the chemical conjugation as well as physical entrapment of adriamycin (ADR, also known as doxorubicin), an anticancer drug, in PEG-Poly(aspartic acid) micelles (PEG-P(Asp)) [173-177]. These micelles have begun clinical trials in
Figure 2.11 Poly (ethylene glycol) –b – poly (propylene glycol) – b- poly (ethylene glycol) chemical structure (A), Schematic of triblock micelle formation, gray lines represent PEG blocks and black lines represent PPG block (B).
Figure 2.12 Poly (ethylene glycol) – block – poly (aspartic acid), note free carboxylic acid group on poly (aspartic acid ) block for functionalization.
Japan [178]. To achieve this, PEG-PBLA (Poly (β-benzyl L-aspartate) is synthesized from β-benzyl N-carboxy L-aspartate anhydride (BLA-NCA) and α-Methyl-ω-aminopoly(oxyethylene). PEG-P(Asp) is then prepared by debenzylation under alkaline conditions of the PEG-PBLA. Following this step, ADR can be conjugated via amide bond formation [175]. The amount of ADR conjugated onto the PAA block can be tailored to impart water solubility [175]. In addition, the reaction has been optimized to reduce the side reactions of ADR and minimize polydispersity in micelle size [174]. Micelles prepared from drug-conjugates have the added advantage that extended drug release will still be possible in the event of dissociation of the micelle, as the drug must still be cleaved from the polymer chain. As expected, stability of these PEG-P(Asp(ADR)) micelles has been shown to be dependent on the length of the PEG chain, but has also been found to be influenced by the percent ADR content. In addition, micelles which were found to be more stable in vitro had better antitumor activity in vivo [176]. Polymeric micelles with conjugated ADR alone or physically entrapped ADR alone had little antitumor activity as compared to the combination of conjugated and physically incorporated ADR [173, 177]. Adriamycin could be incorporated at higher weight percentages, i.e. 15-20% (w/w), when the hydrophobic interactions between the core forming block and the drug molecules were maximized [179]. PEG-PBLA could also be synthesized with free hydroxyl groups on the outer core of the micelle for further conjugation to a targeting moiety (i.e. antibody, glucose) [158].

Other drugs have been conjugated to PEG-poly(amino acid) block copolymers. Cisplatin has been bound to P(Asp) using the same block copolymer PEG-P(Asp) discussed above [180]. Methotrexate (Mtx) esters of PEG-block-Poly (2-hydroxyethyl-L-
aspartimide) (PEG-PHEA) were found to form stable micelles with a sustained release profile, dependent on the amount of Mtx substitution [181]. A higher percent methotrexate substitution increased the hydrophobicity of the core, decreased the CMC, increased the micelle size, and extended the release profile. The release of the methotrexate ester was slow, with only 20% release of the drug over a 10 day period, due to the unfavorable conditions in the micelle core for hydrolysis of the ester bonds [182]. Amphotericin B has been incorporated into PEG-PAA micelles and has shown to increase antifungal activity as compared to free drug alone [183]. This may be caused by a stabilization effect of the micelles from autooxidation or an enhanced fungal membrane interaction with the micelles. Moreover, PEG-PAA micelles conjugated to the anti-tuberculosis drug isoniazid were able to decrease the minimum inhibitory concentration by more than 5 times as compared to conventional delivery [184].

In recent years much work has been done using mPEG-PLA micelles for drug delivery. Several groups have studied the characteristics of the micelles, while others have focused on drug loading and release. Stolnick et. al. found that while polymer concentration had no effect on micelle size, the molecular weight of the PLA block was the main determinant of diameter [164]. Govender et. al. also found that the micelle diameter increased with an increased PLA molecular weight [166]. An increase in PEG content increases the degradation rate, and consequently the release rate, including the burst release [166]. This is indicative of the delicate balance of tailoring the structure and properties of the micelles with the block molecular weights. The structure the block copolymer forms during the self-assembly is dependent on the weight fraction of the hydrophilic block relative to the total polymer molecular weight [3]. In order for
spherical micelles to form, the hydrophobic block, in this case PEG, must have a weight fraction of more than 55%. Whereas for worm-like micelles, discussed previously, the hydrophilic block should comprise 45-55% weight fraction, with lower weight fractions yielding vesicles [140].

The PLA core of the block copolymer micelles were found to form a glassy center such that polymer chains do not migrate between unimers and micelles in a manner similar to surfactants [164]. This is a good indication that micelles will maintain their structural integrity upon dilution in the body. In addition, the CMC of polymeric micelles is significantly lower than that of surfactant micelles. However, surfactant micelles are orders of magnitude smaller than polymeric micelles. The polydispersity of micelle diameters was found to be affected primarily by the molecular weight distribution of the polymers used [18]. Yasugi et. al. were able to form micelles with diameters of ~30 nm, from an mPEG-PLA copolymer with both block molecular weights at 5000 g/mole. The CMC of these micelles were approximately 3 μg/ml [18]. Lee et. al. used a modified mPEG-PLA to make micelles with carboxylic acid groups incorporated onto the PLA backbone [185]. These micelles made from this novel copolymer were characterized for size, CMC, and structure using NMR, and they were found to have diameters of ~35 nm and an average CMC of ~2 μg/ml [185]. The incorporated functional groups were able to hydrogen bond with drug molecules and increase the percent incorporation of the drug, papaverine, from 4% to 14.9% (w/w) in the micelle core [185]. mPEG-PLA micelles with incorporated paclitaxel were found to reduce the toxicity and increase antitumor efficacy compared to current paclitaxel formulations [186]. Yoo et. al. were able to conjugate the hydrophilic anticancer drug, doxorubicin, to
mPEG-PLA via an acid cleavable hydrozone bond [187]. The micelles formed from this conjugate were approximately 90 nm in diameter and had a CMC of 1.3 μg/ml [187]. Due to the acid cleavable linkage, these micelles had a high rate of release at low pH, which is indicative of a release rate after cellular internalization. Block copolymer micelles were also made from mPEG-PLGA-doxorubicin conjugates via an amide linkage [188]. The micelles made from the conjugate polymer show a more sustained release profile than those made with incorporated free drug. In addition, doxorubicin delivery from these micelles was shown to be more cytotoxic to Hep2G, a cancer cell line, than free doxorubicin, suggesting that micelles were actively taken up by the cells as opposed to a passive diffusion mechanism.

2.8 Prodrugs

2.8.1 Polymeric prodrugs

In many cases an active substance is linked to a polymeric molecule via a covalent bond which is naturally hydrolyzable in vivo, in order to prolong the drug release [189, 190]. In 1975 Ringsdorf proposed the design for the polymeric prodrug [191]. The benefits of using a polymeric prodrug include: prolonging the action of a drug, controlling a release of a drug, taking advantage of the EPR effect, altering a drug's biodistribution, and altering the cellular uptake properties of a drug [191]. There have been several different approaches to creating polymeric prodrugs, although most of them have included creating degradable bonds, either directly between drug and polymer or with a linker connecting the drug to a polymer. Other polymeric prodrugs use pharmacological compounds as monomers and incorporate the drug into the polymer backbone [192, 193]. A large class of these polymeric prodrugs, poly(amino acids), were
discussed earlier in this chapter. Polymeric prodrugs have been used extensively with anti-tumor agents because of their advantages due to the enhanced permeation and retention effect at tumor sites [194, 195]. Many of these drug-polymer conjugates have entered Phase I/II clinical trials [194].

Over the last several years, doxorubicin has been linked to PLGA and mPEG-PLGA/PLA via an ester linkage, an amide linkage, or a hydrozone linkage and formulated into solid polymer nanoparticles and block copolymer micelles [13, 14, 187, 188]. The use of these conjugates was shown to increase both percent incorporation and incorporation efficiency. The presence of the polymeric prodrug was able to increase the time over which doxorubicin was released from 5 days for free drug to one month [14]. The use of the poly (amino acid), poly(aspartic acid), as a polymeric prodrug was discussed at length previously in this chapter.

In many cases polymeric prodrugs have been synthesized for microsphere formulation for depot injection. One example of this is the conjugation of the antiviral drug idoxuridine conjugated via spacer molecules to PLA prior to formation of microspheres, or potentially solid polymer nanoparticles [189]. Polymeric prodrugs have been created with the lipid lowering drug, gemfibrozil, and PHEA [196]. Oh et. al. created PLGA prodrug microparticles with a model drug, Fmoc(Trp(Boc)), and found a significantly reduced burst effect. In the past, prodrugs were often made by linking an active drug to a macromolecule. However, while this does extend the drug’s release profile in the body, often the drug is no longer water soluble and is injected in some type of oil compound [77]. Polymeric drug delivery vehicles offer the versatility to deliver these conventional prodrugs and further extend release [77, 197].
2.8.2 Haloperidol and clinical relevance

One of the goals for the development of a long-term delivery system is to reduce the side effects associated with the peaks that result from conventional oral dosing. Haloperidol is a dopamine D2 antagonist antipsychotic and is used primarily to treat schizophrenia. For haloperidol, this is particularly important due to the emergence of motor side effects at higher plasma concentrations. Ideally, a continuous, low concentration of haloperidol would decrease these side effects. Depot injections, in which drug is sequestered within the body, were introduced in the 1960s as long-term methods for the delivery of antipsychotic agents [197]. Most of these incorporate older antipsychotic agents and are synthesized by esterification of the active drug to a fatty acid to create a prodrug and are dissolved in oil for intramuscular injection. The resulting formulations are referred to as decanoates. Although these formulations serve to increase compliance and improve the steady-state delivery of medication, they are irreversible and constrained by the requirement of injection. There is the potential for adverse side effects that are occasionally manifested and must be endured for the remainder of the treatment interval due to the inability to remove depot medications. Prolonged pain also occasionally persists at the injection site [198] and as a result, many patients cease to return for continued clinical treatment.

Medication adherence is a major concern during pharmacological treatment of individuals with serious psychiatric illnesses. Up to 55% of individuals with chronic psychotic disorders such as schizophrenia have significant difficulties adhering to treatment recommendations [199]. Nonadherence often results in deterioration in social function and more intensive interventions including rehospitalization such that
nonadherence has been shown to be the most important predictor of rehospitalization among a state hospital population [200]. As such, the National Institutes of Mental Health have called for the design and evaluation of interventions to help with adherence behaviors [201]. Consequently, antipsychotic drugs, such as haloperidol, are excellent candidates for a long-term drug delivery vehicle. Most importantly, the vehicle must increase the bioavailability such that toxic side effects can be reduced and patient adherence improved.
2.9 References


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CHAPTER 3: RESEARCH GOALS

The first objective of this work was to covalently link the biocompatible and biodegradable block copolymer, mPEG-PLA, to a model compound, haloperidol, via a biodegradable ester linkage. Furthermore, the goal was to determine if this polymeric prodrug could be used to extend the release profile of haloperidol from nanoscale block copolymer micelles. The overall intent of this project was to understand the properties and characteristics of drug-polymer conjugates as they relate to block copolymer micelles. More specifically, the aims of this work were to synthesize and characterize the prodrug structure, to formulate micelle-like structures, both with the control polymer and the prodrug, to encapsulate free haloperidol in both control and prodrug micelles, and finally to characterize the release profile of haloperidol from the micelles. The polymeric prodrug was characterized for structure, percent conjugation, and molecular weight. The micelle-like structures were evaluated on their size, structure, and stability, including any changes that occur with control polymer versus polymeric prodrug. Haloperidol loading in micelles was characterized for the percent incorporation and efficiency of control polymer micelles loaded with free drug, prodrug micelles alone, and prodrug micelles loaded with free drug. Each of these groups of micelles were characterized for their release profiles. The specific aims of this work were:

1. To synthesize a biodegradable polymeric prodrug with a model psychoactive compound, and characterize the new molecule.
2. To formulate block copolymer micelles as nanostructured drug delivery vehicles with the polymeric prodrugs synthesized in aim 1 and characterize for size, polydispersity, morphology, aggregation number and stability.

3. To evaluate the effects of different degrees of conjugation on the stability, size, and degree of drug loading on the micelles.

4. To evaluate release characteristics of block copolymer micelles characterized in aims 2 and 3 and optimize them for linear release over a sustained period of time.
CHAPTER 4: SYNTHESIS AND CHARACTERIZATION OF THE mPEG-PLA-HALOPERIDOL POLYMERIC PRODRUG

4.1 Introduction

Block copolymers of PEG and PLA have been widely used for synthesizing drug delivery carriers such as solid polymer nanoparticles, micelles and polymersomes [1-4]. PEG is a nonionic, biocompatible polymer with a large exclusion volume, which aids in prevention of opsonization of particles and therefore longer systemic circulation. PEG is used in this work with a monomethoxy group capping one end of the polymer (known as mPEG) allowing for functionalization or polymerization on a single end. PLA is a commonly used biodegradable polymer. PLA degrades hydrolytically into lactic acid monomers which is a byproduct of normal metabolism that can either be excreted or further metabolized to carbon dioxide and water in the Krebs cycle [5, 6]. PLA occurs naturally as the pure enantiomeric poly(L-lactic acid) (LPLA) with a semicrystalline structure. However, most types of PLA used for biological applications exist in the racemic D,L form (DLPLA) and are amorphous polymers. Ring-opening polymerization is currently the preferred method for synthesis of PLA due to shorter reaction times and higher monomer conversion rates. This polymerization is generally carried out in bulk, in the presence of a catalyst, in the temperature range from 100 – 150°C [7].

Frequently drug molecules, proteins, nucleic acids or polymer chains can be covalently linked to one another for the purpose of increasing bioavailability, extending release or improving the ease of delivery. This is often accomplished using specific compounds, known as coupling agents. In the past the coupling agent bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP) was used for peptide synthesis, including the often difficult reaction with N-methylated amino acids [8]. In
work with nucleoside coupling, it was found that phosphonium salt coupling agents, such as PyBroP, when used together with the catalyst 4-dimethylaminopyridine, (DMAP), was an efficient coupling reagent combination for esterification [9]. These esterification reactions were shown to be faster than previous methods using carbodiimide coupling agents. Phosphonium salts have been shown as an effective coupling agent to activate a carboxylic acid group for condensation reactions, such as the one performed in this work, including esterification to tertiary alcohols [10]. Finally, PyBroP/DMAP has been used to create a biodegradable ester linkage between PLGA and the anticancer drug doxorubicin [11]. Based on its history with complex coupling reactions the PyBroP/DMAP combination was chosen for the esterification between mPEG-PLA-COOH and haloperidol.

In this work, the hydroxyl end group on the mPEG polymer was functionalized to create an electrophilic carboxylate end group via reaction with succinic anhydride [12]. A ring-opening polymerization was carried out with lactide, with the carboxylate as the propagating functional group, to produce an mPEG-PLA-COOH block copolymer [7]. The number average molecular weight of this polymer was determined by $^1$H nuclear magnetic resonance (NMR) spectroscopy. The number average molecular weight, $M_n$, is defined as the total weight, $w$, of all the molecules in a polymer sample divided by the total number of moles of polymer [13].

$$\overline{M}_n = \sum N_x M_x \quad (2.1)$$

$N_x$ is the mole fraction of molecules of size $M_x$. The number average is just the sum of individual polymer molecular weights divided by the number of chains. The polymer end group was then conjugated to the free hydroxyl group on haloperidol via the coupling
agent PyBroP. This conjugation was confirmed using both $^1$H and $^{13}$C NMR spectroscopy.

4.2 Experimental section

4.2.1 Materials

Lactide (3,6 dimethyl-1, 4-dioxane-2, 5 dione) and deuterium oxide were supplied by Aldrich (St. Louis, MO). mPEG 5000, stannous 2-ethyl-hexanoate, succinic anhydride, anhydrous pyridine, anhydrous dichloromethane (DCM), 4-dimethylaminopyridine (DMAP), diethyl ether, triethylamine (TEA), acetone, haloperidol, and DMSO-d6 were purchased from Sigma Chemical Co. (St. Louis, MO). Bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP) was provided by Fluka (Buchs, Switzerland). All chemicals were used as supplied.

4.2.2 Preparation of succinylated mPEG

mPEG was succinylated by dissolving succinic anhydride (approximately 0.22 grams) and mPEG (approximately 10 grams) in 50 ml of anhydrous pyridine in a ratio of 1.1:1 moles of succinic anhydride to moles of the mPEG hydroxyl end group. The reaction was carried out at 50°C in a shaking water bath for 5 hours. The pyridine was removed using a rotary evaporator and the resulting succinylated mPEG was washed several times in deionized water to remove excess solvent. An aqueous solution of the succinylated mPEG was frozen at −20 °C and lyophilized. Fourier Transform Infrared (FTIR) spectroscopy and NMR spectroscopy were performed to confirm the succinylation reaction.
4.2.3 FTIR Spectroscopy

Freeze dried samples were ground into a powder and mixed with KBr. The KBr to polymer ratio was between 1:20 and 1:50. A Nicolet economy sample press was used to obtain optically clear pellets. Pellets were analyzed using transmission FTIR in a Mange IR560 (Nicolet, Madison, WI). Dry air was used as the chamber purge stream for all samples. The scanning resolution was set to 1 nm with a total of 1024 scans per sample. The background was obtained against a pure KBr pellet.

4.2.4 Preparation of the block copolymer

mPEG-PLA-COOH copolymer was synthesized by a bulk, ring-opening polymerization [1]. Briefly, lactide and 2 mole % succinylated mPEG were dissolved at 135 °C under a nitrogen stream. Stannous 2-ethyl-hexanoate at 0.2% (w/v) was added and the reaction was carried out under vacuum at temperatures ranging from 130-150 °C for 90 minutes. The reaction temperature was held as close to 140°C as possible. However, literature shows the range from 130-150°C to be an acceptable temperature range for PLA bulk polymerization. The reaction mixture was dissolved in 20 ml anhydrous DCM and precipitated in ten fold the amount of cold diethyl ether. Excess ether was evaporated in the hood overnight. The polymer was dried further in a 25 °C vacuum oven overnight and stored in an airtight container at –20 °C. The structure and molecular weight were determined by 1H NMR after dissolving approximately 20 mg of polymer in 1 ml anhydrous deuterated DMSO. Gel permeation chromatography (GPC) was performed on the block copolymer to confirm apparent polymer molecular weight. The molecular weight was obtained using a 4 mg/ml polymer solution in tetrahydrofuran
(THF) using two PL-Gel mixed-column gel columns of 5 μm particle size (Polymer Labs, Amherst, MA) calibrated with a narrow distribution of polystyrene standards ranging from 580 g/mol to 1,290,000 g/mol.

### 4.2.5 NMR Spectroscopy

NMR was performed at 25°C on a Varian Unity Inova 300 Mhz instrument and spectra were analyzed using Vnmr 6.1b software (Varian, Inc., Palo Alto, CA). All samples were analyzed using 40 scans. Polymer solutions were analyzed as approximately 20% (w/v) solutions.

### 4.2.6 Preparation of mPEG-PLA-conjugate

500 mg of the copolymer and 25 mg haloperidol were lyophilized for two hours prior to the reaction. The coupling reaction was carried out at a 1:1 molar ratio of mPEG-PLA-COOH end group to haloperidol. mPEG-PLA-COOH and haloperidol were dissolved in 20 ml of anhydrous DCM. The flask was purged with nitrogen for 5 minutes. The DCM and all reactant solutions were measured and added to the reaction using needle transfer to ensure anhydrous conditions. In addition, the reaction was sealed with an airtight septum until completion. The PyBroP and DMAP were dissolved in anhydrous DCM as 35 mg/ml and 10 mg/ml solutions respectively. Under a nitrogen stream 1 ml of each reactant solution and 25 μl of TEA were added to the reaction. The reaction was stirred at 200 rpm for 24 hours at room temperature. After completion of the reaction, 20 ml of anhydrous DCM was added and the solvent was removed by rotary evaporation. The dried polymer was washed with an excess of ethanol (~ 150 ml) to remove unreacted haloperidol. Prodrug structure and degree of conjugation were
determined by dissolving approximately 30 to 50 mg of the conjugate polymer in deuterated DMSO and analyzing with $^1$H and $^{13}$C NMR.

4.3 Results and discussion

The overall synthetic route to achieve the mPEG-PLA-haloperidol conjugate structure is shown in Figure 4.1. Succinylated mPEG ($M_w$ 5000) was first prepared by reaction with succinic anhydride in anhydrous pyridine. The FTIR spectra of mPEG and the succinylated mPEG are shown in Figure 4.2. The peak present in the succinylated mPEG spectrum at 1741 cm$^{-1}$ is characteristic of the carboxylic acid bond which arises from the new succinic acid end group. This peak is not present in the mPEG spectrum. The $^1$H spectra for mPEG and succinylated mPEG are shown in Figures 4.3A and 4.3B respectively. In Figure 4.3B there are peaks present at 2.2 and 2.4 ppm that are not present in the $^1$H spectra mPEG prior to functionalization. These peaks are not representative of the protons belonging to the succinic anhydride ring. If this were the case they would appear as a single characteristic peak at 2.6 ppm for all four protons since as a part of succinic anhydride they are not distinct from one another. These peaks are representative of the two CH$_2$ groups in the succinic acid group, the peak at 2.2 ppm represents the protons closest to the carboxylate end group and the peak at 2.4 ppm belongs to the protons closest to the ester linkage between the succinic acid and the mPEG polymer.

The di-block copolymer was synthesized by ring-opening polymerization with D,L-lactide. The $^1$H NMR spectra of mPEG-PLA-COOH can be seen in Figure 4.4. The
Figure 4.1 Schematic representation of synthesis of polymeric prodrug
Figure 4.2 FTIR of mPEG and succinylated mPEG
Figure 4.3 (A) $^1$H NMR of mPEG ($\delta = 3.5$ ppm, -OCH$_2$CH$_2$--; $\delta = 3.2$ ppm, -CH$_3$). (B) $^1$H NMR spectrum of succinylated mPEG in CDCl$_3$ ($\delta = 7.4$ ppm).
Figure 4.4 $^1$H NMR spectra of mPEG-PLA-COOH, inset – corresponding polymer structure.
Mₙ of the copolymer batches was determined by ¹H NMR to range from 7000 to 9000 g/mol. The feed ratio for the lactide polymerization was typically set up to synthesize a PLA chain of approximately 5000 g/mol per each PEG chain. Excess lactide monomer collected around the neck of the flask during the nitrogen purge and when the vacuum was applied which accounted for some loss of monomer. Moreover, it was found that if the polymerization went for at least 2 hours PLA block molecular weights were obtained closer to the predicted values. Consequently, the polymerization time of 90 minutes may not be sufficient for the full conversion of monomer when the propagating group is the carboxylate. However it was desirable to have PLA block molecular weights in the 2000 to 3000 g/mol range which was achievable in the 90 minute time frame. To determine the molecular weight the area of a single proton on the NMR spectra was determined by dividing the area of the monomethoxy peak from mPEG by three. This area was used to determine the number of PLA repeating units by dividing the determined area of a proton into the –CH peak at 5 ppm. Based on the absorbance versus retention time the peak molecular weight on a single batch of copolymer was verified by GPC to be ~ 8700 g/mol (Figure 4.5A). The Mₙ of this batch was determined to be 7300 g/mol by NMR. The peak molecular weight in a molecular weight distribution falls in between the Mₙ and the Mₚ. The weight average molecular weight, or Mₚ, gives more influence to larger chains, so the Mₚ is generally larger [14]. The equation for this is:

\[
\overline{M_w} = \frac{\sum N_x M_x^2}{\sum N_x M_x} \quad (2.2)
\]

Therefore, the weight average is always larger than the number average. A sample molecular weight distribution is shown in Figure 4.5B.
Figure 4.5 (A) GPC molecular weight distribution of mPEG-PLA-COOH. (B) Representative molecular weight distribution for a typical polymer sample with $M_n < M_p < M_w$. 
Haloperidol was conjugated to mPEG-PLA-COOH via an ester linkage between the hydroxyl group in haloperidol and the carboxylate end group in mPEG-PLA-COOH. The mechanism for the esterification using the coupling reagent combination of PyBroP/DMAP is shown in Figure 4.6. The $^1$H and $^{13}$C NMR spectra and the corresponding chemical structure of haloperidol are shown in Figures 4.7 and 4.8 respectively. In addition, the $^{13}$C spectrum of mPEG-PLA-COOH is shown in Figure 4.9.

The conjugation of haloperidol to mPEG-PLA-COOH was confirmed by $^1$H and $^{13}$C NMR. In the proposed esterification reaction, the hydroxyl proton and the carboxylic acid OH group would be eliminated from haloperidol and polymer end group respectively in a condensation reaction to form the new ester linkage. The elimination of the hydroxyl proton is evident in the $^1$H NMR spectra shown in Figure 4.10. The hydroxyl proton of haloperidol ($\delta = 4.8$ ppm) visible as a singlet in Figure 4.10A, and was no longer present in the conjugate spectra, Figure 4.10C. A $^1$H spectrum of a 1:1 molar ratio of haloperidol to the mPEG-PLA-COOH carboxylic acid end group showed a clear singlet of the hydroxyl proton peak at 4.8 ppm, this can be seen in Figure 4.10B. In Figure 4.11 the elimination of the carboxylic acid proton from mPEG-PLA-COOH ($\delta = 13$ ppm) can be seen. In Figure 4.11A the broad peak at 13 ppm is characteristic of a carboxylic acid proton. Carboxylic protons are notorious for being difficult to see; yet in this spectrum the peak is clear. However, in the conjugate spectrum in Figure 4.11B this peak is no longer evident indicating the elimination of this proton from the conjugate structure.

Figures 4.12A and 4.12B show $^{13}$C spectra of haloperidol and the haloperidol - polymer conjugate respectively. Hydroxyl carbons characteristically appear from 50-65 ppm. The hydroxyl carbon in haloperidol shifts to 57 ppm. After esterification with
Figure 4.6 Schematic mechanism for mPEG-PLA-COOH & haloperidol coupling.
Figure 4.7 $^1$H NMR spectra of haloperidol. Inset – corresponding haloperidol chemical structure.
Figure 4.8 $^{13}$C NMR spectra of haloperidol. Inset – corresponding haloperidol chemical structure.
Figure 4.9 $^{13}$C NMR spectra of mPEG-PLA. Inset – corresponding polymer structure.
Figure 4.10 NMR spectra showing elimination of –OH proton from haloperidol. (A) Singlet from hydroxyl proton in haloperidol, 4.8 ppm. (B) Physical mixture of haloperidol and mPEG-PLA-COOH. (C) $^1$H spectra of conjugate polymer, note absence of hydroxyl singlet at 4.8 ppm from haloperidol (inset).
Figure 4.11 (A) $^1$H spectra of mPEG-PLA-COOH, note presence of carboxylic acid proton at 13 ppm. (B) NMR spectra showing elimination of COOH proton from conjugate polymer.
Figure 4.12 (A) $^{13}$C NMR spectra of haloperidol, inset - hydroxyl carbon peak at 57 ppm.

(B) $^{13}$C spectra of mPEG-PLA-haloperidol, note shift of hydroxyl carbon after conjugation to 66 ppm (inset).
haloperidol, one would expect a downfield shift of the hydroxyl carbon because ester carbons characteristically appear further downfield. After conjugation the peak at 57 ppm disappeared and there was a new peak downfield at 66 ppm attributed to the conjugated carbon.

The synthesized polymeric prodrug was washed in an excess of ethanol. The solubility of haloperidol in ethanol is approximately 16 mg/ml. Approximately 10 fold the solubility limit was used to wash the polymeric prodrug. The percent conjugation was determined by the $^1$H NMR spectrum based on the peak intensity ratio of the methylene protons of mPEG (OCH$_2$CH$_2$: $\delta = 3.5$ ppm) and the 2 protons on the aromatic ring closest to the fluorine atom ($\delta = 8.1$ ppm). The polydispersity index of the mPEG polymer, as reported by Sigma, was 1.1. Polydispersity index is determined by dividing the weight average molecular weight by the number average molecular weight. A monodisperse polymer will have a polydispersity index of one. Based on a molecular weight of 500 g/mol, a good approximation can be made that there are 454 protons that make up the peak at 3.5 ppm. A sample was washed once in a ten-fold excess of ethanol and the percent conjugation was determined. After a second wash in ethanol the percent conjugation was found to be unchanged. Therefore, one ethanol wash was found to be sufficient to remove excess haloperidol. On average $64.8 \pm 21\%$ (n=6) of the polymer end groups were conjugated to haloperidol molecules with the highest percentage being 95% and the lowest being 41%. In order to optimize the reaction it was found that when the nitrogen purge was carried out for 5 minutes prior to addition of the coupling agent/catalyst pair, maximizing the anhydrous conditions, the conjugation percentage could be increased.
4.4 Conclusions

The first specific aim of this research was to synthesize a new biodegradable ester linkage between a carboxylic acid on the end of a PEG-PLA block copolymer and a free hydroxyl group on haloperidol. This was accomplished by incorporating a carboxylate onto the end of mPEG for further polymerization with lactide to create mPEG-PLA-COOH. The incorporation of the succinic acid was confirmed using FTIR and NMR spectroscopy. The subsequent polymerization and molecular weight was determined by NMR spectroscopy and confirmed using GPC. The conjugation of haloperidol to the mPEG-PLA-COOH was carried out via the coupling agent, PyBroP/DMAP, under anhydrous conditions. The degree to which these conditions were maintained seemed to be a determinant of the percent conjugation. The presence of the new ester bond was confirmed by $^1$H and $^{13}$C spectroscopy.
4.5 References


CHAPTER 5: SYNTHESIS, CHARACTERIZATION, AND DRUG LOADING OF BLOCK COPOLYMER MICELLES

5.1 Introduction

Nanoscale particles are of great interest for drug delivery applications. Many types of drugs can be incorporated into biodegradable polymer particles. As discussed previously, these biodegradable polymers can be engineered to maintain a steady state concentration of drug in the plasma over an extended period of time. Decreasing administration frequency can improve patient adherence to a pharmaceutical regimen. In addition, maintenance of steady state drug levels can avoid the peaks and valleys of conventional dosing thereby avoiding the potentially toxic side effects of pharmaceuticals at their peak concentrations. Moreover, nanoparticles can improve the bioavailability of certain drugs, either by solubilization of hydrophobic drugs inside polymer particles, or by protecting protein based drugs from proteolysis in the digestive track. By increasing the bioavailability of drugs, the dose can be decreased and again, some toxic side effects may be avoided. However, many nanoparticles have failed to reach their full potential for drug delivery due to poor drug loading, a high initial burst release, and rapid clearance by the immune system. Block copolymer micelles have a great deal of potential for delivery of hydrophobic drugs. They have a small diameter, typically less than 100 nm, with a narrow size distribution that is dependent on the molecular weight of the polymer blocks. The hydrophilic block forms the outer shell of the particles and prevents protein adsorption on to the particle surface through steric hindrance and osmotic repulsion [1]. This can delay recognition and clearance by the immune system leading to longer systemic circulation. In addition, micellization is a spontaneous
process; consequently the ease of preparation may also benefit any future scale up procedures.

Prior work was performed to synthesize and characterize the block copolymer conjugate, mPEG-PLA-haloperidol. In this chapter the formation of micelles from the mPEG-PLA-COOH control polymer and the mPEG-PLA-haloperidol conjugate polymer was analyzed with $^1$H NMR and cryogenic transmission electron microscopy. Both the control and the conjugate polymer micelles were formed with and without free haloperidol incorporated. Micelles were characterized for their size using dynamic light scattering (DLS) and their aggregation number using static light scattering (SLS). Stability is an important parameter when using micelles for drug delivery. The best measure of micelle stability is the CMC, which can be determined using fluorescent spectroscopy with the fluorescent probe pyrene. The fluorescence properties of pyrene change when it transfers from the aqueous environment to the hydrophobic microenvironment within the micelle core. Fluorescence spectroscopy can be used to show whether micelles remain intact below their CMC. Surfactant micelles are known for their dynamic nature, where there is equilibrium between unimers and micelles, and below the CMC micelles dissociate, and only unimers exist in solution. If this were to happen with these polymeric micelles, as soon as micelles were disseminated systemically they would be diluted below their CMC, dissociate and release all the drug loaded in the core. However, polymeric micelles are more stable than their small molecule surfactant counterparts.

Proton NMR was used to determine the total haloperidol incorporated into the micelle core. In the past, polymeric prodrugs have been used to increase drug loading
and extend release of pharmaceuticals [2, 3]. By conjugating drug to the polymer prior to formation into micelles, the rapid release of drug on or near the surface, known as the burst release, may be avoided. In this work, haloperidol was chemically conjugated to the PLA end group. While this may provide the opportunity to reduce the burst release and lengthen the overall release profile, for certain drugs, including haloperidol, the limited amount of drug that can be incorporated in this way may not be enough to reasonably meet dosing requirements. Consequently, it was thought that one could potentially use the favorable hydrophobic interactions between the conjugated haloperidol and free haloperidol to enhance drug incorporation.

There are others factors that may impact drug loading. By saturating the continuous phase with the sparingly soluble haloperidol during micelle preparation, the partition coefficient of the drug between the aqueous phase and the PLA core can be shifted to incorporate more drug molecules. It has been elucidated that free drug loading in the core is also, to some extent, controlled by the Flory-Huggins interaction parameter between the core-forming block and the drug molecule [4, 5]. Furthermore, it has been found that hydrogen bonding between drug and polymer end groups can enhance drug loading [6]. Consequently, there are a number of factors that may impact free drug incorporation into the micelle core, and conjugation may be a powerful tool in achieving the ideal drug loading and release properties. It is imperative that there is a full characterization of micelles made with both control and conjugate polymer, and with and without free drug incorporation, to elicit any changes in micelle structure or properties.

The specific goals of this work were to formulate block copolymer micelles with the control and conjugate polymer, and characterize for size, polydispersity, morphology,
aggregation number and stability. In addition, to evaluate the effects of different degrees of conjugation on the stability and size of the micelles, and to determine the degree of free haloperidol loading in control and conjugate micelles.

5.2 Experimental section

5.2.1 Preparation of micelles

Micellization is a spontaneous process in which a block copolymer is dissolved in a common solvent for both blocks. This solution is added to a larger volume of a selective solvent for one block that induces the spontaneous formation of micelles. Conjugate and unconjugated copolymer (100 mg) were dissolved in 10 ml acetone. The polymer solution was added to 100 ml of deionized water stirring at 400 rpm. In order to load haloperidol into the micelle core, varying weight percentages of haloperidol were added to the polymer/acetone solution. The polymer/drug solution was added to an aqueous solution of 100 μg/ml of haloperidol. Unincorporated free haloperidol was removed from lyophilized micelles by resuspension of micelles in water and passage through a PD-10 desalting column (Amersham Biosciences, GE Healthcare, Piscataway, NJ). PD-10 desalting columns are prepacked columns containing Sephadex™ G-25 medium for separation of high molecular weight substances (> 5000 g/mol) from low molecular weight compounds (<1000 g/mol). Micelles were lyophilized and stored at –20°C.

5.2.2 Dynamic light scattering

Dynamic light scattering (DLS) is also sometimes referred to as quasi-elastic light scattering (QELS) and photon correlation spectroscopy (PCS). To determine the
hydrodynamic diameter and polydispersity of the micelles dynamic light scattering (DLS, Brookhaven 90Plus apparatus, Holtsville, NY) was performed. The apparatus was used with a 15 mW, solid state laser operating at a 678 nm wavelength and a BI-9000AT autocorrelator. Particles suspended in a liquid are subject to Brownian motion, defined as the random motion of particles suspended in a liquid or gas. Laser light hits the samples and particles scatter light in all directions. A photomultiplier tube measures the scattered photons. The intensity of the photons appear to fluctuate randomly and are compiled by an autocorrelation function. To determine the autocorrelation function, at a moment in time, \( t \), particles in solution will scatter light with a particular intensity at a set angle, in this case that angle is 90º. After some time change \( \Delta t \) scattering intensity of the particles will change as a result in the change of particle orientation. If \( \Delta t \) is very small, then the intensity will not change significantly, since the particles have not had enough time to move around in solution. As \( \Delta t \) increases, the chances of the intensity being the same will decrease dramatically. This dependence of intensity autocorrelation on time is directly related to the ability of particles to randomly move, or to diffuse through their medium. The measured autocorrelation functions are analyzed for the first and second cumulants of a quadratic fit. This provides measures of the apparent diffusivity and polydispersity respectively. Hence, we can relate the decay in the autocorrelation directly to diffusion coefficient and the hydrodynamic diameter through the Stokes-Einstein equation:

\[
D_h = \frac{k_B T}{3\pi \eta D} \tag{5.1}
\]
where $D_h$ is the hydrodynamic diameter of the particles, $k_B$ is the Boltzmann constant, $T$ is the temperature ($25^\circ C$), $\eta$ is the solvent viscosity, and $D$ is the diffusivity from the first cumulant.

Another important measure is the polydispersity which provides a rough measure of the width of size distribution, and should not be confused with the standard deviation. The polydispersity is defined as the relative variance, or the second moment, it is the intensity weighted relative variance of the diffusion coefficient. When the polydispersity is closer to zero it is an indication that the sample is more monodisperse.

5.2.3 Static light scattering

Static light scattering has long been used to measure the average of the intensity of light over time that has been compared to fluctuations due to thermal motion of polymer particles. Aggregation number, or total molar mass of the micelles, was also determined using the Brookhaven 90Plus apparatus. Static light scattering can be used to determine the size, morphology and molecular weight of colloidal solutions [7]. Analysis was performed using the Brookhaven Zimm plot software with the Debye plot graph type on several concentrations of micelles above the CMC. The DyBye plot uses the following equations, where inverse of the molar mass is the intercept of the plot:

$$H \cdot c / \Delta R(\Theta, c) = 1/ M_w + 2A_2c \quad (5.2)$$

$$H = \frac{4\pi^2 n^2 \lambda_0}{(N_o \lambda_o^4)} \quad (5.3)$$

$H$ is an optical constant where $n_o$ is the refractive index of the solvent, $dn/dc$ is the specific refractive index increment of the solution, $N_o$ is Avogadro’s number, and $\lambda_o$ is the wavelength of the laser which is equal to 633 nm. In the equation used to create the
DeBye plot, \( c \) is equal to the polymer concentration, \( \Delta R \) is the Rayleigh ratio which is calculated at different concentrations and is proportional to the corrected, scattered light intensity (the proportionality constant is determined by calibration against a substance with a known Rayleigh ratio), \( \Theta \) is the scattering angle in degrees which is fixed at 90°, \( M_w \) is the molar mass, and \( A_2 \) is the second virial coefficient. The intercept of the equation is \( 1/M_w \).

### 5.2.4 Fluorescence spectroscopy

The critical micelle concentration (CMC) was determined using pyrene as a fluorescent probe. Fluorescence spectroscopy using the localization of pyrene in the micelle core is the most sensitive and most commonly used method for determination of polymeric micelle CMC. Micelle solutions varying in concentration from 0.1 \( \mu \)g/ml to 500 \( \mu \)g/ml were equilibrated with a fixed concentration of pyrene, 0.04 \( \mu \)g/ml. Fluorescence measurements were carried out using a steady state fluorescence spectrometer model A-710 (Photon Technology International, Ontario, Canada). The bandpass was set to 2 nm, the excitation wavelength was 330 nm and the fluorescence emission was measured in 1 nm intervals from 350 to 450 nm with a 0.25 second integration time for all tests. The inner filter effect was negligible because measurements were taken on dilute solutions so there was no turbidity to keep laser light from passing through the samples. The CMC was determined by plotting the intensity ratio of the 3\(^{rd}\) to the 1\(^{st}\) most intense peaks or \( I_3/I_1 \) which in this case was 381/377 nm, against the log of concentration of the micelle solutions. The flexion point in this plot was taken as the CMC.
5.2.5 Nuclear magnetic resonance (NMR) spectroscopy

NMR was performed at 25°C on a Varian Unity Inova 300 MHz instrument and spectra were analyzed using Vnmr 6.1b software (Varian, Inc., Palo Alto, CA). All samples were analyzed using 40 scans. Polymer solutions were analyzed as approximately 20% (w/v) solutions.

5.2.6 Cryogenic transmission electron microscopy

The shape and size of the micelles were also determined by cryogenic transmission electron microscopy (cryo-TEM). Samples for cryo-TEM were prepared in a controlled environment vitrification system (CEVS) at controlled temperature of 25 °C, and at saturation to avoid evaporation of water during specimen preparation [8]. An 8 μl drop of the solution was placed on a TEM grid covered with a perforated film (Ted Pella). The sample was blotted to form a 100-250 nm thick liquid film and immediately plunged into liquid ethane at its freezing point (-183° C), producing a vitrified specimen. Vitrified samples were studied using a Philips CM120 transmission electron microscope operated at 120 kV, using an Oxford CT-3500 cryo-holder maintained at below -178 °C. Images were recorded digitally in the minimal electron dose mode by a side-mounted Gatan 791 MultiScan CCD camera with the Digital Micrograph software package [9]. Contrast and brightness enhancement were done using the Adobe Photoshop 7.0 ME package.

5.2.7 Determination of drug loading

To determine the total haloperidol incorporation, approximately 20 to 40 mg of lyophilized micelles were dissolved in deuterated DMSO. From the NMR spectra a peak
area ratio was determined between the methylene proton peak from the PEG repeating unit ($\delta = 3.5$ ppm) and a doublet of doublets that results from the 2 protons on the aromatic ring closest to the fluorine atom on haloperidol ($\delta = 8.1$ ppm). This peak area ratio is equivalent to a molar ratio between haloperidol in the micelle core and the polymer chains that make up the micelles. With the knowledge of the micelle sample weight a percent incorporation (w/w) could be determined. The incorporation efficiency was determined by the actual haloperidol incorporation divided by the theoretical haloperidol incorporation. However, for consistency, the haloperidol used to saturate the aqueous continuous phase for free drug incorporation, was not taken into account for efficiency determination.

5.3 Results and discussion

Control and conjugate block copolymer spontaneously formed micelles upon addition of an organic phase solution of polymer in acetone, into the continuous aqueous phase. The initial indication that micelles formed was that upon addition of the block copolymer solution, which is insoluble in water, to the aqueous phase, no precipitate formed. This indicates that the hydrophobic block that causes the block copolymer to be insoluble in water has been sequestered away from the aqueous phase. The formation of mPEG-PLA-COOH micelles was confirmed using $^1$H NMR of lyophilized micelles that were dispersed in deuterium oxide (Figure 5.1). The predominant peak was the mPEG peak, which is to be expected because the PEG block makes up the corona of the micelle-like structure and is soluble in water. Due to the limited mobility of the PLA chains in the micelle core, the intensity of the proton peaks that were generated by the PLA chain ($\delta = 5.0$ ppm and 1.8 ppm) were dramatically reduced compared to those peaks in the
Figure 5.1 $^1$H NMR spectrum of mPEG-PLA-COOH micelles resuspended in D$_2$O.
DMSO solution (Figure 5.2). The absence of the PLA peaks could be attributed to either the PEG shell shielding the PLA core, or these results may indicate that PLA has formed a solid core. Stolnik et. al. has shown that the reduction of PLA peaks is indicative of the latter. $^1$H NMR on solid PLGA nanoparticles, resuspended in D$_2$O, show a similar reduction in peak intensity of PLGA demonstrating that this phenomena can be attributed to the formation of a solid state [10]. In addition, NMR was performed on surfactant micelles and their respective unimers, which showed all peaks for both segments visible with equivalent intensity in both micelle and unimers, indicating the dynamic nature of these micelles [10].

The $^1$H spectrum for the conjugate micelles is shown in Figure 5.3. These results were consistent with those for control micelles. In addition, no haloperidol was present in the NMR spectrum indicating that the conjugated haloperidol was contained inside the micelle core.

Traditionally, drug loading in micelles is accomplished by codissolving drug and copolymer in the organic phase prior to the formation of micelles in the aqueous phase [11]. Unfortunately, when following this procedure with mPEG-PLA-COOH and haloperidol, there was no detectable drug in the micelle core. Briefly, the moles of haloperidol entrapped in the micellar core were determined by dividing the weight of the micelle sample by the molar ratio of polymer to haloperidol, multiplied by the polymer molecular weight plus the haloperidol molecular weight.

$$Haloperidol(moles) = \frac{Micelle \_ sample \_ weight(g)}{Molar \_ ratio \times polymer \_ MW + haloperidol \_ MW}$$ (5.4)
Figure 5.2 $^1$H NMR of mPEG-PLA-COOH in DMSO-$d_6$. 
Figure 5.3 $^1$H NMR spectrum of conjugate micelles resuspended in D$_2$O.
This number provided the moles of haloperidol incorporated into micelles. In order to aid in the loading of free haloperidol into the micelles, the aqueous phase was saturated with 100 μg/ml of haloperidol prior to the addition of the copolymer and drug mixture. This forces haloperidol to partition into the micellar core and increases the percent haloperidol incorporation to 23.75 % (w/w) for the micelles with free drug alone and 15% (w/w) for the combination micelles (prodrug polymer and free haloperidol). Drug incorporation and efficiency values can be seen in Table 5.1. As stated previously, when no saturated aqueous phase was used, there was no detectable haloperidol in the micelle core. However, when micelles were made with conjugate polymer (92% conjugation efficiency), with no saturated aqueous phase, they were found to contain 3.6 % (w/w) haloperidol. Haloperidol was able to be loaded into the micelle core by either conjugating the drug to the polymer or by saturating the aqueous phase with haloperidol prior to micelle formation. Micelles were formed with a fixed mPEG block length of 5000 g/mol and PLA block lengths ranging from 2000 to 4000 g/mol. In Table 5.2 the difference in incorporation efficiency with PLA block lengths of 2300 and 400 g/mol can be seen. These results are consistent with the results of other groups that show an increased drug loading capacity with larger hydrophobic block lengths [12].

Solubilization is the ability of micelles to enhance aqueous solubility of hydrophobic substances that are otherwise only slightly soluble in water. The amount of compound that can be solubilized in a micelle core is partially controlled by the Flory-Huggins interaction parameter between the solubilizate, haloperidol, and the core forming block, PLA [5]. The thermodynamics of haloperidol partitioning into the micelle core is discussed in greater detail in section 2.6.1. In hindsight, this information would be a key
Table 5.1 Drug incorporation into micelles.

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Percent Incorporation % (wt drug/wt polymer)</th>
<th>Incorporation efficiency % (wt % drug final/wt % drug initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG-PLA-haloperidol</td>
<td>3.6</td>
<td>82</td>
</tr>
<tr>
<td>mPEG-PLA-COOH + free haloperidol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No saturated aqueous phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mPEG-PLA-COOH + free haloperidol</td>
<td>24</td>
<td>86</td>
</tr>
<tr>
<td>Haloperidol 100 µg/ml saturated aqueous phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mPEG-PLA-haloperidol + free haloperidol</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Haloperidol 100 µg/ml saturated aqueous phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA block molecular weight</td>
<td>Haloperidol incorporation efficiency</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td></td>
</tr>
<tr>
<td>2300 g/mol</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>4000 g/mol</td>
<td>86%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2 Impact of PLA molecular weight on haloperidol incorporation efficiency
parameter in designing the best suited drug-polymer system. To determine the Flory-Huggins interaction parameter, the solubility parameter of the core forming block is subtracted from the solubility parameter of the drug, the units for the solubility parameters are \((J/cm^3)^{1/2}\). These solubility parameters can be calculated theoretically or determined experimentally. To experimentally determine the solubility parameter, solvency testing can be used in which polymer solubility is compared in solvent groups with different hydrogen bonding characteristics [13]. The midpoint of the solubility range is the solubility parameter. The difference in solubility parameters is then multiplied by the molar volume, which is the volume of a molecule per mole, and divided by the Boltzmann constant multiplied by the absolute temperature.

It was originally hypothesized that the conjugation of the hydrophobic molecule haloperidol to the end of the PLA block would increase the loading of free haloperidol into the micelle core. In actuality it is unlikely that the addition of an end group would change the Flory Huggins parameter of the PLA block significantly enough to impact loading. It is possible that the presence of a covalently bonded drug molecule may have a steric effect on the core packing that could potentially impact the loading and release of free drug. However, it is more likely that the loss of the carboxylic acid end group and it’s ability to hydrogen bond with haloperidol was the cause for the decreased drug loading capacity for the combination micelles versus the free drug micelles. It has been shown that haloperidol loading in PLA solid polymer nanoparticles is enhanced when the carboxylic acid end group remains uncapped [6].

Micelles were found to have diameters ranging from 28 to 52 nm as determined by DLS (Table 5.3). DLS is a fast, reliable method that does not require preliminary
<table>
<thead>
<tr>
<th>Micelle Type</th>
<th>Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control micelles (mPEG-PLA-COOH)</td>
<td>28.73 ± 1.45</td>
</tr>
<tr>
<td>Conjugate micelles (mPEG-PLA-haloperidol)</td>
<td>49.67 ± 4.29</td>
</tr>
<tr>
<td>Free drug micelles (mPEG-PLA-COOH + free haloperidol)</td>
<td>52.53 ± 1.66</td>
</tr>
<tr>
<td>Combo micelles (mPEG-PLA-haloperidol + free haloperidol)</td>
<td>52.23 ± 1.45</td>
</tr>
</tbody>
</table>

Table 5.3 Micelle diameter by dynamic light scattering (DLS).
calibration or standards. The autocorrelation function of the light scattering intensity provides a measure of the particle size and polydispersity. This dependence of intensity autocorrelation on time is directly related to the ability of particles to randomly move, or to diffuse through their medium. Hence, we can relate the decay in the autocorrelation directly to diffusion coefficient and therefore the hydrodynamic diameter through the Stokes-Einstein equation. The polydispersity distribution from DLS of mPEG-PLA-COOH micelles can be seen in Figure 5.4. Cryo-TEM on control micelles showed a bimodal size distribution with a population having an approximate diameter of 28 nm and a larger population of approximately 13 nm (Figure 5.5A and 5.5B, respectively). The conjugate micelles shown in Figure 5.5C are consistent with the larger population of the control micelles. Polydispersity in the micelle diameter can be attributed to a molecular weight distribution. The micelle size represented in the cryo-TEM images appeared to be smaller than that detected by DLS. Hydrodynamic radii of particles determined using DLS are typically found to be significantly larger than those determined by cryo-TEM [15]. This is seen because the particle radius is based on the intensity of scattered light so the size is skewed towards larger particles. One additional possibility for this difference is the inability to view the corona of the micelle. However, one would see the corona if the micelles were very densely packed.

Micelle size is dependent on the core block molecular weight. A more detailed discussion of micelle formation and parameters can be found in section 2.6. In this work slight variation in micelle diameter, ranging from 25 to 40 nm, was observed with changes in PLA molecular weight ranging from 2000 to 4000 g/mol. As shown in Table 5.3, a relative increase in micelle diameter is seen when haloperidol was loaded into the
micelle core either covalently or as free drug. Moreover, it was of interest to determine the effect the percent conjugation had on micelle diameter. Changes in micelle diameter with percent conjugation can be seen in Figure 5.6. Diameter of micelles increase with degree conjugation of at lower percentages. However, at approximately 45% conjugation the size dependence appears to level off and diameters remain constant at ~ 70 nm up to 95% conjugation.

Aggregation numbers for control, free drug and conjugate micelles are reported in Table 5.4. The aggregation number represents the number of polymer chains that make up the micelle. Aggregation numbers are calculated using at least 4 concentrations of micelles above the CMC. The molecular weight of the micelle was calculated using a Debye plot, and that molecular weight is divided by the molecular weight of a single polymer chain to provide the aggregation number. In 1908 Mie showed that light scattering techniques could be applied to analyze colloidal solutions. It is crucial that the colloidal solutions be dilute such that particles are far enough apart to be treated as independent sources [7]. The molar mass was corrected for the weight fraction of haloperidol in the micelle core. The conjugate micelles have a similar aggregation number, $588 \pm 18$, to the control micelles, $593 \pm 65$. This indicates that the presence of chemically conjugated haloperidol does not significantly alter the micelle structure. However, micelles made with higher haloperidol incorporation, namely the free drug incorporated micelles and the combination of free drug and conjugate micelles have aggregation numbers of $271 \pm 37$ and $356 \pm 64$ respectively. It is evident in Table 5.1 that the both control and conjugate micelles with free drug incorporated have a
Figure 5.4 Typical DLS size distribution of control micelles. The dashed line represents the mean diameter based on intensity of 48.5 nm.
Figure 5.5 Cryo-TEM images of control and conjugate micelles. Two populations of structures were observed in the mPEG-PLA-COOH samples with few micelles of ~ 28 nm in diameter (A) and a large population of smaller micelles of ~ 13 nm in diameter (B). mPEG-PLA-haloperidol micelles (C) are similar in size and appearance to the main micelles formed by the polymer alone.
Figure 5.6 Micelle diameter versus percent conjugation.
<table>
<thead>
<tr>
<th></th>
<th>Aggregation number (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control micelles (mPEG-PLA-COOH)</td>
<td>592 ± 65</td>
</tr>
<tr>
<td>Conjugate micelles (mPEG-PLA-haloperidol)</td>
<td>588 ± 18</td>
</tr>
<tr>
<td>Free drug micelles (mPEG-PLA-COOH + free haloperidol) <em>Haloperidol 100 μg/ml saturated aqueous phase</em></td>
<td>271 ± 37</td>
</tr>
<tr>
<td>Combo micelles (mPEG-PLA-haloperidol + free haloperidol) <em>Haloperidol 100 μg/ml saturated aqueous phase</em></td>
<td>356 ± 64</td>
</tr>
</tbody>
</table>

Table 5.4 Micelle aggregation number.
significantly higher weight percentage of drug molecules in the core. This is expected since the conjugate micelles alone are limited in the amount of drug that can be incorporated to one drug molecule per polymer chain. The decreased aggregation number for these micelles can be attributed to the increased amount of haloperidol limiting the conformation and packing of the micellar core.

The CMC of a copolymer gives an indication of the thermodynamic stability of the micellar system upon dilution. One of the concerns regarding the use of micellar systems for drug delivery is their stability in the body at a very high dilution. Consequently a lower CMC is favorable for the stability in vivo. The CMC of mPEG-PLA-COOH, mPEG-PLA-COOH plus free drug, mPEG-PLA-haloperidol, and combination micelles was determined by using pyrene as an extrinsic fluorescent probe. The fluorescent properties of pyrene change when there is a transfer from the aqueous environment to the hydrophobic microenvironment of the micellar core. To determine the CMC, the ratio of intensity at 381 nm and 377 nm in the excitation spectrum were used. These spectra, and the changes that result with concentrations above and below the CMC, are shown in Figure 5.7. The peak at 381 nm shifts both upfield and in relative intensity as the pyrene moves from the aqueous environment to the hydrophobic core. The intensity ratio of the peaks at 381 nm to 377 nm was plotted against the log of polymer concentration. Examples are shown in Figures 5.8-5.10. The CMC is determined by taking the flexion point of the sigmoidal curve. The CMC of control micelles, free drug micelles, conjugate micelles, and combination micelles were 4.3 ± 2.3, 13 ± 1.3, 11 ± 3.7, and 9.7 ± 1.9 µg/ml respectively and can be seen in Table 5.5. All of these micelle formulations induce only slight changes on the CMC. Consequently we
can deduce that these changes in micelle formulations did little to affect micelle stability. The CMC was taken for conjugate micelles at varying degrees of percent conjugation. These results can be seen in Table 5.6. At a low percent conjugation, 31%, the CMC is closer to values typically seen for control micelles. Similar to the trend seen with diameter and percent conjugation, CMC values for conjugation percents ranging from 50 to 90% remain consistent.

One of the most important parameters when using micelles for drug delivery is their stability. It was previously mentioned that $^1$H NMR was used to show that PLA formed a solid core. It has been hypothesized by many that this “frozen” nature of the micelle core would prevent dissolution of micelles into unimers below the CMC [10, 16]. One way to determine if micelles remain intact after dilution below their CMC is to evaluate their fluorescence intensity. Since the fluorescence intensity of pyrene would change after it was forced back into an aqueous environment one would expect to see a shift in the intensity ratio to the lower part of the CMC curve. Control micelles made at a concentration ten fold above the CMC were diluted to a concentration ten fold below their CMC, and maintained an intensity ratio indicative of intact micelles (Figure 5.11). While spherical micelles are known to form with PEG weight fractions of 55% and greater, the phenomena of micelles remaining intact below the CMC was only observed in this work with PEG weight fraction of 68% and greater (i.e. lower PLA block molecular weights). Consequently, for this drug delivery application higher weight fractions of PEG were found to be more suitable.
Figure 5.7 Fluorescence emission spectra of pyrene as a function of polymer concentration in water (μg/ml). (A) mPEG-PLA-COOH; (B) mPEG-PLA-haloperidol; (C) mPEG-PLA-COOH plus 20 wt% free haloperidol; and (D) combination of mPEG-PLA-haloperidol conjugate plus 20 wt % free haloperidol.
Figure 5.8 CMC determination of control polymer micelles.

4.3 ± 2.3 μg/ml

Intensity ratio (381/377 nm) vs. log conc. mPEG-PLA-COOH (μg/ml)
Figure 5.9 CMC determination of control polymer with free drug incorporated.
Figure 5.10 CMC determination of conjugate polymer micelles.

Int. ratio (381/377 nm)

log concentration mPEG-PLA-haloperidol (µg/ml)

13 ± 1.3 µg/ml
<table>
<thead>
<tr>
<th>Micelle Formulation</th>
<th>CMC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control micelles (mPEG-PLA-COOH)</td>
<td>4.3 ± 2.3</td>
</tr>
<tr>
<td>Conjugate micelles (mPEG-PLA-haloperidol)</td>
<td>13 ± 1.3</td>
</tr>
<tr>
<td>Free drug micelles (mPEG-PLA-COOH + free haloperidol)</td>
<td>11 ± 3.7</td>
</tr>
<tr>
<td>Combo micelles (mPEG-PLA-haloperidol + free haloperidol)</td>
<td>9.7 ± 1.9</td>
</tr>
</tbody>
</table>

Table 5.5 Critical micelle concentration of different micelle formulations.
Table 5.6 CMC dependence on conjugation percent.

<table>
<thead>
<tr>
<th>% Conjugation</th>
<th>CMC conjugate micelles (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>3.4</td>
</tr>
<tr>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>68</td>
<td>13</td>
</tr>
<tr>
<td>90</td>
<td>11</td>
</tr>
</tbody>
</table>
Figure 5.11 Stability of micelles (50 μg/ml) diluted below the CMC (0.5 μg/ml). The red circle on the graph represents this sample. Pyrene remains sequestered in the micelle core below the CMC.
5.4 Conclusions

In this work polymer micelles were made with a small size of less than 100 nm in diameter and corona core structure, with PEG forming a brush on the surface of the particle. These characteristics meet two main criteria for nanoscale drug delivery systems, small size and the potential to extend the particles half-life in vivo.

In addition, stability, which is another important criteria for the use of micelles as a drug delivery system, was addressed. These micelles were found to form a solid core upon synthesis, indicating the lack of a dynamic nature where unimers and micelles exist in equilibrium. Furthermore, using fluorescence spectroscopy, the micelles were found to retain their structure following dilution below their CMC. These micelles were found to load haloperidol either through covalently linking drug to the PLA block or by saturating the aqueous phase with haloperidol prior to micelle formation.

While all the methods used for determining the micelle properties are accepted and well known techniques, there are other means for gathering information about micelle characteristics that may be useful in the future. Small angle x-ray scattering (SAXS) can be used to see structural dimension such as core/shell morphology and measuring shell thickness. The molar mass of a micelle and the second virial coefficient, which is reflective of the thermodynamic interaction between the solvent and the solute, were determined by SLS in this work. These parameters can also be determined by osmometry. Viscometry can be used to determine the hydrodynamic volume of a micelle. While pyrene is perhaps the most widely used determinant of CMC, this can also be determined using sedimentation velocity or size exclusion chromatography.
Another interesting question to answer would be where drug associates in the core and how that changes with the conjugate polymer. Despite the fact that the core of the micelle is hydrophobic, there is still an anisotropic distribution of water in the micelle core, with the highest concentration being near the surface (the interface with the hydrophilic block), and decreasing in concentration towards the center of the core [17]. As a result of this, the spacial positioning of drug molecules within the micelle core is dependent on the drug’s polarity [17].
5.5 References


CHAPTER 6: EVALUATION OF HALOPERIDOL RELEASE FROM MICELLES

6.1 Introduction

One of the goals for the development of a long-term delivery system is to reduce the side effects associated with the peaks in drug plasma concentration that result from conventional dosing. Long-term delivery systems that utilize polymer matrices to administer drug can aid in the reduction of side effects by increasing drugs solubility in vivo, increasing uptake of the drug through mucosal barriers, and thus increasing drugs bioavailability. Consequently these systems reduce the necessity for high doses to achieve a therapeutic concentration of drug. Moreover, long-term delivery systems that attain a linear release profile are less likely to have peak drug plasma concentrations outside the therapeutic level also resulting in a reduction of side effects. One class of drugs that benefits from a long-term delivery application is antipsychotic medication. Up to 55% of individuals with chronic psychotic disorders such as schizophrenia have significant difficulties adhering to treatment recommendations [1]. A long-term delivery system would decrease administration frequency, thereby increasing the potential for patient compliance. Haloperidol is a dopamine D2 antagonist antipsychotic and is used primarily to treat schizophrenia. For haloperidol, the reduction in side effects that can be achieved with long-term delivery is particularly important due to the emergence of motor side effects at higher plasma concentrations. A continuous, low concentration of haloperidol would decrease these side effects. In addition, a reduction in side effects would address the pharmaceutical adherence concerns associated with chronic medication.
One strategy for achieving extended, controlled release is block copolymer micelles. Micelles offer the opportunity to deliver drugs over an extended period of time as compared to conventional dosing. Their small size and hydrophilic outer shell afford them stealth properties that prevent rapid clearance by the immune system and allow for systemic circulation. The core forming polymer, PLA, is widely used for drug delivery because of its biodegradable properties and nontoxic by products. Previously, it was shown that haloperidol could be conjugated to the PLA end of the mPEG-PLA-COOH block copolymer. Furthermore, the conjugate polymer, as well as the control polymer, was formulated into micelles both with and without free drug incorporated. These micelles were found to have a solid PLA core, and therefore remain intact after dilution below their CMC. For small molecules, such as surfactants, after dilution below this concentration, micelles dissociate into unimers. When block copolymer micelle-like structures are made from PEG-PLA, PLA can entangle, forming a solid core, altering the dissociation kinetics and allowing the micelles to remain stable structures below their CMC. This added stability may have an impact on the use of micelles for long-term drug release vehicles.

In addition to micelle stability, another important factor in optimizing release from nanoscale micelles is controlling the burst release. This burst release is part of what is frequently referred to as a biphasic release profile [2]. During the first phase, or the 24 hours known as the burst release, the structural integrity of the particles is maintained. Burst release is characterized by the release of drug that is incorporated on or near the core/corona interface. This is controlled by diffusion rather than particle degradation.
The second phase, or the linear release, is characterized by pore formation and particle deformation [3].

These types of PEG-PLA block copolymer micelles have been shown to extend the release of several different drugs. mPEG-PLA micelles, loaded with paclitaxel, were found to release the majority of their loaded drug over a 7-day period, releasing about 20% in the first 24 hours, and then approximately 10% each day thereafter [4]. Micelles made from doxorubicin conjugated to mPEG-PLGA release around 20% of their drug load in the first 24 hours, but continue to release drug out to 14 days, as opposed to complete release of free doxorubicin within 2 days [5]. Doxorubicin release was extended to greater than 25 days when it was conjugated via an acid cleavable hydrozone linkage at pH 7. However, at a lower pH, doxorubicin release was complete at 15 days [6]. PEG-PLA micelles were synthesized with carboxylic acid functional groups incorporated along the PLA block backbone. This configuration significantly increased papaverine incorporation and extended release as compared to unmodified polymer [7]. However, a significant burst release (approximately 40%) was still evident in the slowest release profile.

It is the goal of this work to evaluate the release characteristics of micelles made with drug-conjugate polymer, micelles with free drug physically incorporated, and a combination of drug-conjugate polymer micelles with free drug incorporated, known as combination micelles. In previous work it was shown that combining the prodrug polymer and free haloperidol does not increase the total amount of drug that can be loaded into the micellar core, but the presence of conjugate drug may have an effect on the core structure and therefore potentially the drug release rate [8]. Presumably there is
a limit to the amount of haloperidol that will be able to pack into the micelle core. The presence of a covalently bonded drug molecule may have a steric effect on the core packing that could potentially impact the release rate of the drug. In addition, covalently linked haloperidol will be released when the ester linkage is cleaved in the same manner as the polymer backbone. This has been shown in other systems to extend release over physically entrapped drug [5]. In this work the comparative release rates of free haloperidol versus conjugated haloperidol from micelles will be determined. Additionally, in vivo behavioral studies were performed to assess haloperidol bioactivity from drug loaded micelles on ketamine induced hyperlocomotion 4 days after injection.
6.2 Experimental section

6.2.1 Release experiments

Conjugate and control micelles were synthesized with and without free drug incorporated as described in Section 5.2.1. Micelles were weighed (10-20 mg) and resuspended in 5 ml of PBS and sealed in regenerated cellulose dialysis membrane with a molecular weight cutoff of 1000 g/mol (SpectraPor 6, Spectrum Laboratories Inc., Rancho Dominguez, CA). The dialysis bags were submerged in 250 ml of PBS and incubated at 37 °C (pH 7.4). At predetermined time intervals 3 ml samples of the incubation medium were drawn and filtered using a 0.45 μm syringe filter (Millex® PVDF durapore) for HPLC injection. Fresh PBS was added back to the dialysate to maintain a constant volume. Samples were drawn at 1, 2, 4, 8, and 24 hours after the start of the release study. In addition, samples were drawn each day thereafter for 7 days. A control experiment was performed to determine the permeability of free haloperidol through the dialysis membrane.

6.2.2 High performance liquid chromatography (HPLC)

HPLC were used to determine very small quantities of haloperidol from in vitro release studies. The sample buffer and mobile phase was made up of acetonitrile (38%) and 10 mM ammonium acetate (62%) with the pH adjusted to 4.8. HPLC (Empower software, Waters Corp., Bedford MA) analysis was performed using a Symmetry C18 column with a 5 μm particle size and a photodiode array detector (PDA). The spectra for haloperidol were extracted at 254 nm. The flow rate was 1 ml/min, the injection volume was 50 μl and the retention time for haloperidol was approximately 6.7 minutes. A
calibration curve of haloperidol dissolved in PBS was generated with 5, 2.5, 1, 0.5, and 0.1 μg/ml concentrations.

### 6.2.3 In vivo behavioral testing

Low dose ketamine induced hyperlocomotion is used as both an animal model for schizophrenia and as a bioassay for antipsychotic activity of co-administered agents. Drug loaded micelles were made according to procedures outlined in Section 5.2.1. C57BL/6J male mice aged 8-10 weeks were injected with various treatment conditions on day zero. Animals were habituated to the locomotor apparatus for 30 minutes on each of the three days prior to ketamine administration. A control saline injection was administered prior to ketamine to determine baseline activity. Ketamine (10 mg/kg) was administered in acute intraperitoneal injection 4 days after micelle treatment. Treatment groups consisted of no treatment (n=8 mice), daily acute 1 mg/kg haloperidol (n=8), 5 and 10 mg/kg/day free drug micelles (n=7 per dose), 1 mg/kg/day conjugate micelles (n=8), and 1 mg/kg/day combination micelles (n=16).

Locomotor activity was measured in a “home cage” activity monitoring system (MedAssociates, St. Albans, VT). This system allows for a standard, clean home cage to be placed in a photobeam frame (30x24x8) with two levels of sensors arranged in an 8-beam array strip with 1.25 inch spacing. A computer detection system monitors interruptions of the photobeams for the ambulations parameter. Total ambulations were determined by the number of photobeam interruptions the animal made while moving about the cage, but did not include movement across the horizontal plane. Data were recorded on Med Associates personal computer-designed software and monitored at 5-
minute intervals for a total of 30 minutes per activity monitoring session. Mice received 3 days of habituation to the apparatus prior to 10 mg/kg ketamine exposure.

An ANOVA was used to evaluate the effects of drug treatment on locomotor activity. Treatment was designated the independent variable and number of ambulations was the dependent variable. Significant interaction effects were followed by Fisher LSD post hoc analyses.
6.3 Results and discussion

6.3.1 *In vitro* release

*In vitro* release profiles of haloperidol from free drug micelles, conjugate micelles, and combination micelles were evaluated over a 7 day period. The drug loading of the micelles evaluated ranged from 5 to 12.5 % (w/w) (Table 6.1). As shown in Figure 6.1, free drug micelles released their drug load the fastest over 72 hours, followed by combination micelles and conjugate micelles, over 96 and 120 hours respectively. While the lowest weight percentage of drug was loaded into the conjugate micelles, these micelles were the closest to approach a linear release profile, which has the potential benefit of achieving a constant infusion profile.

One of the main concerns for nanoscale drug delivery is preventing or controlling the burst release. The *in vitro* burst release profiles from the various composition micelles is shown in Figure 6.2. Interestingly, for the first 8 hours of the release period, the fastest release occurs with the combination micelles. However, at the 24 hour time point, the free drug micelles had released the largest fraction of drug, approximately 70%, while the conjugate micelles had released approximately 37% of loaded drug, while the combination micelles fell in between the two at 56% release of loaded drug. It is possible that during the first 8 hours of release from combination micelles, the free drug dominated the release profile, after a certain time frame the cleavage and release of the covalently linked drug took over and extended the release profile. It was originally hypothesized that the conjugate polymer altered the core packing of the micelles, potentially creating pockets of free drug. One explanation for these results is that these pockets actually increase the burst release of free drug.
<table>
<thead>
<tr>
<th>Micelles</th>
<th>Drug incorporation percent (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free drug micelles – PLA 4000 g/mol</td>
<td>10 %</td>
</tr>
<tr>
<td><em>Saturated aqueous phase</em></td>
<td></td>
</tr>
<tr>
<td>Free drug micelles – PLA 2300 g/mol</td>
<td>12.5 %</td>
</tr>
<tr>
<td><em>Saturated aqueous phase</em></td>
<td></td>
</tr>
<tr>
<td>Conjugate micelles</td>
<td>5 %</td>
</tr>
<tr>
<td>Combination micelles</td>
<td>9 %</td>
</tr>
<tr>
<td><em>Saturated aqueous phase</em></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1 Drug incorporation values for release studies
Figure 6.1 Release of haloperidol from free drug, conjugate, and combination micelles over 7 days.
Figure 6.2 Free drug, conjugate, and combination micelle release over the initial 24 hour period.
6.3.1.1 *In vitro* micelle stability

The hydrophilic polymer, PEG, which forms the corona, imparts the stability of micelles in an aqueous media. This stability is an important characteristic when using these micelles for a drug delivery application because micelle introduction to the body will certainly constitute dilution to below their CMC. It could have potentially dangerous implications if micelles dissociate after introduction to the body, releasing drug intended for a 7 day delivery period within the first hours after administration. In order for spherical micelles to form, the hydrophobic block, in this case PEG, must have a weight fraction of more than 55% [11]. However, greater PEG weight fractions may further stabilize the micelles. Release studies were performed with free drug micelles synthesized with PEG weight fractions of 55% and 68%. The release profiles of free drug micelles made with PLA block molecular weights of 2300 and 4000 g/mol, having PEG weight fractions of 68 and 55% respectively, are shown in Figure 6.3. The drug incorporated for both groups of micelles were 12.5 and 10% (w/w) respectively (Table 6.1). There was a rapid release of loaded haloperidol from the 55% PEG (4000 g/mol PLA) micelles within 8 hours of incubation in release media, indicating dissociation of micelles into polymer chains after introduction into the release medium. This release curve for these micelles was similar to that of the diffusion of free haloperidol from the dialysis membrane. However, the higher PEG weight fraction micelles, 68%, with the lower PLA block molecular weight (2300 g/mol), released loaded free drug over three days indicating release of haloperidol from intact, stable micelles.

One potential explanation for the rapid release from the 55% PEG micelles is that due to the low weight percentage of PEG, there was a lack of necessary stabilization of
Figure 6.3 Comparative release rates for free drug micelles made with PLA block molecular weights of 2300, 4000 g/mol, and free haloperidol release from the dialysis membrane.
the hydrophilic corona. However one may assume that greater entanglement due to a longer hydrophobic block would impart more stability to the micelles. Consequently, another potential explanation for the rapid release from the 55% PEG micelles can be offered. A weight fraction of 55% PEG is near the edge of a morphological transition for self-assembly from spherical micelles to cylindrical micelles. This may allow for different structures to form from PLA blocks that are at higher ends of the molecular weight distribution. The stability of the alternate morphologies is unknown and could account for the difference in release profiles.

6.3.1.2 Discussion of release study design

Measuring the in vitro drug release from micelles presented some technical challenges. Typically when release studies on nanospheres are performed, the release medium is removed and filtered using a syringe filter. The syringe filter is then back flushed into the release medium to return intact particles to the study; the smallest syringe filters available have a pore size of 0.22 μm. This is approximately three times larger than the micelles used in this work. The challenge with this work was the evaluation of the release of covalently linked haloperidol from both the micelle core and the polymer end group. There was the potential of releasing varied length lactic acid oligomers conjugated to haloperidol. There are techniques available for separating micelles or polymer chains from released drug such as using centrifuge units fitted with molecular weight cutoff membranes. Unfortunately, the lowest MWCO commonly available for these units is 3500 g/mol, and since the predominant PLA block length used in this work was 2300 g/mol, once cleaved from the mPEG chain, a PLA-haloperidol oligo of any length could pass through a 3500 MWCO membrane. The most common technique for
analysis of micelle release is incubating an aqueous micelle suspension enclosed in a dialysis bag, in PBS, and sampling the incubation medium. Theoretically, the small drug molecules will pass freely into the incubation medium and the polymer will remain in the dialysis bag. To some extent this posed a similar challenge for analysis of the conjugate micelles. However, dialysis membrane is available with smaller molecular weight cutoffs. The goal was to find the smallest MWCO dialysis membrane that would allow for analysis of the release of haloperidol in which the permeation rate of haloperidol through the dialysis membrane did not affect the release rate. Dialysis membranes with MWCO of 500, 1000, and 3500 were analyzed. Dialysis membrane with a MWCO of 500 was found to inhibit the permeation of free haloperidol. This was to be expected since the molecular weight of haloperidol is 375.9 g/mol. Thus, it was found that the 1000 MWCO to be the smallest dialysis membrane that would allow for permeation of haloperidol. As can be seen in Figure 6.4, the permeability of free haloperidol ($5 \times 10^5$ ml/mm*h) is greater than that of micelle released haloperidol ($1 \times 10^5$ ml/mm*h). The calculated permeability coefficient is equal to the slope of the curve and is calculated based on the linear, early time points in the curve [12]. $C_b(t)$ is the concentration of drug in the dialysate at time, $t$, $V_b$ is the volume of the dialysate, $L$ is the thickness of the dialysis membrane, $C_a$ is the concentration of drug within the dialysis membrane, and $A$ is the cross sectional area of the membrane. The difference between the permeability of free haloperidol from the dialysis membrane is indicative of the negligible effect of the dialysis membrane on the release rate.

There were two concerns regarding the use of dialysis membrane for evaluation of conjugated haloperidol release. First, some amount of the haloperidol that will pass
Figure 6.4 Comparison of permeability of free haloperidol vs. release of haloperidol from free drug micelles and combination micelles (1000 MWCO membrane).
through the membrane could be haloperidol-lactic acid oligomers. Since haloperidol alone crosses the blood brain barrier by passive diffusion, it is unclear if a macromolecule that includes lactic acid units would allow for passage into the brain and bioactivity. Further pharmacokinetic and pharmacodynamic studies would be necessary to determine if a haloperidol-lactic acid conjugate are able to cross the blood brain barrier and maintain bioactivity. The second concern was that the flux of the haloperidol-lactic acid conjugates across the dialysis membrane would be different than released free haloperidol. This may skew the release rate for conjugate and combination micelles.

Unfortunately, there was no change to the UV absorbance of free haloperidol versus conjugated haloperidol, consequently the haloperidol that was released as a conjugate could not be quantified. In addition, the change to the molecular weight of haloperidol with one or two lactic acid monomer units is very small and would be difficult to detect by size exclusion chromatography. As free haloperidol, that were analyzed using the 1000 MWCO dialysis membrane. Because of all these concerns, the release studies were performed on three groups of micelles made at the same time, using the same batch of polymer: free drug micelles, conjugate micelles, and combination micelles. In doing this, the relative release rates of the micelle groups could be analyzed.

6.3.2 Theoretical haloperidol release

The dialysis method for analyzing drug release allowed for the potential measurement of both free haloperidol and haloperidol conjugated to a few lactic acid units. To better understand the release of covalently bonded drug from the polymer end group, the ideal situation would be to measure the complete release of cleaved
haloperidol from the PLA end group. Haloperidol was conjugated to PLA via an ester bond, similar to those bonds that make up the PLA backbone, which are cleaved by hydrolysis. PLA has been thought to degrade by random chain scission according to first-order kinetics [13-15].

The first order rate constant for PLA degradation was determined experimentally by Delgado et al. to be 0.02 h\(^{-1}\) [14]. The number of ester bonds remaining at each time point can be determined by the following equation:

\[
C_E(t) = C_E e^{-kt} \quad (6.1)
\]

where \(C_E\) is the number of ester bonds, \(k\) is the first order rate constant and \(t\) is time in hours. The average number of repeating units for the PLA chains that made up the micelle core was approximately 30. Consequently, in order to theoretically determine the release of covalently linked haloperidol, one must determine the probability that at each time point the ester bond broken was a bond between lactic acid and haloperidol. Several assumptions must be made, such as: the concentration of water remained constant, there was a negligible effect of autocatalysis, the polymer was water insoluble, and that the probability of each bond breaking was equal. In Figure 6.5 the derivation used to determine the concentration of haloperidol released, \(C_d\) (a dimensionless concentration indicating fractional haloperidol released) is shown. A probability factor, \(P_b\), was included into the initial equation (6.2), which states that the rate of haloperidol released was equal to 1/30\(^{th}\) of the rate of ester bond loss. The plot of predicted haloperidol release, \(C_d\) versus time, is shown in Figure 6.6. According to the predicted
\[ Rh = -P_b k C_E \quad (6.2) \]

\[ \frac{dC_d}{dt} = -P_b k C_E \quad (6.3) \]

Rh – rate of haldol released

P_b – probability that the bond broken is a haldol bond \(1/30\)

k – rate constant \(0.02 \text{ h}^{-1}\)

C_T = total concentration of ester bonds

C_d = concentration of drug released

C_E = concentration of ester bonds unbroken

C_b = concentration of ester bonds broken

\[ C_T = C_E + C_d + C_b \quad (6.4) \]

\[ C_b = 29 C_d \quad (6.5) \]

\[ C_E = C_T - 30 C_d \quad (6.6) \]

\[ \frac{dC_d}{dt} = -P_b k (C_T - 30 C_d) \quad (6.7) \]

\[ x = C_T - 30 C_d \quad (6.8) \]

\[ \frac{dx}{dt} = \frac{dx}{dC_d} \frac{dC_d}{dt} \quad (6.9) \]

\[ \frac{dx}{dC_d} = -30 \quad (6.10) \]

\[ \frac{dx}{dt} = -30 \frac{dC_d}{dt} \quad (6.11) \]

\[ \frac{dx}{dt} = 30 P_b k x \quad (6.12) \]

\[ \int_0^x \frac{dx}{x} \left(-\frac{1}{30}\right) = \int_0^t P_b k dt \quad (6.13) \]

\[ \frac{1}{30} x = e^{-P_b k t} \quad (6.14) \]

\[ \frac{1}{30} (C_T - 30 C_d) = e^{-P_b k t} \quad (6.15) \]

\[ C_d = \frac{1}{30} C_T - e^{-P_b k t} \quad (6.16) \]

\[ C_d = 1 - e^{-P_b k t} \quad (6.17) \]

Figure 6.5 Calculations for theoretical release of conjugated haloperidol
Figure 6.6 Theoretical prediction of conjugated haloperidol release over 7-days due to random chain scission using a probability factor.
release, half of the conjugated haloperidol molecules would be released at approximately 1000 hours or 42 days. Obviously this was not consistent with experimental data in which 50% of conjugated haloperidol was released in approximately 2 days (Figure 6.1). However, when a similar derivation was completed to plot the total concentration of ester bonds broken, $C_b$ over time, removing the probability factor, the half-life of this concentration is approximately 41 hours (Figure 6.7). This was more consistent with the release rate of covalently linked haloperidol from micelles. The unexpected slow release of haloperidol when using the probability factor, indicating that there was a one in thirty chance the ester bond broken was a drug conjugate bond, requires another look at the assumptions made prior to the calculations. The first assumption that the concentration of water was constant cannot be true if the micelles were to remain intact upon immersion into the release medium. When micelles are suspended in water there is a concentration gradient, with the highest concentration of water near the interface of the micelle corona and the core [16]. In addition, the assumption that each ester bond has equal reactivity to hydrolysis may not be true. Degradation models have shown that the hydrolysis of PLA, unlike PCL, is not entirely random, but controlled by chain-end scission of the ester bonds [17]. This accelerated release hydrolysis of the chain-ends may account for a faster release of covalently linked haloperidol from PLA than if the release was completely random chain scission.
Figure 6.7 Theoretical PLA chain-end scission and release of conjugated haloperidol.
6.3.3 *In vivo* bioactivity

Low doses of ketamine, an antagonist of the N-methyl-D-aspartate type glutamate receptor, can induce changes which closely resemble the symptoms of schizophrenia [18, 19]. In addition, certain antipsychotic drugs such as haloperidol can modulate the effects of ketamine induced psychosis [18]. Ketamine induced psychosis is modeled in mice by using locomotive activity tasks, which are also blocked by antipsychotic medication such as haloperidol. It was found that a dose of 10 mg/kg ketamine significantly increased locomotor activity above baseline (F(1, 48)=122.1, p<0.001). Haloperidol reduced locomotor activity across all conditions (F(5, 48)=52.8, p<0.001). Micelles were administered on day zero and the effect of the haloperidol released from these micelles was analyzed against the 10 mg/kg ketamine dose 4 days post injection. There was a significant ketamine by haloperidol interaction (F(5, 48)=7.4, p<0.001) indicating that 1mg/kg and 0.1 mg/kg acute haloperidol treatment, 1mg/kg/day conjugate micelles, and 1mg/kg/day combination micelles attenuated the hyperlocomotion induced by ketamine. It is shown in Figure 6.8 that when haloperidol was administered as 0.1 and 1 mg/kg acute injections, a reversal of the affect of ketamine was seen (*), as well as a detrimental decrease in baseline locomotion (#). The conjugate micelles and the combination micelles both attenuated the effect of ketamine (*), indicating the presence of haloperidol 4 days post injection, without the impaired baseline locomotion of the acute haloperidol injection. There was no reduction of activity in the 10mg/kg/day free drug micelles (p=0.117), 5 mg/kg/day free drug micelles (p=0.753). This data is consistent with the *in vitro* release data. The effect of haloperidol on the ketamine induced hyperlocomotion
Figure 6.8 *In vivo* bioactivity of free drug, conjugate, combination micelle and acute haloperidol delivery by the effect on ketamine induced hyperlocomotion.
was not analyzed until four days post injection. Based in the *in vitro* release data, the combination and conjugate micelles were the only groups still releasing haloperidol at that time point, whereas the free drug micelles had released their entire drug load by 3 days. The gray bars in Figure 6.8 represent the locomotor activity after the 10 mg/kg ketamine injection and the black bars represent the baseline locomotions. It would be preferable for the haloperidol released from micelles to have fully reversed ketamine induced hyperlocomotion to baseline rather than attenuation of this effect. Further work must be done to alter the dose to achieve reversal of the effect of ketamine.

Another important result is the difference between the attenuation of hyperlocomotion between the acute injection and conjugate and combination micelles. After the acute haloperidol injection, the locomotor activity was reduced below baseline (Figure 6.8). However, while the attenuation of hyperlocomotion was seen with conjugate and combination micelles, the baseline activity was unaffected. One of the goals of a long-term delivery system is to deliver a drug over an extended period of time with a reduction in potentially harmful side effects. These results show the potential for the long-term delivery of haloperidol with a reduction in the associated motor side effects.

**6.4 Conclusions**

In this work, conjugate polymer micelles were shown to extend the release of haloperidol over a 5-day period as compared to free drug micelles, which released their incorporated drug over a 3-day period. Interestingly, combination micelles fell in between the two, releasing their loaded drug over a 4-day period. The fast release of haloperidol from combination micelles over the initial 8-hour period indicates that the conjugate polymer may have altered the core packing allowing for a more rapid release of
the physically entrapped drug. One hypothesis for this is that the altered core conformation created more pores for the diffusion of water into the core which allowed for a faster cleavage of the covalently bound drug within four days rather than five. Overall, the use of conjugate polymer micelles has great potential for the controlled release of drug. In addition, with future scale-up procedures in mind or applications to a more expensive drug, using a saturated aqueous solution to load free drug may not be efficient. This system could be applied to another compound in which the drug does not readily partition into the micellar core by traditional techniques.

*In vivo* behavioral studies were performed to assess haloperidol bioactivity from drug loaded micelles on ketamine induced hyperlocomotion. Results were consistent with *in vitro* release data, showing that conjugate and combination micelles continued to release drug 4 days post injection, attenuating the effects of the ketamine induced hyperlocomotion. Furthermore, results indicate that the sedative side effects of haloperidol were reduced with the micelle delivery systems as compared to the acute haloperidol injection.
6.5 References


CHAPTER 7: CONCLUSIONS AND FUTURE RECOMMENDATIONS

7.1 Conclusions

The overall goal of this work was to develop a nanoscale polymeric prodrug delivery strategy and to elucidate the effect the drug conjugate had on the characteristics of the system, including drug loading and release. The polymeric prodrug, mPEG-PLA-haloperidol, was synthesized via a coupling agent and confirmed with $^1$H and $^{13}$C NMR. Taking advantage of the self-assembly properties of amphiphilic block copolymers, nanoscale drug delivery vehicles were formulated from both the control and conjugate polymer. Spherical polymer micelles were made with a small size of less than 100 nm in diameter and corona core structure, with PEG forming a brush on the surface of the particle. These micelles were found to form a solid core, indicating the lack of a dynamic nature where unimers and micelles exist in equilibrium. Furthermore, using fluorescence spectroscopy, the micelles were found to retain their structure following dilution below their CMC. Haloperidol was able to be loaded into the micelle core by either conjugating the drug to the polymer or by saturating the aqueous phase with haloperidol prior to micelle formation.

Conjugate polymer micelles were shown to extend the release of haloperidol over a 5-day period as compared to free drug micelles, which released their incorporated drug over a 3-day period. Interestingly, combination micelles fell in between the two, releasing their loaded drug over a 4-day period. The burst release for the conjugate micelles was significantly reduced from the initial release from free drug and combination micelles. Ketamine induced psychosis was modeled in mice by using locomotive activity tasks, which are also blocked by antipsychotic medication such as
haloperidol. Using this model, the bioactivity of haloperidol was assessed from free drug, combination, and conjugate micelles four days post injection. Results were consistent with \textit{in vitro} release data, showing that conjugate and combination micelles continued to release drug 4 days post injection, attenuating the effects of the ketamine induced hyperlocomotion. Furthermore, the attenuation of the effect of ketamine induced hyperlocomotion by the conjugate and combination micelles did not exhibit similar sedative side effects below baseline locomotions as the acute haloperidol injections. These results are indicative that the sedative side effects of haloperidol were reduced with the micelle delivery systems as compared to the acute haloperidol injection.

\textbf{7.2 Recommendations}

While the initial goals of this work were met, there are still questions surrounding the use of block copolymer micelles for drug delivery applications. The remainder of this chapter will be devoted to recommendations for future work in overcoming some of the limitations of this system.

\textbf{7.2.1 Micelle characterization}

Small angle neutron scattering (SANS) can be used to see structural dimension such as core/shell morphology and shell thickness. Drug loading and release data indicate that the conjugate polymer alters the micelle core packing. It may be of interest to see if the potentially altered conformation has an affect on the micelle core/shell morphology. This information could help in designing the optimum conjugate polymer composition.
Drug release from many types of polymer delivery systems has been extensively studied. The mechanisms of polymer degradation and drug release were reviewed in Section 2.3. However, the specific mechanism for drug release from block copolymer micelles has not been elucidated. With solid polymer nanoparticles, it is known that some percentage of loaded drug molecules can be adsorbed onto or entrapped near the particle surface that causes the burst release. Based on the characterization of drug-loaded micelles in this work, it is known that haloperidol was incorporated into the PLA core, and was not present in the PEG corona. It has been hypothesized that drug incorporates into the micelle core in different places depending on the polarity of the drug molecule [1]. Determining the location of haloperidol within the micelle core may be helpful in understanding drug release from micelles. This can be done with drug molecules of differing polarity to shed some light on the release mechanism from block copolymer micelles.

The benefit of conjugating a single drug molecule on to the end of a single polymer chain, and its ability to extend release, has been demonstrated in this work. However, depending on a drug’s dosing requirements, a maximum of a single drug molecule per chain may not be enough to deliver a sufficient amount of drug. Polymers such as PLA have been modified to incorporate carboxylic acid functional groups along the backbone [2]. Techniques such as these could be used to conjugate a higher percentage of drug molecules per micelle.

As techniques for creating more complex polymeric prodrugs are explored, more information is needed as to the mechanism of polymeric prodrug degradation and subsequent release. The theoretical calculations in Chapter 6 suggest that the release of
conjugated haloperidol from PLA may not be due entirely to random chain scission. The propensity of the drug-polymer bond to hydrolyze may be greater than that of the polymer backbone ester bonds. Consequently, if this can be properly modeled, release rates for various forms of polymeric prodrugs can be designed accordingly.

7.2.2 Delivery and circulation

While the delivery of an antipsychotic medication such as haloperidol can benefit from a long-term delivery application, it may be interesting to see if this system would cross over to other pharmaceutical compounds. Namely, hydrophilic compounds which would not be able to partition into the hydrophobic core of polymeric micelles without first having conjugated the drug. Polymer conjugate micelles incorporating hydrophilic compounds have been investigated with anticancer agents [3, 4]. However, it may be interesting to investigate if there is an application of the conjugate chemistry used in this work to hydrophilic compounds. This would provide information about the long-range applicability of this work to other areas of drug delivery. Furthermore, nanoscale protein administration is an interesting and challenging area of drug delivery. Proteins, which typically have a hydrophilic or amphiphilic nature, would also need to be conjugated to polymer to efficiently be incorporated into block copolymer micelles. Polymeric prodrug micelles offer the opportunity to encapsulate proteins in polymer matrices without exposure to harsh solvents and the high energy inputs frequently used to formulate nanoparticles. Moreover, once encapsulated in the micelle core, the hydrophilic outer shell could serve to protect proteins from enzymatic degradation.

When designing a drug delivery system for chronic medication, oral delivery would be the preferred method of administration. In order to use a long-term delivery
system to improve compliance, it would be preferable to not be constrained by an injection or a doctor’s visit. However, meeting the goal of oral administration has been difficult. While small size does allow for a small amount of passive oral uptake of particles, it is not enough to improve the oral bioavailability of drugs. Realistically, if particles were free to move across the intestinal epithelia, we would be unprotected from systemic infection by many bacteria and viruses. Consequently, an active targeting approach is necessary to transport particles across the intestinal epithelia. Another challenge for the use of nanoscale particles is their long-term circulation capabilities. Particles can be engineered to release drug for weeks in vitro. However, it is clear that even with the presence of a PEG brush on the surface of particles, this extended circulation time has not yet been achieved. Consequently, work must be done to find ways to either further extend the systemic circulation of particles, or to continue ongoing work to target particles directly to the intended treatment site prior to being cleared.

One method for further extending release from nanoscale drug delivery vehicles is by incorporating them into hydrogels. Hydrogels are three dimensional water swollen polymer networks. Hydrogels made from poly (vinyl alcohol) – poly (vinyl pyrrolidone) (PVA-PVP) networks have been shown to incorporate and extend the release of hydrocortisone loaded microparticles [5]. With future goals of extending release of pharmaceuticals past one week, combining these techniques might prove useful. Furthermore, complexation hydrogels can be used to protect cargo from the low pH environment of the stomach, swelling in the higher pH of the upper small intestine [6]. This may improve the oral delivery capabilities of nanoparticles by releasing them directly at the site of oral uptake.
One of the key benefits of using PEG as the hydrophilic block of polymeric micelles is its bifunctional capability. A hydrophobic polymer, such as PLA or PCL, can be polymerized on one end, while the other end is available for functionalization. Bifunctional PEG polymers are commercially available with one hydroxyl end group and an amine group on the other. The presence of multiple functional groups may require additional steps of protection throughout the polymerization and drug polymer conjugation, but ultimately the goal will be to end up with a PEG brush that can have specific moieties added to the PEG end group exposed to the aqueous media. For example, antibodies can be conjugated to the PEG end group to target micelles to specific cells. Since micelles are an ideal size for cellular uptake, this could increase the bioavailability of loaded drug even further. Another example for functionalization is the carbohydrate epitope, sialylated Lewis antigen A (SLAA), which has been identified on human M cells and facilitate oral uptake in the GALT [7]. This can also potentially be applied to the systemic circulation of particles. Work has been done to explore the use of the protein CD47, the marker of self on red blood cells, for the extension of systemic circulation [8].
7.3 References


VITA

Meredith L. Hans was born on August 17, 1977 in Plainview, New York. She graduated from Oyster Bay High School in Oyster Bay, NY in 1995. After high school she enrolled in the Cell Biology/Biochemistry program Bucknell University. While trying to determine her final career path, Meredith spent the spring and summer semesters of her junior year as a visiting student at the Cold Spring Harbor Laboratories under the supervision of Dr. Winship Herr. Upon returning to Bucknell she began undergraduate research with Dr. Marie Pizzorno. After receiving her BS she worked as a research trainee at the Mount Sinai Medical Center in the laboratory of Dr. Barry Coller. In the fall of 1999 she began as a research technician in the laboratory of Dr. Janet Robishaw at the Weis Center for Research at the Geisinger medical center in Danville, PA. In the fall of 2001 Meredith entered Drexel University as a Masters student and after joining the laboratory of Dr. Anthony Lowman she decided to pursue her PhD. During her time at Drexel she coauthored numerous papers and book chapters, as well as presenting her work at national and international meeting. She received her PhD on the 25th of October, 2005.