THESIS

PREPARATION AND CHARACTERIZATION OF POLY LACTIC-CO-GLYCOLIC
NANOPARTICLES ENCAPSULATED WITH GENTAMICIN FOR DRUG DELIVERY
APPLICATIONS

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ABSTRACT

PREPARATION AND CHARACTERIZATION OF POLY LACTIC-CO-GLYCOLIC NANOPARTICLES ENCAPSULATED WITH GENTAMICIN FOR DRUG DELIVERY APPLICATIONS

Wound treatment has always been a popular topic around the world. Since the emergence of nanotechnology, the development and design of novel wound dressing materials have been dramatically improved. The use of nanoparticles encapsulated with antibiotics to deliver drugs has been shown to be a potentially effective approach to control bacterial infections at a wound position. Recently, biodegradable and biocompatible polymers have drawn lots of attention for the manufacture of drug-loaded nanoparticles in the pharmaceutical industry. In this work, poly-lactic-co-glycolic acid (PLGA) was used in nanoparticle synthesis due to its biodegradability, biocompatibility, and nontoxicity. For this work, gentamicin was loaded into the PLGA nanoparticles as an antibiotic because it is a broad-spectrum antibiotic effective in wound treatments. PLGA nanoparticles were prepared while gentamicin was loaded in the nanoparticles via a double emulsion evaporation method. Poly vinyl alcohol (PVA) was a surfactant that was an important factor in determining the most probable nanoparticle size and morphology. When the PVA concentrations were 9% and 12%, the nanoparticles demonstrated a spherical structure with a porous surface. The porous surface of a nanoparticle was promising for the purpose of releasing encapsulated antibiotics. Another important factor in determining the formation of nanoparticles was the PLGA concentration. Poly lactic-co-glycolic acid (PLGA) was the main material affecting PLGA nanoparticles’ properties. PLGA nanoparticles would have various release profiles,
morphology, and size distribution with different PLGA concentrations. The results suggested that
different PLGA concentrations can endow PLGA nanoparticles with various properties which can
lead to different applications of PLGA nanoparticles.
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Chapter 1

INTRODUCTION

Wound treatments against bacterial infections relate every one of us in our daily lives. The treatment of cuts and scrapes has always been developing and advancing throughout human history. The application of antibiotics is a quick and effective method to fight against infections in wound care and management. Efficient and timely delivery of antibiotics to the wound is critical to the success of wound treatments [Stebbins et al., 2004]. Antibiotics can be taken by mouth (orally), directly into veins (intravenously), or applied directly to skin (topically). In oral delivery, antibiotics are absorbed by the human digestive system and transported throughout the body via the circulatory system. By comparison, topical application of antibiotics may be more effective in delivering antibiotics to infected sites and cause nearly no disruption to uninfected sites. As topical antibiotics act only on the area of application, there is less likelihood of unwanted effects to the whole body, such as nausea and diarrhea. Topical antibiotics are also thought to reduce the chances of bacterial resistance (bacteria changing to become more resistant to medication) [Wei, 2012]. Topical antibiotics are usually available in two pharmaceutical forms, ointments and creams. In addition, wound dressings have been widely used along with topical antibiotics to facilitate the wound healing process. However, ointments or creams can have unwanted effects, and the most common is an allergic reaction on the skin (contact dermatitis), which can cause redness, itching and pain at the site where the topical antibiotic is applied [Chereddy et al., 2016].

Therefore, there is a need to develop a novel method for delivering antibiotics, preventing antibiotic-resistance, and avoiding allergic reactions in wound treatments as well as providing effective delivery of antibiotics to fight against bacterial infections. One attractive approach is to
apply a novel drug delivery system in wound care products. Recently, nanotechnology has demonstrated great potential to this end. It was reported that nanoparticles could be used to encapsulate antibiotics with varying release rates [Xiong et al., 2014]. Tailoring nanoparticle release rate and morphology could allow nanoparticles to target specific bacteria in different situations. The objective of my project is to develop nanoparticles that can encapsulate antibiotics and have them release antibiotics as a response to the presence of bacteria. The antibiotic-encapsulating nanoparticles could be integrated into fibers for the development of “smart” wound dressings. The nanoparticles can carry drugs directly to the infected sites in the wound, protecting uninfected sites from infection. This novel system increases efficiency of drug delivery and treatment.

Nanoparticles not only have increased efficiency, but also have some other outstanding advantages as a drug delivery system. While drugs are encapsulated in nanoparticles they are well protected from contamination. Due to the small sizes of nanoparticles, nanoparticles can deliver drugs through blood capillary walls and this allows for access into cells [Cho et al., 2008]. Nanoparticles are able to carry and transport drugs to targeted positions directly without having any interactions with other cells, thereby avoiding imparting the drugs’ side effects elsewhere within the body [Hans and Lowman, 2002]. Nanoparticles are able to preserve the bioactivity of drugs for a long time (about one year), which is favorable for scale-up production., Drug release rate can be controlled by controlling the size of nanoparticles, which is significant to the efficiency of drug delivery. With all these advantages, nanoparticles have been found to have superior potential in drug delivery applications. Recognizing this potential, Cho saw conventional drug delivery systems in cancer treatments have many problems such as low water solubility, nonspecific targeting, and poor therapeutic indices. By modifying ligands on a nanoparticle’s
surface, the nanoparticles can react with a specific ligand on a tumor cell destroying the cell. Cho provided a new approach to cancer treatments by utilizing ligand-tailored nanocarriers made of polymers such as poly-(L-glutamate)-Taxol, poly-(L-glutamate)-Camptothecin (CT-2106), and poly(amidoamine)-doxorubicin [Cho et al., 2008].

Such advantages to nanoparticles show their benefits to be used as a drug delivery system. Despite these advantages, not enough research has been done to explore the release profiles of nanoparticles. In this project, poly (lactic-co-glycolic acid) (PLGA) was used to develop nanoparticles for investigating their release profiles and physical properties. Those nanoparticles were loaded with antibiotics for treating bacterial infections in wounds. PLGA was chosen in developing antibiotic-loaded nanoparticles in this work because it demonstrated high biodegradability, biocompatibility, and nontoxicity [Posadowska, et al., 2014]. The nanoparticles were characterized using scanning electron microscopy and dynamic light scattering to study particle size distribution and morphology. The drug release response of the PLGA nanoparticles were studied through UV-vis spectrophotometry to evaluate the efficiency of this potential drug delivery application.
Chapter 2

LITERATURE REVIEW

2.1 Nanoparticles in drug delivery system

Advancements in nanotechnology have promoted a revolution in the field of drug delivery. The use of nanoparticles in drug delivery has been significantly studied in the last decade due to its stability and ease of surface modification. These superior properties endow nanoparticles standing up with the human body temperature and keeping the bioactivity of encapsulated drug molecules for a period time. The synthetic materials for nanoparticles used in drug delivery can be classified into two categories: (1) synthetic biodegradable polymers that includes relatively hydrophobic materials such as the α-hydroxy acids, polyanhydrides and others; (2) naturally occurring polymers, such as complex sugars and inorganics [Kamaly et al., 2006]. Biodegradable polymer-based nanoparticles have been widely used in medical treatments as a result of two main advantages: nanosized and biodegradability. Solid lipid nanoparticles and lipid microparticles have been developed as alternating carry of therapeutic agents [Almeida et al., 2007] in a treatment. Solid lipid nanoparticles and lipid microparticles can incorporate hydrophilic and hydrophobic agents to fulfill medical treatments in one delivery system. In addition, the solid lipid nanoparticles can deliver drugs by an oral fashion due to highly stability and biodegradability. For example, the therapeutic agents in the nanoparticles are protected from enzymatic degradation.
2.2 Biodegradable polymers for drug delivery

Biodegradable polymers have been proven they can be used in drug delivery, including polylactic acid (PLA), poly-caprolactone (PCL), poly (lactic-co-glycolic acid) (PLGA), chitosan, and gelatin, primarily due to biodegradability and biocompatibility. For example, polylactic acid (PLA) is a biodegradable and biocompatible material that would easily be degraded in carbohydrate metabolism after it enters the human body.

Polycaprolactone (PCL) is also commonly used in drug delivery systems because PCL can be degraded due to a hydrolysis of ester linkages in physiological conditions. The degradation rate of PCL can be slow, which is favorable in encapsulating long-term implantable devices to the human body.

Chitosan, a modified natural carbohydrate, also has been used for drug delivery system.

Gelatin is a polyampholyte having cationic and anionic groups along with hydrophilic groups. Gelatin has been used in a lot of medical products due to its biodegradable, bioactive, nontoxic, and outstanding mechanical properties.

Among all the polymers, poly (lactic-co-glycolic acid) (PLGA) is the most popular for drug delivery system due to its bioactive, biocompatible, nontoxic, and biodegradable properties. PLGA was used in the PLGA nanoparticles synthesis, and the reasons why I chose PLGA will be talked in the next section
2.3 Poly Lactic-co-Glycolic Acid (PLGA)

Poly (lactic-co-glycolic acid) (PLGA) is obtained through ring-opening copolymerization of poly lactic acid (PLA) and poly glycolic acid (PGA), and PLGA has been widely used for therapeutics entrapment due to its biodegradability and biocompatibility. PLGA degrades via hydrolysis in the human body, resulting in the original lactic acid and glycolic acid. Both lactic acid and glycolic acid can participate in metabolism in human body. They are metabolized in the tricarboxylic acid cycle and can be eliminated as by-products of carbon dioxide and water. Therefore, minimal systemic toxicity is yielded when PLGA is used in drug delivery systems. A variety of PLGA with different molecular weight can be synthesized by varying the ratio of lactide acid to glycolic acid [Champion et al. 2007]. Figure 1 shows a genetic structure of PLGA. The block structure and molar ratio between PLA and PGA determine the crystallinity of PLGA (from amorphous to crystalline) and hence affect the drug release capability of PLGA nanoparticles in drug delivery applications. PLA is more hydrophobic than PGA due to extra methyl groups on the side chain of the PLA. Therefore, a relatively hydrophobic PLGA can be made by using a high ratio of PLA to PGA, resulting in low water absorption and a slow degrade rate [Makadia and Siegel, 2011] in drug delivery. In addition, molecular weight, glass transition temperature, and
solubility have strong impacts on release rate and release behaviors of incorporated drug molecules. When PLGA has a high ratio of PGA, the time required for degradation will be low.

PLGA nanoparticles have been used for drug delivery in cells and tissue recently. PLGA nanoparticles can be used to deliver proteins, peptides, and other low molecular weight compounds [Panyam J.& Labhasetwar V., 2002]. The using of therapeutic PLGA nanoparticles can increase the efficiency of drug release to targeted positions. One application is using the nanoparticles to deliver drugs in cancer treatments. Nanoparticles can carry drugs directly to target positions by modifying ligands on the outer shell surface. Another application is to use PLGA nanoparticles as a sustainable system to deliver genes, providing a sustainable release of genes and avoiding degradation due to a digestive enzyme such as lysosomal enzyme.

2.4 Synthesis of PLGA nanoparticles

Different synthesis methods of PLGA nanoparticles have been developed. The choices of methods are mainly determined by polymers as well as drugs. Different methods have a great effect on PLGA nanoparticles properties such as nanoparticles size distribution and molecular weight distribution. In the following section, we will discuss a few methods that have been reported previously in literature.

The methods can be divided into two categories: (1) bottom-up methods including precipitation polymerization, interfacial polymerization and emulsion polymerization; (2) top-down methods including emulsion evaporation, salting out techniques, solvent displacement, and emulsion diffusion. The mechanism of bottom-up methods is that individual monomers will form into nanoparticles through polymerization. The bottom-up methods have advantage in controlling
the size of nanoparticles, and the reaction rate can be managed based on the concentration of monomers. In addition, bottom-up methods can be applied to various monomers, so different kinds of nanoparticles can be synthesized from these methods. However, bottom-up methods have an unavoidable disadvantage of by-products left in nanoparticles. Nanoparticles synthesized through bottom-up methods are started with a single monomer, and the monomer will grow by chemical reactions to form nanoparticles. Sometimes, the monomers will decompose to different molecules, and these molecules will polymerize to other polymers instead of nanoparticles. The residual by-products might be toxic and have a negative impact on the release of drugs from nanoparticles.

Therefore, top-down techniques are developed to prevent the presence of by-products in the yield of PLGA nanoparticles. In addition, the size of PLGA nanoparticles synthesized in top-down methods can be controlled via reducing the molecular weight of polymers [Astete and Sabliov, 2006]. Therefore, the top-down techniques are efficient and have become popular in the development of PLGA nanoparticles for drug delivery applications.

Emulsion evaporation is a conventional top-down method and has been widely used for PLGA nanoparticles synthesis. A narrow size distribution around 100 nm can be resulted through using an emulsion evaporation method. In this method, the PLGA is first dissolved into an organic solvent, resulting in an organic phase. The organic phase is then mixed with an aqueous phase in which a selected surfactant is added to increase stability of the emulsion. The nanodroplets can be created when the organic solvent evaporates from the emulsified PLGA solution in the aqueous phase. Stirring the emulsion is needed in emulsification because a high shear stress rate applied to the solution can reduce the size of nanodroplets. After the emulsification, PLGA nanoparticles are formatted via the evaporation of organic solvent. Active components such as antibiotics or
drugs can be encapsulated in the nanoparticles by either single emulsion (oil in water (o/w) or water in oil (w/o)) or double emulsion (w/o/w). The single emulsion method is usually used to encapsulate hydrophobic components. The double emulsion method is primarily used for hydrophilic components entrapment [Astete and Sabliov, 2006]. Low entrapment efficiency and a large mean size of nanoparticles have been found when the single emulsion method was used [Ficheus et al. 1998]. The large mean size of nanoparticles production might be due to a destabilizing effect that is usually caused by coalescence of small inner droplets with globule interface or coalescence between droplets with globule and Ostwald ripening. Nanoparticle size can be reduced if a strong shear stress is applied. The low entrapment efficiency is due to the leakage of active components (drugs). The entrapment efficiency can be improved by increasing polymer concentration and surfactant concentration. Surfactant will play an important role when controlling the nanoparticle size and entrapment efficiency. Data from several experiments has shown that various concentrations of surfactant leading to a range of mean nanoparticle size distribution. It has been found that when the concentration of surfactant is lower than a critical point of 1.2 mg/L, the mean size of PLGA nanoparticles will dramatically drop to a small size. [Astete and Sabliov, 2006]. However, When the concentration of surfactant is too low (0.4mg/ml), the size of PLGA nanoparticles is, instead, increased to 69.1nm. The reason of this phenomena can be explained by decreasing surfactant concertation leading to an increase of surface tension, so the size of nanoparticles will increase.
2.5 Drug release and release rate in nanoparticle drug delivery

One of key properties in nanoparticle drug delivery system is the drug release property. Drug release rate is defined as the rate of a drug molecule encapsulated in nanoparticles transferring from inner matrix to the outer surface, resulting in a drug release to the environment matrix. In common, the drug release should be stable, sustainable, and at an appropriate rate. Currently, drug release rate of nanoparticles is determined by the diffusion rate of drug molecules via four different mechanisms including water-filled pores, degradation of nanoparticles, osmotic pumping, and diffusion through polymer matrix [Fredenberg et al. 2011]. Diffusion through water-filled pores is highly dependent on nanoparticle structures. Porosity, tortuosity, and diffusion coefficient of fluid in the pores determine the effective diffusion coefficient of drug molecules. Pores need to have sufficient radius for drug molecules passing through. Continuous porous structure from inner to outer surface is another essential condition. Diffusion through water-filled pores has been proved as the primary mechanism of drug release at the beginning of releasing process. [Fredenberg et al. 2011]. In addition, molecule weight, degree of connections between pores, and polymer chain mobility have influences on diffusion rate.

Diffusion through polymers occurs when nanoparticles encapsulate small hydrophobic drugs [Fredenberg et al. 2011]. Diffusion through polymers is not dependent on porous structures as much as that in diffusion through water-filled pores. However, temperature is an important factor affecting the diffusion rate of drug release. Diffusion through polymers is the most common diffusion mechanism taking place in polymers. Diffusion through polymers only depends on temperature, and the temperature in solution cannot be unitized, so temperature gradient will be existing in a solution. Therefore, diffusion through polymers will be the most common process throughout the whole releasing process. In addition, physical state of nanoparticles, such as sizes,
plays a critical role in determining the rate of diffusion through a polymer. Previous experiments have shown that when the size of PLGA nanoparticles is small and the molecule weight of PLGA is low, so Higuchi model can be applied. Higuchi model bases on the assumptions that initial drug concentration is higher than drug solubility, drug diffusion only happens in one dimension, and nanoparticles are small. Within the Higuchi model, the diffusion coefficient is nearly constant and only governed by the Higuchi equation which is \( Q = K_H \sqrt{t} \), where \( Q \) is the total drug release in time of \( t \), and \( K_H \) is the diffusion coefficient. Base on the Higuchi equation, the diffusion coefficient can be calculated from the Higuchi equation. [Mittal et al, 2007].

Osmotic pressure is originated due to water absorption and is able to drive the release of drugs, which is called osmotic pumping [Fredenberg et al. 2011]. The condition that allows osmotic pressure exiting is that the influx and efflux rate of water is equal, and the polymer of nanoparticles is hydrophobic. Unlike diffusive transport depending on both the length and the area of pores, osmotic transport is only affected by the length of channels. When the length of channels is more than 60μm, osmotic pumping become dominated. During the osmotic pumping, the degradation and erosion can be neglected due to low degradation of such polymers.

The degradation of nanoparticles widely occurs in polymer-based nanoparticles, such as PLGA nanoparticles. They would biodegrade by themselves over time, which leads to the release of drugs encapsulated in particles. Degradation is the main mechanism of drug release in nanoparticles. Degradation is a release mechanism without drug transport. The drug is released when degradation occurs to polymer-based nanoparticles, such as PLGA nanoparticles [Fredenberg et al. 2011]. However, the shortcoming of degradation of nanoparticles is found in nanoparticles reservation. Drug molecules released due to degradation is the main mechanism for low molecule weight nanoparticles. Previous research has shown that the degradation of polymers
domains the final period of drug release, so degradation of nanoparticles is always treated as a rate controlling release mechanism. Polymers degradation result into transferring drug molecules to nanoparticles surface to be released. So, degradation release mechanism will be the main release mechanism in the final stage for PLAG nanoparticles drug release.

More than one release mechanism usually occurs simultaneously and the primary mechanism dominating the release rate changes with time throughout the entire release process. There are many factors that have effects on the drug release profiles. The factors include nanoparticle size, ratio of surface area to volume of a nanoparticle, concentration of solution matrix, polymers crystallinity, molecular weight of polymers, side chain property, glass temperature of polymers, temperature, and the properties of encapsulated drugs, and all these factors have influences on the drug release profiles. In this work, Ultraviolet–visible spectroscopy (UV-vis) will be used to study the release rate of antibiotics from PLGA nanoparticles. UV-vis is a common technique for the study of drug release. UV-vis results will be combined with conventional dissolution tests to determine drug release rate [Weerd J. & Kazarian S., 2004]. UV-vis can provide interpretation of experimental observations of drug release profiles.
Chapter 3

MATERIALS AND METHODS

3.1 Materials

Filtered water (Fisher Chemical) was used to solve polyvinyl alcohol (PVA, Mw 3100, produced by SIGMA) as the surfactant, and poly (D, L-lactide-co-glycolic acid) (PLGA, 75:25, ALORICH) was solved in dichloromethane (DCM, 99.8%, ALORICH) to form an oil phase. Gentamicin sulfate salt (SIGMA) was used as antibiotics within nanoparticles for its broad-spectra effectiveness in treating bacterial infections. For organic cleaner, Denatured ethanol (70%, Decon) was used.

3.2 PLGA Nanoparticles Synthesis

PLGA nanoparticles were synthesized via single emulsion and double emulsion evaporation methods. In a single emulsion method, two phases including an aqueous phase and an oil phase were prepared separately. At first, hydrophilic gentamicin was solved in water to make the aqueous phase 1 [$w_1$]. Then, PVA serving as the surfactant was dissolved in water to make the aqueous phase 2 [$w_2$]. PLGA was dissolved in DCM to make the oil phase [$o$]. These three solutions were mixed together and then ultrasonication (Fisherbrand™-Model 505) was used to disperse the mixture to generate nanoparticles. The ultrasonication was conducted for a few sections before the size of nanoparticles became as small as needed. During the sonication process,
using the optical microscopy to observe the morphology of nanoparticles was essential. Base on the size of nanoparticles, sonication would be expanded as needed.

In the double emulsion evaporation method, the preparation of oil phase and aqueous phases were as the same as those in the single emulsion method. The two phases were mixed followed by ultrasonication. The PVA surfactant solution was prepared separately. After the mixing of the oil and aqueous phases was completed, the mixture would be added into the surfactant solution with constant stirring. During the process, the solvent diffused from PLGA matrix to the outer liquid phase, resulting in precipitation of obtained nanoparticles. The stirring continued for several hours before water in the solution had evaporated to make the solution a condensed solution, resulting in the aggregation of nanoparticles. Centrifugation would be used to separate the nanoparticle precipitation from the solution. After centrifugation, the precipitation needed to be washed using water. Sonication can be used to disperse the aggregation of nanoparticles. Through water washing process, the left out PVA can be washed away from the solution. The key difference between the single emulsion method and the double emulsion method is the double emulsion method can yield small nanoparticles. Finally, lyophilization would be done to preserve nanoparticles. During lyophilization, the washed nanoparticles were mixed with a suitable cryoprotectant such as mannitol.

A number of factors had effects on nanoparticles’ properties within the synthesis. First, the surfactant of PVA had a strong effect on the properties of nanoparticles. Different concentrations of PVA solutions would be used, ranging from 3% to 20% by weight percentage. Second, the amount of water used to solve gentamicin sulfate salt was another key factor. In this work, a volume of 200μl water was used to mix with 25mg gentamicin sulfate. Third, sonication in the synthesis affected the development of nanoparticles. Sonication generated energy in the solution,
which was able to disperse nanoparticles dramatically to make them small. Sonication with 35% amplitude was used to disperse the solution for 3 minutes. In addition, the concentrations of PLGA were experimented ranging from 80mg to 120mg dissolving in 6ml dichloromethane respectively. Controlled factors method was used in this proposal to collect all experiment data. Quantitative analysis was conducted via using MATLAB to investigate the relationships between synthesis factors and nanoparticles properties.

### 3.3 Nanoparticle Characterization

The obtained nanoparticles were characterized by determining particle size distribution and morphologies. Scanning Electron Microscopy (SEM) was used to investigate the size and the morphology of obtained nanoparticles.

Dynamic Light Scattering (DLS) was used to measure the size and size distribution of nanoparticles. Nanoparticles undergo Brownian motion in a solution. In a DLS measurement, the distance between scatters changes constantly with time when a light source hit on solution. Diffusion coefficient of nanoparticles in solutions was obtained over time. Stokes-Einstein relation and time autocorrelation function were applied, the size and size distribution of nanoparticles were calculated.

### 3.4 Drug degradation and release rate study

PLGA nanoparticles will degrade with time. The study of nanoparticles degradation is important for characterizing nanoparticles. In our experiments, the degradation rate was
represented by weight change of nanoparticles. The weights of nanoparticles precipitate were measured at different time. Therefore, the degradation rate can be showed via plotting the relationship between weight change and time.

Gentamicin was encapsulated in PLGA nanoparticles. The release of gentamicin is critical for the drug delivery application and is studied as a function of time. The release rate of gentamicin was determined by release rate of gentamicin. In our work, a UV-vis spectroscopy was used to determine the concentrations of gentamicin. The UV-vis spectroscopy utilizes visible light to determine the concentration of a chemical compound in a solution. The drug release responses of PLGA nanoparticles drug carriers was studied at a temperature of 25 °C. In our experiments, PLGA nanoparticles water solution was equally distributed into several test tubes. After let nanoparticles degrade for a designed period of time, gentamicin encapsulated in nanoparticles would be release to the solution. Then, the amounts of gentamicin sulfate salt in the aqueous solution can be quantified using UV-vis spectrophotometry. The concentrations of gentamicin in the solution of every time potion can be collected, so the releasing curve of gentamicin can be obtained. Thus, the release rate of nanoparticles with time can be measured.
Chapter 4

MANUSCRIPT

Overview

Poly-lactic-co-glycolic acid (PLGA) was mixed with gentamicin sulfate, used as an antibiotic, via a double emulsion method, resulting in gentamicin-loaded PLGA nanoparticles that exhibited a potential application for smart wound dressings. The particles’ morphology, particle degradation rate, and drug release profiles were investigated by using Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), and Ultraviolet-visible spectroscopy (UV-vis). The SEM images have shown that the PLGA nanoparticle formation was primarily determined by the concentration of PLGA and concentration of surfactant. Uniform and spherical PLGA nanoparticles loaded with gentamicin were prepared at a concentration of 1.67 %g/ml PLGA and 12 % (w/v) surfactant. The particle degradation rate and gentamicin release profiles were determined using UV-vis. The results suggested that the nanoparticles synthesized with different PLGA concentrations would exhibit various release profiles, morphologies, and size distributions.

4.1 Introduction

Wound treatment against bacterial infection has always been a concern for medical professionals. The main purposes of using wound dressings are to facilitate the healing process and to prevent bacterial infections in wound sites. The use of antibiotics has been proven to be the most effective and fastest approach for controlling bacterial infections [Stebbins et al., 2014].
Therefore, finding an efficient and timely way to deliver antibiotics to the wounds is critical for success in wound treatment [Stebbins et al., 2004]. There are usually three approaches to deliver antibiotics: taken by mouth (orally), injection into veins (intravenously) and application to the skin (topically). Because the topical antibiotics only act on wound sites, some typical unwanted side effects, such as nausea or diarrhea, can be avoided. In addition, it is evident that the use of topical antibiotics can reduce the possibility of bacterial resistance [Wei, 2012]. However, topical antibiotics are still not used as commonly as other antibiotics due to ineffective and inefficient application to wounds.

Despite the uncommon application of topical antibiotics, conventional wound dressings, which are inherently topical, made of synthetic or natural materials such as cotton or polyurethane only can control bacterial infections to some extent [Selig et al., 2012]. Recently, there has been an increasing market demand for high-performance wound dressings to innovate. One innovation is to use a new drug delivery system, such as nanoparticles. Using nanoparticles to deliver drugs, such as topical antibiotics, directly to wounds would promote faster healing. There are several advantages making the use of nanoparticles become so popular. Antibiotics can be encapsulated in a nanoparticle, and hence the bioactivity of antibiotics can be preserved for a given time which can lead to scalable production. Due to the small size of nanoparticles, they can deliver drugs through blood capillaries and allow for access into cells [Cho et al., 2008]. In addition, the drug release rate can be efficiently tuned by controlling the size distribution and morphology of nanoparticles. These properties can be influenced by the chosen material.

Different materials have been reported in the synthesis of nanoparticles, including chitosan, gelatin, polycaprolactone, and poly-lactic-co-glycolic acid (PLGA) [Wei, 2012]. PLGA is attractive because PLGA has several advantages such as biodegradability, biocompatibility and
nontoxicity, and has been used in many applications in the pharmaceutical industry [Makadia and Siegel, 2011]. If PLGA is introduced to the circulatory system it can be decomposed into carbon dioxide and water, suggesting no harm to the body [Panyam and Labhasetwar, 2003]. Recently, PLGA nanoparticles have been used to deliver drugs in cells and tissue engineering. PLGA nanoparticles can be loaded with protein, peptides, and low molecular weight compounds for lots of therapeutic applications [Panyan J. & Labhasetwar V., 2002]. In addition, PLGA nanoparticles can serve as a sustainable system to deliver genes or antibiotics with a stable release rate [Cho et al., 2008]. The efficiency of drug release to targeted positions can be dramatically increased by using therapeutic PLGA nanoparticles.

There are two methods commonly used to synthesize PLGA nanoparticles. The first method is a chemical process including mini-emulsion polymerization, emulsion solvent evaporation, and interfacial polymerization. The second method is a physicochemical process including multiple emulsion techniques, emulsion solvent diffusion, layer by layer process, and spray drying [Iqbal et al., 2-15]. The emulsion solvent evaporation technique was developed by Ogawa in 1998 [Ogawa et al., 1998], and a variety of methods based on the technique have been developed since. One example is a double emulsion evaporation method that has been demonstrated effectively in synthesizing PLGA nanoparticles for hydrophilic-drug entrapment. The double emulsion evaporation method is an easy-to-control process and cost-effective, requiring no special instruments [Ruan et al., 2002]. Another example is a Water-in-Oil-in-Water approach, a small amount of water (w1) is dispersed in an oil or organic phase (o) leading to a primary emulsion (w1/o). Then, the primary emulsion is dispersed in another continuous aqueous phase (w2) forming large droplets, resulting in a double emulsion.
In this paper, PLGA nanoparticles were developed using a double emulsion method for encapsulation of antibiotics. Gentamicin sulfate was chosen as the antibiotic for the drug delivery system because gentamicin is a broad-spectrum antibiotic for both gram positive and gram-negative bacteria. Synthesis parameters such as PLGA concentration and surfactant concentration demonstrated significant impacts on the size and morphology of PLGA nanoparticles. The degradation rate of PLGA nanoparticles and the release rate of gentamicin were determined via UV-vis. The results showed that the nanoparticles synthesized at 9% and 12% PVA concentrations have spherical shapes and well distributed porous surface structures. Nanoparticles synthesized with different PLGA concentrations have various release profiles and degradation rates. The author hopes that the information in this paper will contribute to the further innovation of high-performance wound dressings incorporating antibiotic-encapsulated nanoparticles.

4.2 Experimental Section

4.2.1 Materials

Poly vinyl alcohol (Mw = 89000-98000), gentamicin sulfate salt, and the poly-lactide-co-glycolic (lactide: glycolic 75:25, Mw 4000-15000), and dichloromethane were purchased from Sigma-Aldrich. Dissolute water (pH=7, 7732-18-5) was purchased from Fisher Chemical. The chemicals were used without further purification.
4.2.2 Gentamicin-loaded PLGA Nanoparticle Synthesis

The PLGA nanoparticles containing gentamicin were synthesized via a double emulsion evaporation method adopted from Astete in 2006 [Astete and Sabilov, 2006]. The synthesis was performed step-wise as shown in Figure 2.

**Step 1:** Oil phase and aqueous phase were prepared, respectively before the two phases were mixed.

**Step 2:** The mixed solution was sonicated, resulting in the primary emulsion.

**Step 3:** The primary solution was mixed with water phase 2 (PVA solution), resulting in the double emulsion solution.

**Step 4:** The emulsion solution was under stirring to allow solvent evaporation, resulting in nanoparticle precipitation.

Figure 2. Schematic illustration of the gentamicin-loaded PLGA nanoparticle synthesis.
As shown in Figure 1, the PLGA was first dissolved in dichloromethane, resulting in an oil phase \([o]\). The gentamicin sulfate was dissolved in water. Polyvinyl alcohol (PVA) was dissolved in water as a surfactant. A small amount of PVA solution was mixed with the gentamicin solution, resulting in the water/aqueous phase 1 \([w1]\). The PVA solution was used as the water/aqueous phase 2 \([w2]\). First, \(o\) was mixed with \(w1\), resulting in a primary emulsion solution. Second, the primary emulsion was mixed with \(w2\), leading to a double emulsion of nanodroplets. PVA as a surfactant has two different ligand parts; one part is hydrophilic and the other is hydrophobic. PVA aided the formation of double emulsion through reduction of surface tension between \(o\), \(w1\), and \(w2\). In the double emulsion evaporation method, the dichloromethane from \(o\) diffused to \(w2\), resulting in the PLGA nanoparticles precipitating around \(w1\) [Bilati et al., 2005]. The surfactant remained at the interface during the diffusion process and helped nanoparticles encapsulate antibiotics. The PLGA nanoparticles were formed after the solvent from \(o\) completely diffused to \(w2\). Our procedure followed closely to Astete’s procedure.

In our experiments, PVA powder was dissolved in distilled water while stirring for 1 hour on a hot plate at room temperature. The concentrations of PVA solutions were 3%, 5%, 7%, 9%, and 12% (weight/volume). The temperature of the hot plate was gradually raised to 80°C. Then, the solution was stirred at 40°C for overnight. A 20μm Whatman quantitative filter paper (VWR®) was used to filter out any undissolved powder. The filtered solution was sonicated via a sonic dismembrator (Fisherbrand™-Model 505) at 25% amplitude for 30 seconds to improve the consistency of the PVA solution. 20mg gentamicin sulfate was dissolved in 200μL distilled water and then the gentamicin solution was mixed with a 75μL PVA solution, resulting in \(w1\), and the mixing solution was kept in a refrigerator overnight to complete dissolve. Different masses of PLGA (80mg, 90mg, 100mg, 110mg, and 120mg) were dissolved in 6ml dichloromethane (DCM)
resulting in oil phases with PLGA concentration of 1.33%, 1.5%, 1.67%, 1.83%, 2% (g/ml). The oil was mixed with w1 and the mixture was sonicated for 3 minutes at 35% amplitude yielding a primary emulsion solution. This was to disperse the mixture to have small nanodroplets. The 50ml PVA solution was stirred at 450rpm to create our w2. The primary emulsion solution was added dropwise into w2 while stirring, resulting in a double emulsion solution. After the double emulsion solution was stirred for 4 hours, nanoparticles precipitated after the solvent (dichloromethane) had diffused to w2. The mixture was divided amongst 10 15ml plastic tubes, resulting in 5ml of solution in each tube. Each was put into centrifugation at 6000rpm for 10 minutes. Each tube was decanted and rinsed with 5ml dissolute water to wash away any residue of nonparticulate PLGA or PVA. The rinse was performed three times in each tube, and then each tube was allowed to dry for 10 hours at room temperature. For nanoparticle degradation studies, the nanoparticles were immersed again in dissolute water after the third rinse and without letting them dry.

4.2.3 Particle Size and Morphology

The morphology and size distribution of PLGA nanoparticles made at different PLGA and PVA concentrations were investigated via scanning electron microscopy (SEM) and dynamic light scanning (DLS), respectively. In sample preparation for SEM imaging, the nanoparticles were first dispersed in dissolute water, and then drop casted onto a silica wafer that was left until the water had completely evaporated. The silica wafer coated by 3nm of gold, and then examined via a JEOL 6500 FE-SEM to study the particle morphology. 3ml of highly dilute nanoparticle solution was put into a cuvette for evaluation of particle size distribution with a Malvern Zetasizer Dynamic Light Scanning device. A size distribution profile was compiled from DLS measurements. The
SEM and DLS measurements were used to determine the highest yield of PLGA nanoparticles by PVA concentration. The particles made at the 12% PVA concentration were further used to study nanoparticle degradation and gentamicin release rate which were both functions of PLGA concentration.

4.2.4 PLGA nanoparticle Degradation

As the PLGA nanoparticles are expected to deliver encapsulated gentamicin, the nanoparticle degradation rate was important to investigate. The method used to determine the mass change of PLGA nanoparticles in solution as a function of time during degradation was derived by Soppimath [Soppimath et al., 2001]. The PLGA nanoparticles made at 12% PVA were chosen in the study of degradation rate. The obtained particle suspension was further diluted in dissolute water and kept at room temperature to allow breakdown from spherical PLGA nanoparticles. Suspension samples were taken every two hours, from $t = 0$ hours to $t = 10$ hours, after degradation. The sample solutions were centrifuged, decanted and dried for 10 hours. The mass of the precipitate was measured. The ratio of the remaining weight to the original weight was calculated in Equation 1:

$$W_i = \frac{w_i}{w_0} \quad \text{Equation 1}$$

where $w_0$ is the weight of precipitate at 0 hours; $w_i$ is the weight of collected precipitate after the solution was kept in the tube for a period of time; $W_i$ is the weight ratio of remaining precipitate compared to the original precipitate.
The degradation experiments were repeated for nanoparticles obtained at different PLGA concentrations (1.33% g/ml, 1.5% g/ml, 1.67% g/ml, 1.83% g/ml, 2% g/ml). The weight ratio was calculated for each concentration of nanoparticles. The data was analyzed through code written in MATLAB to compile and visualize the calculations. The weight ratios were plotted as functions of time. A two-factor exponential decay function was chosen to approximate the behaviors of degradation. The chosen function is Equation 2:

\[ W = At^B \quad \text{Equation 2} \]

where \( W \) was the weight ratio at different time; \( A \) and \( B \) were constants; and \( t \) was the time of degradation.

### 4.2.5 Gentamicin Release rate

The gentamicin was expected to release when the PLGA nanoparticles began degrading. Xiong suggested in his paper more work should be done to explore the release rate due to various nanoparticle properties [Xiong et al., 2014]. In this paper, UV-vis was used to measure gentamicin concentrations in solution over time for release rate study. Before carrying out the release rate determination experiments, a calibration curve of gentamicin should be obtained through UV-vis. A range of gentamicin aqueous solution concentrations from 0.25% to 2% (w/w) were prepared. The calibration curve was based on the absorbance of gentamicin at 196 nm and is a function of gentamicin concentration. After the calibration curve was determined, the concentration in any solution within 0.25% to 2% could be quantified. The release rate study was conducted with 12% PVA and different PLGA concentrations. The samples used in the UV-vis absorbance measurements were the liquid solutions after centrifuging because the gentamicin was dissolved
in the water after it was released from the nanoparticles. The liquid samples were measured with UV-vis, and the absorbance at 196 nm was used to quantify the gentamicin concentrations released from the nanoparticles according to the calibration curve. The release rate of gentamicin was presented as a function of time (from 0 hours to 10 hours).

A numerical fitting method was also used to analyze the release rate of gentamicin. The concentration of gentamicin as a function of time was described in an exponential function as shown in Equation 3:

\[ C = At^B \quad \text{Equation 3} \]

where the \( C \) was the concentration of gentamicin released from the PLGA nanoparticles.

4.3 Results and Discussion

4.3.1 PLGA nanoparticle size and morphology

\textit{PVA (surfactant) concentration}

PVA was used as the surfactant and had significant impacts on the size and morphology of nanoparticles when gentamicin was particle-encapsulated. The nanoparticle morphology and size were assessed via SEM images and DLS measurements, respectively. Figure 3 (a-e) showed SEM images of the nanoparticles made at different PVA concentrations.
The SEM images suggested that the nanoparticles synthesized with 9% and 12% PVA concentrations showed roughly uniform spherical structures while there were almost no spherical particles obtained at 3% or 5% PVA concentrations. The results demonstrated a key role of the PVA (surfactant) in the formation of nanoparticles, which was in great agreement with previous findings [Champion et al., 2007]. PVA has two different kinds of ligands; one is hydrophobic and the other one is hydrophilic. When the w1-o-w2 emulsion solution was formed, the PVA was aligned in a fashion where the hydrophobic parts bonded with the oil phase (the PLGA), and the hydrophilic parts attached to the water phase. The PVA reduced the surface tension between the two liquid phases and prevented phase separation [Ficheux et al., 1998]. The water-PVA-oil mixture (as shown in the 4th step of Figure 2) eventually developed nanoparticles after the organic solvent for dissolving PLGA had diffused to the outer water solution. Therefore, when the
concentration of PVA was increased, more uniformly spherical particles were formed. Figure 4 (a-d) showed the size distribution of nanoparticles made at different PVA concentrations.

The DLS size distributions show the average particle sizes of PLGA nanoparticles made at 3%, 5%, 7%, 9%, and 12% PVA concentrations were 32nm ± 2.51nm, 350nm ± 30.4nm, 980nm ± 140.9nm, 1050nm ± 117.4nm, and 1000nm ± 140.9nm, respectively. The results suggested that large particles were prominent at high PVA concentration. However, the SEM images revealed that the particle synthesis was not sufficient at 3% or 5% PVA because too few spherical particles were identifiable. Figure 5 showed a relationship between the PVA concentration and the average
particles size, which indicated that the PVA played an important role in particle formation. The mean nanoparticle size was increased when increasing the PVA concentration. When PVA concentrations were 9% and 12%, the slope was almost flat, indicating the PVA was not a dominant factor any more.

The results of particle size and morphology suggested that the PVA concentration should be at 9% or 12% to ensure better distributed size and greater spherical uniformity of PLGA nanoparticles. 12% PVA was used for the PLGA concentration study.

**PLGA concentration**

The formation of emulsion-feasible PLGA nanoparticles was also affected by the concentration of PLGA used in the synthesis. The morphology of nanoparticles was examined using SEM and the images are shown in Figure 6 (a-e).
The SEM images revealed a porous structure in all PLGA nanoparticles as shown in Figure 6 (a-e), which would be beneficial for drug release applications [Fredenberg et al., 2011]. In addition, the pore size on the particle surfaces decreased with an increase of PLGA concentration.

Figure 7 (a-e) show the size distributions of nanoparticles made at different PLGA concentrations. The plots are obtained through calculating the average intensity at different particle size with error bar.
The average sizes of nanoparticles synthesized at 1.33% g/ml, 1.5% g/ml, 1.67% g/ml, 1.83% g/ml, and 2.00% g/ml PLGA were 1500nm ± 102.3nm, 1600nm ± 123.1nm, 1000nm ± 140.9nm, 1800nm ± 160.7nm, and 2400nm ± 217.8nm, respectively. Figure 8 showed the particle size as a function of PLGA concentration, indicating the particle size generally increased with an increase in PLGA concentration, but there was a short span in which size decreased with increasing concentration (1.5% to 1.67%). The smallest nanoparticles were obtained when 1.67% g/ml PLGA was used in the synthesis process. Interestingly, nanoparticles made at 1.67% g/ml PLGA demonstrated smaller pores.

Figure 7. Size distributions of nanoparticles with various amounts of PLGA dissolved in 6ml dichloromethane. (a) 80mg/6ml = 0.08g/6ml = 1.3% g/ml (w/v); (b) 1.5% g/ml; (c) 1.67% g/ml; (d) 1.83% g/ml; (e) 2.00% g/ml.
The nanoparticle percent yield was the overall weight of nanoparticles that were synthesized in every batch divided by the mass of PLGA. Figure 9 showed the nanoparticle percent yield as a function of PLGA concentration in the synthesis.

Figure 8. the relationship between various concentrations of PLGA and size of nanoparticles.

Figure 9. Percentage yield of nanoparticles under various PLGA concentrations. The Black line was the experimental curve, and the red line was a curve fit.
The nanoparticles percent yields were 68.34%, 58.89%, 96.33%, 32.12%, and 38.16%. The results suggested the highest percent yield of nanoparticles when 1.67% g/ml PLGA was used in the synthesis. Overall, the 1.67% g/ml PLGA was a critical concentration that could produce the smallest pores and the highest percent yield in the double emulsion method. The author expected the results may have been caused by the ratio of PVA to PLGA, and believes it is worth further study.

4.3.2 PLGA nanoparticle degradation

Gentamicin was encapsulated in PLGA nanoparticles during the double emulsion solution evaporation. The gentamicin could be released via the degradation of particles. Therefore, it was important to understand the degradation rate and mechanisms of PLGA nanoparticles. The degradation rate was represented by the change in weight ratio. The calculated weight ratio was the weight of particles at time $t$ relative to the initial weight of particles at time $t_0$. The degradation rate was studied at different PLGA concentrations, 1.33% g/ml; 1.5% g/ml; 1.67% g/ml; 1.83% g/ml; 2% g/ml, used in particle synthesis. Figure 10 shows the weight ratio of nanoparticles as a function of time from $t = 0$ hr. to $t = 10$ hr. at different PLGA concentrations.
The plots of the nanoparticle weight ratio as a function of time show different degradation rates. The degradation of the nanoparticles at lower PLGA concentrations (1.33%, 1.5%, and 1.67%) was faster than that at higher PLGA concentrations (1.83% and 2.00%), especially within the first 2 hours. The weight ratios were all below 50% after 10 hours which means most of the gentamicin had been released. The particles made with 1.33% PLGA show below 10% weight after 10 hours which means nearly completed degradation.

A better understanding of the relationships between the PLGA concentrations and the degradation rates of the nanoparticles was needed. Exponential decay curves were fit to the degradation rate data using MATLAB. The fitting curves are shown in Figure 11.

Figure 10. The weight ratio of different PLGA nanoparticles in degradation from t = 0 hour to t = 10 hour with respect to the dry precipitate weight at t = 0.
The standard deviation values for the fits are 0.92, 0.923, 0.946, 0.662, 0.871, respectively. The degradation of the PLGA nanoparticles and the release of gentamicin accounted for most of the lost weight. Usually, it took time before the PLGA nanoparticles started to degrade, so the lost weight was mainly caused by the release of gentamicin within the first few hours. There are four main release mechanisms for the gentamicin: diffusion through polymers, osmotic pumping, diffusion through water-filled pores, degradation of nanoparticles [Fredenberg et al., 2011]. Some research has shown more than one release mechanism can occur simultaneously, and the primary mechanism can change during the release process [Fredenberg et al., 2011]. Thus, a single release mechanism cannot represent the whole degradation process, so each curve should be discussed individually throughout the degradation process. Before any conclusions can be drawn, the gentamicin release rate should be investigated.

Figure 11. Curve fits of the nanoparticle degradation weight ratios. The solid blue lines show the experimental data. The red dash lines are the curve fits. The particles were made at different PLGA concentration (a) 1.33 %; (b) 1.5 %; and (c) 1.67 %; (d) 1.83%; (e) 2%.
Diffusion through polymers is the most common release mechanism throughout the whole release process. Temperature and concentration gradient are the key parameters. Even though this mechanism exists throughout the release process, the release rate caused by diffusion through polymers can be small [Fredenberg er at., 20011].

Osmotic pumping is originated due to water absorption leading to driving the release of drugs. Gentamicin release through osmotic pumping is affected by the pore channel length. Fredenberg has shown that nanoparticles have small pore size and long channel length likely release gentamicin through osmotic pumping [Fredenberg er at., 20011].

The degradation of nanoparticles widely occurs in polymer-based nanoparticles, and it is the main release mechanism, so it is treated as the control mechanism. This mechanism dominates the release rate in the last few hours. Nanoparticles with larger diameter are likely to degrade [Fredenberg er at., 20011].

Diffusion through water-filled pores highly depends on the pore structure of nanoparticles. Nanoparticle diameter and pore size are the main factors of diffusion through water-filled pores. It has been shown that nanoparticles with smaller diameter and larger pore size likely to release gentamicin through this release mechanism. Diffusion through water-filled pores can be the main release mechanism at the beginning of release process [Fredenberg er at., 20011].

4.3.3 Gentamicin release rate

The gentamicin release rate was assessed by measuring the change of gentamicin concentrations over time. The concentration of gentamicin was measured via UV-vis. The gentamicin absorbance calibration curve is shown in Figure 12. A linear relationship is shown
from 0 to 0.1 % gentamicin in water, which was used to calculate the gentamicin concentrations in the subsequent release study.

The relationship between absorptance and concentration of gentamicin below 0.1% can be described by a first order linear function. Using the function, the absorptance can be used to calculate the concentration of gentamicin. In subsequent experiments, a 10-hour period time was enough for the degradation of nanoparticles in order to calculate the concentration of gentamicin released.

To complete the release study, the gentamicin-loaded PLGA nanoparticles were investigated from $t = 0$ hr. to $t = 10$ hr. in 1 hour increments up to hour 2 and in 2 hour increments afterward. The absorptance of released gentamicin in solution was measured via UV-vis. The concentration of gentamicin was calculated using the linear calibration curve from Figure 12 (b). The release rates of different PLGA concentrations are shown in Figure 13.
To better understand these release rates over time, exponential functions were used to fit the data. Figure 14 shows the curve fits of different PLGA concentrations using MATLAB.

Figure 13. The concentration of gentamicin released from different PLGA nanoparticles made at different PLGA concentrations.

Figure 14. Fitting curves of gentamicin concentrations during release. The blue lines show experimental data. The red dash lines are the fitting curves. (a) – (b) represents the nanoparticles made at different PLGA concentrations (1.33%g/ml, 1.5%g/ml, 1.67%g/ml, 1.83%g/ml, and 2%g/ml). R values are 0.968, 0.97, 0.958, 0.974, 0.978, respectively.
It was clear to see that the exponential functions can fit the experimental data lines which was in agreements with nanoparticles degradation behaviors, but the first-order decay cannot represent the whole release process due to multiple release mechanism involved throughout the entire release process. Combining the degradation rate and release rate was the most appropriate way to analyze the release properties of the nanoparticles.

4.3.4 Discussion and Analysis

The whole release process is very complicated. Within the first 3 to 4 hours, the release rate highly depends on the extant nanoparticle structure and the type of release mechanism. Between 4 and 7 hours, there is a complicated interaction between the nanoparticle degradation state and the dominant release mechanism. In the last three hours, the degradation becomes the dominant factor in the release of the remaining gentamicin. To analyze nanoparticles at different PLGA concentrations, the release and degradation rates should be considered at the same time. Figure 15 showed the release and degradation rates of PLGA nanoparticles made at 1.33%g/ml PLGA.

Figure 15. Fitting curves of gentamicin concentrations during release. The blue lines showed experimental data. The red dash lines were the fitting curves. (a) – (b) represents the nanoparticles made at different PLGA concentrations (1.33%g/ml, 1.5%g/ml, 1.67%g/ml, 1.83%g/ml, and 2%g/ml).
Figure 15 (c) shows that nanoparticles synthesized with 1.33%g/ml PLGA have a large pore size. Thus, the release rate is likely controlled by the diffusion through water-filled pores within the first 4 hours based on the observed porosity. Figure 15 (a) and (b) show a faster degradation rate and faster release rate for the first 4 hours and 2 hours, respectively, which is in agreement with this understanding. The release rate decreased within 2 to 4 hours may be due to the smaller gentamicin concentration difference between inner particles and the outer liquid solution. From 4 to 8 hours, the release rate may be based on gentamicin diffusion combined with PLGA nanoparticle degradation. As the gentamicin concentration gradient became smaller, the release rate caused by diffusion through the polymer competed with the release rate due to diffusion through water-filled pores. After 8 hours, nanoparticle degradation increased significantly, so the dominating mechanism would be degradation of the nanoparticles.

When PLGA concentration was 1.5%g/ml, the nanoparticles presented a similar release profile to 1.33%g/ml PLGA nanoparticles as shown in Figure 16.
Base on an examination of the morphology of the nanoparticles synthesized with 1.5% g/ml PLGA, it can be seen that the average pore size on a nanoparticle’s surface was still large enough for gentamicin likely to diffuse through water-filled pores. According to the degradation rate and release rate, this was likely to be the main release mechanism within the first 4 hours. After 4 hours, a combination of diffusion through water-filled pores and polymers both affected the release rate. After 8 hours, degradation of nanoparticles became dominant.

For particles made with a PLGA concentration of 1.67% g/ml, the release rate was unique compared to other nanoparticles. As shown in Figure 17 (c), the nanoparticle had better distributed pores structure. The nanoparticles have a greater probability of similarly spherical shape. According to the DLS experiments, nanoparticles synthesized with 1.67% g/ml PLGA had the smallest average particle size. The release rate shown in Figure 17 (b) was well fitted with an exponential function within the first 4 hours. Therefore, it can be assumed that the main release mechanism was likely diffusion through polymers base on the Higuchi model [Mittal et al., 2007]. Previous experiments by Mittal have shown that when the average size of PLGA nanoparticles is
small and the initial drug concentration is higher than drug solubility in the outer aqueous phase, the Higuchi model can be applied [Mittal et al., 2007]. Between hours 4 and 6, the release rate decreased, but the degradation rate increased. One hypothesis was that the nanoparticles started to degrade which led to an increase in degradation rate as shown in Figure 17 (a). The release rate decreased because of a decrease in the gentamicin concentration gradient. After 6 hours, the main release mechanism was nanoparticle degradation.

![Figure 18](image.png)

Nanoparticles synthesized with 1.83%g/ml PLGA possessed the profiles as shown in Figure 18. Within the first two hours, diffusion through water-filled pores was likely the main release mechanism. After 2 hours, the main release mechanism may have changed to osmotic pumping because of water absorption. Base on the DLS data and SEM image, the nanoparticles had a large diameter and small pore size, which may have lead to long pore channels. By the 5th hour, the degradation curve well matched with the fitting curve which meant it was a first order decay. Thus, nanoparticles started to degrade after 5 hours. One idea for the nanoparticles degrading so early was their large average size.
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Based on the degradation rate curve and release rate curve of nanoparticles synthesized with 2% g/ml PLGA in Figure 19, it looked like the release rate and degradation rate were composed of two parts. According to the size distribution from DLS and SEM image, nanoparticles synthesized with 2% PLGA have the largest average diameter and a small average pore size, which may lead to long pore channels. Therefore, osmotic pumping may be the main release mechanism within the first 5 hours. From the degradation curve, the degradation rate can be well fitted with an exponential function meaning it was a first order decay beginning at the 6th hour. As a result, the main release mechanism should be the nanoparticle degradation from 6 hours to 10 hours.

4. Conclusion

PLGA nanoparticles can be prepared and loaded with gentamicin via a double emulsion evaporation method with different parameters such as PVA (surfactant) concentration, PLGA concentration, and stirring time used to control the formation of the nanoparticles. In our experiments, the percent yield of nanoparticles was increased with an increase in PVA
concentration. The nanoparticles were mostly spherical, and all were porous. The effects of PLGA concentration on nanoparticle formation were not linear. In the range of 1.33% to 2% PLGA concentrations, the nanoparticle percent yield first increased and then decreased after 1.67%. The nanoparticles made at 1.67% PLGA concentration demonstrated a well distributed porosity and the smallest average size.

Different PLGA nanoparticles had various release properties. The release rate changed with time throughout the release process, and no single release mechanism can represent nanoparticles’ release rates. Within the scope of this study, it was impossible to determine which nanoparticles had the best release properties. Besides, the demand of nanoparticle release properties can be different under various circumstances. Thus, further study should be conducted to quantify the release properties of different nanoparticles, so the most desirable nanoparticles can be applied to specific situations.
Chapter 5

FUTURE WORK

PLGA nanoparticles can be prepared with tailorable properties, and loaded with gentamicin, suggesting a great potential for smart wound dressing applications. To evaluate the feasibility of the gentamicin-loaded PLGA nanoparticles in smart wound dressings, future work includes:

1. Release rate determination: Though the average release rates were determined for the different nanoparticles, instantaneous release rates based upon specific mechanisms should be calculated. One possible way to quantify release rate is using Monte Carlo Simulation. Using Monte Carlo Simulation to set up models for the release mechanisms will present a better model of fit for nanoparticle release properties.

2. Bacterial degradation test: The gentamicin-loaded PLGA nanoparticles should be tested for their efficacy against the common bacterium Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive) as model organisms. The bacterial inhibition rate of the nanoparticles should be investigated following the agar test as described in American Associate Textile Colorist and Chemist (AATCC) standard testing 100.

3. Incorporation in fibers: The PLGA nanoparticles should be incorporated into electro-spun fibers to develop nonwoven fabrics for wound dressing materials. The morphology and mechanical properties of the nanoparticle-loaded fibers should be studied.

4. Nonwoven efficacy test: The AATCC standard testing 100 should be adopted to test the bacterial efficacy of the nanoparticle-loaded nonwoven fabrics. The feasibility of developing smart wound dressings using the PLGA nanoparticle-loaded fibers should be investigated.
Bibliography


Gentamicin loaded PLGA nanoparticles as local drug delivery system for the osteomyelitis.


