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Transport characteristics using nor-dihydroguaiaretic acid (NDGA)-polymerized collagen fibers as a local drug delivery system

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Transport Characteristics Using Nor-Dihydroguaiaretic Acid (NDGA)-Polymerized Collagen Fibers as a Local Drug Delivery System

by

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ABSTRACT

Dexamethasone and dexamethasone 21-phosphate were loaded into NDGA-polymerized collagen fibers and release rate studies were performed to calculate their diffusion coefficients.

Dexamethasone loaded fibers were placed in a PBS solution for specified time intervals (1, 3, 6, 7, 12, 24, 30, and 48 hours) after which the eluant was removed and analyzed by capillary zone electrophoresis (CZE). CZE is a tool that can be utilized for quantitative analysis of chemical compounds. This data was incorporated into mathematical models to determine the diffusion coefficient. The diffusion coefficient (D) for dexamethasone in NDGA-polymerized collagen fibers is $D = 1.86 \times 10^{-14}$ m^2/s.

Similarly, dexamethasone 21-phosphate loaded fibers were placed into a PBS solution and analyzed using CZE at these specified intervals (15, 30, 45, 60, and 75 minutes). Applying this data to the mathematical model provided a diffusion coefficient for dexamethasone 21-phosphate in NDGA-polymerized collagen fibers of $D = 2.36 \times 10^{-13}$ m^2/s.

In an effort to control drug delivery from these fibers a polylactic-co-glycolic acid (PLGA) coating was applied to the fibers. This coating helped sustain delivery of dexamethasone 21-phosphate for over a 100 day period. CZE experiments were again

conducted in conjunction with another mathematical model to characterize release. A semi steady-state diffusion coefficient was estimated to be $D = 4.59 \times 10^{-14}$ m^2/s.

Chapter 1

Background

1.1 Diabetes

Diabetes is a disease that affects the blood glucose levels. These levels are augmented to a higher state because of a deficiency in insulin production and utilization. In 2002, the 6th leading cause of death in the United States was diabetes. This disease afflicted 20.8 million Americans in 2005. Diabetes can cause serious complications in the human body, and people of similar age with this disease are twice as likely to experience premature death. Every day 613 Americans will die from this disease. Regrettably, the cause for diabetes is still unknown; however, genetics and environmental factors such as health and diet contribute greatly to this disease[3].

Diabetes mellitus is a chronic metabolic disorder affecting the way the body uses glucose. When food is consumed and digested it is broken down into glucose, a simple sugar that is the main source of energy for the body. Glucose is absorbed into the blood stream where cells utilize it for energy and growth. However, glucose alone cannot be absorbed into the cells; it requires the presence of insulin. Insulin is a hormone produced by the pancreas whose primary function is to help the cells metabolize and use glucose. During the digestion phase the body produces the appropriate amount of insulin required to move glucose from the bloodstream to our cells. However, this systemic disease can limit or cause no insulin production to occur and can even alter the cells response to the

insulin. When this happens there is nothing present to fuel the cells or metabolize the excess glucose, resulting in Hyperglycemia[7]. This can lead to numerous potential problems throughout the body (Table 1).

Table 1. The numerous complications diabetics may face[4].

Complications	
Hypertension	Affects more than 70% of all diabetics
Heart disease and stroke	2 to 4 times as likely to experience than someone who does not have this disease
Diabetic retinopathy, blindness	Leading cause of blindness (ages 20-74)
Diabetic nephropathy, kidney failure	Leading cause of kidney failure
Nervous system disease (digestion problems, Carpal Tunnel Syndrome, lack of feeling or pain in appendages, etc…)	Affects more than 60% of all diabetics
Lower-extremity amputations	60% of all cases are diabetics
Pregnancy	15-20% increased chance of miscarriage 5-10% will have major birth defects
Biochemical imbalances can lead to: Diabetic ketoacidosis Hyperosmolar (nonketonic) comas	Acute life threatening events

Since diabetes primarily targets insulin production and influences glucose levels it is evident that maintaining and monitoring these levels is of the utmost of importance. There are two main types of blood tests administered: the A1c blood test and self-monitoring of blood glucose (SMBG) test. Both of these are used to monitor glucose levels and provide vital results to aid in the adjustment of treatments. The A1c blood test measures the glycerated hemoglobin percentage. The protein, hemoglobin, is a component in red blood cells that transfers oxygen from the lungs to the body. The excess presence of glucose caused from diabetes links up and glycates with the molecules of the

hemoglobin. This forms a compound known as HbA_{1c} that can be measured by the A1c test as a percentage, which shows an average of the glucose control over a two to three month period[7].

The second method, the SMBG test, is self-administrated about two to four times a day. This provides the patient with a better understanding of how their bodies' glucose levels fluctuate. Changes in medicine, diet, stress, physical activity, health, or routines can alter the state of your blood glucose. By monitoring these levels the diabetic patient will learn how their body reacts and can make self-adjustments when needed. These daily results are compared to the physician's A1c test to see if accuracy is being achieved. This also allows the physician to see possible trends and to adjust treatments appropriately.

However, both test methods are fairly effective for the monitoring of glucose but each presents limitations. The A1c is by far the most accurate method of the two but must be administered by a physician and then sent to a lab for analysis. It is only taken every two to three months and within this time frame drastic changes in glucose levels may occur. In most cases SMBG test would pick up these changes; however, studies show that patient testing techniques are not without error. An estimated 31% of SMBG users, due to improper testing techniques, have results that vary by 10-20% of the actual glucose value and 53% perform errors that cause results to be off up to 10%. Furthermore, FDA guidelines allow glucose meters on the market to vary up to 20% of actual blood glucose levels. So, with all this variation how accurate are the readings patients are receiving? It seems a better monitoring technique is needed, one which has the accuracy of the A1c testing but the frequency of SMBG testing. A technique to monitor glucose levels without the influence of human error[1, 15].

1.2 Glucose Sensor

A possible solution to the inadequacies of the SMBG and A1c tests would be to develop an implantable glucose sensor for the body (figure1). This biosensor would revolutionize current monitoring techniques and significantly contribute to the control and understanding of diabetes. Current monitoring techniques use discrete measurement methods collecting data points from a system that is constantly changing. These test methods contribute to delays from the acquired level to actual glucose level due to the setup and test time. Similarly, patterns or rapid fluctuations in the patient's glucose levels will not show. To develop an effective means to continuously monitor glucose levels would be of great benefit to the medical community. Through continuous monitoring of blood glucose levels diabetics would be able to administer insulin in a timely manner, knowing precisely when levels are not where they should be. This in itself would be a great tool furthering the effectiveness of insulin delivery and proactively preventing the frequencies of attacks from occurring. It is clear there is a need for these sensors, and there is a potential market. In 2002 the American Diabetes Association reported that U.S. healthcare costs for diabetes exceeded 132 billion dollars. According to Business Communications Company Inc. who performed a market study in 2002, predicted that by 2007 glucose monitor market will exceed 8 billion dollars worldwide. Clearly, the need for improvement is present and with increasing technological advancements an implantable glucose sensor is a feasible solution.

In general, this sensor will need to be tiny, as it is to be implanted in human tissue; it will need to provide accurate readings with a rapid response-time; and also be biocompatible with the human body. Miniaturization is no longer an issue as

technological advancements have lead to substantial improvements in sensor designs. Current glucose sensor elements occupy an area in the range of less than 1mm^2. These sensors can accurately provide continuous response times given that the sensor is intact and not influenced by outside factors. Furthermore, sensors are becoming increasingly more biocompatible as our understanding of material properties and the human response to these increases. However, the body's greatest ally is the enemy to the biosensor; the human body's complex defense and healing mechanisms. This intricate system has lead to substantial failures in designing an effective implantable glucose sensor.

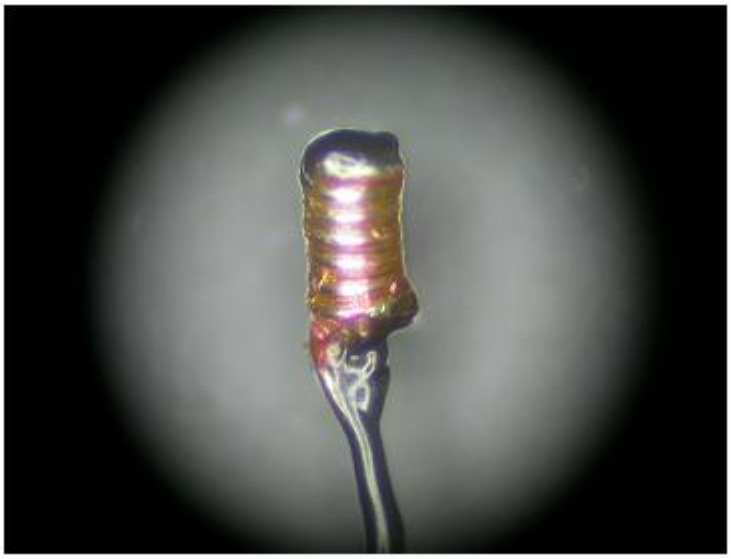

Figure 1. Implantable glucose sensor[9].

1.3 Sensor Complications

These failures often occur due to the interaction between the biosensor and the body's immune system response. There are two reactions that occur when implanting sensors that contribute to their failure: the implantation process and the foreign body response. During the implantation phase a wound is created at the surgical site. Various

techniques have been studied to minimize tissue and cell damage from surgical incisions to insertion using small gauge needles. However, all these techniques will contribute to a certain amount of damage at the insertion site that cannot be avoided. When thinking of the biosensors' size one must consider that although it is very small, it is much larger than the cells and blood capillaries from which it will need to acquire data making insertion damage inevitable. Consequently, one must minimize the implantation site as much as possible and ensure the sensor is contaminant free to eliminate risk of infection. Similarly, one must prevent the body's defenses from rejecting the sensor. As the body begins the wound-healing phase, trying to stop loss of blood, prevent infection and restore function to the injured implantation site; inflammatory cells like neutrophils and macrophages detect the presence of a foreign body. Since phagocytosis, the breakdown of foreign objects, is nearly impossible the macrophages form into giant cells encapsulating the site. The giant cells will form a collagen shell around the implant preventing normal interaction with the body by isolating it from surrounding tissue. This will lead to chronic inflammation resulting in potential sensor failure and inaccurate sensor readings.

There is a viable solution to this problem, which deals primarily with the biocompatibility of materials. Biocompatible materials are ideally ones, which are not rejected by the body, ones that elicit very little foreign body response and cause little to no irritation in the body. However, no current implant materials have been developed that will not induce some sort of biological response. Since this cannot be avoided, the only answer is to reduce the reaction that will occur. If one can modify the outer layer of the biosensor to a more tolerable material then there will be less interaction between the body's defenses and the device. Essentially, the body would be deceived into accepting

the device as normal. However, this is a very complicated task. It would be even more effective to combine a special outer layer to the sensor, which could lessen the foreign body response through the use of preventive agents to help the sensor gain acceptance[6].

1.4 Control Inflammation by Using a Drug Delivery System

The use of preventive agents could substantially improve the function of the sensor. By combining the sensor with a drug delivery coating one, could use the medicinal properties of the agent to prevent the body's foreign body response. Dr. T.J. Koob has developed a biocompatible fiber that ideally suits this purpose (figure 2). By surrounding the sensor with a collagen based nor-dihydroguaiaretic acid (NDGA) fiber potential negative interaction between the body and sensor could be limited. Using this fiber as a delivery system an anti-inflammatory agent could be administered by the process of diffusion. A substantial candidate for this is dexamethasone. This synthetic glucocorticoid is widely used as an anti-inflammatory and an immunosuppressive drug. Therefore, surrounding the fiber with dexamethasone loaded NDGA fibers should provide an effective means for controlling inflammation and drastically increase the life of the sensor.

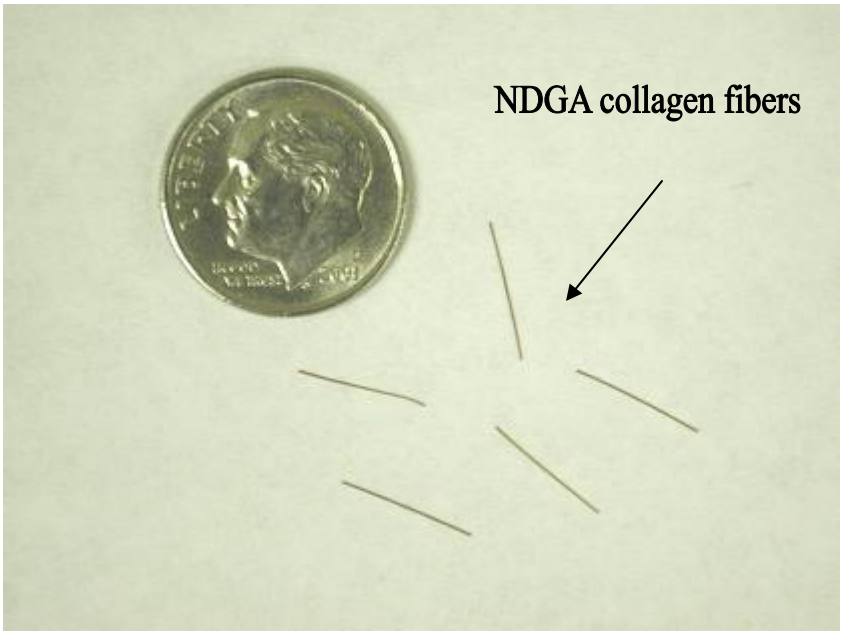

Figure 2. NDGA collagen fibers.

1.5 Purpose

Now that an effective system has been hypothesized to help extend biosensor function the mechanisms that control our process must be understood. Diffusion is a passive transport process where the driving potential is due to the species concentration gradient. The higher concentration of dexamethasone will diffuse to the lower concentration until a balance is achieved (figure 21 appendix. page 51). Diffusion will occur until the drug is depleted from the fiber[8]. The dexamethasone loaded NDGA collagen fibers will administer the drug at a specific rate. This diffusion coefficient will help one understand how much of the drug could potentially be delivered into the body for a certain length of time. However, calculations of this rate have never been performed in this media. This paper aims to illustrate a novel drug delivery system and model transport characteristics for three different cases presenting the various analytical and

experimental techniques performed to obtain these rates. The three cases examined were: i.) dexamethasone diffusing through the NDGA collagen fiber ii.) dexamethasone-21 phosphate disodium diffusing through NDGA collagen fibers iii.) dexamethasone-21 phosphate disodium diffusing through a polylactic-co-glycolic acid (PLGA) coating that surrounds the fiber. Understanding these rates will help to optimize an effective drug deliver system.

Chapter 2

NDGA Collagen Fibers

2.1 Background

In attempt to produce a material for use in tendon repair, Dr. Thomas J. Koob developed NDGA collagen based fibers. These fibers were created with similar mechanical properties to the actual human tendon, modeling an elastic solid. More significantly to this research, the fibers are biologically based and biocompatible[10]; a key factor in their potential use as a sensor coating and drug delivery tool. The main component of these fibers is collagen, a chemical protein found throughout the body that aids in strengthening and connecting tissues. Since this protein is found throughout the body it is a prime candidate as a potential biomaterial. Extracted fetal bovine collagens at 37°C in physiological buffers will re-nature into collagen fibrils (figure 3). These synthetic fibrils are weak because native cross-linking pathways do not manifest *in vitro* formation. A cross-linking agent is needed for the collagen fibers to increase the tensile strength and to lower the potential inflammatory response. The anti-oxidant, NDGA meets these criteria (figure 4). NDGA is a di-catechol extracted from the creosote bush and when cross-linked resolved strength and biocompatibility issues[11].

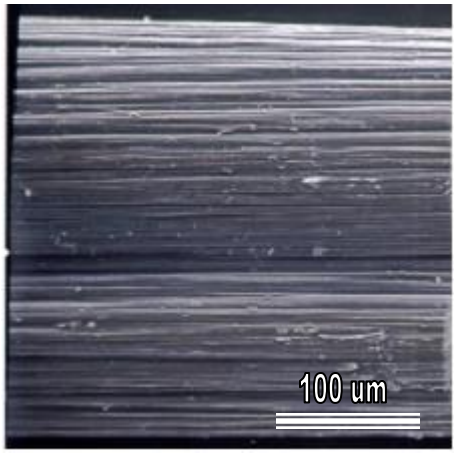

Figure 3. SEM image of two NDGA collagen fibers side by side.

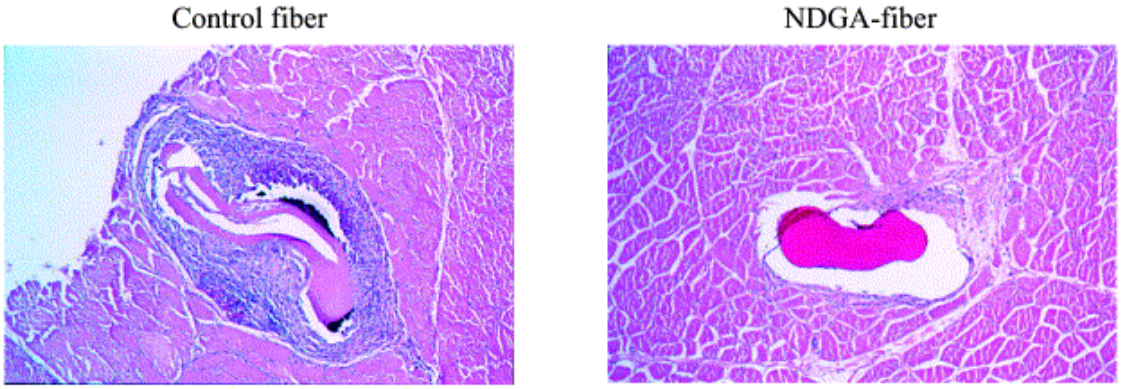

Figure 4. 10x magnification of H & E stained cross-section of implanted fibers at 6 weeks. These were implanted in the paravertebral musculature of rabbits. It is evident that the control fiber (non cross-linked collagen fiber) has well-organized capsules of cells surrounding it. It is also fragmented and has begun to degrade. The NDGA fiber has barely any encapsulating cells surrounding it, with the exception of the right corner. It is completely intact except for fragmentation that was caused during sectioning[10].

Any biomaterial incorporated into a host must not elicit harm to the body or cells. However, during the fabrication process, NDGA and residual products from cross-linking were found to be cytotoxic *in vitro*. However, by washing the fibers in 70% ethanol cytotoxicity can be eliminated[12]. To ensure that non-cytotoxic and biologically based biocompatible fibers are fabricated, an intricate protocol must be followed.

2.2 Fiber Fabrication Process

The fiber fabrication process (figure 23 appendix, page 56) is a very delicate and intricate procedure. It is essential to follow the required steps to produce high strength, biocompatible fibers. The entire process takes four days and can be broken up into daily procedures. Refer to appendix (Detailed Fiber Fabrication Protocol, page 53) for a more detailed procedure.

The fibers were made using purified pepsin-solubilized type I fetal bovine tendon collagen. The 0.13% w/v collagen solution was placed in 0.32-ml/cm dialysis tubing and then washed every 30 minutes in de-ionized water for at least 7 hours. The tubing assemblies were then transferred to a PBS solution of pH level 7.4 and incubated at 37°C for 16 hours. This extrusion process permits the collagen to re-nature and promotes fibril alignment. Following this step, the fibers were then hung dry; strengthening the weakened fibers. Once the fibers are dry, NDGA cross-linking can occur. Oxidized sodium phosphate buffer, having a pH level of 9.0, is combined with NDGA (Cayman Chemical, Ann Arbor, MI) to form the cross-linking agent. The amount of oxygen present accelerates the cross-linking reaction. The fibers are then agitated in this NDGA solution overnight. The final day encompasses washing and drying the NDGA treated fibers. The fibers are washed in 70% ethanol to remove any un-reacted, soluble NDGA intermediates. The procedure is sufficiently repeated to ensure all unbound NDGA is removed. The fibers are finally straightened and hung vertically in tension to dry overnight, completing the fabrication process.

2.3 Dexamethasone Loading

With the fabrication process complete the fibers are ready to be loaded with a drug agent. The drug loading procedure is fairly simple and could potentially be applied to other drug agents. In the first case, ten dried fibers were loaded with dexamethasone. These samples (diameter of 0.08 mm) were cut into 10 mm lengths and placed into 1.5 ml tubes. These tubes contained 200 µl of 10 mg/ml of dexamethasone (SIGMA, St. Louis, MO) in a 70% ethanol solution (10 fibers/tube, number of tubes, n = 10). The fibers were incubated in this mixture for 18 hours at room temperature. The solution was then removed and the fibers were allowed to air dry for 24 hours. Once dried, the fibers were washed in 200 µl of PBS, with pH level of 7.4, to remove any residual dexamethasone left from drying. The fibers were then incubated at 25°C in 200 µl of PBS in the dark, as it is light sensitive. The PBS was removed at specific time periods (1, 3, 6, 7, 12, 24, 30, and 48 hours) and replaced with fresh PBS. This removed dexamethasone was analyzed by Capillary Zone Electrophoresis (CZE) for drug elution amounts (refer to ch.3).

2.4 Dexamethasone 21-phosphate Loading

In the second case, ten dried fibers of length 10 mm and diameter of 0.08 mm were placed into a 1.5 ml tubes. These tubes contained 200 µl of 10 mg/ml of dexamethasone 21-phosphate disodium salt (SIGMA, St. Louis, MO) in a 3% acetic acid solution (10 fibers/tube, number of tubes, n = 10). The fibers were then incubated at room temperature for 18 hours in this solution. The mixture was discarded and the fibers were air dried for 24 hours. The dried fibers were then washed with PBS, with a pH level of 7.4, to remove any residual dexamethasone 21-phosphate not incorporated into the fibers.

The fibers were then stored in the dark at 25°C, in 200 µl of PBS. The PBS was removed at specific time intervals (15, 30, 45, 60, and 75 minutes) and analyzed on the CZE. Fresh PBS replaced the removed solution, which was analyzed on the CZE for dexamethasone 21-phosphate content (refer ch.3).

2.5 PLGA Coating of NDGA Collagen Fibers

In the third examined case dexamethasone 21-phosphate loaded fibers (n = 15) were coated with PLGA (figure 22 appendix, page 51). To coat the fibers, 0.5g of PLGA crystals, which were stored at –20°Celsius, were dissolved in 1g of chloroform. The chloroform had a purity of 99% and is anhydrous. Place the solution on a rocker for at least 2 hours to ensure complete PLGA crystal breakdown. Allow the solution to sit overnight to dissipate any air bubbles from the mixture. On the following day, dip coat the fibers in PLGA (50:50) in chloroform (PLGA/chloroform = 54%) uniformly. Remove them from the solution and hang them to air dry at 25°C for 5 days. This will provide a PLGA coating to the fibers with an average diameter of 0.306 mm (n = 30). The coated fibers were then incubated in 200 µl of PBS (3 fibers/tube). The PBS was removed at varying time intervals and examined on the CZE for dexamethasone 21-phosphate content (refer to ch.3). The PBS was replaced with fresh PBS after every analysis.

Chapter 3

Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) is a tool that can be utilized for quantitative analysis of chemical compounds. The system has the ability to separate analytes based on their charge and size. The CZE machine usually consists of two reservoirs and a capillary filled with a homogeneous buffer solution. Supplying a high-voltage across the capillary creates an electric field. This electric field produces an electro-osmotic flow in the capillary causing the cations in the solvent to migrate towards the cathode. This migration also allows separation of the chemical compound because of the electrophoretic mobility of the analyte. Using various wavelengths, depending on your sample, the migration rates can be detected and quantified using UV methods of detection. This data is then sent to a computer and displayed as an electropherogram, which displays the response as a function of time. The output is displayed as peaks based of the analytes retention times. The consequential profile provides a very fast, highly efficient separation method.

By taking the drug loaded fibers and analyzing them *in vitro*, in sink conditions, concentration levels can be found. Since the fibers are in sink conditions, the PBS washes described earlier, provide a solution containing PBS and the eluted dexamethasone agents. Using the CZE machine the amount of eluted drug can be calculated at each time interval. The PBS eluant was analyzed on a Dionex Capillary Electrophoresis System I. Using a sodium borate buffer (10 mM of sodium borate, 50 mM of boric acid, pH 8.0) the eluant

was diluted (2 fold). It was then loaded from a height of 50 mm for 10 s by gravity into a 75 μm inner diameter x 80 cm long hollow glass capillary. This capillary was then electrophoresed at 20,000 V. Dexamethasone agents can be detected at 246 nm[13]. Prior to loading samples, calibration of the CZE was performed for dexamethasone and dexamethasone 21-phosphate. Standards were dissolved directly into the CZE buffer at increasing concentrations providing a relationship between peak area output and concentration. Running each of the experimental samples in the CZE provided the elution amounts determined from peak area. This provided data for the amount of dexamethasone and dexamethasone-21 phosphate released with respect to each time interval.

Chapter 4

Mathematical Model

4.1 Transient Mathematical Model

From a research standpoint, it is vital to understand how various chemicals and agents react within other media. When there is a different species concentration in a mixture, mass transfer will occur. The primary mechanism governing this mass transfer or drug release from the fibers is diffusion. In the first two examined cases fibers were placed into a well-stirred reservoir of PBS. This represents a sink condition as would occur in the body. The sink condition ensures a balance will not be achieved between concentrations inside and outside the fibers, as the volume is sufficiently large allowing complete diffusion. To understand this better one can use mathematical models to help illustrate the occurring process. To begin a few assumptions must be made to properly model the specified case. The formations of the fibers are solid and uniform in nature, meaning there is no other material inside the fibers to warrant a composite case. The fibers are cylindrical in formation having a length of 10 mm. The hydrated radius of the fibers is on average 0.058 mm. The length to radius ratio is sufficiently large enough to assume the case of diffusion through a cylinder of infinite length. Secondly, this ratio also means diffusion through the cylinder will happen radially; diffusion with respect to length is insubstantial. Also, diffusion here is a transient process, thus is time dependent. Based off these assumptions and simplifications, a governing equation can be formulated

that describes the concentration of a diffusing substance from a long cylinder with a uniform distribution under steady-state conditions:

$$\frac{\partial c}{\partial t} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r}\right] \qquad (1)$$

Here, c is the concentration of the drug in the fiber, t is the time following immersion into the reservoir, D is the diffusion coefficient of the drug in the fiber, and r is the radial distance within the fiber. Certain boundary conditions must be assumed to solve this equation: 1) the drug distribution is initially uniform in the fiber ($c = c_i$ at $t = 0$ for $0 < r < R$ where R is the radius of the fiber and c_i is the initial concentration); and 2) the drug concentration at the surface of the fiber is zero throughout the release ($c = 0$ for $t > 0$ at $r = R$). The solution for equation (1) is[19]:

$$c(r,t) = \frac{2c_i}{R}\sum_{n=1}^{\infty}\frac{J_0(\alpha_n r)}{\alpha_n J_1(\alpha_n R)}\exp(-\alpha_n{}^2 Dt) \qquad (2)$$

This solution provides an expression where concentration is a function of radial distance and time. This allows concentration profiles to be formulated for dexamethasone and dexamethasone 21-phosphate eluted from the NDGA collagen fibers.

The amount of diffusing substance per unit area, M_t, which has left the cylindrical fibers in time, t equals:

$$M_t = -\int_0^t D\left(\frac{\partial c}{\partial r}\right)_{r=R} dt \qquad (3)$$

If M_∞ is the amount of diffusing substance per unit area that is left as t approaches infinity, then for short times this equation becomes:

$$\frac{M_t}{M_\infty} = \frac{4}{R}\left(\frac{Dt}{\pi}\right)^{0.5} \qquad (4)$$

The amount, $M_{\infty,}$ is also the same as the initial amount loaded into the fibers. The amount, $M_{\infty,}$ is also the same as the initial amount loaded into the fibers. By combining equation (4) with the slope from experimental data plots for M_t/M_∞ vs. $t^{1/2}$ (cumulative drug release versus the square root of time), the diffusion coefficient, D, can be calculated[5].

4.2 Composite Mathematical Model

For the 3rd case in which diffusion occurs from the NDGA fibers through the PLGA membrane, a different mathematical model was used. Since the PLGA coating degrades with time, a steady diffusion coefficient cannot be calculated using the previously mentioned method. Instead, an analysis must be used that looks at each time interval independently to solve for a time dependent diffusion coefficient.

This model uses the assumption that the PLGA coating is the main factor controlling diffusion of dexamethasone 21-phosphate; the NDGA collagen fiber acts only as a storage vessel for the drug. This assumption is valid as the diffusion rate for

dexamethasone 21-phosphate in the fiber is much greater than in PLGA, which will be discussed later in chapter 5. To solve for the time dependent diffusion rate, each time interval was viewed as an individual diffusion case. Since each time interval was relatively short, a quasi-steady-state assumption was made. The mathematical model was assumed to be for a hollow cylinder of infinite length under steady state conditions with constant drug concentrations on each surface:

$$0 = \frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r} \qquad (5)$$

Two boundary conditions were assumed here: 1) the inner surface of the PLGA coating has a concentration equal to the fibers concentration ($c = c_i$ at $r = R_i$ where c_i is the concentration in the fiber and R_i is the inner radius, that of the fiber); and 2) the outer surface of the PLGA coating has a concentration ($c = c_e$ at $r = R_e$ where R_e is the outer radius of the coated fiber and c_e is the concentration in the PLGA coating) (see figure 5). Applying these boundary conditions and solving equation (5) leaves an expression for concentration as a function of radius:

$$c(r) = c_e + \frac{(c_e - c_i)\ln\left(\frac{r}{R_e}\right)}{\ln\left(\frac{R_e}{R_i}\right)} \qquad (6)$$

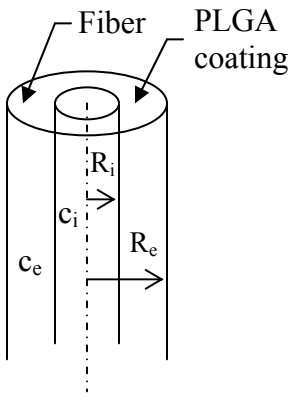

Figure 5. Diagram of the fiber and the model parameters.

Fick's 1st Law, denoted below, relates the rate of diffusion to the concentration gradient as the driving force behind mass transfer.

$$J = -D\frac{dc}{dr} \qquad (7)$$

Here, J represents mass flux. This expression can be related to the fibers geometry through the cross-sectional area, A.

$$M = JA = -D\frac{dc}{dr}A \qquad (8)$$

By integrating equation (8) with respect to time, an expression for the amount of diffusing substance, M_t, which diffuses through the length, L, of the cylindrical fibers in time, t, can be obtained:

$$M_t = -2\pi R_e LD\left(\frac{d}{dr}c(r)\right)_{r=R_e} t \qquad (9)$$

Taking the derivative of equation (6) provides an expression for:

$$\frac{d}{dr}c(r) = \frac{c_e - c_i}{\ln\left(\frac{R_e}{R_i}\right)R_e} \qquad (10)$$

If we assume perfect sink conditions, the concentration in the PLGA membrane will go to zero. Applying this simplification to equation (10) and substituting this new expression into equation (9) yields:

$$M_t = \frac{2\pi LDc_i t}{\ln\left(\frac{R_e}{R_i}\right)} \qquad (11)$$

This formula can be re-arranged into a numerical expression to calculate the diffusion coefficient at each time interval. The fibers concentration, c_i, will now be a function of the total concentration left in the fiber at the specific time being examined. Similarly, M_t will be a function for the amount of diffusing substance at each time. Lastly, the time, t, will be the time period. This leads to an expression for the individual diffusion coefficient at each specific elution period:

$$D(t) = \frac{M_t}{2\pi L c_i (\Delta t)} \ln\left(\frac{R_e}{R_i}\right) \qquad (12)$$

Refer to appendix (Program Diffusion through PLGA membrane Program, page 86) for calculations and for a more in depth derivation of formulas.

Chapter 5

Dexamethasone Loaded Fiber Results

Using the CZE machine a standard curve for dexamethasone was created by dissolving varying concentrations of dexamethasone in PBS. The different concentrations were diluted (2 fold) into a sodium borate buffer and then electrophoresesed as described in chapter 3. The obtained peak areas provided a linear relationship for concentration (figure 6).

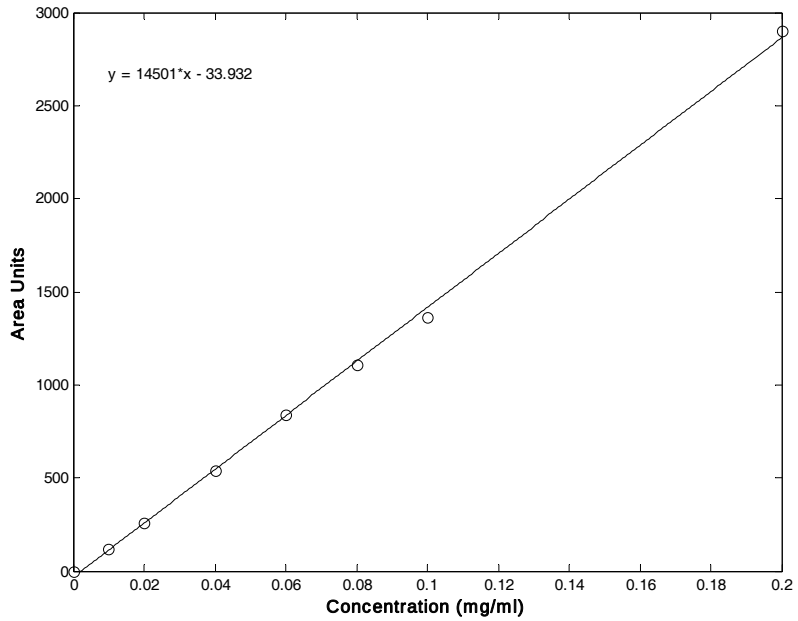

Figure 6. Standard curve for dexamethasone.

The dexamethasone-loaded fibers were placed in a PBS solution for specified time intervals (1, 3, 6, 7, 12, 24, 30, and 48 hours). After each incubation period the

eluant was removed and analyzed using CZE. This provided an accurate method for determining the dexamethasone content, which was eluted into the PBS solution at each period (figure 7).

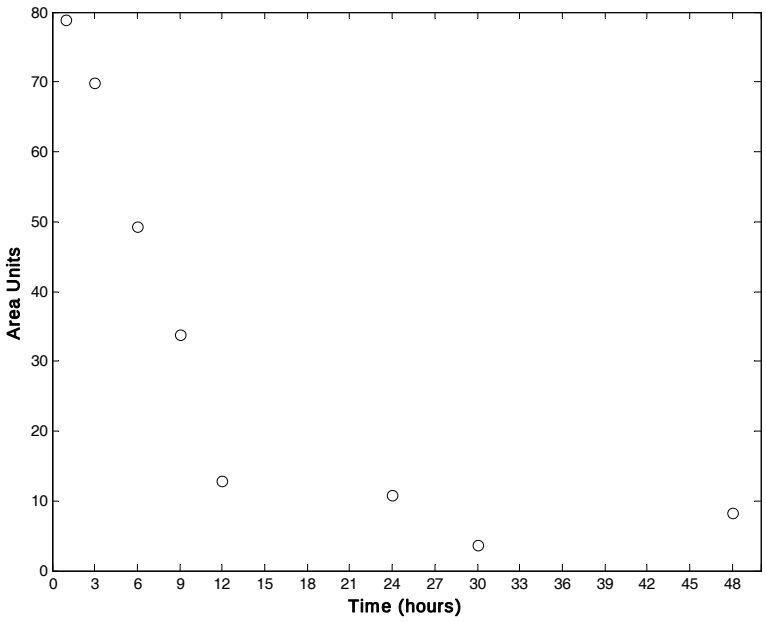

Figure 7. CZE data obtained from each incubation time.

Using the standard curve (figure 6) with the above figure's data provides a direct correlation between time and concentration levels. The peak areas for each sample were converted to their equivalent concentration using this relationship (figure 8).

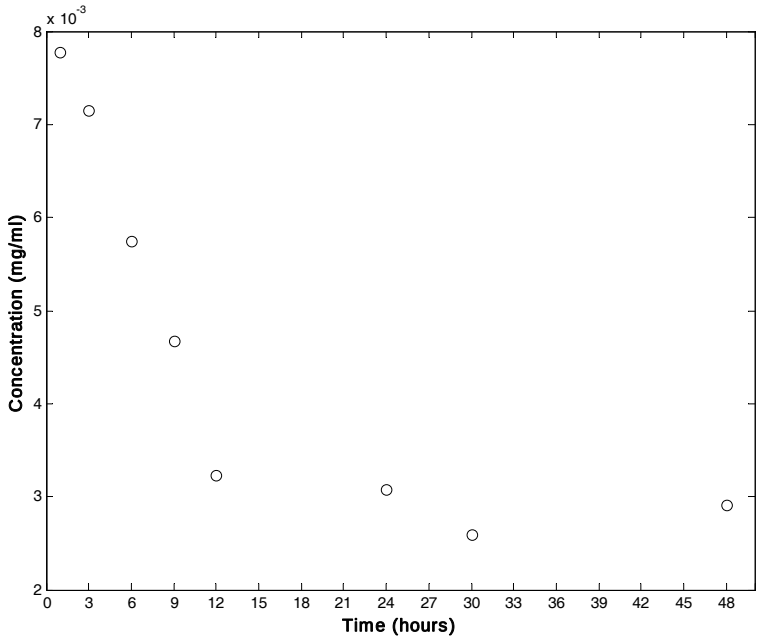

Figure 8. Relationship between dexamethasone concentration and time.

Simply taking the corresponding concentration at each time interval and adding the following concentration can form a cumulative relationship. This shows the cumulative release of dexamethasone concentration for each time until depletion (figure 9). The outlying bars represent standard deviation. Refer to appendix (Program Dexamethasone – Concentrations, page 57) for calculations.

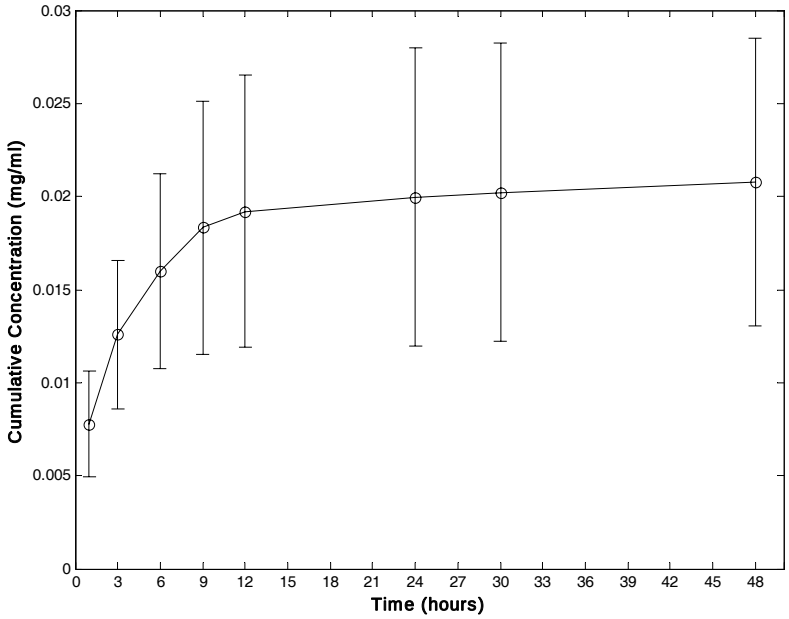

Figure 9. Cumulative concentration release against time for dexamethasone.

The fibers on average were loaded with a concentration of 0.021 mg/ml of dexamethasone as verified by CZE. Based off the release data the fiber segments were loaded with 4.2 μg of dexamethasone. Knowing the initial concentration allows the previous figure to be converted to a cumulative mass release versus time plot. The cumulative mass release plot is then taken against the square root of time (figure 10). This ensures the data is in the proper format to apply equation (4) from the mathematical model. Taking the slope for the short times from this plot satisfies,

$$slope = \frac{M_t / M_\infty}{\sqrt{t}} \qquad (12)$$

which allows one to solve for the diffusion coefficient.

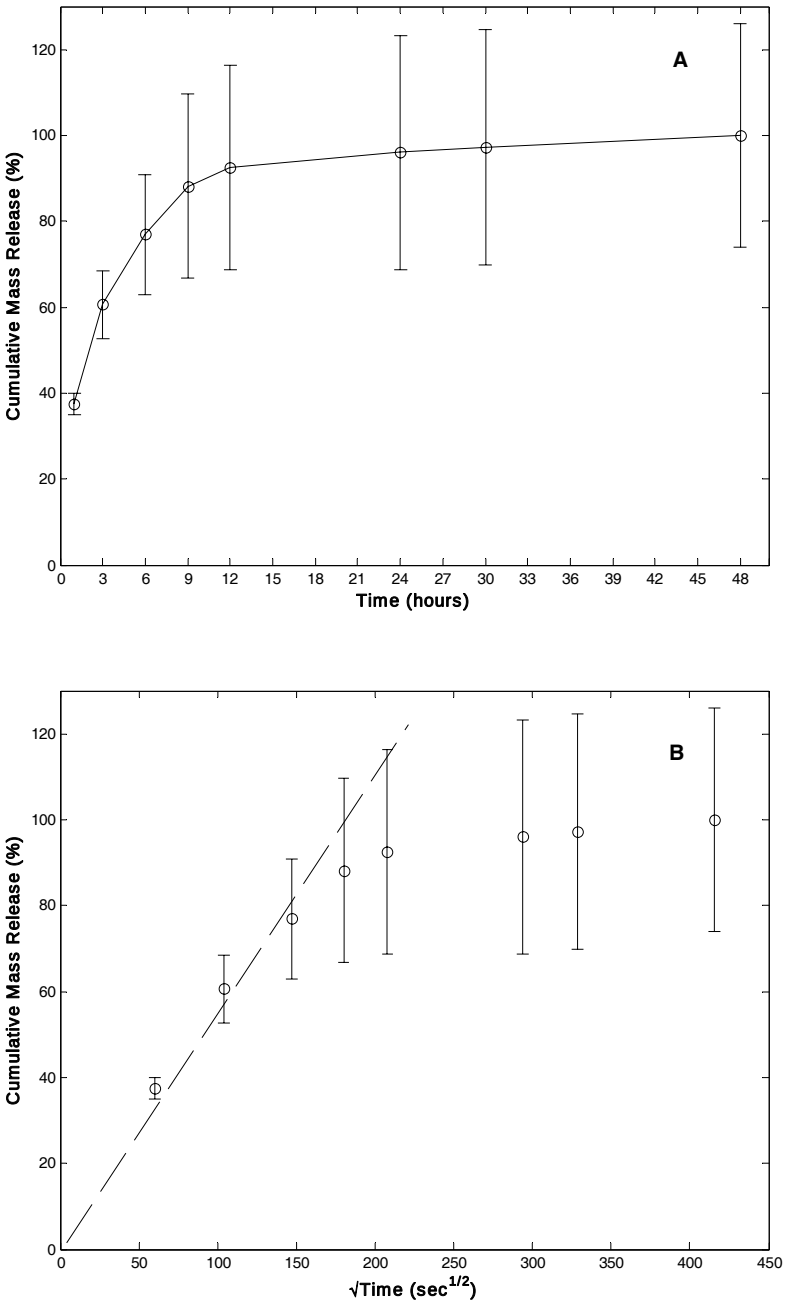

Figure 10. (A) The cumulative percent of dexamethasone released per fiber into PBS versus time. (B) The cumulative percent of dexamethasone released per fiber into PBS versus the square root of time. Dashed line indicates the slope for short times.

The rather large standard deviation is probably because of the insolubility of dexamethasone in PBS. The first 6 hours of release of dexamethasone was linear with

respect to the square root of time. Approximately 60% of the drug was released in the first 3 hours; by 6 hours nearly 77% had been released. Using the slope the diffusion coefficient was estimated to be $D = 1.86 \times 10^{-14}$ m^2/s. Refer to appendix (Program Diffusion Coefficient for Dexamethasone Calculations, page 84).

Chapter 6

Dexamethasone 21-phosphate Loaded Fiber Results

Using the CZE machine a standard curve for dexamethasone 21-phosphate was created by dissolving varying concentrations of dexamethasone 21-phosphate in PBS. The different concentrations were diluted (2 fold) into a sodium borate buffer and then electrophoresesed as described in chapter 3. The obtained peak areas provided a linear relationship for concentration (figure 11).

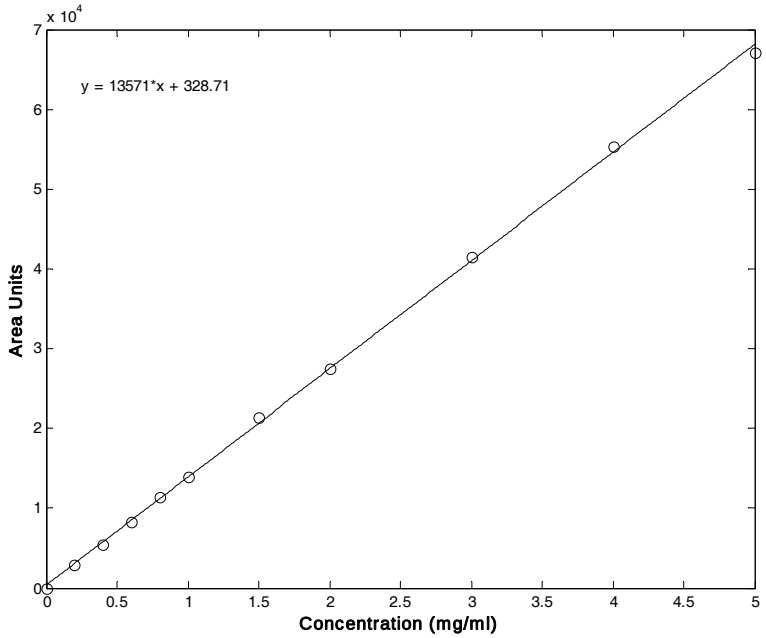

Figure 11. Standard curve for dexamethasone 21-phosphate.

The dexamethasone 21-phosphate loaded fibers were placed in a PBS solution for specified time intervals (15, 30, 45, 60, and 75 minutes). After each incubation period the

eluant was removed and analyzed using CZE. This provided an accurate method for determining the dexamethasone 21-phosphate content, which was eluted into the PBS solution at each period (figure 12).

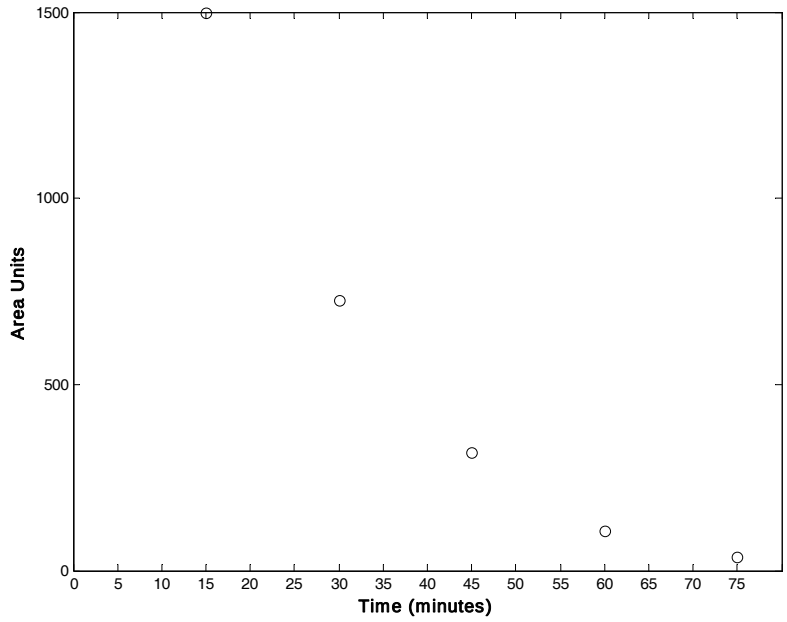

Figure 12. CZE data obtained from each incubation time for dexamethasone 21-phosphate.

Using the standard curve (figure 11) with the above figure's data provides a direct correlation between time and elution concentration levels. The peak areas for each sample were converted to their equivalent concentration using this relationship (figure 13).

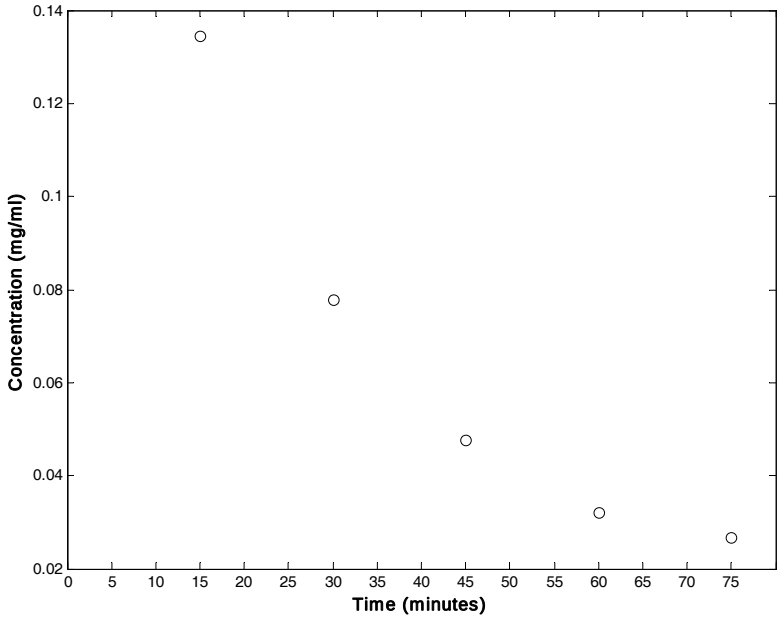

Figure 13. Relationship between dexamethasone 21-phosphate concentration and time.

Taking the corresponding concentration at each time interval and adding the following concentration a cumulative relationship can be formed. This shows the cumulative release of dexamethasone 21-phosphate concentration for each time until depletion (figure 14). The outlying bars represent standard deviation. Refer to appendix (Program Dexamethasone 21-phosphate Concentrations, page 63) for calculations.

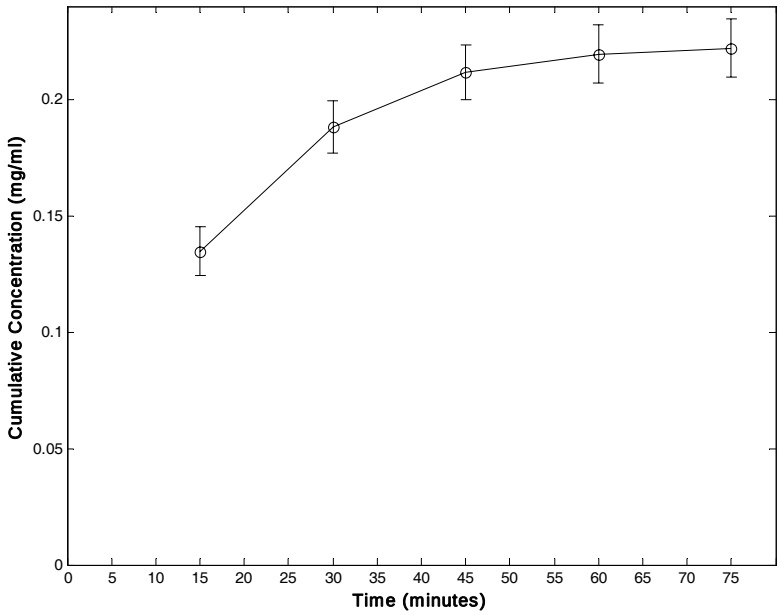

Figure 14. Cumulative concentration release against time for dexamehatsone 21-phosphate.

The fibers on average were loaded with a concentration of 0.222 mg/ml of dexamethasone 21-phosphate as verified by CZE. Based off the release data the fiber segments were loaded with 44.4 µg of dexamethasone 21-phosphate. Knowing the initial concentration allows the previous figure to be converted to a cumulative mass release versus time plot. The cumulative mass release plot is then taken against the square root of time (figure 15). This ensures the data is in the proper format to apply equation (4) from the mathematical model. Taking the slope for the short times from this plot satisfies equation (11) from chapter 4. With this slope, the diffusion coefficient can be solved.

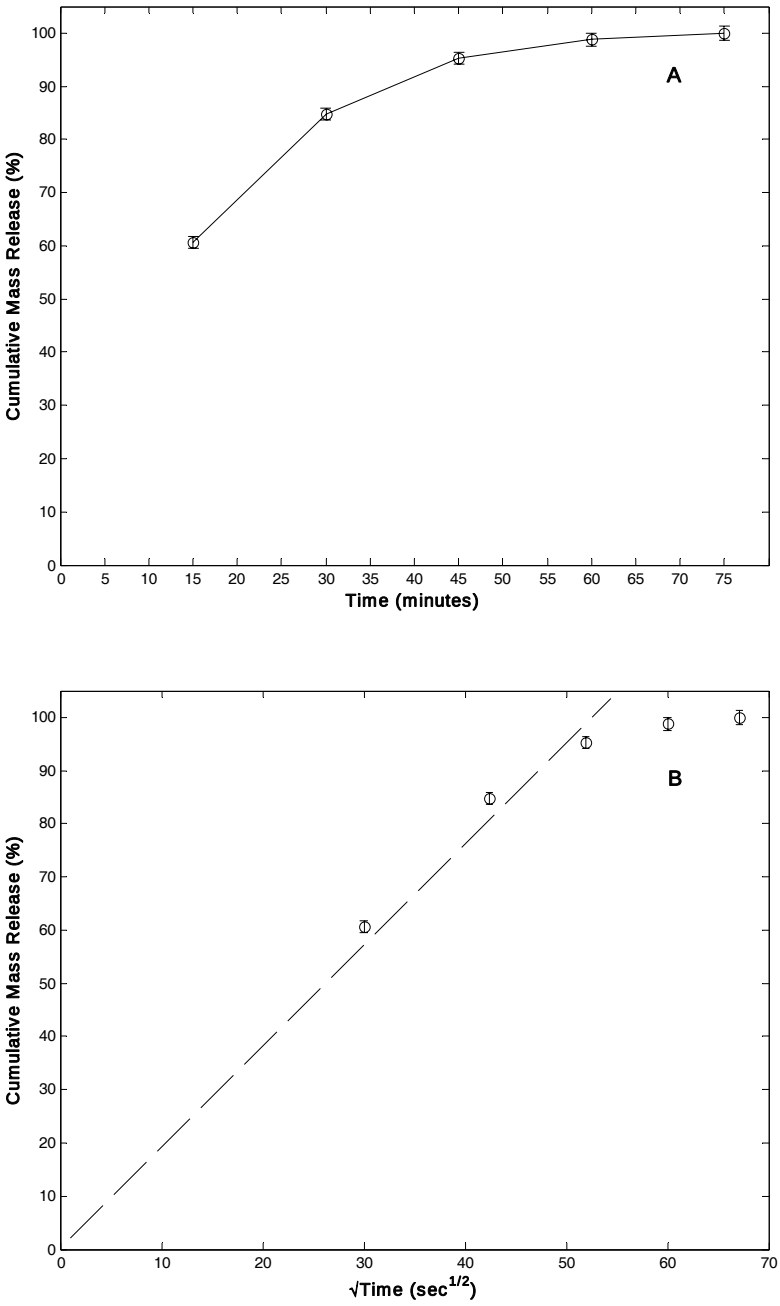

Figure 15. (A) The cumulative percent of dexamethasone 21-phosphate released per fiber into PBS versus time. (B) The cumulative percent of dexamethasone 21-phosphate released per fiber into PBS versus the square root of time. Dashed line indicates the slope for short times.

The standard deviation is substantially smaller than for the dexamethasone in PBS case. This is most likely due to the solubility of dexamethasone 21-phosphate in PBS.

During the first 45 minutes release of dexamethasone 21-phosphate with respect to the square root of time was fairly linear. Approximately 60% of the drug was released in the first 15 minutes; by 45 minutes nearly 95% had been released. Using the slope the diffusion coefficient was estimated to be $D = 2.36 \times 10^{-13}$ m^2/s. Refer to appendix (Program Diffusion Coefficient for Dexamethasone 21-phosphate Calculations, page 85).

Chapter 7

Dexamethasone 21-phosphate Loaded PLGA Coated Fiber Results

For this 3rd case dexamethasone 21-phosphate was diffusing through a PLGA membrane into PBS. Since the same agent was being analyzed using the CZE machine, as in chapter 5, the same standard curve was used (refer to figure 11). The dexamethasone 21-phosphate loaded PLGA coated fibers were again placed in a PBS solution and analyzed at various time periods (1, 2, 5, 8, 12, 17, 23, 30, 37, 44, 51, 58, 65, 72, 79, 86, 93, 100, and 107 days). After each incubation period the eluant was removed and analyzed using CZE and then replaced with fresh PBS. This provided an accurate method for determining the dexamethasone 21-phosphate content, which was eluted into the PBS solution during each period (figure 16).

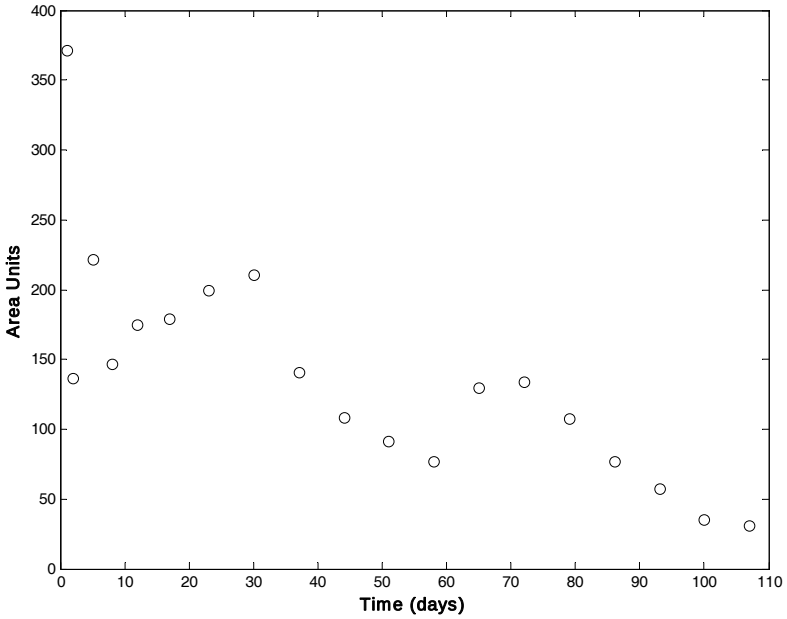

Figure 16. CZE data obtained for each incubation period.

To use the mathematical model described in chapter 4 it is necessary to calculate the concentration and the cumulative concentration levels at each time period. This can be achieved as in the previous chapters using the standard curve (figure 11). Forming a relationship between the two sets of data provides a direct correlation between time and concentration levels. The peak areas for each sample were converted to their equivalent concentration using this relationship (figure 17). The outlying bars in the figures represent standard deviation for the data sets.

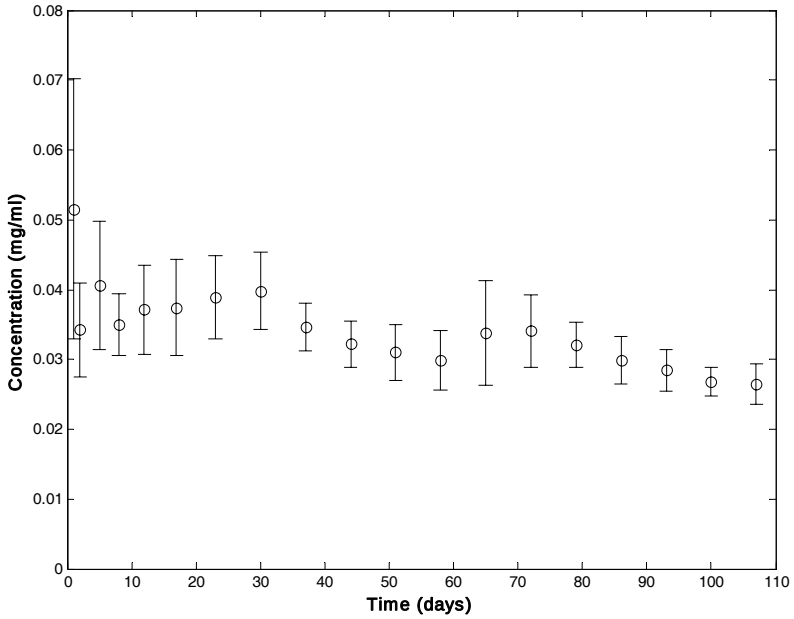

Figure 17. Concentration eluted at each time interval for the dexamethasone 21-phosphate loaded PLGA coated fibers.

Taking the corresponding concentration at each time interval and adding the following concentration a cumulative relationship was formed. This shows the cumulative release of dexamethasone 21-phosphate concentration for each time until depletion from the coated fiber (figure 18). Refer to appendix (Program PLGA DEX21 – Concentrations, page 68) for calculations.

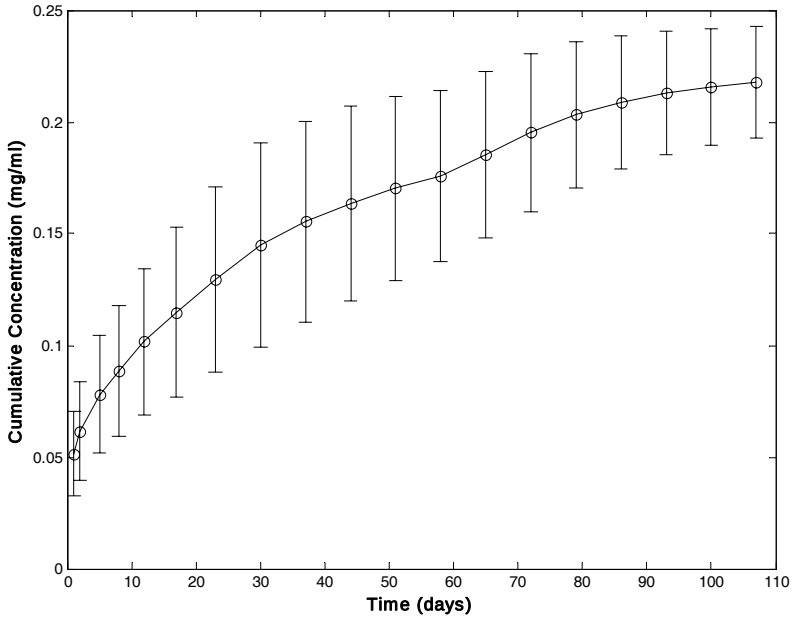

Figure 18. Cumulative concentration release against time for PLGA coated fibers.

On average the fibers were loaded with a concentration of 0.222 mg/ml of dexamethasone 21-phosphate as verified by CZE having an equivalent mass of 44.4 μg per fiber segment. Knowing the initial concentration allows the previous figure to be converted to a cumulative mass release versus time plot (figure 19). This set of data is not necessary in the calculation of the diffusion coefficient; however, this plot helps provides a better understanding of the diffusion process. By day 17, approximately 52% of the drug was released from the fiber through the PLGA membrane. Release was measured till day 107 at which time ~98% of the agent had been released. Due to the increasing rate of release in the later time intervals it is safe to assume that the fibers were very near to complete elution by 107 days. This can be verified below by the diffusion rate trends.

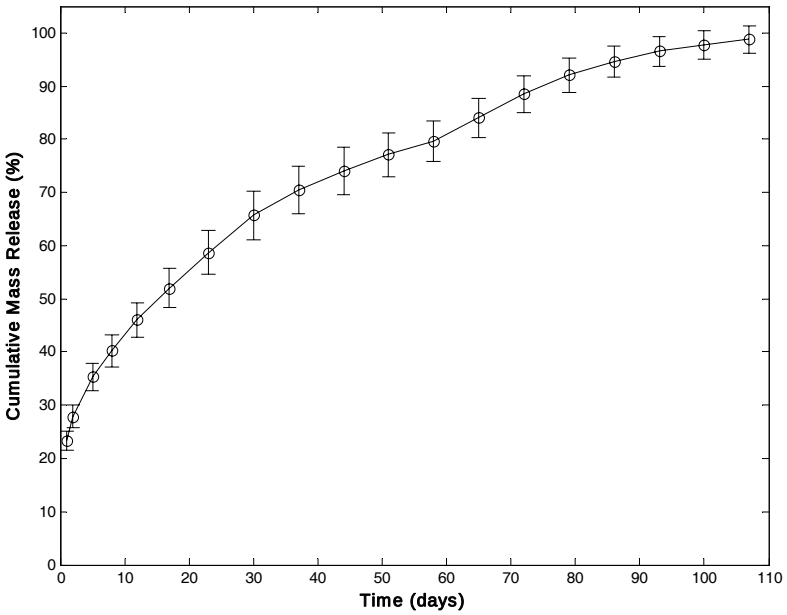

Figure 19. The cumulative percentage of dexamethasone 21-phosphate released from the PLGA membrane surrounding the fiber into PBS versus time.

Using the concentration and cumulative concentration data with chapter 4's equation (12) individual time interval diffusion coefficients can be calculated. Refer to the program (Program Diffusion through PLGA membrane Program, page 86) in the appendix. Plotting these coefficients illustrates how diffusion is varying with respect to time (figure 20).

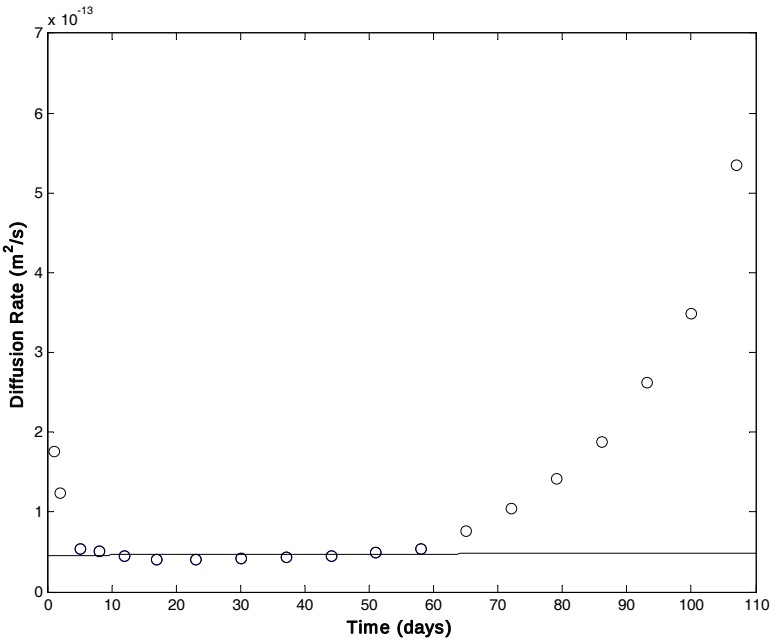

Figure 20. Diffusion rates at each time interval for dexamethasone 21-phosphate through a PLGA membrane.

Analyzing (figure 20), it is evident that for a sustained period dexamethasone 21-phoshate is diffusing through the PLGA membrane at an almost steady rate. From day 5 until day 58 it appears that nearly steady-state diffusion occurred. This model was linearized and the steady-state diffusion coefficient for dexamethasone 21-phosphate through a PLGA membrane was estimated to be $D = 4.59 \times 10^{-14}$ m^2/s, a value not previously reported. For the first two days the diffusion rate was faster because there is an initial burst of release for the dexamethasone 21-phosphate. This is mainly due to the residual drug left on the outside of the fiber. After 58 days the fiber's coating began to degrade releasing the agent at an increasing rate as time progressed. These two results seem quite accurate and follow what was expected for release from the polymer, PLGA.

Chapter 8

Discussion

8.1 Dexamethasone

The dried NDGA collagen fibers weigh on average 0.169 mg/fiber, have a diameter of ~0.08 mm, and a length of 10 mm. When placed in a dexamethasone solution overnight the fibers swell and absorb the drug. The hydrated fiber's diameter increases to 0.117 mm on average. After 2 days nearly all of the dexamethasone was released into the PBS solution, an estimated 0.021 mg/ml of dexamethasone (figure 9). The diffusion coefficient of dexamethasone in the NDGA collagen fibers was found to be $D = 1.86 \times 10^{-14}$ m^2/s, a value that has not been previously reported. The diffusion coefficient of dexamethasone in the NDGA collagen fiber was compared to the diffusion coefficient for dexamethasone in other media from the literature. The diffusion coefficient of dexamethasone in the NDGA collagen fiber is less than that in cellulose acetate but greater than in the poly(ether urethane), Tecoplast (Table 2).

Table 2. Diffusion coefficients of dexamethasone in various media.

Medium	*D [m^2/s]*	*Reference*
Water	$6.82x10^{-10}$	Stokes-Einstein equation
Subcutaneous tissue	$4.11\pm1.77\ x10^{-10}$	Moussy et al. 2006 [16]
Subcutaneous tissue	$4.01\pm2.01\ x10^{-10}$	Moussy et al. 2006 [17]
Brain	$2.0x10^{-10}$	Saltzman and Radomsky, 1991 [18]
Cellulose acetate membrane	$3.15x10^{-11a}$	Barry and Brace, 1977 [2]
NDGA collagen fibers	$1.86\ x\ 10^{-14}$	This study.
Tecoplast	$7.0\ x\ 10^{-17}$	Lyu et al., 2005 [14]
PTMC	$2.26\ x\ 10^{-21b}$	Zhang et al., 2006 [21]
$mPEG_3$-$PTMC_{11}$	$4.8\ x\ 10^{-22c}$	Zhang et al., 2006 [21]
Tecothane75D	$3.0\ x\ 10^{-23}$	Lyu et al., 2005 [14]

[a] Interpolated for 37°C

[b] poly(trimethylene carbonate)

[c] monomethoxy poly(ethylene glycol)-*block*-poly(trimethylene carbonate)

8.2 Dexamethasone 21-phosphate

The dexamethasone 21-phosphate loaded NDGA collagen fibers were of the same dimensions as the dexamethasone loaded fibers when hydrated. The primary difference between these two agents is their capability for loading and their solubility. Protonated free amines in the collagen phase bind with the negatively charged phosphate groups in the dexamethasone 21-phosphate. This binding process enables the fibers to be loaded with an estimated 0.222 mg/ml of drug when loaded in a 3% acetic acid solution. This is

nearly 11 times greater than when the fibers are loaded with dexamethasone in ethanol. The two agents are loaded using different solvents (ethanol versus 3% acetic acid). In water dexamethasone is nearly insoluble having a solubility of 10 mg/100 ml. However, it is highly soluble in ethanol[3]. On the other hand, dexamethasone 21-phosphate is water-soluble. However, dexamethasone 21-phosphate is loaded in the 3% acetic acid (v/v, in water) because it alters the pH levels causing the collagen phase to become positively charged favoring ionic interaction with the negatively charged phosphate groups increasing the loading potential. When examining the data for dexamethasone 21-phosphate elution, it is evident that the release rate is much faster than for dexamethasone. After 75 minutes the dexamethasone 21-phosphate had left the fiber. This rapid elution is due to the solubility of this drug in PBS and the neutralization of the collagen in PBS.

The diffusion coefficient of dexamethasone 21-phosphate in the NDGA collagen fibers was found to be $D = 2.36 \times 10^{-13}$ m^2/s, a value that has not previously been reported. The diffusion coefficient for dexamethasone 21-phosphate in the NDGA collagen fiber is approximately 12 times greater than for dexamethasone in the NDGA collagen fiber.

8.3 PLGA Coated Dexamethasone 21-phosphate Loaded Fibers

Clearly, dexamethasone 21-phosphate shows a greater capacity for loading in the NDGA collagen fibers. However, since this agent is water-soluble the release rate is too rapid and does not demonstrate substantial benefit for drug delivery applications. To use this anti-inflammatory drug the release rate must be controlled in a sustained manner,

which is why a PLGA membrane was applied to the fibers. This membrane increased the fibers average diameter to 0.306 mm (n=30). The preliminary results for the 3rd case show that after 100 days the coated fibers continue to release dexamethasone 21-phosphate (figure 18). The PLGA membrane also sustains a nearly steady state rate of release for the first 58 days. This steady state diffusion coefficient was estimated to be D = 4.59 x 10^{-14} m^2/s, a value not previously reported. This rate is approximately 5 times slower than that of the uncoated fiber loaded with dexamethasone 21-phosphate. The rate is based off of the diffusional distance, which corresponds to the thickness of the fibers coating. These preliminary results illustrate the potential that PLGA coated NDGA collagen fibers possess for a drug delivery system.

Chapter 9

Conclusion

9.1 Summary

It is evident that there is a substantial need for a method to continuously monitor blood glucose levels via an implantable sensor. Applying an effective drug delivery system for anti-inflammatory and immunosuppresant agents *in vivo* will increase the biosensors acceptance by the host, increase functionality and lifespan[20]. This paper has shown that NDGA collagen fibers can be loaded with a therapeutic agent and release of this agent can be determined and controlled. The loading process is principally a mechanical process. Therefore, loading the fibers with other agents (or combinations of agents) should be a viable option. By altering the fiber length or thickness during fabrication potential loading volumes can be increased and by utilizing different chemistries drug retention in the fibers can be improved. If further control of release is required different biopolymer membranes could be applied. Similar to the fibers, the thickness of the coatings can be adjusted to promote the optimum rate of diffusion. Previous studies have demonstrated that the NDGA collagen fibers are biocompatible *in vitro* and *in vivo*[12]. Thus, NDGA collagen fibers exhibit a great deal of potential for *in vivo* applications and clearly represent a novel drug delivery system.

9.2 Future Works

Now that an effective system has been proposed to deliver anti-inflammatory agents, the next step will be to incorporate these fibers into the implantable glucose sensor. The next proposed project would be to apply these fibers to glucose sensors, which our lab has developed, and implant these for *in vivo* testing. This will hopefully provide insight into how effective the dexamethasone 21-phosphate is at reducing inflammation and fibrosis around the implanted sensor and show if the sensitivity and lifespan of the sensor is improved. If the results from this experiment show promise, drug loading amounts and diffusion rates may be adjusted during fabrication to model the most efficient system for use with the sensor. Further studies may include the loading of different agents into the fibers, such as Vascular Endothelial Growth Factor (VEGF). VEGF should increase blood vessel growth; thus, has potential for increasing sensitivity in the sensor.

References

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Appendices

Appendix A: Additional Information and Figures

Diffusion

- Diffusion is a passive transport process in which the driving potential is the species concentration gradient.
- The higher concentration will permeate through the fiber to the lower concentration until a balance is achieved.

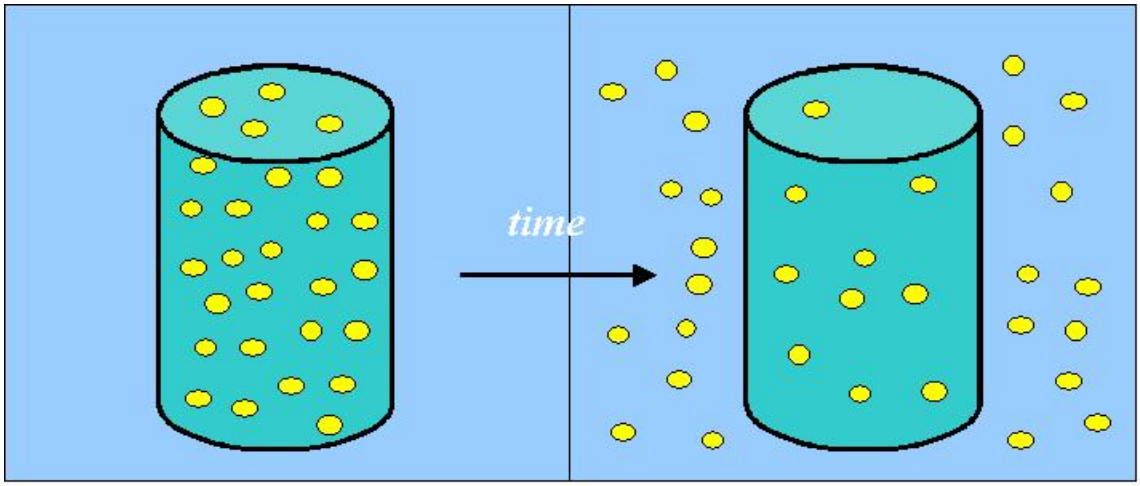

Figure 21. This figure illustrates the process at which the agents are diffusing from the fibers.

Appendix A: (Continued)

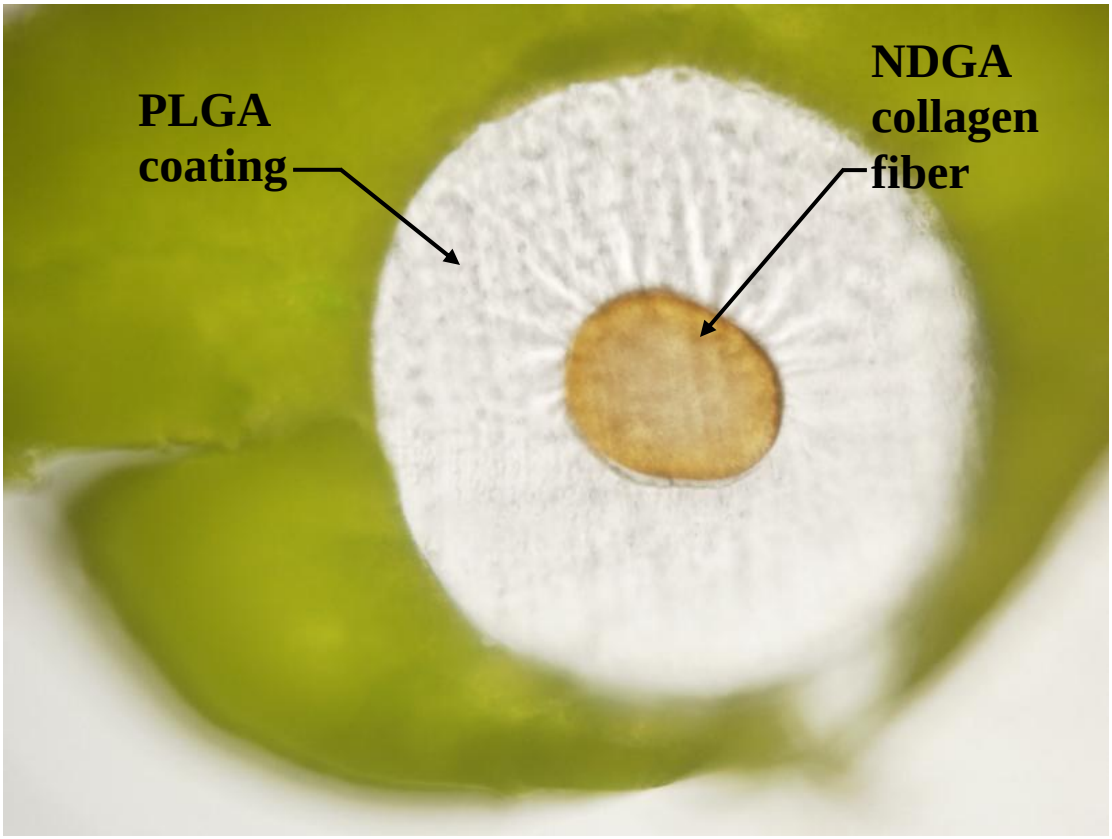

Figure 22. Cross-section of PLGA coated fiber.

Appendix A: (Continued)

Detailed Fiber Fabrication Protocol

The fiber fabrication process is a very delicate and intricate procedure. It is essential to follow the required steps to produce high strength, biocompatible fibers. The entire process takes four days and can be broken up into daily procedures. The following is a more detailed account of the fabrication procedure.

The first day covers initial setup and collagen production. To begin attach 0.32ml/cm hydrated dialysis tubing to the end of a 5 ml Ependorf Repeater pipet tips. It is essential not to crimp or hit the dialysis tubing on any sharp edges, as it is very fragile and important to the collagen formation process. Use a piece of silicon tubing to hold the 41.5cm length dialysis tubing onto the repeater tip. The collagen solution used is 0.13% w/v in 3% acetic acid. This 0.13% w/v yields the strongest fibers feasible, with around a 250 Newton tensile strength. Load the collagen solution into the dialysis tubing. Make sure to seal the end of the tubing, so as not to lose the solution. Aspirate any air bubbles, as this will weaken the collagen fibril formation. Make sure the tubing assemblies are hung in tension to prevent imperfections in fibril alignment and place them in a 4-liter graduated cylinder of de-ionized water. Change the water every 30 minutes for at least 7 hours. This washing step is necessary as it dialyses the acetic acid from the collagen solution. Any remaining acetic acid left in the tubing will breakdown the collagen preventing fibril alignment and formation. Once the washing is complete transfer the tubing assemblies into 4-liters of freshly made PBS, pH 7.4. Place this into a 37°C incubator overnight.

Appendix A: (Continued)

The second day encompasses drying the fibers. After 16 hours of incubation remove the PBS filled graduated cylinder. During this time period the collagen will have re-natured and formed fibrils. Once again, transfer the tubing assembly into a 4-liter graduated cylinder filled with de-ionized water for 30 minutes to remove any salt that was absorbed during incubation. Transfer the fibers to a flat pan filled with 1cm of de-ionized water. At this stage in fabrication the fibers are extremely fragile. Ensure the fibers will not twist or kink, as this will promote weaknesses in the drying phase. The drying device is essentially a motor drive the lifts a jack at variable speeds. Attached to the jack is a Styrofoam block that overhangs the pan. Attach the fiber ends to a bamboo toothpick by overlapping them. Place the toothpick approximately 4cm out of the water into the Styrofoam block. Allow the fibers to dry here for about 2 hours, until their diameter is about 1mm. Once the fiber is dry the strength will increase dramatically. Running the lifting device at rate of 1.4mm/min ensures exposed fibers will dry and strengthen enough to support the hydrated fibers that are being lifted from the pan.

On the third day NDGA cross-linking takes place. Remove the dried fibers from the lifting device and use sewing thread to bunch the fibers together at one end. Making sure the fibers remain aligned and in slight tension using the thread. Place the fibers into a long glass tube with stoppers at each end. Create a 27ml solution of 0.1 M sodium phosphate buffer increase the pH level to 9.0 using NaOH. Sparge this solution for two minutes. While the buffer is sparging, dissolve 90mg of NDGA in 0.4 M NaOH. Mix both of these solutions together and place contents inside the glass tube that houses the

Appendix A: (Continued)

fibers. Set glass tube on a rocker overnight. This step will help the fibers to cross-link evenly with the NDGA.

The final day is used to wash the fibers and dry them. Begin by removing the NDGA solution and briefly wash the fibers with 5ml of 70% ethanol. Empty contents of the glass tube and then fill 2/3 of tube with ethanol again. Seal the tube and replace it back onto the rocker for about 20 minutes. Drain again and perform final wash refilling tube with ethanol and placing on rocker for 60 minutes. Finally, drain the tube and remove fibers carefully. Hang vertically for drying. Ensure the fibers are separated while drying and in tension. Allow fibers to dry overnight.

Appendix A: (Continued)

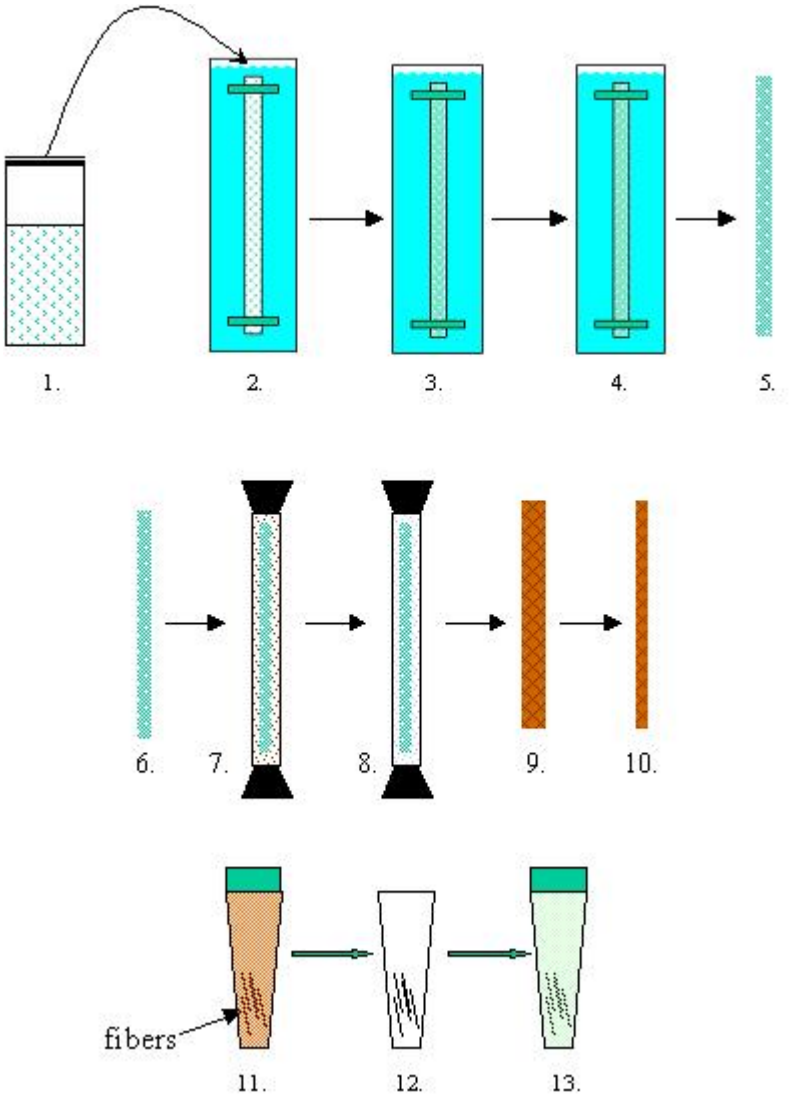

Figure 23. Fabrication procedure. Top panel: 1) Take 0.13%(w/v) collagen in 3% acetic acid and place in dialysis tubing. 2) Dialyze in de-ionized H_2O for ~7 hrs. 3) Incubate at 37 °C for 16 hrs in 4L of PBS solution 4) Dialyze in de-ionized H_2O again. 5) Extrude and dry fibers; NDGA Cross-linking. Middle panel: 6) Place the dry fibers into a glass tube with NDGA/sodium phosphate buffer solution. 7) Cap tube and place on rocker overnight. 8) Wash fibers in 70% EtOH to remove unbound NDGA. 9) Remove NDGA treated fibers and hang to dry. 10) NDGA cross-linked collagen fibers; Drug Loading and Elution. Bottom panel: 11) Dexamethasone loaded in 70% EtOH solution or dexamethasone 21-phosphate loaded in 3% acetic acid overnight. 12) Discard solution and dry fibers for one day. 13) Place drug loaded fibers in PBS solution and use Capillary Zone Electrophoresis to measure drug elution at specified time intervals.

Appendix B: Maple Programs

```
> restart;
```

Program Dexamethasone - Concentrations

This program calculates first the *concentrations at corresponding times (a)***,** then the *cumulative concentrations (b) including standard deviations*.

This equation was obtained from the standard curve of DEX in PBS calculated by Tian Davis' experiments. Using data provided by her June 5th, 2006 excel sheet. This formula represents how much DEX is eluted (y in area units) depending on the concentration loaded into the fibers (x in mg/ml).

y:=14501*x-33.932

This equation is then rewritten in terms of x:

```
> x:=(y+33.932)/14501;
```

$$x := \frac{y}{14501} + 0.002339976553$$

This equation was then used with corresponding data from Tian Davis' experiment 6 from June 1st, 2006 email. In experiment 6 Tian found a relationship between the DEX eluted in PBS to the time. Using the data from this experiment we shall formulate a relationship between the concentration (mg/ml) vs. time (hours).

case 1: at time 0 there was no area units present.

```
> y:=0;
```

$$y := 0$$

```
> Concentration[time=0]:=evalf(x);
```

$$Concentration_{time = 0} := 0.002339976553$$

case 2: at time = 1 hr, 78.81 area units were eluted.

```
> y:=78.81;
Concentration[time=1]:=(y+33.932)/14501;
```

$$y := 78.81$$

$$Concentration_{time = 1} := 0.007774774153$$

case 3a: at time = 3 hr, 69.8 area units were eluted.

Appendix B: (Continued)

```
> y:=69.8;
Concentration[time=3]:=(y+33.932)/14501;
```

$$y := 69.8$$

$$Concentration_{time = 3} := 0.007153437694$$

case 4a: at time = 6 hr, 49.34 area units were eluted.

```
> y:=49.34;
Concentration[time=6]:=(y+33.932)/14501;
>
```

$$y := 49.34$$

$$Concentration_{time = 6} := 0.005742500517$$

case 5a: at time = 9 hr, 33.81 area units were eluted.

```
> y:=33.81;
Concentration[time=9]:=(y+33.932)/14501;
```

$$y := 33.81$$

$$Concentration_{time = 9} := 0.004671539893$$

case 6a: at time = 12 hr, 12.91 area units were eluted.

```
> y:=12.91;
Concentration[time=12]:=(y+33.932)/14501;
```

$$y := 12.91$$

$$Concentration_{time = 12} := 0.003230259982$$

case 7a: at time = 24 hr, 10.74 area units were eluted.

```
> y:=10.74;
Concentration[time=24]:=(y+33.932)/14501;
```

$$y := 10.74$$

$$Concentration_{time = 24} := 0.003080615130$$

case8a: at time = 30 hr, 3.7 area units were eluted.

```
> y:=3.7;
```

Appendix B: (Continued)

```
Concentration[time=30]:=(y+33.932)/14501;
```

$$y := 3.7$$

$$Concentration_{time = 30} := 0.002595131370$$

case9a: at time = 48 hr, 8.27 area units were eluted.

```
> y:=8.27;
Concentration[time=48]:=(y+33.932)/14501;
```

$$y := 8.27$$

$$Concentration_{time = 48} := 0.002910282049$$

These are the *cumulative concentrations*:

case 1: at time 0 there was no area units present.

case 2: at time = 1 hr, 78.81 area units were eluted.

```
> y:=78.81;
CumulativeConcentration[time=1]:=(y+33.932)/14501;
dev:=78.81+(7.485608414);
UpperStd[time=1]:=(dev+33.932)/14501;
DevDiff:=.8290987408e-2-.7774774153e-2;
```

$$y := 78.81$$

$$CumulativeConcentration_{time = 1} := 0.007774774153$$

$$dev := 86.29560841$$

$$UpperStd_{time = 1} := 0.008290987408$$

$$DevDiff := 0.000516213255$$

case 3b: at time = 3 hr, 69.8 more area units were eluted.

```
> y:=78.81+69.8;
CumulativeConcentration[time=3]:=(y+33.932)/14501;
dev:=148.61+(23.99038928);
UpperStd[time=3]:=(dev+33.932)/14501;
DevDiff:=.1424263080e-1-.1258823529e-1;
```

$$y := 148.61$$

Appendix B: (Continued)

$$CumulativeConcentration_{time=3} := 0.01258823529$$

$$dev := 172.6003893$$

$$UpperStd_{time=3} := 0.01424263080$$

$$DevDiff := 0.00165439551$$

case 4b: at time = 6 hr, 49.34 more area units were eluted.

```
> y:=78.81+69.8+49.34;
CumulativeConcentration[time=6]:=(y+33.932)/14501;
dev:=197.95+(42.08267656);
UpperStd[time=6]:=(dev+33.932)/14501;
DevDiff:=.1889281267e-1-.1599075925e-1;
```

$$y := 197.95$$

$$CumulativeConcentration_{time=6} := 0.01599075925$$

$$dev := 240.0326766$$

$$UpperStd_{time=6} := 0.01889281267$$

$$DevDiff := 0.00290205342$$

case 5b: at time = 9 hr, 33.81 more area units were eluted.

```
> y:=78.81+69.8+49.34+33.81;
CumulativeConcentration[time=9]:=(y+33.932)/14501;
dev:=231.76+(64.53328512);
UpperStd[time=9]:=(dev+33.932)/14501;
DevDiff:=.2277258707e-1-.1832232260e-1;
```

$$y := 231.76$$

$$CumulativeConcentration_{time=9} := 0.01832232260$$

$$dev := 296.2932851$$

$$UpperStd_{time=9} := 0.02277258707$$

$$DevDiff := 0.00445026447$$

case 6b: at time = 12 hr, 12.91 more area units were eluted.

Appendix B: (Continued)

```
> y:=78.81+69.8+49.34+33.81+12.91;
CumulativeConcentration[time=12]:=(y+33.932)/14501;
dev:=244.67+(71.77189252);
UpperStd[time=12]:=(dev+33.932)/14501;
DevDiff:=.2416205037e-1-.1921260602e-1;
```

$$y := 244.67$$

$$CumulativeConcentration_{time = 12} := 0.01921260602$$

$$dev := 316.4418925$$

$$UpperStd_{time = 12} := 0.02416205037$$

$$DevDiff := 0.00494944435$$

case 7b: at time = 24 hr, 10.74 more area units were eluted.

```
> y:=78.81+69.8+49.34+33.81+12.91+10.74;
CumulativeConcentration[time=24]:=(y+33.932)/14501;
dev:=255.41+(82.22359015);
UpperStd[time=24]:=(dev+33.932)/14501;

DevDiff:=.2562344598e-1-.1995324460e-1;
```

$$y := 255.41$$

$$CumulativeConcentration_{time = 24} := 0.01995324460$$

$$dev := 337.6335902$$

$$UpperStd_{time = 24} := 0.02562344598$$

$$DevDiff := 0.00567020138$$

case 8b: at time = 30 hr, 3.7 more area units were eluted.

```
> y:=78.81+69.8+49.34+33.81+12.91+10.74+3.7;
CumulativeConcentration[time=30]:=(y+33.932)/14501;
dev:=259.11+(82.27916504);
UpperStd[time=30]:=(dev+33.932)/14501;
DevDiff:=.2588243328e-1-.2020839942e-1;
```

Appendix B: (Continued)

$$y := 259.11$$

$$CumulativeConcentration_{time = 30} := 0.02020839942$$

$$dev := 341.3891650$$

$$UpperStd_{time = 30} := 0.02588243328$$

$$DevDiff := 0.00567403386$$

case 9b: at time = 48 hr, 8.27 more area units were eluted.

```
> y:=78.81+69.8+49.34+33.81+12.91+10.74+3.7+8.27;
CumulativeConcentration[time=48]:=(y+33.932)/14501;
dev:=267.38+(77.96223872);
UpperStd[time=48]:=(dev+33.932)/14501;
DevDiff:=.2615504025e-1-.2077870491e-1;
```

$$y := 267.38$$

$$CumulativeConcentration_{time = 48} := 0.02077870491$$

$$dev := 345.3422387$$

$$UpperStd_{time = 48} := 0.02615504025$$

$$DevDiff := 0.00537633534$$

An average of 267.38 area units eluted which is equivalent to a total of 0.02077870491 mg/ml

Appendix B: (Continued)

```
> restart;
```

Program Dexamethasone 21-phosphate Concentrations

This program calculates first the *concentrations at corresponding times (a)***,** then the *cumulative concentrations (b)* and standard deviations**.**

This equation was obtained from the standard curve of dexamethasone 21-phosphate (DEX21) in PBS calculated by Tian Davis' experiments. Using data provided by her June 13, 2006 excel sheet called 'Exp 15 std'. This formula represents how much DEX21 is eluted (y in area units) depending on the concentration loaded into the fibers (x in mg/ml).

$$y:=13571*x-328.71$$

This equation is then rewritten in terms of x:

```
> x:=(y+328.71)/13571;
```

$$x := \frac{y}{13571} + 0.02422150173$$

This equation was then used with corresponding data from Tian Davis' experiment 8 from July 7th, 2006 email. In experiment 8 Tian found a relationship between the DEX21 eluted in PBS to the time. Using the data from this experiment we shall formulate a relationship between the concentration (mg/ml) vs. time (minutes).

case 1: at time 0 there was no area units present.

```
> y:=0;
```

$$y := 0$$

```
> Concentration[time=0]:=evalf(x);
```

$$Concentration_{time = 0} := 0.02422150173$$

case 2: at time = 15 minutes, 1498.27 area units were eluted.

```
> y:=1498.27;
Concentration[time=15]:=(y+328.71)/13571;
```

$$y := 1498.27$$

$$Concentration_{time = 15} := 0.1346238302$$

case 3a: at time = 30 minutes, 727.55 area units were eluted.

```
> y:=727.55;
```

Appendix B: (Continued)

```
Concentration[time=30]:=(y+328.71)/13571;
```

$$y := 727.55$$

$$Concentration_{time = 30} := 0.07783214207$$

case 4a: at time = 45 minutes, 317.15 area units were eluted.

```
> y:=317.15;
Concentration[time=45]:=(y+328.71)/13571;
>
```

$$y := 317.15$$

$$Concentration_{time = 45} := 0.04759118709$$

case 5a: at time = 60 minutes, 106.52 area units were eluted.

```
> y:=106.52;
Concentration[time=60]:=(y+328.71)/13571;
```

$$y := 106.52$$

$$Concentration_{time = 60} := 0.03207059170$$

case 6a: at time = 75 minutes, 35.5 area units were eluted.

```
> y:=35.5;
Concentration[time=75]:=(y+328.71)/13571;
```

$$y := 35.5$$

$$Concentration_{time = 75} := 0.02683737381$$

Here, case 2 is redone because the standard deviation was needed.

case 2: at time = 15 minutes, 1498.27 area units were eluted.

```
> y:=1498.27;
Concentration[time=15]:=(y+328.71)/13571;
dev:=1498.27+142.4087626;
UpperStd[time=15]:=(dev+328.71)/13571;
devDiff:=.1451174388-.1346238302;
```

Appendix B: (Continued)

$$y := 1498.27$$

$$Concentration_{time = 15} := 0.1346238302$$

$$dev := 1640.678763$$

$$UpperStd_{time = 15} := 0.1451174388$$

$$devDiff := 0.0104936086$$

These are the *cumulative concentrations (b)* and also the standard deviations for the data**.** Notice case 1 and 2 were omitted because they are the same.

case 3b: at time = 30 minutes, 727.55 more area units were eluted.

```
> y:=1498.27+727.55;
CumulativeConcentration[time=30]:=(y+328.71)/13571;
dev:=2225.82+151.9352698;
UpperStd[time=30]:=(dev+328.71)/13571;
devDiff:=.1994300545-.1882344705;
```

$$y := 2225.82$$

$$CumulativeConcentration_{time = 30} := 0.1882344705$$

$$dev := 2377.755270$$

$$UpperStd_{time = 30} := 0.1994300545$$

$$devDiff := 0.0111955840$$

case 4b: at time = 45 minutes, 317.15 more area units were eluted.

```
> y:=1498.27+727.55+317.15;
CumulativeConcentration[time=45]:=(y+328.71)/13571;
dev:=2542.97+159.5902465;
UpperStd[time=45]:=(dev+328.71)/13571;
devDiff:=.2233638085-.2116041559;
```

Appendix B: (Continued)

$$y := 2542.97$$

$$CumulativeConcentration_{time = 45} := 0.2116041559$$

$$dev := 2702.560246$$

$$UpperStd_{time = 45} := 0.2233638085$$

$$devDiff := 0.0117596526$$

case 5b: at time = 60 minutes, 106.52 more area units were eluted.

```
> y:=1498.27+727.55+317.15+106.52;
CumulativeConcentration[time=60]:=(y+328.71)/13571;
dev:=2649.49+168.421221;
UpperStd[time=60]:=(dev+328.71)/13571;
devDiff:=.2318636225-.2194532459;
```

$$y := 2649.49$$

$$CumulativeConcentration_{time = 60} := 0.2194532459$$

$$dev := 2817.911221$$

$$UpperStd_{time = 60} := 0.2318636225$$

$$devDiff := 0.0124103766$$

case 6b: at time = 75 minutes, 35.5 more area units were eluted.

```
> y:=1498.27+727.55+317.15+106.52+35.5;
CumulativeConcentration[time=75]:=(y+328.71)/13571;
dev:=2684.99+171.8312121;
UpperStd[time=75]:=(dev+328.71)/13571;
devDiff:=.2347307650-.2220691179;
```

$$y := 2684.99$$

$$CumulativeConcentration_{time = 75} := 0.2220691179$$

$$dev := 2856.821212$$

$$UpperStd_{time = 75} := 0.2347307650$$

$$devDiff := 0.0126616471$$

Appendix B: (Continued)

An average of 2684.99 area units were eluted which is equivalent to a total of 0.2220691179 mg/ml

Appendix B: (Continued)

```
> restart;
```

Program PLGA DEX21 - Concentrations

This program calculates the *concentrations at corresponding times (a),* and the *cumulative concentrations at corresponding times (b).* Both include standard deviation calculations.

This equation was obtained from the standard curve of DEX21 in PBS calculated by Tian Davis' experiments. Using data provided by her June 13, 2006 excel sheet called 'Exp 15 std'. This formula represents how much DEX21 is eluted (y in area units) depending on the concentration level (x in mg/ml).

$$y:=13571*x-328.71$$

This equation is then rewritten in terms of x:

```
> x:=(y+328.71)/13571;
```

$$x := \frac{y}{13571} + 0.02422150173$$

This equation was then used with corresponding data from Tian Davis' experiment 14. In experiment 14 Tian found a relationship between the DEX21 eluted from the PLGA coated fibers into PBS with respect to time. Using the data from this experiment we shall formulate a relationship between the concentration (mg/ml) vs. time (days).

case 1: at time 0 there was no area units present.

case 2: at time = 1 day, 371.2 area units were eluted.

```
> y:=371.2;
Concentration[time=1]:=(y+328.71)/13571;
dev:=254.0424943+371.2;
UpperStd[time=1]:=(dev+328.71)/13571;
devDiff:=.7029345621e-1-.5157394444e-1;
```

$$y := 371.2$$

$$Concentration_{time = 1} := 0.05157394444$$

$$dev := 625.2424943$$

$$UpperStd_{time = 1} := 0.07029345621$$

$$devDiff := 0.01871951177$$

Appendix B: (Continued)

case 3a: at time = 2 days, 136.33333 area units were eluted.

```
> y:=136.333333;
Concentration[time=2]:=(y+328.71)/13571;
dev:=91.40963966+136.333333;
UpperStd[time=2]:=(dev+328.71)/13571;
devDiff:=.4100309282e-1-.3426743298e-1;
```

$$y := 136.333333$$

$$Concentration_{time = 2} := 0.03426743298$$

$$dev := 227.7429727$$

$$UpperStd_{time = 2} := 0.04100309282$$

$$devDiff := 0.00673565984$$

case 4a: at time = 5 days, 221.93333 area units were eluted.

```
> y:=221.9333333;
Concentration[time=5]:=(y+328.71)/13571;
dev:= 124.4473115+y;
UpperStd[time=5]:=(dev+328.71)/13571;
devDiff:=.4974509209e-1-.4057500061e-1;
```

$$y := 221.9333333$$

$$Concentration_{time = 5} := 0.04057500061$$

$$dev := 346.3806448$$

$$UpperStd_{time = 5} := 0.04974509209$$

$$devDiff := 0.00917009148$$

case 5a: at time = 8days, 146.66667 area units were eluted.

```
> y:=146.6666667;
Concentration[time=8]:=(y+328.71)/13571;
dev:= 59.99907407+y;
UpperStd[time=8]:=(dev+328.71)/13571;
devDiff:=.3944998458e-1-.3502886056e-1;
```

Appendix B: (Continued)

$$y := 146.6666667$$

$$Concentration_{time = 8} := 0.03502886056$$

$$dev := 206.6657408$$

$$UpperStd_{time = 8} := 0.03944998458$$

$$devDiff := 0.00442112402$$

case 6a: at time = 12days, 175.266667 area units were eluted.

```
> y:=175.2666667;
Concentration[time=12]:=(y+328.71)/13571;
dev:= 85.8860356+y;
UpperStd[time=12]:=(dev+328.71)/13571;
devDiff:=.4346494011e-1-.3713629553e-1;
```

$$y := 175.2666667$$

$$Concentration_{time = 12} := 0.03713629553$$

$$dev := 261.1527023$$

$$UpperStd_{time = 12} := 0.04346494011$$

$$devDiff := 0.00632864458$$

case 7a: at time = 17days, 178.733333 area units were eluted.

```
> y:=178.7333333;
Concentration[time=17]:=(y+328.71)/13571;
dev:= 93.18154324+y;
UpperStd[time=17]:=(dev+328.71)/13571;
devDiff:=.4425796747e-1-.3739174219e-1;
```

$$y := 178.7333333$$

$$Concentration_{time = 17} := 0.03739174219$$

$$dev := 271.9148765$$

$$UpperStd_{time = 17} := 0.04425796747$$

$$devDiff := 0.00686622528$$

case 8a: at time = 23days, 199.333333 area units were eluted.

Appendix B: (Continued)

```
> y:=199.3333333;
Concentration[time=23]:=(y+328.71)/13571;
dev:= 81.15999288+y;
UpperStd[time=23]:=(dev+328.71)/13571;
devDiff:=.4489008372e-1-.3890968486e-1;
```

$$y := 199.3333333$$

$$Concentration_{time = 23} := 0.03890968486$$

$$dev := 280.4933262$$

$$UpperStd_{time = 23} := 0.04489008372$$

$$devDiff := 0.00598039886$$

case 9a: at time = 30days, 210.8 area units were eluted.

```
> y:=210.8;
Concentration[time=30]:=(y+328.71)/13571;
dev:= 74.98762861+y;
UpperStd[time=30]:=(dev+328.71)/13571;
devDiff:=.4528020253e-1-.3975462383e-1;
```

$$y := 210.8$$

$$Concentration_{time = 30} := 0.03975462383$$

$$dev := 285.7876286$$

$$UpperStd_{time = 30} := 0.04528020253$$

$$devDiff := 0.00552557870$$

case 10a: at time = 37days, 141.266667 area units were eluted.

```
> y:=141.2666667;
Concentration[time=37]:=(y+328.71)/13571;
dev:= 47.21487525+y;
UpperStd[time=37]:=(dev+328.71)/13571;
devDiff:=.3811005394e-1-.3463095326e-1;
```

Appendix B: (Continued)

$$y := 141.2666667$$

$$Concentration_{time = 37} := 0.03463095326$$

$$dev := 188.4815420$$

$$UpperStd_{time = 37} := 0.03811005394$$

$$devDiff := 0.00347910068$$

case 11a: at time = 44days, 108.133333 area units were eluted.

```
> y:=108.1333333;
Concentration[time=44]:=(y+328.71)/13571;
dev:= 44.58985934+y;
UpperStd[time=44]:=(dev+328.71)/13571;
devDiff:=.3547514498e-1-.3218947265e-1;
```

$$y := 108.1333333$$

$$Concentration_{time = 44} := 0.03218947265$$

$$dev := 152.7231926$$

$$UpperStd_{time = 44} := 0.03547514498$$

$$devDiff := 0.00328567233$$

case 12a: at time = 51days, 91.8 area units were eluted.

```
> y:=91.8;
Concentration[time=51]:=(y+328.71)/13571;
dev:= 53.45226323+y;
UpperStd[time=51]:=(dev+328.71)/13571;
devDiff:=.3492463806e-1-.3098592587e-1;
```

$$y := 91.8$$

$$Concentration_{time = 51} := 0.03098592587$$

$$dev := 145.2522632$$

$$UpperStd_{time = 51} := 0.03492463806$$

$$devDiff := 0.00393871219$$

Appendix B: (Continued)

case 13a: at time = 58days, 76.933333 area units were eluted.

```
> y:=76.93333333;
Concentration[time=58]:=(y+328.71)/13571;
dev:= 57.3349806+y;
UpperStd[time=58]:=(dev+328.71)/13571;
devDiff:=.3411526887e-1-.2989045268e-1;
```

$$y := 76.93333333$$

$$Concentration_{time = 58} := 0.02989045268$$

$$dev := 134.2683139$$

$$UpperStd_{time = 58} := 0.03411526887$$

$$devDiff := 0.00422481619$$

case 14a: at time = 65days, 129.466667 area units were eluted.

```
> y:=129.4666667;
Concentration[time=65]:=(y+328.71)/13571;
dev:= 102.1144782+y;
UpperStd[time=65]:=(dev+328.71)/13571;
devDiff:=.4128591444e-1-.3376145212e-1;
```

$$y := 129.4666667$$

$$Concentration_{time = 65} := 0.03376145212$$

$$dev := 231.5811449$$

$$UpperStd_{time = 65} := 0.04128591444$$

$$devDiff := 0.00752446232$$

case 15a: at time = 72days, 133.66667 area units were eluted.

```
> y:=133.6666667;
Concentration[time=72]:=(y+328.71)/13571;
dev:= 69.89714666+y;
UpperStd[time=72]:=(dev+328.71)/13571;
devDiff:=.3922141429e-1-.3407093557e-1;
```

Appendix B: (Continued)

$$y := 133.6666667$$

$$Concentration_{time = 72} := 0.03407093557$$

$$dev := 203.5638134$$

$$UpperStd_{time = 72} := 0.03922141429$$

$$devDiff := 0.00515047872$$

case 16a: at time = 79days, 107.4666667 area units were eluted.

```
> y:=107.4666667;
Concentration[time=79]:=(y+328.71)/13571;
dev:= 43.57279987+y;
UpperStd[time=79]:=(dev+328.71)/13571;
devDiff:=.3535107705e-1-.3214034829e-1;
```

$$y := 107.4666667$$

$$Concentration_{time = 79} := 0.03214034829$$

$$dev := 151.0394666$$

$$UpperStd_{time = 79} := 0.03535107705$$

$$devDiff := 0.00321072876$$

case 17a: at time = 86days, 76.7333333 area units were eluted.

```
> y:=76.73333333;
Concentration[time=86]:=(y+328.71)/13571;
dev:= 47.03568385+y;
UpperStd[time=86]:=(dev+328.71)/13571;
devDiff:=.3334161205e-1-.2987571537e-1;
```

$$y := 76.73333333$$

$$Concentration_{time = 86} := 0.02987571537$$

$$dev := 123.7690172$$

$$UpperStd_{time = 86} := 0.03334161205$$

$$devDiff := 0.00346589668$$

Appendix B: (Continued)

case 18a: at time = 93days, 57.0666667 area units were eluted.

```
> y:=57.06666667;
Concentration[time=93]:=(y+328.71)/13571;
dev:= 39.87368946+y;
UpperStd[time=93]:=(dev+328.71)/13571;
devDiff:=.3136470091e-1-.2842654680e-1;
```

$$y := 57.06666667$$

$$Concentration_{time = 93} := 0.02842654680$$

$$dev := 96.94035613$$

$$UpperStd_{time = 93} := 0.03136470091$$

$$devDiff := 0.00293815411$$

case 19a: at time = 100days, 35.5333333 area units were eluted.

```
> y:=35.53333333;
Concentration[time=100]:=(y+328.71)/13571;
dev:= 28.39268294+y;
UpperStd[time=100]:=(dev+328.71)/13571;
devDiff:=.2893198852e-1-.2683983003e-1;
```

$$y := 35.53333333$$

$$Concentration_{time = 100} := 0.02683983003$$

$$dev := 63.92601627$$

$$UpperStd_{time = 100} := 0.02893198852$$

$$devDiff := 0.00209215849$$

case 20a: at time = 107days, 30.8666667 area units were eluted.

```
> y:=30.86666667;
Concentration[time=107]:=(y+328.71)/13571;
dev:= 38.54694108+y;
UpperStd[time=107]:=(dev+328.71)/13571;
devDiff:=.2933635014e-1-.2649595952e-1;
```

Appendix B: (Continued)

$$y := 30.86666667$$

$$Concentration_{time = 107} := 0.02649595952$$

$$dev := 69.41360775$$

$$UpperStd_{time = 107} := 0.02933635014$$

$$devDiff := 0.00284039062$$

These are the *cumulative concentrations at corresponding times (b)* with standard deviations.

Note that case 1 and 2 are omitted because they were already solved in part (a).

case 3b: at time = 2 days, 136.3333 more area units were eluted.

```
> y:=507.5333333;
CumulativeConcentration[time=2]:=(y+328.71)/13571;
dev:=507.5333333+298.679929;
UpperStd[time=2]:=(dev+328.71)/13571;
devDiff:=.8362856549e-1-.6161987571e-1;
```

$$y := 507.5333333$$

$$CumulativeConcentration_{time = 2} := 0.06161987571$$

$$dev := 806.2132623$$

$$UpperStd_{time = 2} := 0.08362856549$$

$$devDiff := 0.02200868978$$

case 4b: at time = 5 days, 221.9333 more area units were eluted.

```
> y:=729.4666667;
CumulativeConcentration[time=5]:=(y+328.71)/13571;
dev:=729.4666667+357.1519751;
UpperStd[time=5]:=(dev+328.71)/13571;
devDiff:=.1042906670-.7797337460e-1;
```

Appendix B: (Continued)

$$y := 729.4666667$$

$$CumulativeConcentration_{time = 5} := 0.07797337460$$

$$dev := 1086.618642$$

$$UpperStd_{time = 5} := 0.1042906670$$

$$devDiff := 0.02631729240$$

case 5b: at time = 8 days, 146.6667 more area units were eluted.

```
> y:=876.1333333;
CumulativeConcentration[time=8]:=(y+328.71)/13571;
dev:=876.1333333+396.7563344;
UpperStd[time=8]:=(dev+328.71)/13571;
devDiff:=.1180163340-.8878073342e-1;
```

$$y := 876.1333333$$

$$CumulativeConcentration_{time = 8} := 0.08878073342$$

$$dev := 1272.889668$$

$$UpperStd_{time = 8} := 0.1180163340$$

$$devDiff := 0.02923560058$$

case 6b: at time = 12 days, 175.2667 more area units were eluted.

```
> y:=1051.4;
CumulativeConcentration[time=12]:=(y+328.71)/13571;
dev:=1051.4+443.6188554;
UpperStd[time=12]:=(dev+328.71)/13571;
devDiff:=.1343842646-.1016955272;
```

$$y := 1051.4$$

$$CumulativeConcentration_{time = 12} := 0.1016955272$$

$$dev := 1495.018855$$

$$UpperStd_{time = 12} := 0.1343842646$$

$$devDiff := 0.0326887374$$

Appendix B: (Continued)

case 7b: at time = 17 days, 178.73333 more area units were eluted.

```
> y:=1230.133333;
CumulativeConcentration[time=17]:=(y+328.71)/13571;
dev:=1230.133333+513.1005749;
UpperStd[time=17]:=(dev+328.71)/13571;
devDiff:=.1526743724-.1148657677;
```

$$y := 1230.133333$$

$$CumulativeConcentration_{time = 17} := 0.1148657677$$

$$dev := 1743.233908$$

$$UpperStd_{time = 17} := 0.1526743724$$

$$devDiff := 0.0378086047$$

case 8b: at time = 23 days, 199.3333 more area units were eluted.

```
> y:=1429.466667;
CumulativeConcentration[time=23]:=(y+328.71)/13571;
dev:=1429.466667+562.1573327;
UpperStd[time=23]:=(dev+328.71)/13571;
devDiff:=.1709773782-.1295539508;
```

$$y := 1429.466667$$

$$CumulativeConcentration_{time = 23} := 0.1295539508$$

$$dev := 1991.624000$$

$$UpperStd_{time = 23} := 0.1709773782$$

$$devDiff := 0.0414234274$$

case 9b: at time = 30 days, 210.8 more area units were eluted.

```
> y:=1640.266667;
CumulativeConcentration[time=30]:=(y+328.71)/13571;
dev:=1640.266667+619.3419178;
UpperStd[time=30]:=(dev+328.71)/13571;
devDiff:=.1907242344-.1450870729;
```

Appendix B: (Continued)

$$y := 1640.266667$$

$$CumulativeConcentration_{time = 30} := 0.1450870729$$

$$dev := 2259.608585$$

$$UpperStd_{time = 30} := 0.1907242344$$

$$devDiff := 0.0456371615$$

case 10b: at time = 37 days, 141.2667 more area units were eluted.

```
> y:=1781.533333;
CumulativeConcentration[time=37]:=(y+328.71)/13571;
dev:=1781.533333+610.5314898;
UpperStd[time=37]:=(dev+328.71)/13571;
devDiff:=.2004844759-.1554965244;
```

$$y := 1781.533333$$

$$CumulativeConcentration_{time = 37} := 0.1554965244$$

$$dev := 2392.064823$$

$$UpperStd_{time = 37} := 0.2004844759$$

$$devDiff := 0.0449879515$$

case 11b: at time = 44 days, 108.1333 more area units were eluted.

```
> y:=1889.666667;
CumulativeConcentration[time=44]:=(y+328.71)/13571;
dev:=1889.666667+593.91928;
UpperStd[time=44]:=(dev+328.71)/13571;
devDiff:=.2072283506-.1634644954;
```

$$y := 1889.666667$$

$$CumulativeConcentration_{time = 44} := 0.1634644954$$

$$dev := 2483.585947$$

$$UpperStd_{time = 44} := 0.2072283506$$

$$devDiff := 0.0437638552$$

Appendix B: (Continued)

case 12b: at time = 51 days, 91.8 more area units were eluted.

```
> y:=1981.466667;
CumulativeConcentration[time=51]:=(y+328.71)/13571;
dev:=1981.466667+558.2319112;
UpperStd[time=51]:=(dev+328.71)/13571;
devDiff:=.2113630961-.1702289195;
```

$$y := 1981.466667$$

$$CumulativeConcentration_{time = 51} := 0.1702289195$$

$$dev := 2539.698578$$

$$UpperStd_{time = 51} := 0.2113630961$$

$$devDiff := 0.0411341766$$

case 13b: at time = 58 days, 76.9333 more area units were eluted.

```
> y:=2058.4;
CumulativeConcentration[time=58]:=(y+328.71)/13571;
dev:=2058.4+521.9679322;
UpperStd[time=58]:=(dev+328.71)/13571;
devDiff:=.2143598800-.1758978704;
```

$$y := 2058.4$$

$$CumulativeConcentration_{time = 58} := 0.1758978704$$

$$dev := 2580.367932$$

$$UpperStd_{time = 58} := 0.2143598800$$

$$devDiff := 0.0384620096$$

case 14b: at time = 65 days, 129.4667 more area units were eluted.

```
> y:=2187.866667;
CumulativeConcentration[time=65]:=(y+328.71)/13571;
dev:=2187.866667+506.5052703;
UpperStd[time=65]:=(dev+328.71)/13571;
devDiff:=.2227604404-.1854378208;
```

Appendix B: (Continued)

$$y := 2187.866667$$

$$CumulativeConcentration_{time = 65} := 0.1854378208$$

$$dev := 2694.371937$$

$$UpperStd_{time = 65} := 0.2227604404$$

$$devDiff := 0.0373226196$$

case 15b: at time = 72 days, 133.6667 more area units were eluted.

```
> y:=2321.533333;
CumulativeConcentration[time=72]:=(y+328.71)/13571;
dev:=2321.533333+481.014241;
UpperStd[time=72]:=(dev+328.71)/13571;
devDiff:=.2307315285-.1952872546;
```

$$y := 2321.533333$$

$$CumulativeConcentration_{time = 72} := 0.1952872546$$

$$dev := 2802.547574$$

$$UpperStd_{time = 72} := 0.2307315285$$

$$devDiff := 0.0354442739$$

case 16b: at time = 79 days, 107.4667 more area units were eluted.

```
> y:=2429;
CumulativeConcentration[time=79]:=(y+328.71)/13571;
dev:=2429+442.8970284;
UpperStd[time=79]:=(dev+328.71)/13571;
devDiff:=.2358416497-.2032061012;
```

$$y := 2429$$

$$CumulativeConcentration_{time = 79} := 0.2032061012$$

$$dev := 2871.897028$$

$$UpperStd_{time = 79} := 0.2358416497$$

$$devDiff := 0.0326355485$$

Appendix B: (Continued)

case 17b: at time = 86 days, 76.73333 more area units were eluted.

```
> y:=2505.733333;
CumulativeConcentration[time=86]:=(y+328.71)/13571;
dev:=2505.733333+405.2431643;
UpperStd[time=86]:=(dev+328.71)/13571;
devDiff:=.2387212804-.2088603148;
```

$$y := 2505.733333$$

$$CumulativeConcentration_{time = 86} := 0.2088603148$$

$$dev := 2910.976497$$

$$UpperStd_{time = 86} := 0.2387212804$$

$$devDiff := 0.0298609656$$

case 18b: at time = 93 days, 57.06667 more area units were eluted.

```
> y:=2562.8;
CumulativeConcentration[time=93]:=(y+328.71)/13571;
dev:=2562.8+377.4835979;
UpperStd[time=93]:=(dev+328.71)/13571;
devDiff:=.2408808192-.2130653599;
```

$$y := 2562.8$$

$$CumulativeConcentration_{time = 93} := 0.2130653599$$

$$dev := 2940.283598$$

$$UpperStd_{time = 93} := 0.2408808192$$

$$devDiff := 0.0278154593$$

case 19b: at time = 100 days, 35.53333 more area units were eluted.

```
> y:=2598.333333;
CumulativeConcentration[time=100]:=(y+328.71)/13571;
dev:=2598.333333+355.7691199;
UpperStd[time=100]:=(dev+328.71)/13571;
devDiff:=.2418990828-.2156836882;
```

Appendix B: (Continued)

$$y := 2598.333333$$

$$CumulativeConcentration_{time = 100} := 0.2156836882$$

$$dev := 2954.102453$$

$$UpperStd_{time = 100} := 0.2418990828$$

$$devDiff := 0.0262153946$$

case 20b: at time = 107 days, 30.86667 more area units were eluted.

```
> y:=2629.2;
CumulativeConcentration[time=107]:=(y+328.71)/13571;
dev:=2629.2+337.9609281;
UpperStd[time=107]:=(dev+328.71)/13571;
devDiff:=.2428613166-.2179581460;
```

$$y := 2629.2$$

$$CumulativeConcentration_{time = 107} := 0.2179581460$$

$$dev := 2967.160928$$

$$UpperStd_{time = 107} := 0.2428613166$$

$$devDiff := 0.0249031706$$

An average of 2629.2 area units were eluted which is equivalent to a total of .2179581460 mg/ml.

Appendix B: (Continued)

Program Diffusion Coefficient for Dexamethasone Calculations

This program uses the method described in "Dextran Retention in the Rat Brain Following Release from a Polymer Implant" by Wenbin Dang and W. Mark Saltzman.

Taking the slope of figure 10 provides this equation.

$$y = 0.0052829x + 0.026786$$

thus, the slope: 0.0052829 / sec^(1/2) = $\frac{M_t / M_\infty}{\sqrt{t}}$

```
> slope:= 0.0052829;
```

$$slope := 0.0052829$$

The hydrated radius (in m) of the fiber, R, is known :

```
> R:=0.00005832;
```

$$R := 0.00005832$$

Using equation (4) $\frac{M_t}{M_\infty} = \frac{4}{R}\left(\frac{Dt}{\pi}\right)^{0.5}$ the diffusion coefficient, D, of Dexamethasone can be solved.

```
> fsolve(slope = (4/R)*(D/Pi)^(1/2));
```

$$0.1863844603\ 10^{-13}$$

Thus, the diffusion coefficient for Dexamethasone in NDGA collagen fiber is 1.863844603 * 10^(-14) m^2/sec

Appendix B: (Continued)

Program Diffusion Coefficient for Dexamethasone 21-phosphate Calculations

This program uses the method described in "Dextran Retention in the Rat Brain Following Release from a Polymer Implant" by Wenbin Dang and W. Mark Saltzman.

Taking the slope of figure 10 provides this equation.

$$y = 0.018813x + 0.016663$$

thus, the slope: 0.0052829 / sec^(1/2) = $\frac{M_t/M_\infty}{\sqrt{t}}$

```
> slope:= 0.018813;
```

$$slope := 0.018813$$

The hydrated radius (in m) of the fiber, R, is known :

```
> R:=0.00005832;
```

$$R := 0.00005832$$

Using equation (4) $\frac{M_t}{M_\infty} = \frac{4}{R}\left(\frac{Dt}{\pi}\right)^{0.5}$ the diffusion coefficient, D, of Dexamethasone can be solved.

```
> fsolve(slope = (4/R)*(D/Pi)^(1/2));
```

$$0.2363638370\ 10^{-12}$$

Thus, the diffusion coefficient for Dexamethasone in NDGA collagen fiber is 2.36368370* 10^(-13) m^2/sec.

Appendix B: (Continued)

```
> restart;
> unprotect(D);
```

Program Diffusion through PLGA membrane Program

This program goes through the derivations necessary to obtain equation (12) and the diffusion coefficient at each time interval.

case: Hollow cylinder of Infinite Length under steady state conditions with constant concentration on each surface. Boundary Conditions:

@ r = Ri, C = Ci
@ r = Re, C = Ce

>

Variable declarations: **Ri** is the inner radius, the radius of the fiber.
Re is the outer radius, the radius of the PLGA coating.
r is the radius at any location in the composite, depends on time.
D is the diffusion coefficient
L is the length of the coated fiber
t is the time
Mt is the amount of diffusing substance
V is the volume of the fiber
Ci is the dex21 fibers concentration
Ce is the concentration in the PLGA coated fiber

Steady State equations:

```
> Eq1:=diff(r*diff(C(r),r),r);
```

$$Eq1 := \left(\frac{d}{dr}\mathrm{C}(r)\right) + r\left(\frac{d^2}{dr^2}\mathrm{C}(r)\right)$$

```
> Eq2:=dsolve(Eq1,C(r));
```

$$Eq2 := \mathrm{C}(r) = \_C1 + \_C2\ln(r)$$

```
> Eq3:=diff(Eq2,r);
```

$$Eq3 := \frac{d}{dr}\mathrm{C}(r) = \frac{\_C2}{r}$$

Solving for using the boundary conditions previously stated.

```
> bc1:=_C2*ln(R[i])+_C1-C[i];
```

$$bc1 := \_C2\ln(R_i) + \_C1 - C_i$$

```
> bc2:=_C2*ln(R[e])+_C1-C[e];
```

$$bc2 := \_C2\ln(R_e) + \_C1 - C_e$$

Appendix B: (Continued)

> **Eq4:=bc2-bc1;**

$$Eq4 := \_C2 \ln(R_e) - C_e - \_C2 \ln(R_i) + C_i$$

> **solve(Eq4,_C2);**

$$\frac{C_e - C_i}{\ln(R_e) - \ln(R_i)}$$

Rewriting this result still in terms of what _C2 equals.

> **_C2:=(C[e]-C[i])/ln(R[e]/R[i]);**

$$\_C2 := \frac{C_e - C_i}{\ln\left(\frac{R_e}{R_i}\right)}$$

Substituting _C2 back into bc2 to solve for _C1.

> **solve(bc2,_C1);**

$$\frac{-\ln(R_e)\, C_e + \ln(R_e)\, C_i + C_e \ln\left(\frac{R_e}{R_i}\right)}{\ln\left(\frac{R_e}{R_i}\right)}$$

Assigning these constants and substituting them into our intial Concentration equation.

> **assign(_C1,_C2);**
> **Eq2;**

$$\mathrm{C}(r) = \frac{C_e - C_i}{\ln\left(\frac{R_e}{R_i}\right)} + \frac{(C_e - C_i)\ln(r)}{\ln\left(\frac{R_e}{R_i}\right)}$$

Rewriting these results yields:

> **Eq2:=C(r)=C[e]+((C[e]-C[i])/ln(R[e]/R[i]))*ln(r/R[e]);**

Appendix B: (Continued)

$$Eq2 := C(r) = C_e + \frac{(C_e - C_i)\ln\left(\frac{r}{R_e}\right)}{\ln\left(\frac{R_e}{R_i}\right)}$$

>

The amount of a diffusing substance, **M[t]**, diffuses through the length of the tubing in time, **t** , can be calculated by integrating Fick's 1st Law w.r.t time.

```
> Eq4:=lhs(Eq3)=subs(r=R[e],rhs(Eq3));
```

$$Eq4 := \frac{d}{dr}C(r) = \frac{C_e - C_i}{\ln\left(\frac{R_e}{R_i}\right)R_e}$$

```
> Ficks:=-2*pi*R[e]*L*int((D*diff(C(r),r)),t);
```

$$Ficks := -2\,\pi\,R_e\,L\,D\left(\frac{d}{dr}C(r)\right)t$$

```
> M[t]:=subs(Eq4,Ficks);
```

$$M_t := -\frac{2\,\pi\,L\,D\,(C_e - C_i)\,t}{\ln\left(\frac{R_e}{R_i}\right)}$$

Since we have perfect sink conditions, **Ce** goes to 0.

```
> M[t]:=subs(C[e]=0,M[t]);
```

$$M_t := \frac{2\,\pi\,L\,D\,C_i\,t}{\ln\left(\frac{R_e}{R_i}\right)}$$

>

The purpose is to calculate **D**, the diffusion coefficient, so rearranging these equations:

```
> M[t]:=unapply(M[t]);
```

Appendix B: (Continued)

$$M_t := (\ ) \rightarrow \frac{2\,\pi\,L\,D\,C_i\,t}{\ln\left(\frac{R_e}{R_i}\right)}$$

```
> D:=(M[t]*ln(R[e]/R[i]))/(2*Pi*L*C[i]*t);
```

$$D := \frac{1}{2}\,\frac{M_t\,\ln\left(\frac{R_e}{R_i}\right)}{\pi\,L\,C_i\,t}$$

We shall now simplify this expression by making it a numerical expression and set it up for each time interval by taking out the constants not affected by each interval.

The time for each case will be represented here by the variable n; however, the specific cases do not follow a specific interval and therefore must be calculated individually. Hours will be what **n** represents.

```
> D[n]:=A*M[t](n)/(C[i](n)*(t(n)-t(n-1)));
```

$$D_n := \frac{A\,\pi\,M_t}{\pi\,C_i(n)\,(t(n) - t(n-1))}$$

Defining what variable **A** represents.

```
> A:=ln(R[e]/R[i])/(2*Pi*L);
```

$$A := \frac{1}{2}\,\frac{\ln\left(\frac{R_e}{R_i}\right)}{\pi\,L}$$

Declaring the constants: Inner Radius, **Ri**; PLGA coated Radius, **Re**; **L**, length of the coated fiber.

The inner radius, **Ri** is equal to the radius of the Dex21 collagen fiber before the coating. This value was obtained from the volume fraction program for dexamethasone (the units are in mm).

```
> R[i]:=0.05832;
```

$$R_i := 0.05832$$

Appendix B: (Continued)

The outer radius, **Re** is equal to the total radius with coating. Using the average of 30 fiber measurements we were able to obtain a total diameter average of 0.372333mm. Therefore the radius would be 0.1861655mm.

> **R[e]:=0.305667;**

$$R_e := 0.305667$$

The length of the coated fiber will be equal to the length of our fibers 10mm, plus the thickness of the coating on the top and bottom of the fibers. The thickness is equal to the radius of the PLGA. Solving for thickness (in mm), then length of the coated fiber, **L** yields:

> **PLGAthickness:= R[e]-R[i];**

$$PLGAthickness := 0.247347$$

> **L:= (PLGAthickness*2)+10;**

$$L := 10.494694$$

Now we have all the components needed to solve for **A** (units are 1/mm).

> **A:=ln(R[e]/R[i])/(2*evalf(Pi)*L);**

$$A := 0.02512205784$$

I shall now convert this to centimeters for easier calculations later.

> **A:=A/10;**

$$A := 0.002512205784$$

The dex21 fibers concentration, **Ci**, is equal to the total concentration, **Ct**, minus the dex21 eluted from the fibers.

Note: The total concentration was obtained from our previous experiments with just Dex21 loaded fibers. This value is assumed to be the same since the fibers were

loaded exactly the same; the only difference is after loading they were coated with PLGA. Here I will declare the total concentration

> **C[tot]:=0.2220691179;**

$$C_{tot} := 0.2220691179$$

Appendix B: (Continued)

To calculate **Mt**, the amount of diffusing substance at each time, we must multiply the concentration at that time (which has been previously calculated, refer to maple program, **Program Dexamethasone 21-phosphate Concentrations)** by the volume of our PBS solution, **V[PBS]** declared here. Units are milliliters.

```
> V[pbs]:=0.2;
```

$$V_{pbs} := 0.2$$

Diffusion calculations at each time interval.
Units: **Mt** is in mg
Ci is in mg/ml
t is in seconds
D is in cm^2/sec

```
> unprotect(Ci);
```

Case1: At time 1 day.

```
> Mt[1]:= V[pbs]*.5157394444e-1;
```

$$Mt_1 := 0.01031478889$$

```
> Ci[1]:= C[tot]-.5157394444e-1;
```

$$Ci_1 := 0.1704951735$$

```
> t[1]:=24*60*60;
```

$$t_1 := 86400$$

```
> D[1]:=(A*Mt[1])/(Ci[1]*t[1]);
```

$$D_1 := 0.1759096738\ 10^{-8}$$

Case2: At time 2 days.

```
> Mt[2]:= V[pbs]*.3426743298e-1;
```

$$Mt_2 := 0.006853486596$$

Appendix B: (Continued)

> **Ci[2]:= C[tot]-.6161987571e-1;**

$$Ci_2 := 0.1604492422$$

> **t[2]:=86400;**

$$t_2 := 86400$$

> **D[2]:=(A*Mt[2])/(Ci[2]*t[2]);**

$$D_2 := 0.1241982185\ 10^{-8}$$

Case3: At time 5 days.

> **Mt[3]:= V[pbs]*.4057500061e-1;**

$$Mt_3 := 0.008115000122$$

> **Ci[3]:= C[tot]-.7797337460e-1;**

$$Ci_3 := 0.1440957433$$

> **t[3]:=(86400*5-86400*2);**

$$t_3 := 259200$$

> **D[3]:=(A*Mt[3])/(Ci[3]*t[3]);**

$$D_3 := 0.5458302404\ 10^{-9}$$

Case4: At time 8 days.

> **Mt[4]:= V[pbs]*.3502886056e-1;**

$$Mt_4 := 0.007005772112$$

> **Ci[4]:= C[tot]-.8878073342e-1;**

$$Ci_4 := 0.1332883845$$

Appendix B: (Continued)

```
> t[4]:=(86400*8-86400*5);
```

$$t_4 := 259200$$

```
> D[4]:=(A*Mt[4])/(Ci[4]*t[4]);
```

$$D_4 := 0.5094292955\ 10^{-9}$$

Case5: At time 12 days.

```
> Mt[5]:= V[pbs]*.3713629553e-1;
```

$$Mt_5 := 0.007427259106$$

```
> Ci[5]:= C[tot]-.1016955272;
```

$$Ci_5 := 0.1203735907$$

```
> t[5]:=(86400*12-86400*8);
```

$$t_5 := 345600$$

```
> D[5]:=(A*Mt[5])/(Ci[5]*t[5]);
```

$$D_5 := 0.4485169259\ 10^{-9}$$

Case6: At time 17 days.

```
> Mt[6]:= V[pbs]*.3739174219e-1;
```

$$Mt_6 := 0.007478348438$$

```
> Ci[6]:= C[tot]-.1148657677;
```

$$Ci_6 := 0.1072033502$$

```
> t[6]:=(86400*17-86400*12);
```

$$t_6 := 432000$$

Appendix B: (Continued)

```
> D[6]:=(A*Mt[6])/(Ci[6]*t[6]);
```

$$D_6 := 0.4056661806\ 10^{-9}$$

Case7: At time 23 days.

```
> Mt[7]:= V[pbs]*.3890968486e-1;
```

$$Mt_7 := 0.007781936972$$

```
> Ci[7]:= C[tot]-.1295539508;
```

$$Ci_7 := 0.0925151671$$

```
> t[7]:=(86400*23-86400*17);
```

$$t_7 := 518400$$

```
> D[7]:=(A*Mt[7])/(Ci[7]*t[7]);
```

$$D_7 := 0.4076289213\ 10^{-9}$$

Case8: At time 30 days.

```
> Mt[8]:= V[pbs]*.3975462383e-1;
```

$$Mt_8 := 0.007950924766$$

```
> Ci[8]:= C[tot]-.1450870729;
```

$$Ci_8 := 0.0769820450$$

```
> t[8]:=(86400*30-86400*23);
```

$$t_8 := 604800$$

```
> D[8]:=(A*Mt[8])/(Ci[8]*t[8]);
```

$$D_8 := 0.4290141624\ 10^{-9}$$

Appendix B: (Continued)

Case9: At time 37 days.

```
> Mt[9]:= V[pbs]*.3463095326e-1;
```

$$Mt_9 := 0.006926190652$$

```
> Ci[9]:= C[tot]-.1554965244;
```

$$Ci_9 := 0.0665725935$$

```
> t[9]:=(86400*37-86400*30);
```

$$t_9 := 604800$$

```
> D[9]:=(A*Mt[9])/(Ci[9]*t[9]);
```

$$D_9 := 0.4321578381\ 10^{-9}$$

Case10: At time 44 days.

```
> Mt[10]:= V[pbs]*.3218947265e-1;
```

$$Mt_{10} := 0.006437894530$$

```
> Ci[10]:= C[tot]-.1634644954;
```

$$Ci_{10} := 0.0586046225$$

```
> t[10]:=(86400*44-86400*37);
```

$$t_{10} := 604800$$

```
> D[10]:=(A*Mt[10])/(Ci[10]*t[10]);
```

$$D_{10} := 0.4563051953\ 10^{-9}$$

Case11: At time 51 days.

Appendix B: (Continued)

> **Mt[11]:= V[pbs]*.3098592587e-1;**

$$Mt_{11} := 0.006197185174$$

> **Ci[11]:= C[tot]-.1702289195;**

$$Ci_{11} := 0.0518401984$$

> **t[11]:=(86400*51-86400*44);**

$$t_{11} := 604800$$

> **D[11]:=(A*Mt[11])/(Ci[11]*t[11]);**

$$D_{11} := 0.4965594449\ 10^{-9}$$

Case12: At time 58 days.

> **Mt[12]:= V[pbs]*.2989045268e-1;**

$$Mt_{12} := 0.005978090536$$

> **Ci[12]:= C[tot]-.1758978704;**

$$Ci_{12} := 0.0461712475$$

> **t[12]:=(86400*58-86400*51);**

$$t_{12} := 604800$$

> **D[12]:=(A*Mt[12])/(Ci[12]*t[12]);**

$$D_{12} := 0.5378167298\ 10^{-9}$$

Case13: At time 65 days.

> **Mt[13]:= V[pbs]*.3376145212e-1;**

$$Mt_{13} := 0.006752290424$$

Appendix B: (Continued)

> **Ci[13]:= C[tot]-.1854378208;**

$$Ci_{13} := 0.0366312971$$

> **t[13]:=(86400*65-86400*58);**

$$t_{13} := 604800$$

> **D[13]:=(A*Mt[13])/(Ci[13]*t[13]);**

$$D_{13} := 0.7656710823\ 10^{-9}$$

Case14: At time 72 days.

> **Mt[14]:= V[pbs]*.3407093557e-1;**

$$Mt_{14} := 0.006814187114$$

> **Ci[14]:= C[tot]-.1952872546;**

$$Ci_{14} := 0.0267818633$$

> **t[14]:=(86400*72-86400*65);**

$$t_{14} := 604800$$

> **D[14]:=(A*Mt[14])/(Ci[14]*t[14]);**

$$D_{14} := 0.1056858135\ 10^{-8}$$

Case15: At time 79 days.

> **Mt[15]:= V[pbs]*.3214034829e-1;**

$$Mt_{15} := 0.006428069658$$

> **Ci[15]:= C[tot]-.2032061012;**

$$Ci_{15} := 0.0188630167$$

Appendix B: (Continued)

> t[15]:=(86400*79-86400*72);

$$t_{15} := 604800$$

> D[15]:=(A*Mt[15])/(Ci[15]*t[15]);

$$D_{15} := 0.1415509713\ 10^{-8}$$

Case16: At time 86 days.

> Mt[16]:= V[pbs]*.2987571537e-1;

$$Mt_{16} := 0.005975143074$$

> Ci[16]:= C[tot]-.2088603148;

$$Ci_{16} := 0.0132088031$$

> t[16]:=(86400*86-86400*79);

$$t_{16} := 604800$$

> D[16]:=(A*Mt[16])/(Ci[16]*t[16]);

$$D_{16} := 0.1879006452\ 10^{-8}$$

Case17: At time 93 days.

> Mt[17]:= V[pbs]*0.2842654680e-1;

$$Mt_{17} := 0.005685309360$$

> Ci[17]:= C[tot]-.2130653599;

$$Ci_{17} := 0.0090037580$$

> t[17]:=(86400*93-86400*86);

$$t_{17} := 604800$$

Appendix B: (Continued)

```
> D[17]:=(A*Mt[17])/(Ci[17]*t[17]);
```

$$D_{17} := 0.2622851584\ 10^{-8}$$

Case18: At time 100 days.

```
> Mt[18]:= V[pbs]*.2683983003e-1;
```

$$Mt_{18} := 0.005367966006$$

```
> Ci[18]:= C[tot]-.2156836882;
```

$$Ci_{18} := 0.0063854297$$

```
> t[18]:=(86400*100-86400*93);
```

$$t_{18} := 604800$$

```
> D[18]:=(A*Mt[18])/(Ci[18]*t[18]);
```

$$D_{18} := 0.3491910129\ 10^{-8}$$

Case19: At time 107 days.

```
> Mt[19]:= V[pbs]*.2649595952e-1;
```

$$Mt_{19} := 0.005299191904$$

```
> Ci[19]:= C[tot]-.2179581460;
```

$$Ci_{19} := 0.0041109719$$

```
> t[19]:=(86400*107-86400*100);
```

$$t_{19} := 604800$$

```
> D[19]:=(A*Mt[19])/(Ci[19]*t[19]);
```

$$D_{19} := 0.5354372330\ 10^{-8}$$

Appendix B: (Continued)

Displaying all the diffusion coefficients up to day 107 (Units m^2/s).

```
> for i from 1 by 1 to 19 do
  D[i]*10^(-4);
  end do;
```

$$0.1759096738\ 10^{-12}$$

$$0.1241982185\ 10^{-12}$$

$$0.5458302404\ 10^{-13}$$

$$0.5094292955\ 10^{-13}$$

$$0.4485169259\ 10^{-13}$$

$$0.4056661806\ 10^{-13}$$

$$0.4076289213\ 10^{-13}$$

$$0.4290141624\ 10^{-13}$$

$$0.4321578381\ 10^{-13}$$

$$0.4563051953\ 10^{-13}$$

$$0.4965594449\ 10^{-13}$$

$$0.5378167298\ 10^{-13}$$

$$0.7656710823\ 10^{-13}$$

$$0.1056858135\ 10^{-12}$$

$$0.1415509713\ 10^{-12}$$

$$0.1879006452\ 10^{-12}$$

$$0.2622851584\ 10^{-12}$$

$$0.3491910129\ 10^{-12}$$

$$0.5354372330\ 10^{-12}$$

>

Using Matlab to plot the diffusion coefficients versus time (figure 20), we were able to calculate the steady-state diffusion rate. After 5 days the diffusion rate stabilizes till day 58, from here a linearization was taken. This provided us with an equation of:

$$y = 3.17e\text{-}22*x+4.59e\text{-}14$$

Appendix B: (Continued)

where y is the diffusion coefficient and x is the time in seconds. The slope of the line can be cancelled out since it is so minute. This provides us with a steady-state diffusion coefficient of 4.59e-14 m^2/sec for diffusion of dexamethasone 21-phosphate through the PLGA coating.

Note: The experimental data we used to calculate these results was only tested for 107 days. According to the data and trend the coated fibers should last slightly longer than this. At 107 days, 98% of the dexamethasone 21-phosphate was eluted. Theoretically, as the experiment continues to progress, the PLGA coating should begin to degrade more rapidly allowing the remaining dexamethasone 21-phosphate to diffuse quicker. From the diffusion results it appears this occurs. At about 65 days the PLGA coating may start to degrade increasing the rate of diffusion. It seems shortly after 107 days all the dexamethasone 21-phosphate loaded should have left the fiber.