Controlled Release of Exendin-4 from PLGA Microspheres with Convective Blood Flow

BEE 4530 Computer-Aided Engineering: Applications to Biomedical Processes

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1 Executive Summary

Type 2 diabetes is a metabolic disease characterized by high blood glucose due to insulin resistance and relative insulin deficiency. Primarily affecting those suffering from obesity, it comprises approximately 90% of all cases of diabetes. Currently, insulin and metformin injections are the most common methods of lowering blood glucose levels in type 2 diabetics. However, there are several disadvantages to these treatments, including the need for several injections a day and the risks associated with improper dosage or deviation from injection schedule. One proposed alternative treatment is to deliver microspheres embedded with exendin-4, an insulin secretagogue with glucoregulatory effects on the body, via a single subcutaneous injection. Bioerodible microspheres allow for a slow, sustained release of drug that will decrease the required frequency of administration and subsequently improve patient compliance.

This paper documents the release of exendin-4 from poly(lactic-*co*-glycolic acid) (PLGA) microspheres into the bloodstream and the phenomena that influence its transport, namely the diffusion through the polymer matrix and bloodstream and the convective mass transfer effected by the flow of blood in the vessel. Because the motivation behind using microspheres as the preferred method of delivery is to eliminate the need for repeated administration, it is necessary to achieve a controlled, prolonged delivery of the desired dosage of exendin-4.

The goal of this study is to find the optimal formulation properties for a steady release of exendin-4 into the bloodstream for an extended period of treatment. To this end, we developed a 2D-axisymmetric geometry in COMSOL to model a single microsphere in the human artery. A time varying boundary condition was implemented to simulate the changing radius of the microsphere, which steadily decreases due to surface degradation. A variety of parameters (e.g. PLGA composition, initial drug concentration, microsphere radius) were simulated using a series of parametric sweeps, and the effects of parametric changes were observed using sensitivity analysis.

We found that the determination of the optimal diffusivity of exendin-4 in the PLGA microsphere depends on the desired balance between steadiness of release rate and total amount released after three weeks. Higher ratios of glycolic acid resulted in undesired bursts of drug release, whereas higher ratios of lactic acid did not result in appreciable rates of diffusion through the polymer matrix and thus did not achieve complete release by the end of the administration period. For any given composition of PLGA, we determined that an initial concentration of 1.505 mol/m^3 (247 mg/mL) provided flux values within the reasonable range for effective delivery of exendin-4 over the desired period of administration.

Our model does not provide conclusive evidence that the delivery of exendin-4 embedded in PLGA microspheres will achieve adequate therapeutic results. However, computational analysis of the concentration profiles attainable in the bloodstream provides a rough estimate of the formulation conditions required for controlled drug release; subsequent experiments will be conducted to evaluate its viability as a safer, less invasive alternative to periodic direct insulin injections for the treatment of type 2 diabetes.

2 Introduction

2.1 *Background*

Type 2 diabetes, also known as adult-onset diabetes, is a chronic disease characterized by high levels of sugar in the blood due to insulin resistance and relative insulin deficiency [1]. Insulin is a peptide hormone that helps regulate blood sugar levels by storing glucose into cells. However, due to insulin resistance or relative insulin deficiency in people with type 2 diabetes, blood sugar does not move into cells and high levels of sugar build up in the blood. These individuals also lack glucose-sensitive regulation of glucagon and maintain constant glucagon levels even after food consumption. To mitigate insulin resistance, patients can undergo exercise and improve their eating habits, but some patients require further treatment. Doctors recommend medications to lower high blood glucose level by administering metformin and insulin injections to suppress glucose production in the liver [1]. However, due to risks associated with wrong dosages or improper administration of the drug, we will explore the viability of an alternate method of drug delivery.

Long-term controlled drug delivery would be optimal in administering the drug for patients. Without the need for frequent injections certain dosages of the drugs, microspheres of biodegradable polymers that provide a sustained-release delivery of the peptide improves patient compliance. Therefore, we are analyzing the sustained release delivery system of exendin-4 encapsulated in PLGA microsphere. Exendin-4 is a glucose-dependent insulinotropic hormone that possesses glucoregulatory effects [2]. This drug helps with glycemic control by glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying, and reduction of food intake [3]. Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetics [4]. Its dual capability of inducing cells on the liver to produce insulin and suppressing glucagon production from α-cell contribute to the reduction in blood glucose levels in patients with type 2 diabetes [3]. Drug burst release in PLGA microspheres may induce hypoglycemia in diabetic patients [3]. However, since exendin-4 is glucose-dependent, it won't induce hypoglycemia. Therefore, exendin-4 is the drug we want to be administered by the PLGA microspheres for the treatment of type 2 diabetes.

Hence, we are modeling drug release from the PLGA microspheres in the blood stream to determine the optimal properties of the microsphere, such as PLGA monomer composition, sphere size, and diffusivity, for the long-term steady drug release of exendin-4. We want to develop a well sustained delivery system for the treatment of type 2 diabetes with exendin-4 by poly(lactic-*co*-glycolic acid) microspheres for user comfort and safety.

2.2 *Design Objectives*

In this project, we model the release of the drug exendin-4 from a biodegradable PLGA microsphere and its concentration profile as it diffuses through the bloodstream. Our design objectives are twofold. The first objective is to simulate a controlled release of drug from the microsphere by means of constant flux at the microsphere-bloodstream interface. In order to do this, several parameters are considered, including the monomer composition of the poly(lactic-*co*-glycolic acid) polymer matrix, the size of the microspheres to be administered, and the initial loading of drug in the sphere. In addition, the degradation of PLGA in the bloodstream, which affects the size of the sphere and concentration of encapsulated drug over time, is modeled and coupled with the drug release rate. The second objective is to ensure that enough exendin-4 is released into the bloodstream to meet an appropriate therapeutic quota, corresponding to standard exendin-4 dosages recommended by physicians. Because the drug will be transported through the bloodstream, the diffusive and convective properties and velocity of the blood flow must be taken into account.

By developing a physically accurate model of the degradation kinetics of PLGA and diffusion of exendin-4 in the blood, we hope to investigate what will be the optimal properties of the microsphere to aid in the development of the pharmaceutical formulation. Completion of these objectives will demonstrate that the delivery of exendin-4 from PLGA microspheres is an acceptable method of administration and a viable alternative to the standard insulin and metformin injection formulations.

3 Problem Formulation

3.1 *Schematic and Model Design*

The computational domain of our COMSOL model consists of two regions: the microsphere and the blood. Although the original problem involves diffusion of exendin-4 and blood flow around the microsphere in three dimensions, the geometry can be simplified for ease and efficiency of computation. For the purposes of this model, diffusion of drug from the sphere and through the bloodstream can be assumed to be spherically symmetrical about the origin. The bloodstream that carries the drug via convective mass transfer flows through the blood vessel in a specified direction, but the flow is axisymmetric in the θ-direction. Therefore, the geometry can be modeled as a semicircle in 2D axisymmetric cylindrical coordinates, with an axis of symmetry that is parallel to the direction of bulk blood flow. We are interested mainly in observing the concentration profile of exendin-4 in regions of the bloodstream relatively close to the microsphere and far away from the vessel walls, so the blood vessel is modeled as a rectangular semi-infinite domain, as shown in Figure 1.

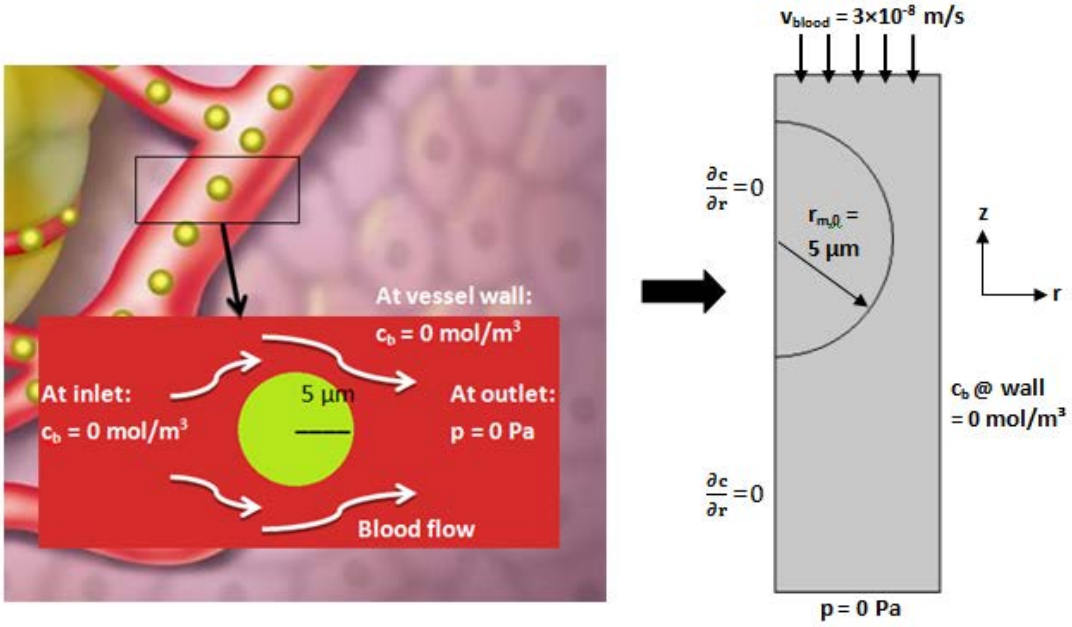

Figure 1. Schematic of the 2D axisymmetric exendin-4 loaded PLGA microsphere in the blood. The microsphere is modeled as a semicircle of radius 5 µm. Blood flowing relative to the sphere imposes a convective boundary condition, and the blood vessel is modeled as a semi-infinite domain due to the large distance of the vessel wall from the sphere. The edges of the blood vessel computational domain are relatively close to the sphere in the region near the blood flow inlet; the geometry was designed as such to reduce inessential computation, as the drug concentration does not diffuse radially very far in this region.

To implement the convective effects the blood flow around the microsphere has on the drug release, we modeled the flow of fluid using the Navier-Stokes governing equations. Because the velocity of the blood is not constant as it flows around the sphere, it is not sufficient to simply model the blood flow as a convective mass transfer boundary condition. Rather, the equations for fluid flow must be coupled with the diffusion equations and solved for the r- and z-components of the blood velocity at every point.

Controlled release of exendin-4 occurs via several different mechanisms. In our system of interest, the primary mechanisms of drug release are diffusion of exendin-4 through the polymeric network, and release of exendin-4 into the blood by erosion of the encapsulating microsphere. The former is described by Fick's law of diffusion and is primarily a function of the polymer's diffusivity, which in turn is determined by the ratio of lactide to glycolide monomers in the PLGA. The latter is due to hydrolytic degradation of ester linkages in the PLGA polymer in the presence of aqueous solution [3].

Erosion, or hydrolytic volume loss of the PLGA matrix, can be further broken down into two types: surface erosion and bulk erosion. In surface erosion, the degradation of the PLGA occurs solely at the surface, causing the microsphere to slowly decrease in size while the polymer density remains relatively constant. Bulk erosion occurs when water diffuses into the polymer matrix and initiates homogenous degradation throughout the sphere from the inside; in this case the sphere slowly becomes less and less dense while its volume stays the same [3]. Due to various complications with implementation of bulk erosion in COMSOL, we assume as a physical approximation that PLGA erosion occurs solely at the surface.

We designed the semicircle in COMSOL as a flexible domain to simulate the shrinking of the sphere. The domain boundary, which represents the surface of the sphere, was made to move uniformly over time according to a specified velocity function. Because there are several mathematical models available in the literature for evaluating the kinetics of drug release from biodegradable polymeric microspheres, we initially thought of modeling the degradation mechanisms as closely and accurately as possible. However, in the end it proved difficult to simulate the net rate of degradation as a single function that could be written in COMSOL. We instead chose to prescribe a mesh velocity that is a function of the drug release (refer to Appendix A.2).

4 Results and Discussion

4.1 *Solution*

Using a time step of $\Delta t = 86400$ s, or 1 day, we modeled the release of exendin-4 from the PLGA microsphere into the bloodstream over the total administration period of 21 days, or three weeks. The following section describes the details behind the development of our model in COMSOL.

The rate of diffusion of drug from the microsphere is coupled with the rate of sphere surface degradation. To model this degradation in COMSOL, we implemented a moving boundary that causes the radius of the semicircle domain to shrink at a specified velocity, as discussed in Section 3.1. The changing volume of the sphere was plotted as a function of time and is shown in Figure 2.

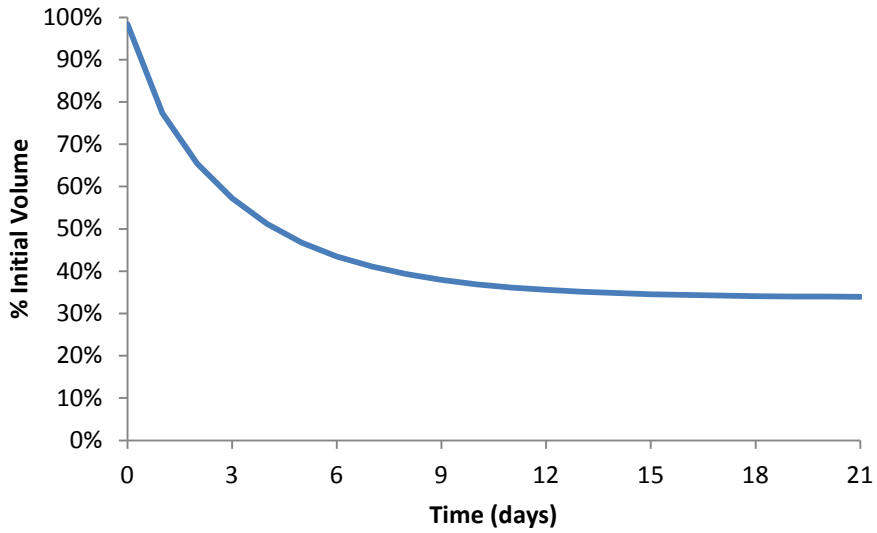

Figure 2. Volume of the microsphere expressed as a percentage of the initial volume versus time. Values were found in COMSOL at each time by computing the volumetric integral of the domain and dividing by the initial volume.

Upon implementation of the moving boundary, we calculated an initial solution using a diffusivity value of D_S = 3.84×10^{-18} m²/s. The resulting concentration surface plot is shown in Figure 3.

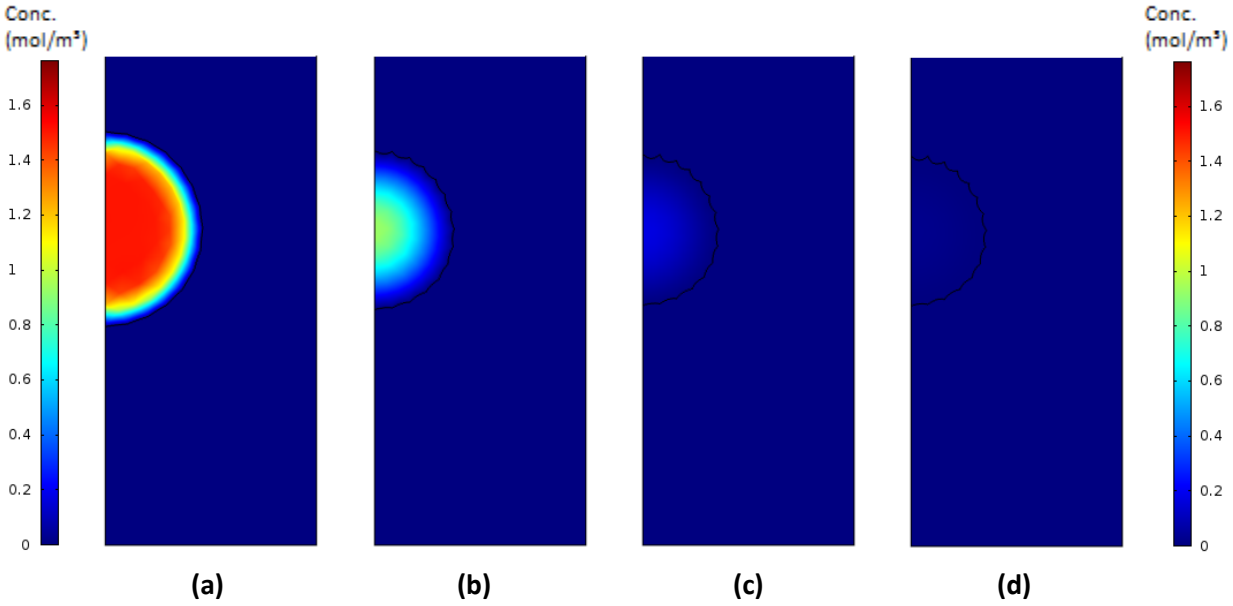

Figure 3a-d. Concentration profiles (in mol/m³) for exendin-4 loaded PLGA microspheres with a diffusivity value of 3.84×10^{-18} m²/s and an initial radius of 5 µm. a) At t = 1 day, the radius of the sphere has shrunk to about 4.5 µm. There is a concentration gradient localized at about 1µm from the surface, while the rest of the sphere maintains the initial concentration of approximately 1.5 mol/m³. b) After t = 7 days, the radius of the microsphere has shrunk to less than 4 µm. The concentration in the sphere has visibly decreased, with a gradient covering a larger distance. c) At t = 14 days, the radius of the microsphere has not changed significantly, and the concentration levels within the sphere have reached close to 0. d) At t=21 days, the radius of the sphere remains unchanged, and almost all of the drug has diffused into the blood.

We had initially thought that the diffusion of exendin-4 from the microsphere would result in a visible layer of drug surrounding the sphere due to spherically symmetric diffusion, and a visible trail of drug carried by convection at the front of the sphere's movement. However, no visible concentration is apparent in the blood right outside the sphere, and the effects of convection on the path of drug release cannot be seen.

Due to the inherent limitations of a color surface plot for a constant range of concentrations, there is no way to tell that drug is diffusing out from these profiles alone. To solve this problem, we decided to monitor drug concentration at a point in the bloodstream near

the microsphere surface, where drug should have just diffused from the sphere. Because the sphere radius changes over time, the measure point must change accordingly to stay close to the surface and get a consistent picture of the drug diffusion. We chose to track a point that stays at a distance of 2% of the current sphere radius from the sphere surface; in other words:

$$\frac{\text{Distance from measure point to origin}}{\text{Radius of sphere}} = 1.02$$

A plot of the changing sphere radius over time, and the corresponding points used to monitor the diffused drug concentration, is shown in Figure 4.

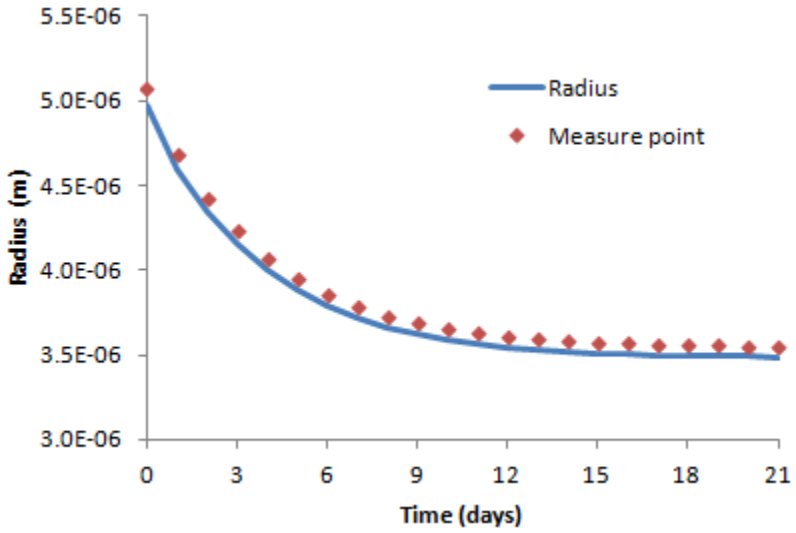

Figure 4. Solid line: radius of the microsphere; diamonds: points at which concentration of exendin-4 is measured. Measure points were determined by multiplying the current radius by a proportion factor of 1.02.

Some complications arose due to the fact that we were unable to specify the location of the 2D cut point as a direct function of the time-varying sphere radius in COMSOL. To circumvent this issue, we manually found the specific measure points of interest at each time step and plotted the corresponding concentration profiles at each of these points (Figure 5). Using this data, we were able to generate a single plot of drug concentration vs. time for a measure point that changes appropriately with the sphere radius at a given time (Figure 6).

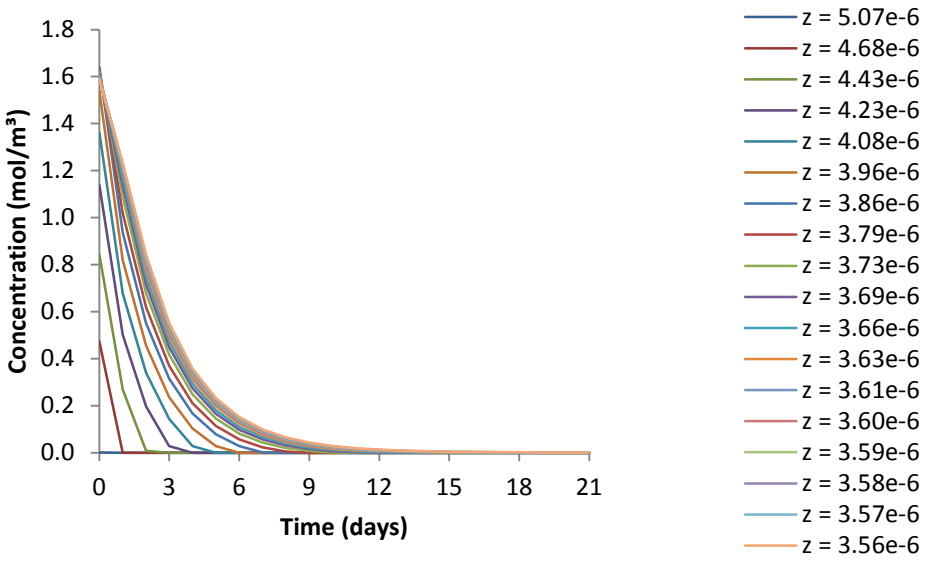

Figure 5. Concentration profiles of exendin-4 at selected distances from the origin. Each data point corresponds to a certain time point based on Figure 4. Concentration values of interest were solely the values at the time points of interest.

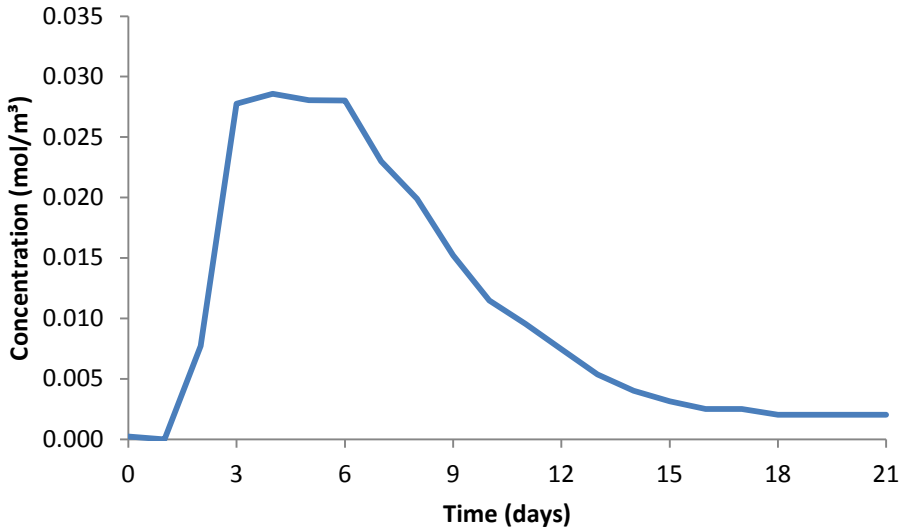

Figure 6. Concentration profile of exendin-4 measured at a point immediately outside the microsphere surface. Points of measurement were chosen such that at any time t, the ratio (Distance from measure point to origin)/(Sphere radius) = 1.02, as shown in Figure 4.

We can see from Fig. 5 that there is a buildup of drug concentration, confirming the release of exendin-4 into the bloodstream around the sphere. As expected, the concentration outside the sphere is zero at t = 0 before the drug has started diffusing, the concentration reaches its peak at intermediate times, and it starts to fall as the concentration of drug remaining in the sphere is depleted.

While the concentration profile outside the microsphere confirms that exendin-4 is, in fact, diffusing out into the bloodstream, it does not provide any information about the direction of the flow. More specifically, the effect of convection on the path of drug release is not clear, and we cannot yet disprove the possibility that the mechanism of drug release might simply be limited to spherically symmetric diffusion.

The reason the flow patterns of exendin-4 in the blood are not readily visible is that the difference between the diffusivity of exendin-4 in the bloodstream (on the order of 10^{-10} m^2/s) and its diffusivity in the PLGA microsphere (on the order of 10^{-18} m^2/s) is enormous, causing exendin-4 to diffuse through the blood much more quickly than it does in the sphere. Also, the relative velocity of the blood flow ensures that any drug that has diffused into the blood is immediately swept away.

To get around this issue, we ran a modified simulation for a case in which the diffusivities of exendin-4 in the microsphere and in the blood are equal. The diffusivity of exendin-4 in the sphere was changed to match that of the blood and the time step was decreased so that the results of the more rapid mass transfer could be observed. The results are shown in Figure 7.

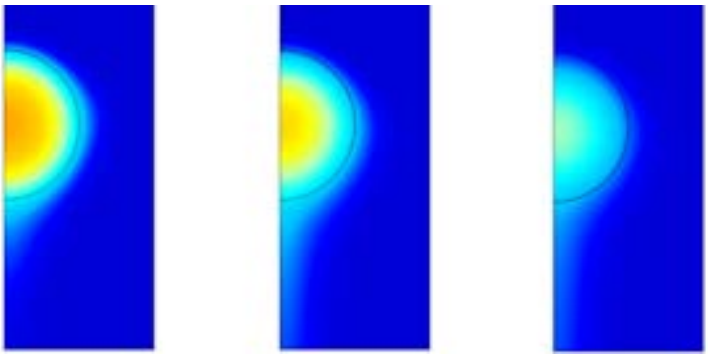

Figure 7. Concentration profiles of exendin-4 at times (a) 500, (b) 1000, and (c) 2000 seconds, for a modified simulation with equal drug diffusivity of 2×10^{-10} m²/s in the sphere and blood. Drug concentration in the blood exhibits a clear taper effect, confirming that blood is flowing over the sphere and that the net transport of exendin-4 is in the direction of the blood flow. Decreasing concentration behavior similar to that of the actual case can be observed within the sphere.

The results of the modified simulation look similar to what we envisioned in our initial expectations. Because changes in magnitude of diffusivity only affect the rate of transport of exendin-4 and not the nature of the physics involved, this simulation proves that the convection due to blood flow facilitates the transport of exendin-4 from the microsphere through the bloodstream. In this way, the simulation can be considered as a form of sensitivity analysis because it shows that the model behaves in the way that it is expected to behave, helping us build confidence in our results.

There were, however, some issues that we could not avoid, many of which are related to the moving boundary of the sphere. The largest problem we encountered was that the COMSOL model could not support the decreasing of the sphere domain size past a certain point. This is presumably due to complications in the mesh distribution when the domain gets too small; when we tried running the simulation for longer periods of time or faster sphere degradation, the results would not converge for sphere volumes that were less than ~35% of its initial value. This prevents us from analyzing drug release behavior for times at which the sphere is close to fully degraded. However, because the PLGA microsphere is likely to break apart into its monomeric or oligomeric units when it becomes sufficiently small [3], we may not be interested in diffusion rates for which the drug is not fully released before the microsphere reaches this size limit.

4.2 *Sensitivity Analysis*

To investigate the effects of various parameters on the outputs of the model, sensitivity analysis was performed on three parameters: diffusivity, blood flow velocity, and initial concentration. For each parameter, three different values were inputted into the model, using the original values as the base cases, and concentration values at the center of the sphere after half of the administration period had passed (1.5 weeks, or 10.5 days) were recorded. The results are shown in Figure 8.

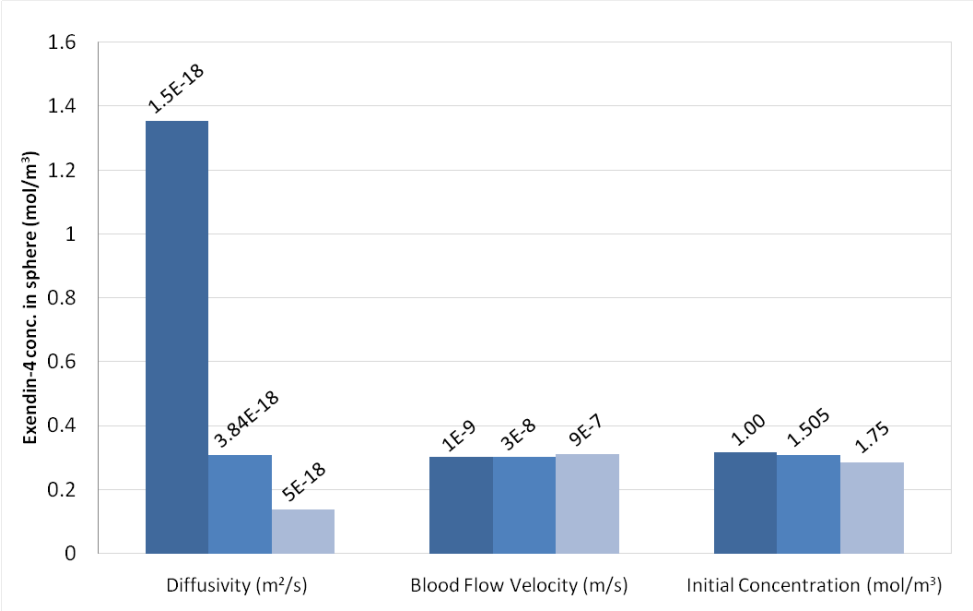

Figure 8. Sensitivity analysis performed on diffusivity, blood flow velocity, and initial concentration, with the concentration of exendin-4 at the center of the microsphere at t = 10.5 days as the monitored parameter. Diffusivity values ranging within the same order of magnitude have result different concentration values, and initial concentration values exhibit moderate effects on later concentration values. Velocity of convective blood flow demonstrates almost no effect on sphere concentration, with velocities spanning 2 orders of magnitude resulting in less than 5% change in concentration.

The model is least sensitive to blood flow velocity. According to literature, blood flow velocity values range anywhere from 10 – 45 cm/sec depending on the type of blood vessel [13]. Theoretically, the amount of change in the microsphere velocity should equal the change in the blood flow velocity, maintaining a constant relative blood velocity. However, slight variations may occur due to differences in drag forces with varying flow velocities, and thus the effects of change in relative velocity were investigated. When performing sensitivity analysis for this parameter using the range of blood velocity found in literature, the results demonstrated minimal

variation. Since all values in this range are within the same order of magnitude, values that spanned multiple orders of magnitude were tested, and these results also demonstrated minimal variation. Thus our model is not highly sensitive to the value of relative blood velocity.

The model is somewhat more sensitive to initial concentration. Since the model assumes an initial concentration value of 1.505 mol/m^3, the simulation was run at lower and higher values of 1.00 mol/m^3 and 1.75 mol/m^3, respectively. However, the results were not as we initially expected, as concentration at the center of the sphere decreased with increasing initial concentration. This may be attributed to the initial burst of drug: a larger initial drug concentration forms a larger initial gradient from the sphere interior to the blood and results in a more rapid initial burst of release, potentially leading to smaller sphere concentrations at later times due to the increased outward flux. Additionally, since these values were recorded at the center of the sphere, they do not necessarily reflect the average concentration within the entire sphere, which if measured exactly may agree more with our initial expectations.

The model is most sensitive to the diffusivity of exendin-4 in PLGA. According to Figure 8, diffusivity has a significant effect on concentration, exhibiting a 90% reduction in concentration with a more than threefold increase in diffusivity. Although diffusivity values can span several orders of magnitude depending on the ratio of PLGA [14], our sensitivity analysis only used values that were within the same order of magnitude. Nonetheless, the results showed extreme effects on concentration, and thus further investigation of the model sensitivity to diffusivity was performed.

Upon further analysis of the effects of varying diffusivity, we found that the cumulative release profile more closely approaches a straight line for smaller diffusivities (Figure 9), indicating a more constant release rate. However, smaller diffusivity also results in a smaller total amount of drug released at the end of the administration period of 3 weeks.

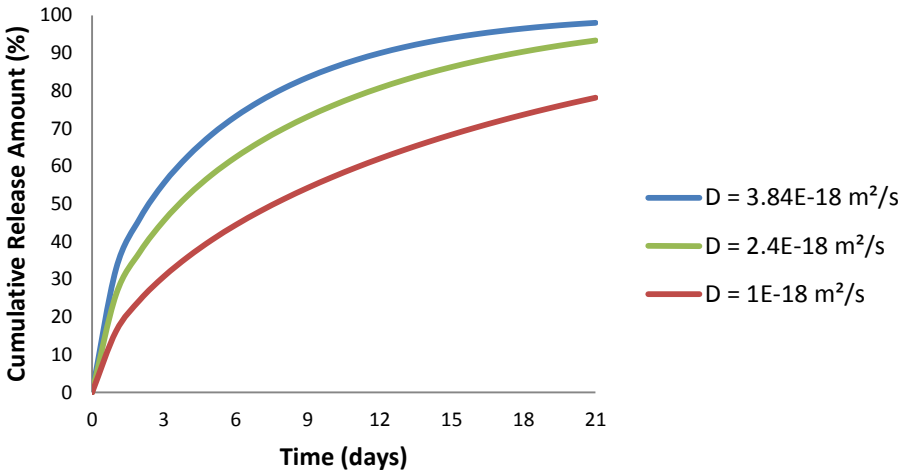

Figure 9. Cumulative percentage of drug release from the microsphere versus incubation time for various diffusivity values. Release rates become more constant as diffusivity decreases; however, total amount of administered drug decreases by the end of 3 weeks.

4.3 *Accuracy Check*

An accuracy check was conducted to compare drug release and/or concentration profiles with results from other papers that have used exendin-4 encapsulated in PLGA microspheres. In Figure 10, obtained from a patented method by Kwak et al. (2012) for preparing a biodegradable polymer microsphere containing a glucose-regulating peptide, the cumulative percent release of exendin-4 is plotted with respect to time [10]. The release profile seems to follow zero-order kinetics with no apparent initial burst of drug, and approximately 100 percent release is achieved within three weeks.

Although the diffusivity value of drug within the polymer is unknown, we can approximate it using the same method we used to calculate our diffusivity value in the sphere. Using equation 1, the diffusivity was calculated to be 9.116×10^{-19} m^2/s. To see if our model could accurately reproduce the experimental results, we implemented this diffusivity value and plotted percent release against time, as shown in Figure 11. The resulting graph exhibits a linear relationship similar to that of the experimental results; however, 100% release is not achieved by the end of the three-week period, and the percent release value of approximately 7% at the initial time suggests an initial burst.

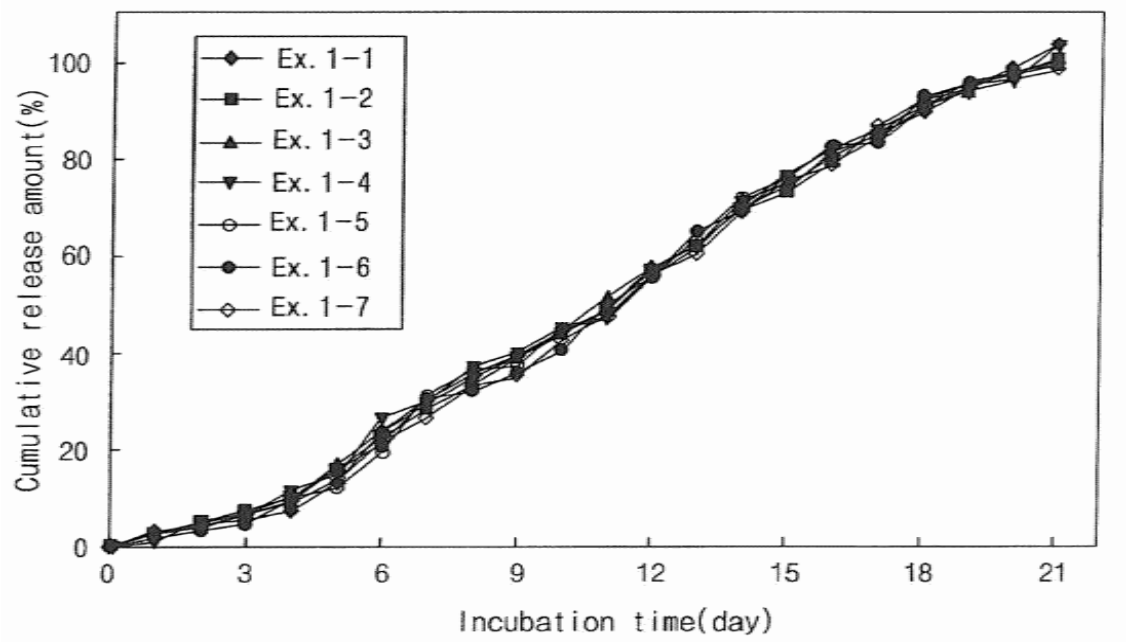

Figure 10. Experimental data for the controlled release rate of exendin-4 from biodegradable microspheres obtained from a patented method by Kwak et al. (2012) 100% drug release is achieved after 3 weeks with approximately zero-order release kinetics and no initial burst of drug.

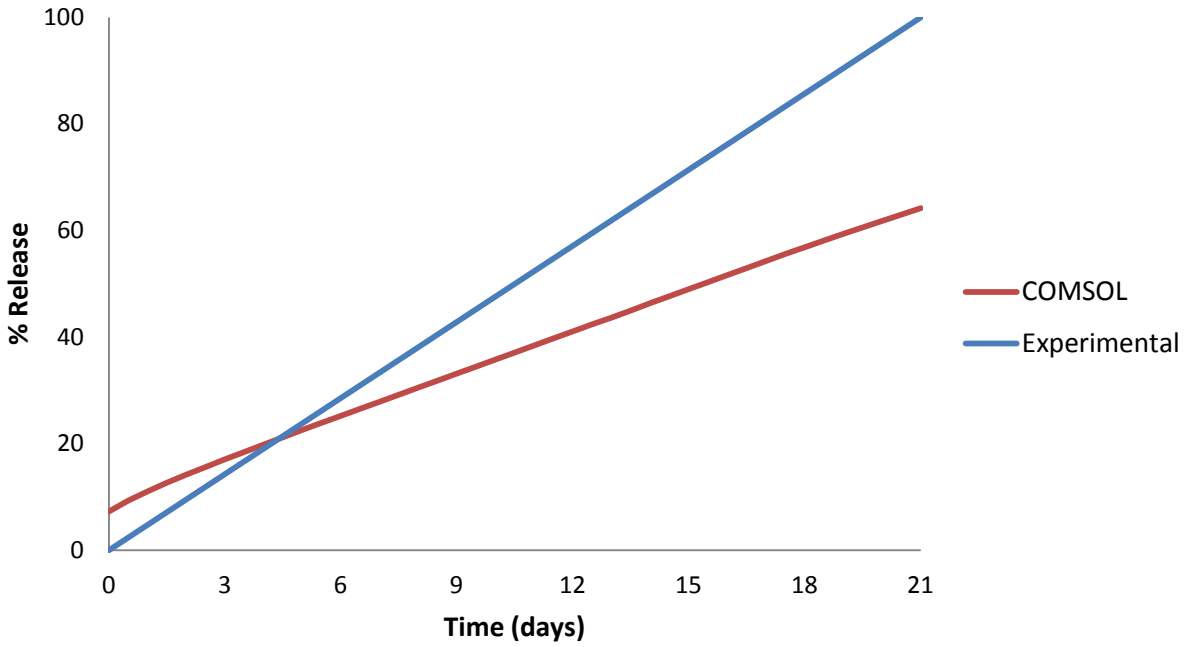

Figure 11. Validation of the drug release rate physics implementation in COMSOL. Both experimental data and analytical solution exhibit linear relationship. Discrepancies in total percent release can be attributed to limitations of the model, i.e. no considerations of bulk erosion or contact between the microsphere and vessel wall.

The differences between the computed and experimental results in Figure 11 are mainly due to modeling limitations. COMSOL cannot model complete degradation of the sphere due to moving boundary limitations, and the model does not account for bulk erosion or contact between the sphere and vessel wall. The model cannot simulate the complete degradation of the sphere, as the moving boundary becomes irregular when the radius approaches small values. Since a large amount of drug is released when the sphere is very small, this limitation greatly impacts the resulting release profile. The model also does not account for several factors, such as contact between the sphere and vessel wall due to collision, and alternative methods of degradation, namely bulk erosion. The exclusion of these factors would further contribute to the error between the model and experimental data. Although the profile produced by the model and the experimental data do not completely coincide, the fact that the computation was able to achieve a linear release profile similar to the experimental results is extremely promising. Therefore, it was determined that the two solutions are sufficiently similar to validate the COMSOL model of the drug release rate physics.

5 Conclusion

5.1 *Design Recommendations/Realistic Constraints*

Our study showed that diffusivity has the most significant influence on the delivery of exendin-4 from the microsphere, and the results showed that there is a tradeoff between the steadiness of release rate and the total amount released at the end of the administration period. Decreasing the diffusivity will cause the release kinetics to approach zeroth order; however, 100 percent release will not be achieved by the end of three weeks. Thus, although varying the PLGA ratio to achieve the desired diffusivity can enhance delivery, the constant release rate requirement will have to be compromised.

The model results serve to demonstrate a range of potentially suitable diffusivities of exendin-4 in PLGA for drug-loaded microsphere fabrication. However, it is ultimately up to the scientists in the next stage of development to determine the optimal combination of release rate and total released amount that will fulfill their requirements for therapeutic viability. The concentration profiles obtained from this model should be used as reference in this decision process.

There are significant advantages to having a constant release rate and total release by the end of the administration period. The advantage of achieving a constant release profile from the PLGA microsphere is the assurance that drug levels in the blood can always be determined, and thus overdose at any point can be avoided. If cumulative drug release is linear, the amount released can be determined based on the time elapsed, and determination of the next administration of microspheres can be planned accordingly. However, having irregular or non-zero order release kinetics makes it much more difficult to determine how much drug has diffused into the blood, and it would be uncertain if the levels in the blood would have therapeutic effects at any time. In a situation as sensitive as increase in blood glucose levels after a meal, it is extremely necessary to ensure that effective amounts of drug are available in the blood to remedy the situation, while maintaining a level that is below excessive amounts.

The advantage of having total release by the end of the administration period is being certain of the initial amount of drug required to be loaded in each microsphere. Knowledge of how much drug is required to achieve total release can serve to minimize costs associated with drug and drug loading. Additionally, total release ensures that there is no residual amount left at

the end of the administration period, and thus there is no buildup of drug with each subsequent dose of microspheres. This would avoid unwanted effects due to overdose and eliminate any complications associated with unknown variables.

A possibility for reducing the need for a tradeoff between these two conditions is to couple an increased initial concentration with a lower diffusivity. In this way, more drug will be available initially and can theoretically achieve therapeutic levels for the duration of the administration period, while still maintaining a constant release profile. However, it is important to note that the use of more drug would increase complications in engineering the pharmaceutical formulation and would result in a more expensive treatment. Moreover, an initial concentration that is too high can result in dangerously rapid bursts of drug release at early times due to the large concentration gradient formed between the sphere and the blood, which can pose health risks to the patient. Therefore, the initial concentration that can be used is constrained by the cost of formulation development and safety issues related to drug dosage.

5.3 *Future Work*

The project was highly limited by the amount of available computing power, as COMSOL could only reach converged solutions for relatively coarse mesh sizes as the sphere computational domain decreased to small sizes. Increased computing power would allow the use of a finer mesh for small microsphere radii to generate more accurate solutions without resulting in computational failure. Furthermore, a better quantitative understanding of PLGA degradation mechanisms should be investigated. While the model accounts for surface erosion, it ignores bulk erosion and possible surface contact of the spheres with the vessel walls [12]. As the physics behind such dynamics are very complex and not fully understood, they are difficult to incorporate into the model but should be considered if more accurate results are desired. A more rigorous analysis of exendin-4 transport would also have to take into account the asymmetry in the microsphere polymeric structure due to the non-uniform distribution of lactide and glycolide monomers in PLGA. Faithful implementation of this asymmetry would require the use of a three-dimensional structure in the model, which could be achieved by simulating the geometries within an actual PLGA microsphere in a 3D modeling program such as SketchUp, and importing this geometry into COMSOL for analysis. Finally, the effect of using larger microspheres or longer administration periods may also be investigated in further studies.

Appendix A: Mathematical Statement of the Problem

A.1 *Governing Equations*

Assumptions/physical approximations:

1. The microsphere is a perfect sphere.
2. Diffusion and surface degradation is axisymmetric.
3. The PLGA microsphere, which has a non-uniform composition ratio of lactide and glycolide monomers, has uniform material properties (diffusivity, density, etc.)
4. PLGA erosion is entirely at the surface; the amount of absorbed water is low such that it does not cause erosion to the interior of the microspheres.

The following equations provide a quantitative description of the problem's underlying physics:

1. Mass transfer equations for diffusion of exendin-4
    a. Within the sphere
    $$\frac{\partial c}{\partial t} = D_S \left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right) + + \frac{\partial^2 c}{\partial z^2}\right]$$
    b. Within the blood
    $$\frac{\partial c}{\partial t} + \left(v_r\frac{\partial c}{\partial r} + + v_z\frac{\partial c}{\partial z}\right) = D_B \left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right) + + \frac{\partial^2 c}{\partial z^2}\right]$$
2. Navier-Stokes equations for blood flow
    a. r – component
    $$\rho_B\left(\frac{\partial v_r}{\partial t} + v_r\frac{\partial v_r}{\partial r} + v_z\frac{\partial v_r}{\partial z}\right) = -\frac{\partial P}{\partial r} + \mu_B\left[\frac{\partial}{\partial r}\left(\frac{1}{r}\frac{\partial}{\partial r}(rv_r)\right) + \frac{\partial^2 v_r}{\partial z^2}\right]$$
    b. z – component
    $$\rho_B\left(\frac{\partial v_z}{\partial t} + v_r\frac{\partial v_z}{\partial r} + v_z\frac{\partial v_z}{\partial z}\right) = -\frac{\partial P}{\partial z} + \mu_B\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial v_z}{\partial r}\right) + \frac{\partial^2 v_z}{\partial z^2}\right]$$

A.2 *Computational Domain, Boundary and Initial Conditions*

Computational Domain

The moving boundary was implemented by making the radius a function of the flux of drug across the boundary and therefore dependent on time. Since the primary effect of blood flow is to maintain a significant concentration gradient by sweeping away any drug that diffuses out, it is effectively changing the mass of drug within the sphere. Thus, coupling the flow to the flux is an accurate approach to implementing the moving boundary. The change in mass due to loss of drug was converted to change in volume using density, and change in radius was obtained from the volume change.

Boundary Conditions

1. At the wall of the blood vessel:

   $c_b = 0$ mol/m^3

   No viscous stress

2. At Inlet:

   $c_b = 0$ mol/m^3

   $v_{blood} = 3\times10^{-8}$ m/s

   Because the sphere is being carried with the flowing blood, the fluid bulk velocity is the relative velocity of the blood with respect to the sphere. It can be estimated as the settling velocity of the sphere, calculated using Stokes' law:

   $$F_d = F_g = (\rho_{PLGA} - \rho_{blood}) \cdot g \cdot \frac{4}{3}\pi \cdot R^3$$

   $$F_d = 6\pi \cdot \mu \cdot R \cdot v_s$$

   $v_s =$ ranges from around an order of magnitude of 10^{-6} m/s to 10^{-9} m/s for microsphere radii ranging from the initial 5 µm to less than 0.5 µm. The coupling of the blood flow with the degrading sphere is very difficult to incorporate into a boundary condition; instead, we assume a constant intermediate value of 3×10^{-8} m/s for the relative velocity of the blood.

3. Pressure at the outlet:

$$p = 0 \text{ Pa}$$

4. Axisymmetry condition: no flux at left boundary (r=0)

Initial Conditions

1. Initial size of microsphere:

$$r_m(t = 0) = r_{m,0} = 5 \text{ µm}$$

2. Initial drug concentration in microsphere*:

$$c_m(t = 0) = c_{m,0} = 1.505 \text{ mol/m}^3 \text{ [3]}$$

3. Initial drug concentration in bloodstream:

$$c_b(t = 0) = 0 \text{ mol/m}^3$$

*The initial drug concentration in the microsphere was calculated using the ratio of drug mass to microsphere mass utilized in the drug loaded microsphere fabrication discussed in DeYoung et. al. [3]. The microsphere mass was divided by the density of PLGA [4], and the drug mass was divided by the resulting volume and converted to moles using the molecular weight of exendin-4 (4186.57 g/mol) [5] to calculate concentration.

A.3 *Material Properties and Input Parameters*

Material properties

Parameter	Description	Value	Units	Reference
D_S	Diffusivity of exendin-4 in sphere**	3.84×10^{-18}	m²/s	See below
D_B	Diffusivity of exendin-4 in bloodstream	2×10^{-10}	m²/s	based on Fig. 12
ρ_{blood}	Density of blood	1050	kg/m³	[7]
μ_{blood}	Viscosity of blood	0.003	Pa · s	[7]
ρ_{PLGA}	Density of PLGA	1260	kg/m³	[4]

**Diffusivity in the sphere was approximated using the equation for Fickian diffusional release from a thin polymer sample found in Siepmann et. al. [6]:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{1/2}$$

where $\frac{M_t}{M_\infty}$ is the percent drug released at time t and L is the characteristic length, which in this case would be the radius of the sphere (5 μm). The percent released at a certain time was approximated using Figure 13 from Liu et. al. [2], which shows the release profiles for microspheres of different PLGA ratios. Because it exhibits the most ideal release kinetics, the 50/50 ratio profile was used to estimate a 13% release after 1 day. Based on these values, a diffusivity of 3.84×10^{-18} m^2/s was calculated. Though this value is very small, other studies have observed even smaller diffusivity values in PLGA for various drugs [14]. Thus we have determined our calculated value to be sufficiently accurate and within reason.

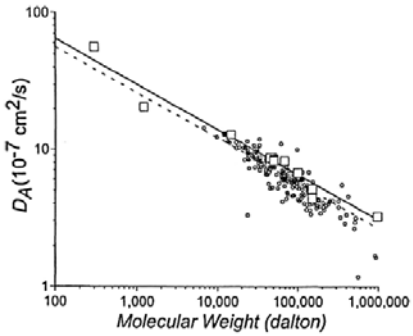

Figure 12. Variation of diffusivity with molecular weight for globular proteins in water. From Salzman (2001).

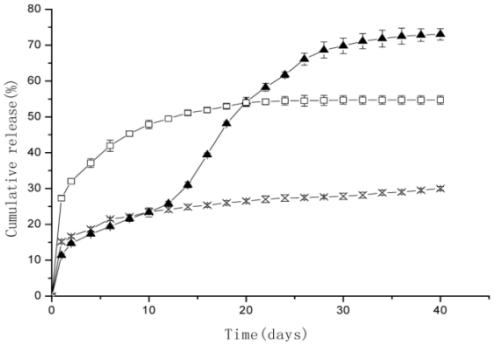

Figure 13. Effect of the polymer type on the release profiles of exenatide loaded PLGA microspheres. PLGA 50/50 (triangle), PLGA 75/25 (square) and PLA (x) (n=3).

Appendix B: Solution Strategy

B.1 *COMSOL Specifications*

Because the problem is being solved with a relatively small number of elements (shown in Section B.2) and we are only dealing with three different physical governing equations, the simulation is best run using a direct solver. In our case, COMSOL is using the direct solver MUMPS.

B.2 *Mesh Convergence*

We performed a mesh convergence analysis to find the minimum number of elements that could be used for computation without introducing significant discretization error into the solution. The parameter chosen to be monitored is the concentration of exendin-4 at a point inside the microsphere at early times, as this displays a large amount of variation over time that can be monitored to check for convergence. Mesh distribution was chosen such that the mesh is finest at and around the surface of the sphere, which is where the greatest change in drug concentration occurs due to the transition in diffusion medium from PLGA to blood.

Mesh convergence was performed at a radius value of 4.2 μm (within the sphere) from t=0 to t=20000 s. Since the surface of the degrading microsphere encounters the most change in concentration, the number of elements in the distribution for the mesh around the microsphere is varied. The greatest number of elements that we could run efficiently given the computational limitations of the software was 4477 elements; the results of this mesh size are somewhat similar to the next greatest number of elements (2370), so we can assume that it is close to converged.

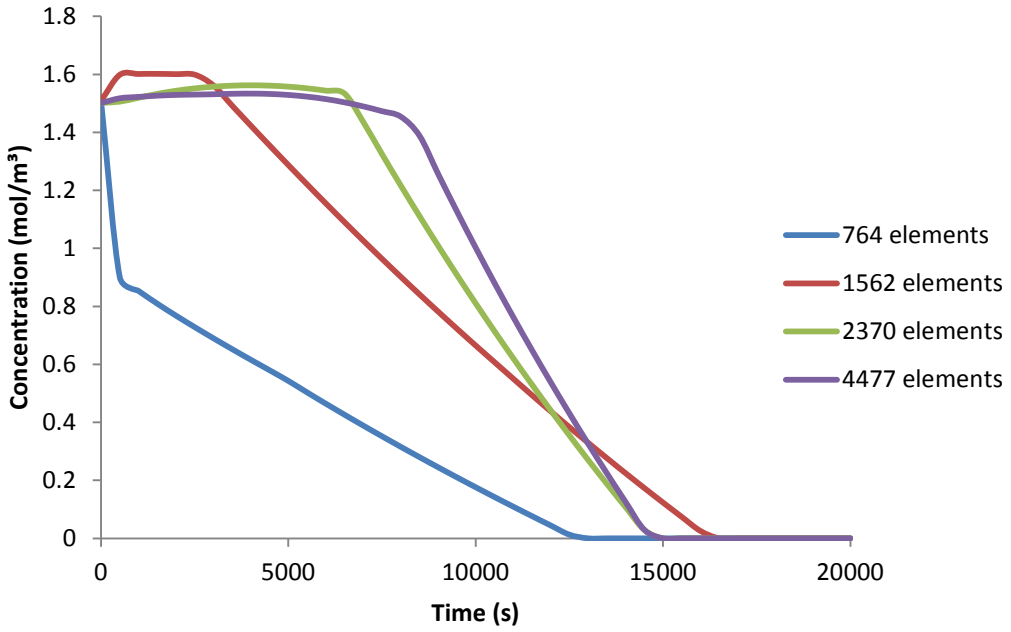

Figure 14. Mesh convergence on concentration profile of exendin-4 at (r=4.2 µm, z=0 µm) for various numbers of free triangular elements. The mesh appears to be close to converging at 2370 elements, but a greater number of elements resulted in computational failure.

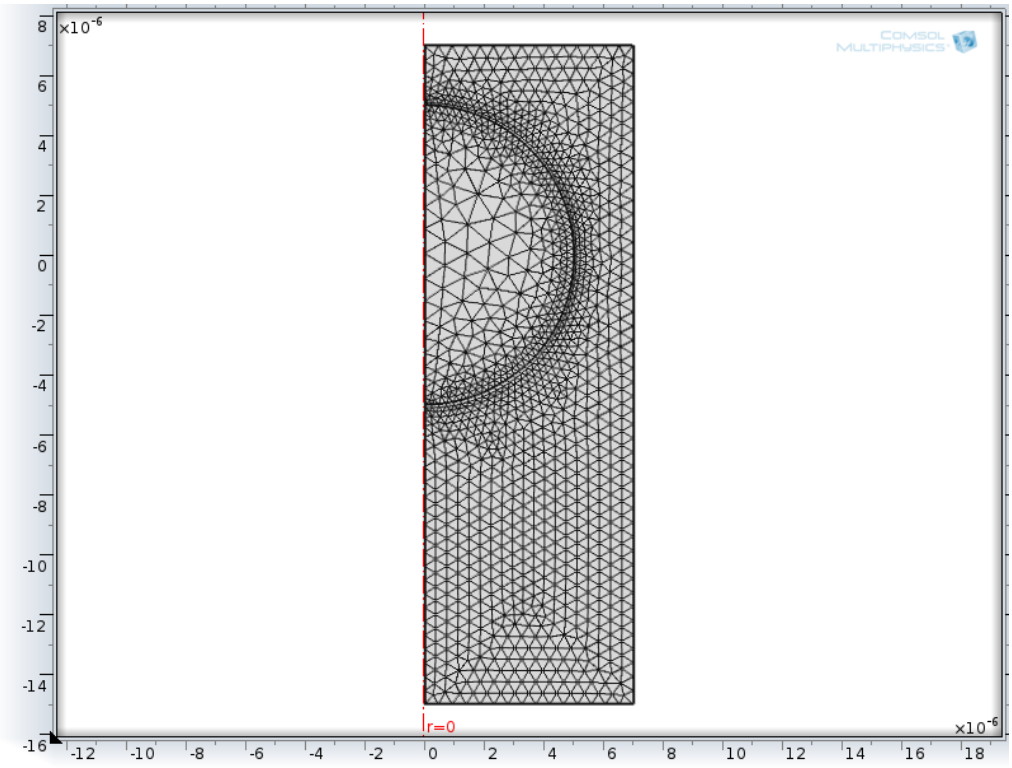

Figure 15. Diagram of final mesh selection, with 2370 elements. Mesh element distribution is finest at the interface between the sphere and the blood.

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