Modeling Treatment of *P. aeruginosa* Biofilms in the Lungs Using Aerosolized Tobramycin

BEE/MAE 4530 – Computer-Aided Engineering: Applications to Biomedical Processes

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Executive Summary

The biofilms produced and maintained by *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients are difficult to treat and can have fatal effects. Antibiotics are necessary to control and eliminate these bacterial biofilms, but in vivo administration may not be the most effective means. Tobramycin, a commonly used antibiotic for treating cystic fibrosis patients, has been commercially developed into a solution that is inhalable via nebulizer. Inhaling this mist form of the antibiotic will allow administration of higher concentrations at the site of infection. The goal of this study was to develop a model using COMSOL Multiphysics to better understand the distribution of tobramycin to bacterial biofilms in the lungs. Like nearly all medications, tobramycin can become toxic at high concentrations. Since filtration from the blood stream is the only significant mechanism of tobramycin elimination, the kidneys are at the greatest risk for toxicity. Therefore the study focused on the possibility of maintaining safe blood serum concentrations while providing sufficient doses to inhibit the bacteria occupying the lungs. The model showed that the bacteria's minimum inhibitory concentration was easily achievable throughout the biofilm while keeping the blood serum concentrations at a safe level.

Introduction

Background

Cystic fibrosis (CF) is a genetically inherited disease that affects one out of 3,500 births in Europe and North America (1). There is no cure for CF and all patients eventually develop chronic obstructive lung disease, which has a 90% mortality rate among CF patients (2). Patients with CF have malfunctioning glands that cause them to have thick and dry mucus, which obstruct biological passageways like the lungs. Normally, the mucus lies on top of a surface-liquid that acts as a lubricant and entrapped particles are removed along with the mucus by beating of the cilia or coughs. The lubrication is partially controlled by chlorine channels, but for CF patients these channels do not function properly and this leads to a lack of lubrication. As a result, stagnant mucus accumulates and bacteria trapped in the mucus proliferate, which can cause serious complications that could lead to death. In fact, life expectancy of people with CF is about 45 years. (3)

Bacteria growing in the mucus are very hard to eliminate without antibiotics because they are immune from the host's defenses in contrast to their free-floating counterpart (4). The most common and lethal bacterial infection in patients with CF is caused by the bacteria *Pseudomonas aeruginosa.* Tobramycin is an antibiotic often administered to treat CF patients with a *P. aeruginosa* infection (5). It is delivered intravenously, but this requires high dosages because intravenous drugs are spread throughout the body and only a small portion of the dose reaches the deep lung. In addition, the dose of tobramycin that can be administered is limited because it is toxic to the kidney. An alternate delivery method is through inhalation of tobramycin particles delivered via nebulizers, which provide mist forms of the drug. Tobramycin Inhalation Solution (TOBI) is one such device already in use. TOBI delivers the drug directly to the lungs so it requires less of the drug than intravenous delivery.

Design Objectives

Our first objective in creating this simulation is to accurately model tobramycin's diffusion through the biofilm and alveolar tissue, which can be done using COMSOL Multiphysics software. By using this model to further evaluate the effects of tobramycin in a biofilm of a cystic fibrosis patient, we will then seek to optimize the dosing of tobramycin. Specifically, we will determine the ideal dosing time and concentration that (a) achieves 95% inhibition of the bacteria with the tobramycin concentration reaching 4 μg/mL in the biofilm (6), (b) does not exceed the toxic trough concentration (the concentration immediately before taking a second dose) of 2 μg/mL in the blood, and (c) does not exceed the toxic peak concentration of 12 μg/mL in the blood (7,8). Meeting these requirements will allow for effective treatment while avoiding kidney damage, keeping in mind that some parameters may vary due to the fact that patients range from infants to elderly adults. Having not only the dose but the proper administration time frame will define the treatment regimen that best alleviates mucus buildup due to biofilm formation in the lungs.

Problem Schematic

To implement this model in COMSOL, an effectively one-dimensional geometry was chosen to represent a thin slice of a high generation alveolus (Figure 1). This model assumes the alveolus is perfectly spherical and fully surrounded by capillary beds. Also, the air and biofilm within each alveolus is homogenous and evenly distributed. Since a high generation alveolus is modeled, the air layer near the biofilm is approximated as stagnant. The thickness of the air layer was chosen by taking half the difference between the alveolar radius and the biofilm thickness. Average biofilm and alveolar wall thicknesses are 33 μm and 4 μm, respectively (9, 10). The height of the blood layer is the radius of an average capillary. This allows the bottom boundary condition to be set as the average blood concentration. (Governing equations, boundary conditions and initial conditions can be found in Appendix A while the finalized mesh and mesh convergence are located in Appendix B.)

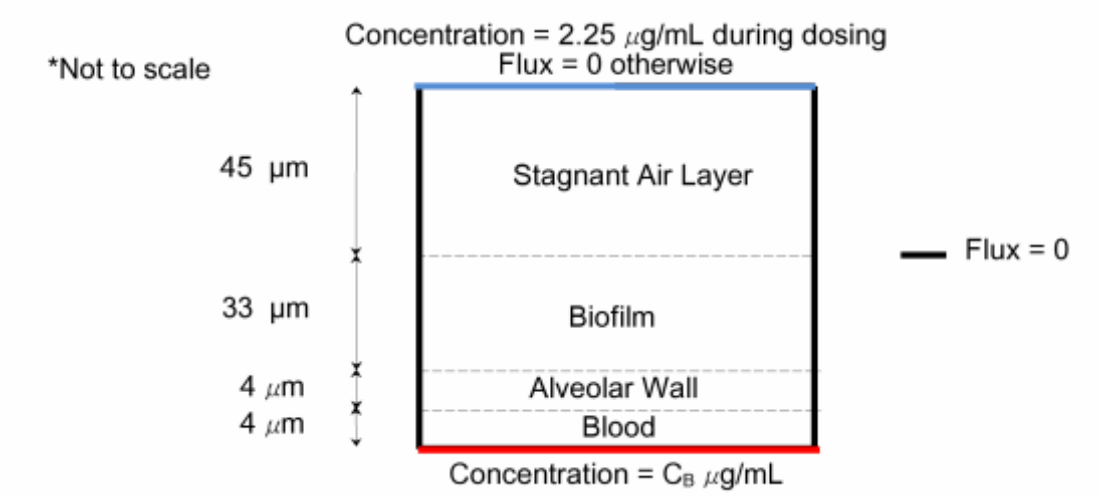


Figure 1. Two dimensional geometry used in COMSOL model to represent one-dimensional transport of antibiotic from air to biofilm to the bloodstream.

Results and Discussion

Diffusion of aerosolized tobramycin was modeled in COMSOL using the schematic outlined above as well as mass transport and balance equations and input parameters described in Appendix A . To perform the finite element analysis in COMSOL, an accurate structured mesh was determined through mesh convergence analysis (Appendix B) for the geometry in Figure 1. The blood and biofilm concentrations were of particular concern in our analysis, since a minimum inhibitory concentration (MIC) of 4 μg/mL must be obtained in the biofilm while keeping the blood serum concentration below the toxic limits. For tobramycin, peak and trough toxic concentrations in the blood are listed in the literature as 12 μg/mL and 2 μg/mL, respectively (8).

To determine initial dosing regimens, the prescription information for a commercialized form of this drug (TOBI) was used. It recommended taking the antibiotic via nebulizer every 8 – 12 hours; and each administration should last approximately 15 minutes. The 60 mg/mL vial of tobramycin solution is converted to approximately 2.25 μg/mL when nebulized, based on a linear approximation from values given in Weber et al. 1993 (5). We assumed that each breath during the administration would bring the nebulized concentration of tobramycin into the model alveolus. While using the nebulizer, the boundary condition of the stagnant airspace in the alveolus is assumed to be held at this constant concentration. Once the dosing is complete, the boundary will become insulated due to the effectively stagnant air in high generation alveoli.

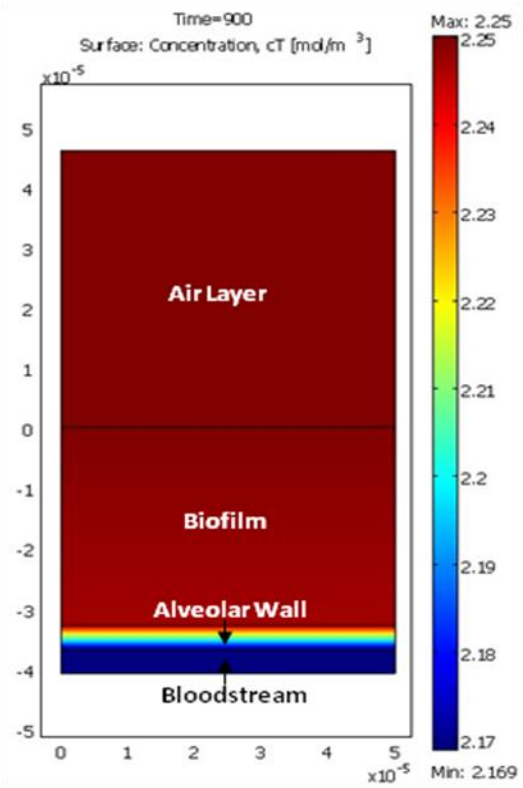


Figure 2. Concentration of tobramycin (μg/mL) after the first 15 minute dose

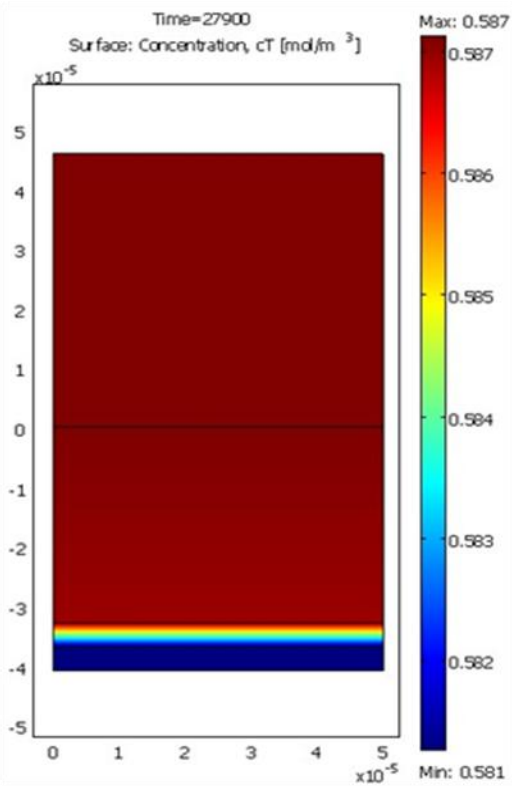


Figure 3. Concentration of tobramycin (μg/mL) 7.75 hrs after the first dose (8hrs from starting dose)

The air and biofilm concentrations equilibrate to the approximate dosage concentration within the 15 minute administration (Figure 2). The lower diffusivity through the alveolar wall creates a concentration gradient, allowing the blood concentration to remain slightly lower. The diffusivity through the biofilm is two orders of magnitude larger than that of the tissue, allowing the drug to move through the biofilm more quickly. This model can only account for simple diffusion without the effects of binding or cellular uptake due to the very limited literature on biofilm transport. Since the model is based on simple diffusion, the biofilm concentration cannot reach 4 μg/mL if the boundary condition is only 2.25 μg/mL. Therefore, a concentration greater than that calculated from the TOBI prescription information must be used for effective treatment in this model.

After the 15 minute dose, the top boundary condition was adjusted as described previously and the model was allowed to run for an additional 7 hours and 45 minutes to determine effects of renal elimination from the bloodstream (Figure 3). After this "off" period, the concentration of drug in the biofilm (measured at the center) decreased significantly by 75.1%. Within the alveolar wall and blood subdomains, similar results were acquired with a 74.7% decrease in the tissue and a 74.4% decrease in the blood.

The combined 8-hour dose and off period were then run repeatedly to determine the dynamics of tobramycin concentration in the biofilm and blood. Simulation of repeated dosing showed that only two doses are required to reach equilibrium peak and trough concentrations for both the center biofilm concentration and the blood concentration (Figure 4 b,c). The concentration of tobramycin in air and biofilm throughout administration of the drug (the first 15

minutes) appears to be constant as a consequence of the constant concentration boundary condition ($c_{T,0}$ = 2.25 µg/mL) and the high diffusivity of the drug through the air and biofilm (Figure 4a). The time it takes for the biofilm concentration to equilibrate with the approximate dosage concentration is less than 30 seconds. Based on our mathematical model, there cannot be any accumulation above the dosage concentration. This means the dosage concentration must be increased in order to achieve the minimal inhibitory concentration for the bacteria.

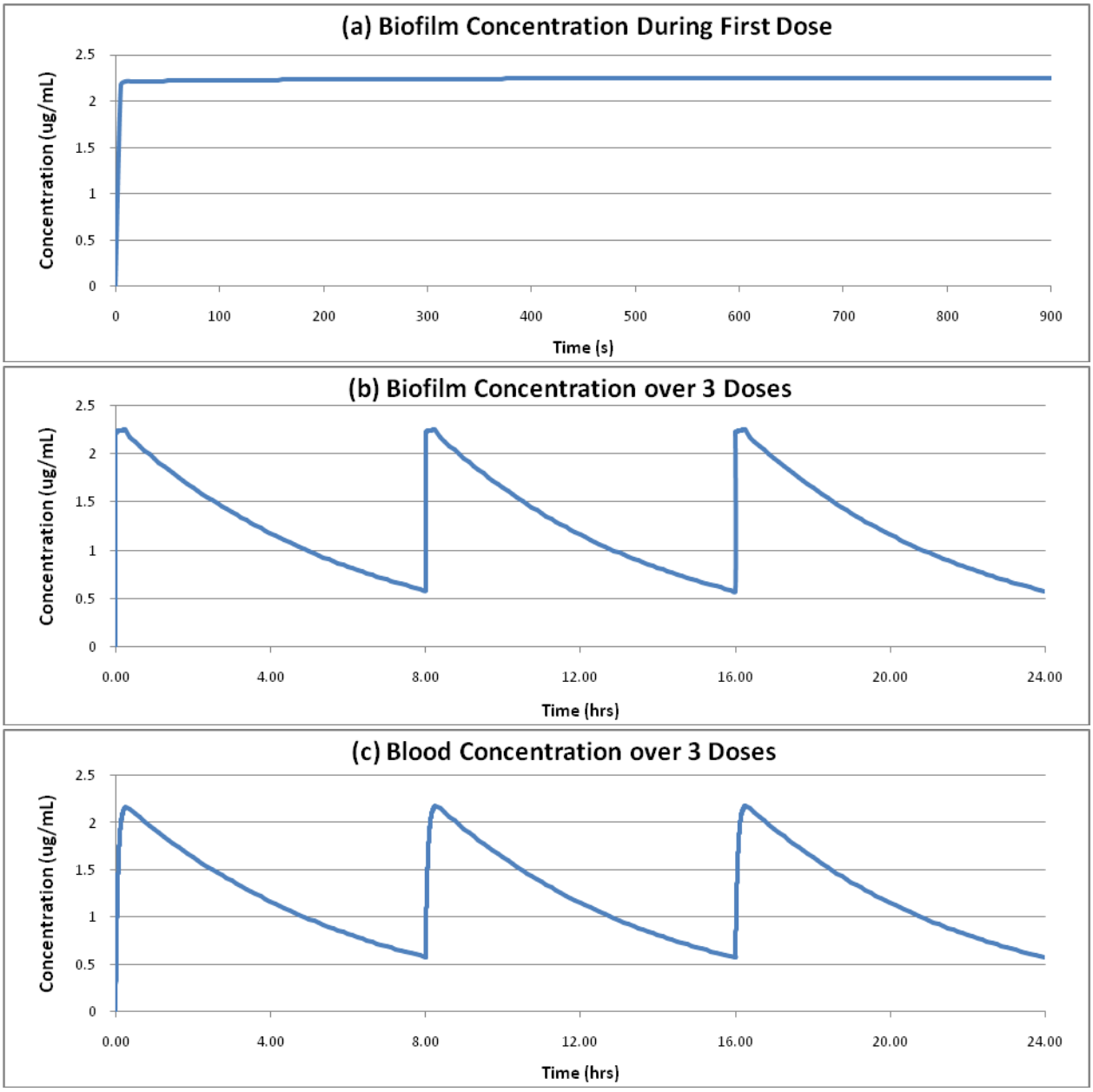


Figure 4. (a) Biofilm concentration (at center of biofilm) following one 15-minute dose. (b) Biofilm concentration over 3 administrations. (c) Blood concentration over 3 administrations.

Accuracy Check

The toxicity of tobramycin is not well defined in the literature. There are references that indicate the potential toxic concentration of tobramycin is 2.0 µg/mL (7), but no time span is indicated. Clearly there must be some time and concentration combination that will produce toxic effects. There have been various studies that have used serum concentrations greater than 2.0 µg/mL for 4 hours or more without noticeable side effects (11,12). Another publication states that toxic peak and trough concentrations of tobramycin are 12 µg/mL and 2.0 µg/mL, respectively (8). These values agree with the literature (11,12) that use higher concentrations without any signs of toxicity, so we will base our analysis and design constraints on this information (Figure 5).

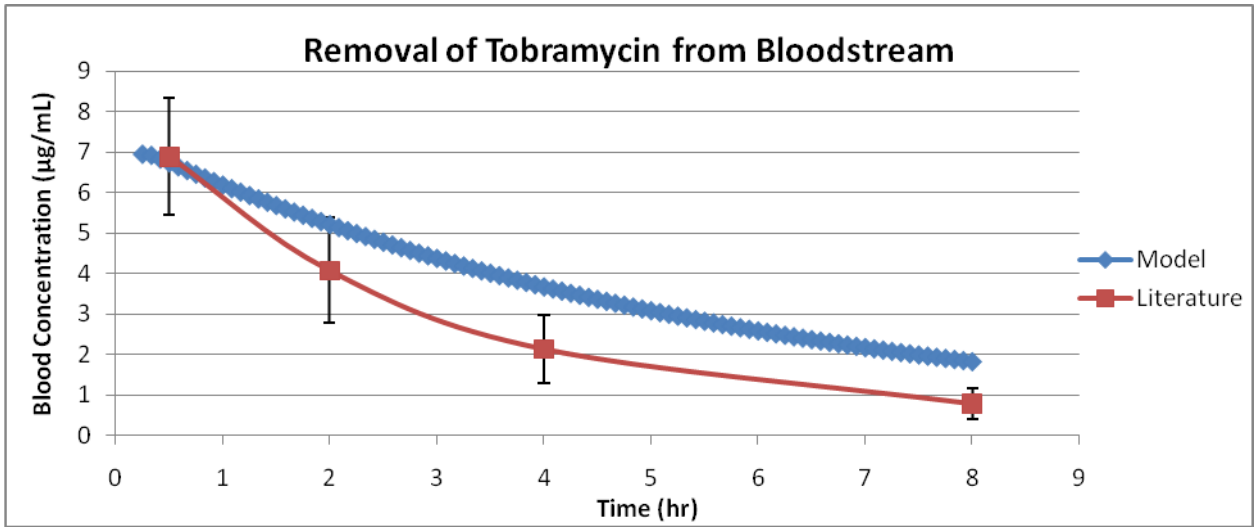


Figure 5. Removal of tobramycin from bloodstream by kidneys over 8 hour period between doses. Dosage concentration used in model was 7.5 µg/mL to match initial concentration in literature (9). Tobramycin was administered intravenously in the study, so there is no drug flux into the bloodstream over time as there is in the model. Therefore, the effective removal rate in the model is lower. Data from literature represents average serum concentration for patients with standard deviation bars.

Sensitivity Analysis

We completed a sensitivity analysis for six parameters in our model including dosage of tobramycin, time of inhalation, number of doses, half-life of tobramycin and diffusivity of tobramycin through biofilm and tissue. Parameters were varied one at a time to determine the relative effect each has on the solution.

The dosage of tobramycin used in commercial products such as TOBI is about 2.25 µg/mL after nebulization. This value was used as the starting point and was varied +/- 10% to determine sensitivity and to ensure the minimum inhibitory concentration (MIC) is achieved in the biofilm without exceeding toxic limits. The inhalation time of commercial products, when using a nebulizer, is described to be around 15 minutes. Again, this was used as a starting point and was varied +/- 10% to determine sensitivity and for optimization. The effects of varying the number of doses are difficult to determine with this model. The only criteria available for killing

the bacteria is a minimum inhibitory concentration (MIC), but no exposure time requirement. The model was run with enough dosages to reach effective steady state (consistent peak/trough concentrations for each dose). The half-life of tobramycin is provided in the literature as a range of values from 1.8 – 2.2 hours. The extremes and mean of this range were used for the sensitivity analysis (mean +/- 10%). The diffusivity of tobramycin through the biofilm and tissue is not well defined in the literature. One publication provides a single value of 3.84×10^{-10} m^2/s for tobramycin diffusion in an *ex vivo* biofilm. This value was varied +/- 20% due to the potential for high variability between biofilms in different patients and biofilms at different stages of biofilm development. A value for tobramycin diffusivity through tissue was not found in the literature. Instead, flux data for amoxicillin through a monolayer of epithelial cells was used to calculate an approximate diffusivity value of 1.62×10^{-12} m^2/s. This parameter likely has a higher potential for error and was varied +/- 20% as well.

The biofilm diffusivity has very little effect on the average biofilm and blood concentrations at both time points (Figure 6 a,b). For a +/-20% change in diffusivity, the largest difference in concentration is 1.94% for the average biofilm and blood concentrations after the 8 hour off-period. The tissue diffusivity has a slightly larger effect on the concentrations, but is still rather small. For a +/-20% change in tissue diffusivity, the largest difference is 2.96% for the average biofilm concentration immediately after the dosage. Since these diffusivities have an insignificant effect on the solution (<5%) with a change of 20%, there is no need to investigate the parameters further. Although these parameters were approximated based on data that does not directly relate to this drug in this model, the sensitivity to these parameters is low enough that error in these parameters should not significantly affect the model.

The administered dose obviously has a significant effect on the concentrations. The dose was varied by +/-10% which resulted in changes of 8.9% - 11.7% in blood and average biofilm concentrations. The elimination constant has the largest effect on both the average biofilm concentration and the blood concentration after the 8 hr off-period. Differences of approximately 15.8% were seen in these concentrations when the half-life was decreased by 10%. Unlike the dose concentration, the dosage administration time did not have a significant effect on the model. From changing the data from a 15 minute administration to a 13.5 minute administration, we found that there is a <2% change in the biofilm and blood concentration after dosage and after 8 hours; similar results were seen with a 16.5 minute administration time (Table 1). These low values show that our model is not sensitive to changes in the drug administration time and the initial specified time is sufficient for our purposes.

The half-life of tobramycin was relatively well defined in the literature, so the mean value of 2 hrs was used in the model. The administration time had a surprisingly small effect on the average biofilm and blood concentrations. Since the biofilm is mostly water, it has a relatively large diffusion coefficient which allows the biofilm concentration to reach steady state quickly compared to administration time.

It should be noted that all blood concentrations found in this analysis fell below toxic peak/trough concentrations stated in literature. Analysis of the aforementioned parameters indicates that optimization of treatment with tobramycin should focus mainly on changing the dosage concentration.

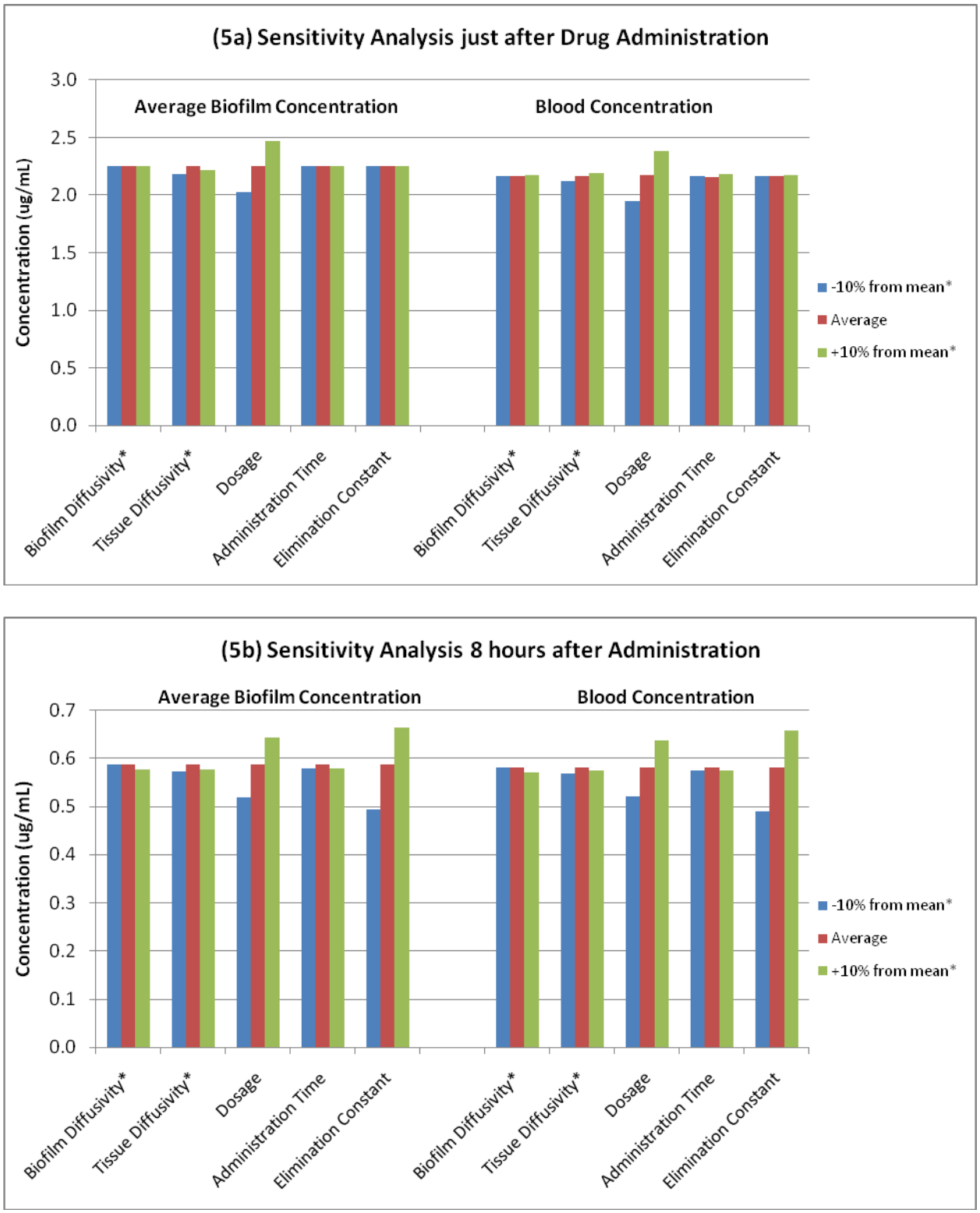


Figure 6. Sensitivity analysis of select parameters just after a 15 minute dosage of tobramycin (a) and after 8 hours following a dosage (b). The parameters were varied +/- 10% from the mean except for the diffusivity values (*) which were varied +/- 20% from the mean.

Table 1. Difference in Solution, Compared with Mean Value, When Each Parameter is Varied

	Biofilm Diffusivity (3.84E-10 m^2/s)		Tissue Diffusivity (1.62E-12 m^2/s)		Dosage (2.25 µg/mL)		Admin. Time (15 min)		Half-life (2 hrs)	
	- 20%	+ 20%	- 20%	+ 20%	- 10%	+ 10%	- 10%	+ 10%	- 10%	+ 10%
Ave. Biofilm Concentration										
after administration	0.02%	0.01%	2.96%	1.29%	10.00%	9.09%	0.01%	0.01%	0.00%	0.00%
after off-period	0.11%	1.94%	2.61%	1.63%	11.68%	8.90%	1.27%	1.32%	15.74%	11.44%
Blood Concentration										
after administration	0.08%	0.15%	2.36%	1.07%	10.17%	8.98%	0.77%	1.27%	0.21%	0.17%
after off-period	0.11%	1.94%	2.24%	1.04%	10.25%	8.90%	1.27%	1.32%	15.83%	11.52%

Conclusion & Design Recommendations

To optimize the dosage, we tested a variety of values to determine how much tobramycin was enough to not only kill the bacteria but sustain such a concentration in the biofilm over an extended time without reaching toxic levels in the body. Since the MIC (4 µg/mL) was not met with the initial dosage of 2.25 µg/mL, a new dose concentration must be found that is high enough to inhibit the bacterial growth while maintaining safe limits (peak <12 µg/mL and trough <2 µg/mL). Consider the prescription information for these commercial antibiotics, which recommends dosing every 8 – 12 hours. If patients abide by this range, the trough concentration will be highest if they take antibiotics every 8 hours. To determine the appropriate dosage, the trough after 8 hours was compared with toxicity limits. Doing this showed that trough concentration increases linearly with the concentration administered (Table 2).

Table 2. Trough blood concentration (µg/mL) after 2 doses

Dose Concentration	Trough Blood Concentration
6	1.530
6.5	1.657
7	1.784
7.5	1.912
8	2.039

If 7 µg/mL or higher was used as the dose concentration, the trough would be too close to or above the toxicity limit. These trough blood concentrations are given based on the average elimination constant for patients in the literature. A patient with less efficient kidneys may not have trough concentrations below the toxic limit. Our sensitivity analysis shows that changes in the elimination constant can result in significant changes in the solution (up to ~16% with 10% decrease in half-life). Therefore, we recommend that 6.5 µg/mL be the maximum dosage concentration. For this dose, the trough blood concentration is 17.2% below the toxic concentration. This will allow patients with less efficient kidneys to remain below toxic limits at a trough concentration of 1.90 µg/mL (Figure 7).

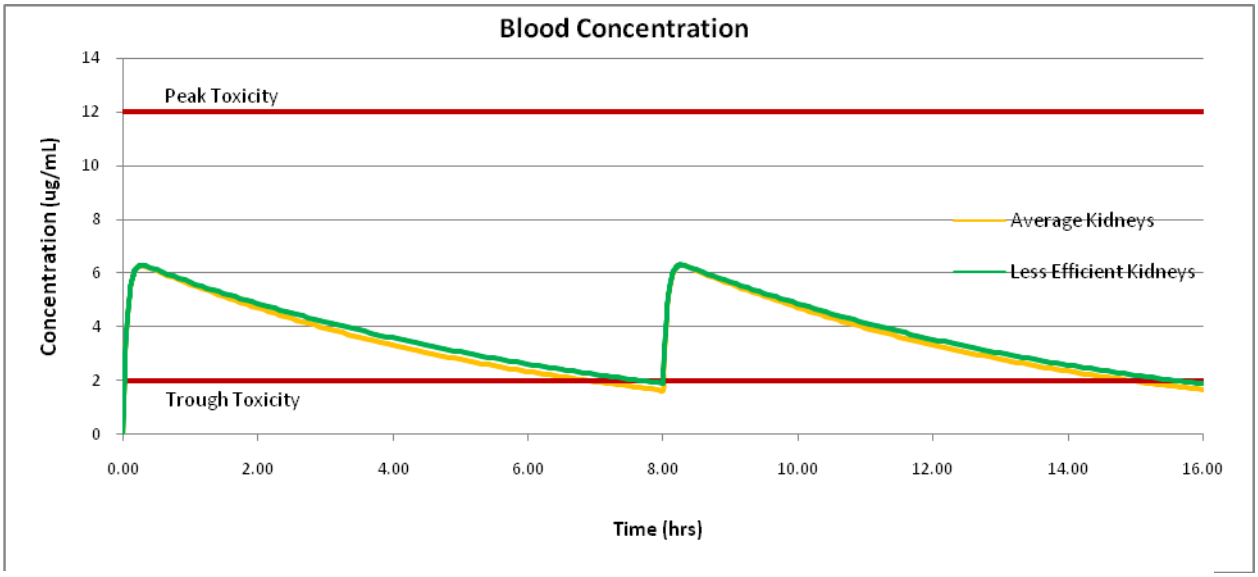


Figure 7. Blood concentration of tobramycin over 2 dose cycles of 6.5 µg/mL. Removal of tobramycin from the blood is compared for average and less efficient kidneys

Inhibition of bacteria is simply defined by a MIC given in the literature. There is generally no exposure time specified for bacterial inhibition. For this model, the only design parameter that may be altered to increase exposure time is the dosage concentration. We will assume that maximizing exposure time for each dose is optimal. At the maximum allowable dosage concentration of 6.5 µg/mL, the bacteria in the biofilm are exposed to concentrations exceeding the MIC for approximately 3 hours per dose (Figure 8). Based on the findings with this model, the administration dosage should be 6.5 µg/mL to ensure bactericidal effects without poisoning the patients kidneys.

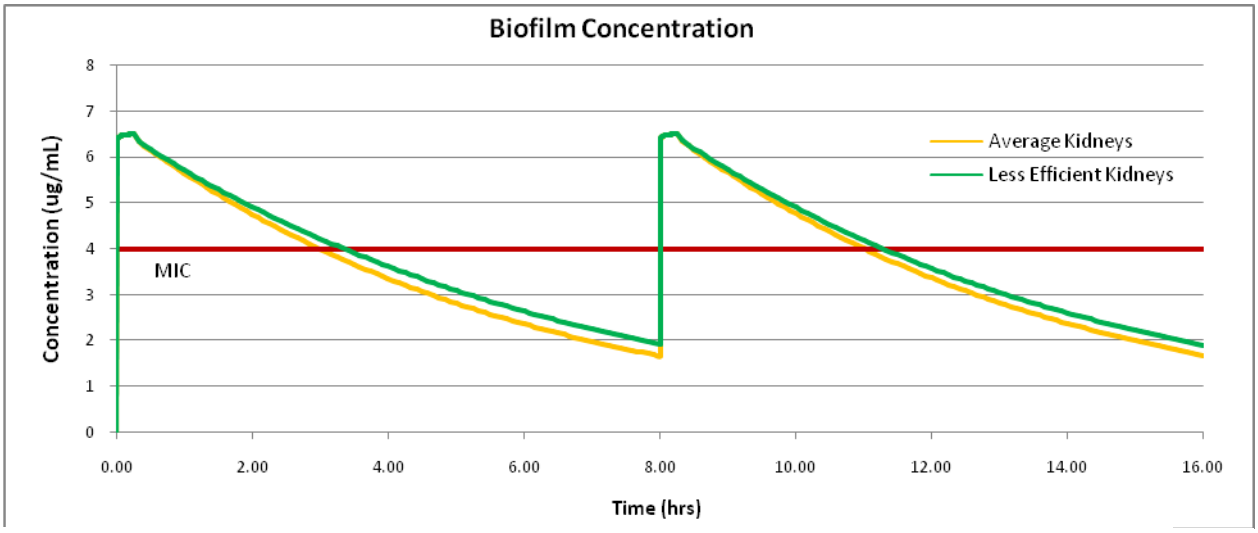


Figure 8. Concentration of tobramycin in the center of the biofilm over 2 dose cycles at 6.5 µg/mL. Removal of tobramycin from the blood is compared for average and less efficient kidneys.

Realistic Constraints

Because tobramycin inhalation solutions and nebulizers have successfully gained FDA approval and been marketed as TOBI and Tobamist, this implies that it has been approved as a safe treatment method, and no manufacturing or economic constraints are apparent. The most significant constraints on the results of this study are the physical assumptions within the model. For simplification, we assumed the air layer above the biofilm was stagnant in high generation alveoli, and thus there was no flux out during of the lungs between administrations. In lower generation alveoli, convection in addition to diffusion may be an important factor. Additionally, biofilm transport of antibiotics is still not well understood. Our model can only account for simple diffusion and is not able to consider binding effects, cellular uptake, or active transport by the cells. The concentration of aerosolized tobramycin may have been inaccurate as well – there was no data in the literature for the conversion of 60 mg/mL solution to a nebulized mist, so it is difficult to compare our dosage recommendation to the currently manufactured dosage (5).

Appendix A: Problem Formulation

A1. Schematic:

See the *Problem Schematic* section on page 4.

A2. Governing Equations:

The drug was modeled as two species: tobramycin in the bloodstream and tobramycin in the other three layers: air, biofilm, and alveolar wall. This is done to perform a mass balance between tobramycin entering the blood and tobramycin being removed by the kidney. The generation term for the concentration in the blood will be based on the flux of tobramycin out of the alveolar wall. This yields the following equations:

(1) Diffusion of tobramycin through the air, biofilm and alveolar wall:

$$\frac{\partial c_A}{\partial t} = D_A\left(\frac{\partial^2 c_A}{\partial x^2} + \frac{\partial^2 c_A}{\partial y^2}\right)$$

where c_A is the concentration of tobramycin in air, biofilm, and alveolar wall. D_A is the diffusivity of the drug in the different layers. The diffusivity values are defined in the parameters section below.

(2) Mass balance of tobramycin concentration in blood:

$$\frac{\partial c_B}{\partial t} = \frac{FA}{V_B} - kc_B$$

where c_B represents the concentration of tobramycin in blood and F is the flux of tobramycin entering the blood from the alveolar wall. A is the total alveolar surface area, and V_B is the volume of blood in the body. k is the rate constant for the removal of tobramycin from the blood by the kidney, determined by a first order rate law using serum half-life from the literature.

A3. Boundary & Initial Conditions:

Boundary Conditions:

- Top boundary condition varies with time. During the 15-minute nebulizer dosage, there is a constant concentration equal to the dosage concentration. For the 8 hours in between doses, there will be a zero flux boundary condition. We are assuming the air in this alveolus to be relatively stagnant, so once the drug is in the lower portion of the alveolar airspace, it will remain there until diffused into the biofilm.
- The two side boundaries are considered insulated.
- The bottom boundary is set at a constant concentration equal to the blood concentration determined by the mass balance above.

Initial Conditions:

- Tobramycin concentration in all regions (air, biofilm, alveolar wall, blood) is zero at t = 0 ($c_{T,0} = 0$, $c_{B,0} = 0$).

A4. Parameters Used in Model:

Parameter	**Value**	**Source**
D_{air}, Diffusivity in air	4.98×10^{-6} m^2/s	Calculated using Graham's law compared with water vapor
D_{film}, Diffusivity in biofilm	$3.83\times10^{-10} - 5.56\times10^{-10}$ m^2/s	References 13 and 14 (**Note:** measured diffusivity of tobramycin through pure water is 5.56×10^{-10} m^2/s)
D_{alv}, Diffusivity in alveolar wall	1.62×10^{-12} m^2/s	Based on Fick's law calculation using flux and concentration data of amoxicillin diffusing through monolayer of epithelial cells (15)
D_{blood}, Diffusivity in blood	5.56×10^{-10} m^2/s	Assumed equal to water
k, elimination constant	-9.627×10^{-5} / s	Based on a half life of 2 hrs and assuming a first order rate law (6)
A, total alveolar surface area	58.9 m^2	Based on alveolar radius, assuming spherical and 300 million alveoli
V_B, total blood volume	5×10^{-3} m^3	

Appendix B: Solution Strategy

B1. Solver:

The model was solved using the Direct (UMFPACK) finite element linear system solver in COMSOL.

B2. Time Stepping:

A time step of 30 seconds was used for the administration period, while a time step of 300 seconds (5 minutes) was used for the off-period of 7 hours 45 minutes.

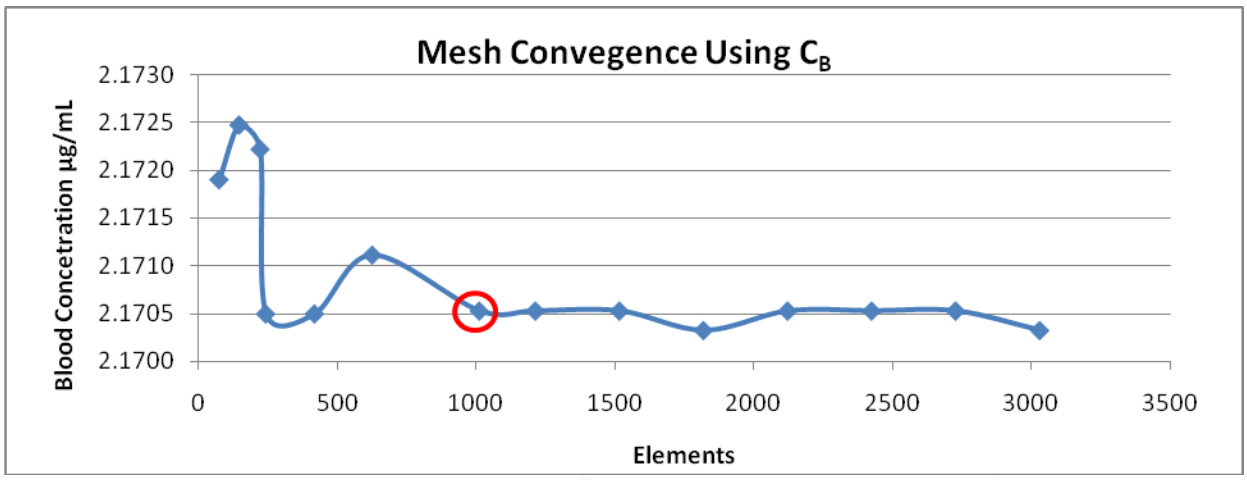
B3. Solver Tolerance:

Because this transient model is a relatively simple diffusion problem, we chose to use the default relative tolerance and absolute tolerance (0.01 and 0.001, respectively) to solve the model.

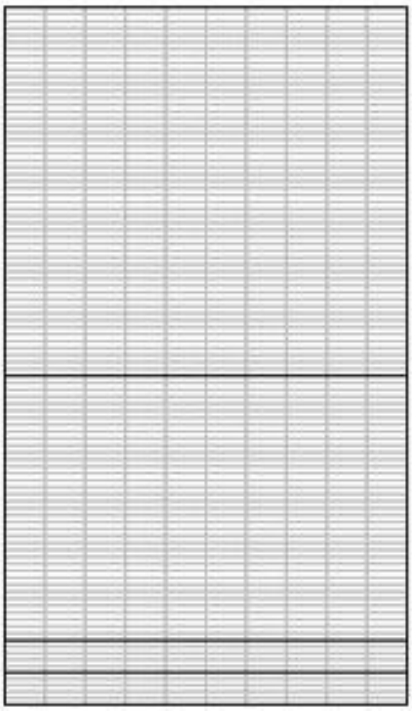
B4. Mesh & Mesh Convergence:

Because this model is essentially a 1-D problem, a structured mesh was chosen. Mesh convergence was performed using tobramycin concentration in blood (c_B) as a point of reference after one 15-minute dosage. The blood concentration was used because it depends on the mesh of the entire model, an average value is not needed, and there is no potential interpolation error since it is a single calculated value. During mesh adjustment, the number of mesh elements in the

y direction was adjusted using COMSOL's presets; in the *x* direction, the number of elements was manually increased by 3 for each new mesh. C_B stabilized at a final mesh size of 1010 elements ("extremely fine", 10 elements wide).



Final mesh: 1010 elements with constrained, 10 horizontal division and "extremely fine" vertical mesh



Appendix C: References

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   (Note: this study used a 20 mg/mL solution. A linear correlation was assumed for the calculation of nebulized tobramycin from 60 mg/mL solution.)
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