

## MODELING THE ABSORPTION, CIRCULATION, AND METABOLISM OF TIRAPAZAMINE

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### Problem Statement:

Tirapazamine (TPZ; 1,2,4-benzotriazin-3-amine 1,4-dioxide) is a hypoxia-activated prodrug currently being studied for antitumor usage. “When activated by one-electron enzymatic reduction, tirapazamine induces radical-mediated oxidative DNA strand cleavage” to selectively kill hypoxic tumor cells (Figure1).<sup>1</sup> Tirapazamine distinguishes tumor cells from healthy cells by their relative oxygenations. Because solid tumors have poorly organized vasculature, cells far from the blood vessels receive an inadequate supply of oxygen. This is an environment fundamentally different from that in the rest of the body, and presents an interesting opportunity for selective targeting. Unfortunately, there is also significant evidence showing that hypoxic tumor cells are often resistant to conventional anticancer treatments like radiotherapy and chemotherapy.<sup>3</sup> The development and study of prodrugs that can circumvent these limitations is therefore of substantial relevance.

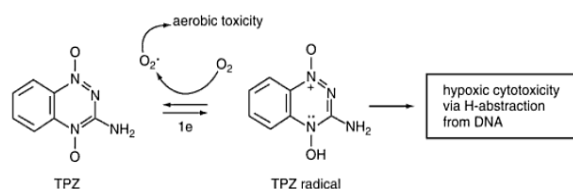


Figure 1. The one-electron enzymatic reduction reaction between the inactive prodrug Tirapazamine and its active form.<sup>2</sup>

The particular pharmacokinetic characteristics of a drug such as tirapazamine determine how it is metabolized in the body. In typical drug studies, concentration of a compound in the blood plasma is important. The aim is to formulate a dose and dosing schedule that will result in a plasma concentration below the toxicity limit and above the minimum effective dose limit; that is, the therapeutic window. In terms of maintaining a constant drug concentration in the plasma, the most effective method to achieve this is would be to employ continuous intravenous infusion. However, this is neither uniformly practical nor convenient for the patient, and other methods, such as an IV bolus dose, oral solution or pill, or patch should be considered.

In biomedical applications, transdermal patches are typically used to deliver low molecular weight drugs over long time courses in a controlled fashion. The drug is usually incorporated into a gel or liquid reservoir in the patch and applied to the skin using an adhesive. During the time that the patch is applied, the drug diffuses from the patch into the body. Because this delivery method relies on diffusion, transdermal drug delivery is feasible only for small molecules. Tirapazamine's relatively low molecular weight ( $M_r = 178.148$  g/mol) in comparison to other common compounds currently incorporated into patches, such as nicotine ( $M_r = 162.26$  g/mol) and nitroglycerin ( $M_r = 227.087$  g/mol), make it an excellent candidate for transdermal drug delivery.

We aimed to mathematically model the drug concentration changes at the absorption site, within the blood, and at the site of metabolism. Specifically, we will examine the case of drug distribution via a tirapazamine-impregnated transdermal patch.

To accomplish this, we chose to model drug absorption, circulation, and metabolism in terms of a three-compartment model. The first compartment corresponds to the transdermal patch itself. From this compartment, the drug diffuses through the skin into the second compartment, the blood. Assuming a well-mixed case, the drug content is then subtracted from this second compartment and shuttled into the third compartment via drug metabolism. The third compartment represents the site of metabolism at hypoxic tumor sites: these locations are

where tirapazamine is converted from prodrug into its active form. This metabolic process is saturable, and therefore follows Michaelis-Menten kinetics.<sup>4</sup>

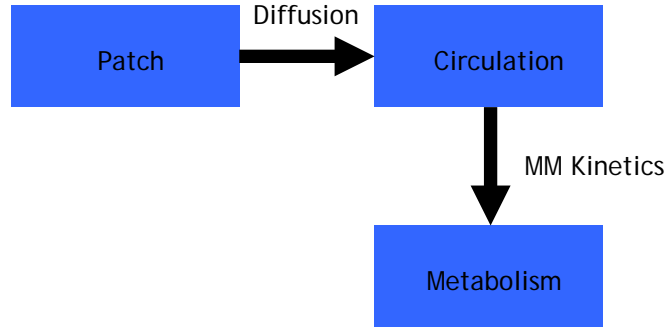


Figure 2. Schematic representation of our three-compartment model.

In mathematical form, our model is represented by a system of three ODEs. Each equation describes the change in drug concentration in their respective compartments over time. P, C, and M represent the three patch, circulation, and metabolism compartments, respectively, described above.  $k_1$  represents the diffusion constant from the patch to the circulation, and  $V_{\max}$  and  $K_m$  are the standard Michaelis-Menten parameters.

$$\begin{aligned}\frac{dP}{dt} &= -k_1 P \\ \frac{dC}{dt} &= k_1 P - \frac{V_{\max} C}{K_m + C} \\ \frac{dM}{dt} &= \frac{V_{\max} C}{K_m + C}\end{aligned}$$

### **Simplified Solution:**

Most drug concentrations are well below  $K_m$  for metabolizing enzymes.<sup>4</sup> Therefore we used this assumption for tirapazamine to simplify the nonlinear system of equations. The rate of metabolism becomes proportional drug concentration and follows a first order rate which simplifies the system of differential equation to:

$$\begin{aligned}\frac{dP}{dt} &= -k_1 P & P(0) &= P_0 \\ \frac{dC}{dt} &= k_1 P - k_2 C \text{ where } k_2 = \frac{V_{\max}}{K_m} & C(0) &= 0 \\ \frac{dM}{dt} &= k_2 C & M(0) &= 0\end{aligned}$$

To solve this system of first order differential equations, we will use the eigenvalue and eigenvector method. Rewriting these differential equations in matrix form, we get:

$$\frac{dA}{dt} = B \cdot A \text{ where}$$

$$A = \begin{bmatrix} P \\ C \\ M \end{bmatrix} \text{ and } B = \begin{bmatrix} -k_1 & 0 & 0 \\ k_1 & -k_2 & 0 \\ 0 & k_2 & 0 \end{bmatrix} \text{ and initial conditions represented as } A(0) = \begin{bmatrix} P_0 \\ 0 \\ 0 \end{bmatrix}$$

To find the eigenvalues  $\lambda$  of the matrix, we will need to determine the values of  $\lambda$  for which the determinant of  $(\mathbf{B}-\lambda\mathbf{I})$  is equal to zero.

$$\det(\mathbf{B} - \lambda\mathbf{I}) = \det \begin{bmatrix} -k_1 - \lambda & 0 & 0 \\ k_1 & -k_2 - \lambda & 0 \\ 0 & k_2 & 0 - \lambda \end{bmatrix} = 0$$

The determinant of the matrix reduces to:

$$(-k_1 - \lambda)(-k_2 - \lambda)(0 - \lambda) = 0$$

Setting each of these factors to zero gives us 3  $\lambda$  values (eigenvalues):

$$(-k_1 - \lambda) = 0 \quad \lambda_1 = -k_1$$

$$(-k_2 - \lambda) = 0 \quad \lambda_2 = -k_2$$

$$(0 - \lambda) = 0 \quad \lambda_3 = 0$$

Now we must find the corresponding eigenvector for each eigenvalue by substituting each eigenvalue into  $\mathbf{B} - \lambda_n\mathbf{I}$  and solving for the vector that satisfies:  $[\mathbf{B} - \lambda_n\mathbf{I}][\mathbf{v}] = 0$

For  $\lambda_1 = -k_1$ :

$$\begin{bmatrix} -k_1 - (-k_1) & 0 & 0 \\ k_1 & -k_2 - (-k_1) & 0 \\ 0 & k_2 & 0 - (-k_1) \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = 0$$

which is equivalent to:

$$(0)v_1 + (0)v_2 + (0)v_3 = 0$$

$$(k_1)v_1 + (-k_2 + k_1)v_2 + (0)v_3 = 0$$

$$(0)v_1 + k_2v_2 + k_1v_3 = 0$$

The first equation tells us that  $v_1$  can be any scalar number so we can set that to 1 to for convenience to find  $v_2$  and  $v_3$ . We can rearrange the second and third equation to be:

$$v_2 = \frac{(-k_1)v_1}{(k_1 - k_2)}$$

$$v_3 = \frac{(-k_2)v_2}{k_1}$$

Substituting  $v_1 = 1$ , we get  $v_2 = \frac{(-k_1)}{(k_1 - k_2)}$  and  $v_3 = \frac{(k_2)}{(k_1 - k_2)}$  so our eigenvector for  $\lambda_1 = -k_1$  is:

$$\mathbf{v}_1 = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = \begin{bmatrix} 1 \\ \frac{(-k_1)}{(k_1 - k_2)} \\ \frac{(k_2)}{(k_1 - k_2)} \end{bmatrix}$$

Repeating the same process to find the corresponding eigenvector for  $\lambda_2 = -k_2$ , we get the following relationships:

$$(-k_1 + k_2)v_1 = 0 \quad \text{Therefore } v_1 = 0$$

$$k_1v_1 + 0v_2 = 0 \quad \text{Since } v_1 = 0, v_2 \text{ can be any scalar number so we choose 1 for convenience}$$

$$v_3 = -v_2 \quad \text{Since } v_2 = 1, v_3 = -1$$

So our eigenvector for  $\lambda_2 = -k_2$  is:

$$\mathbf{v}_2 = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = \begin{bmatrix} 0 \\ 1 \\ -1 \end{bmatrix}$$

Repeating the process once more, we can find the corresponding eigenvector for  $\lambda_3 = 0$ . We get the following relationships:

$$-k_1v_1 = 0 \quad \text{Therefore } v_1 = 0$$

$$k_1v_1 - k_2v_2 = 0 \quad \text{Since } v_1 = 0, v_2 \text{ must also} = 0$$

$$k_2v_2 + (0)v_3 = 0 \quad \text{Since } v_2 = 0, v_3 \text{ can be any scalar number so we choose 1 for convenience}$$

So our eigenvector for  $\lambda_3 = 0$  is:

$$\mathbf{v}_3 = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}$$

Now that we have eigenvalues with their eigenvectors, we can write the general solution of  $\frac{d\mathbf{A}}{dt} = \mathbf{B} \cdot \mathbf{A}$  as the superposition of eigenmodes:

$$\mathbf{A} = c_1 e^{\lambda_1 t} \mathbf{v}_1 + c_2 e^{\lambda_2 t} \mathbf{v}_2 + c_3 e^{\lambda_3 t} \mathbf{v}_3$$

We can then solve for the constants by setting the equation to the initial conditions:  $\mathbf{A}(0) = \begin{bmatrix} P_0 \\ 0 \\ 0 \end{bmatrix}$

and we get  $c_1 = P_0$ ,  $c_2 = P_0 \left( \frac{k_1}{k_1 - k_2} \right)$ , and  $c_3 = -P_0 \left( \frac{k_2 - k_1}{k_1 - k_2} \right)$ .

Substituting our constants back into our equation, the final solutions to the system of differential equations can be reduced to:

$$P(t) = P_0 e^{-k_1 t}$$

$$C(t) = P_0 \left( \frac{k_1}{k_2 - k_1} \right) (e^{-k_1 t} - e^{-k_2 t})$$

$$M(t) = \frac{P_0 (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) - P_0 (k_2 - k_1)}{k_1 - k_2}$$

We used MATLAB to plot these solutions for  $t=10^5$  seconds for comparison to our numerical validation done below:

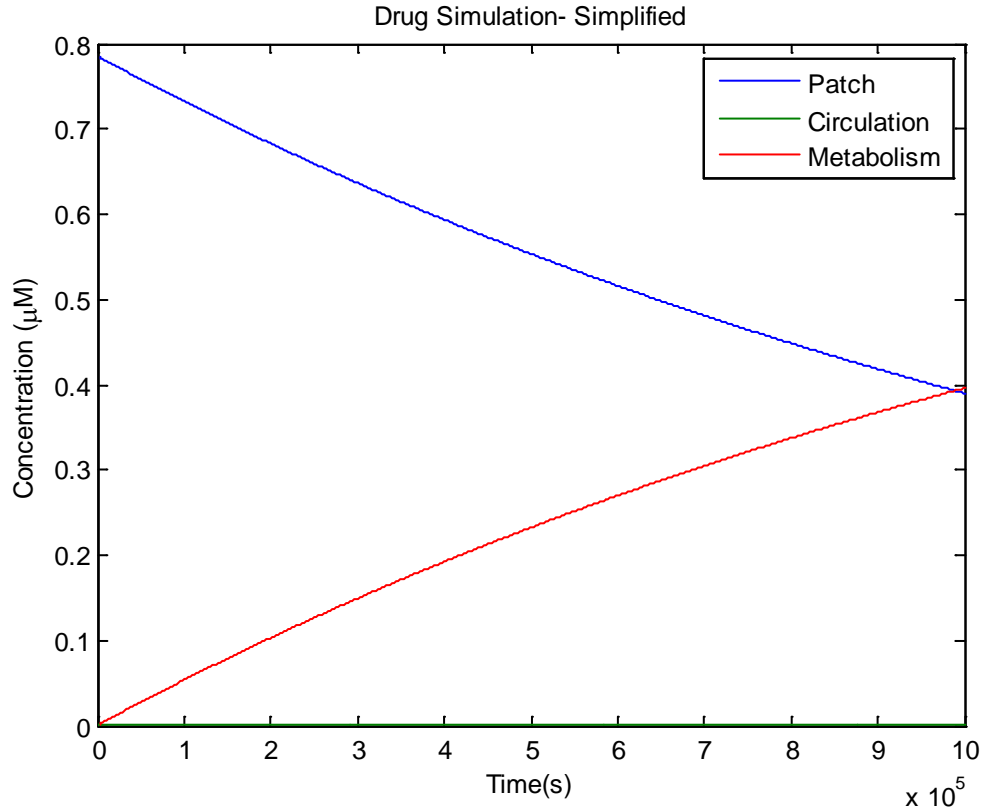


Figure 3. Plot of analytical solutions showing concentration of drug ( $\mu\text{M}$ ) vs. time (s) in the patch (blue), in circulation (green), and being metabolized at the tumor.

### Numerical Validation

The following MATLAB code was used to generate an approximate solution to the unsimplified model.

```
function Drug_Simulation
    format long
    format compact

    % Initial conditions
    t0 = 0;
    tf = 100;
    y0 = [100; 0; 0];

    % Function call
    [t y] = ode45(@yprime,[t0 tf],y0);

    figure
    plot(t, y);
    title('Drug Simulation');
    xlabel('Time (s)');
    ylabel('Concentration (\muM)');
    legend('Uptake', 'Circulation', 'Metabolism')

function dydt = yprime(t,y)
    % Drug transfer coefficients
    k1 = .05;
    Vmax = 1.5;
    Km = 74.8;

    dydt = [-k1*y(1)
            k1*y(1) - Vmax*y(2)/(Km + y(2))
            Vmax*y(2)/(Km + y(2))];
```

The code makes use of another function to insert the system of ODEs into a vector `dydt` to enhance organization and readability. Additionally, we chose to use the `ode45` ODE solver instead of `ode23`. `ode45` is a higher order Runge-Kutta implementation and was recommended by the MATLAB documentation for use before using `ode23`.

Parameters for diffusion, the Michaelis-Menten kinetics, and the initial condition were derived from literature. Using cells obtained from human cervix squamous cell carcinoma (SiHa cells), Kyle and Minchinton determined the diffusion of tirapazamine in these cells to be  $7 \times 10^{-7} \text{ cm}^2/\text{s}$ , and the  $V_{\text{max}}$  of metabolism within these hypoxic cells to be  $1.5 \times 10^{-6} \text{ M/s}$ .<sup>5</sup> In a related study, Wang, et al. determined  $K_m$  for tirapazamine to be  $74.8 \times 10^{-6} \text{ M}$  using a double-reciprocal plot.<sup>6</sup> For modeling purposes, we considered the diffusion through these SiHa cells to be approximately equal to that of skin cells where a transdermal patch might be applied. Additionally, we assumed that distribution to the second compartment, the circulation, was uniform and occurred immediately after diffusion through skin cells. For the initial condition, a Phase III trial of tirapazamine did not provide conclusive data as to the most efficacious dose.<sup>7</sup> Instead, we used the 14 mg nicotine content of NicoDerm® CQ® patches were used as a reference value. As a starting point, we also considered our transdermal patch to have an area of  $1 \text{ cm}^2$ , a thickness of  $0.1 \text{ cm}$ , and that it contained a liquid reservoir of tirapazamine in solution within these dimensions. From these data, and using the molecular weight of tirapazamine, we calculated the initial concentration in the patch to be  $C_0 = 0.786 \text{ mM}$ .

Substituting the parameters and initial condition in our code, we obtained the following plot.

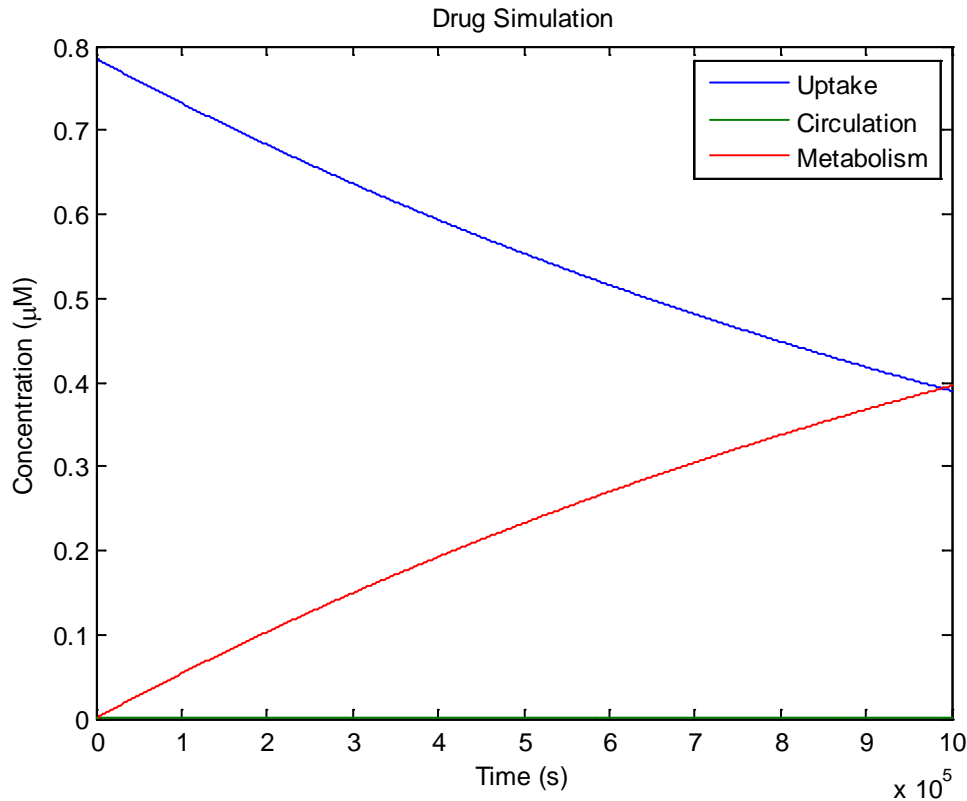


Figure 4. Plot of numerical approximation to the model. As with the plot for the analytical solution, blue represents uptake, green represents circulation, and red represents metabolism.

This plot and the plot of the analytical solution are qualitatively identical, which indicates that the approximation of the Michaelis-Menten term in the circulation and metabolism equations as a first-order rate constant was appropriate. Note that the blue curve, uptake, diminishes to approximately half the initial value in slightly over 11 days, demonstrating the diffusion-mediated release of tirapazamine over a long time course. The red curve rises concurrently as the drug is metabolized into its active form. The green curve is present just above the x-axis. Its value is not zero; the tirapazamine concentration in the circulation is instead very low at all times due to the high

rate of metabolism in comparison to the diffusion of the drug from the patch. Thus, using the values from the literature, tirapazamine prodrug is metabolized into its active form at a much higher rate than it is diffused into the blood.

As a pedagogical example, we use our model to illustrate the case where diffusion and metabolism operate on a more similar order of magnitude.

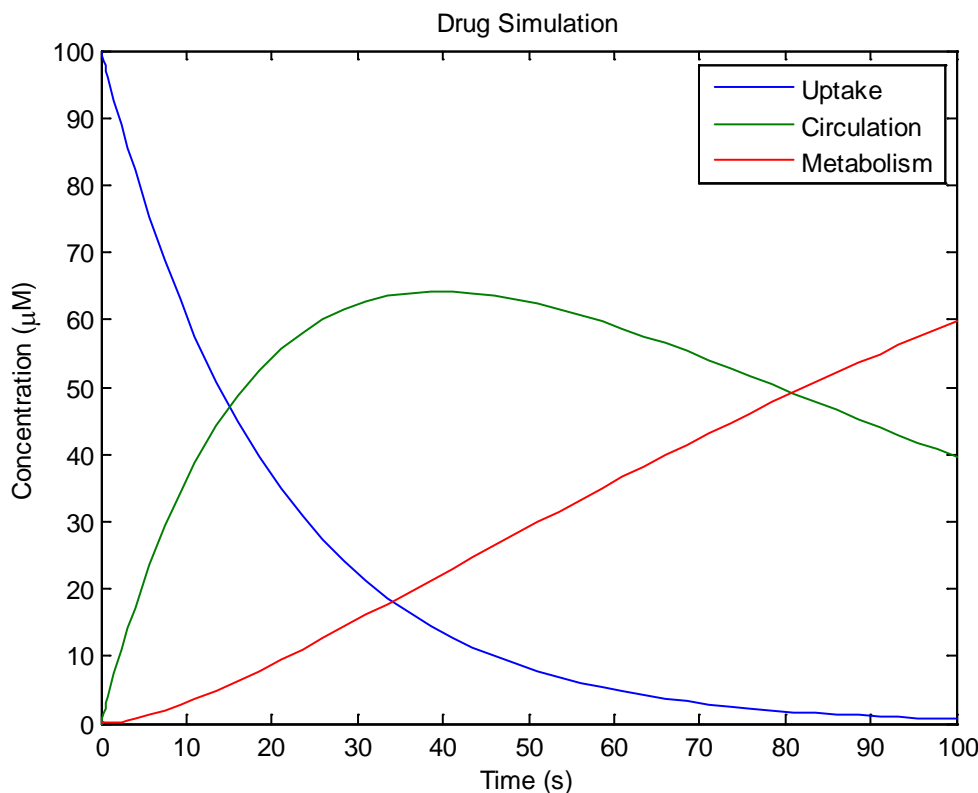


Figure 5. Plot of hypothetical drug with comparable rates for diffusion and metabolism.

In this case, the drug concentration in the circulation is shown to reach a clear  $C_{\max}$  before tapering down as the initial concentration of the drug is exhausted and metabolized. These dynamics are consistent with pharmacokinetic expectations for some drugs.

## **Conclusions**

Due to congruence of the drug concentration plots for the numerical and analytical cases, we conclude that the approximation of the Michaelis-Menten metabolism term as a first-order rate constant was an appropriate choice.

It is worth noting that the numerical validation conducted here was done only for a single drug and a single delivery method. In fact, our model is quite versatile: by altering the relevant parameters, we can conduct *in silico* pharmacokinetic studies on drug delivery and drug metabolism for a number of other compounds. And specifically, we can compare the concentration characteristics for different drug delivery methods by altering the  $k_1$  constant. We only examined the transdermal patch case here, but we can adapt the  $k_1$  value to examine the case of continuous drug infusion, an IV bolus dose, a drug taken orally, etc.

Furthermore, and specifically suited to a bioengineering application, we can adjust our “virtual patch” dimensions and drug content by altering the  $C_0$  value. This would allow us to compare the pharmacokinetic characteristics of various patch sizes and doses to compare their efficacy.

Like many other mathematical models in biology, our model helps demonstrate the power and versatility of *in silico* modeling techniques. With careful design, they can save time and expense and be useful tools in bioengineering and medicine.

## **References**

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