CASE STUDY 1a Kill Curves and Antibiotic Effectiveness

We are studying the relationship between the magnitude of antibiotic treatment and the effectiveness of the treatment. Recall that the extent of bacterial killing by an antibiotic is determined by both the *antibiotic concentration profile* and the *dose response relationship*. Figures 1 and 2 show these plots for the antibiotic ciprofloxacin when used against *E. coli*.[1]

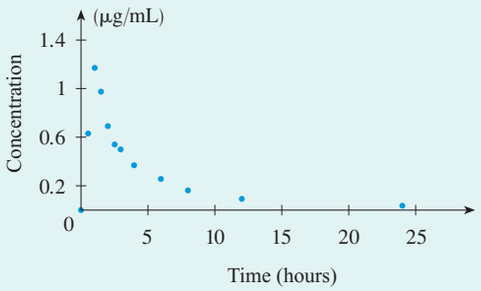

FIGURE 1
Antibiotic concentration profile in plasma of a healthy human volunteer after receiving 500 mg of ciprofloxacin

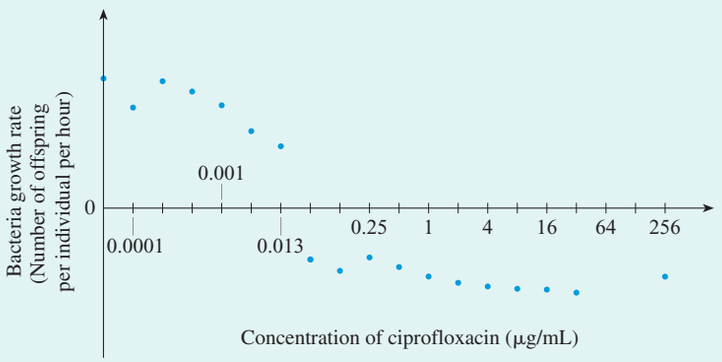

FIGURE 2
Dose response relationship for ciprofloxacin with the bacteria *E. coli*

Now, in the words of Picasso, we are viewing mathematical models as "lies that reveal truth." In other words, we don't expect our mathematical model to capture every detail of the biological system; rather, we simply want it to capture the most important features. To this end, let's describe the main patterns seen in Figures 1 and 2 mathematically.

Figure 1 shows that the antibiotic concentration increases extremely quickly, followed by a slow decay. To simplify matters let's therefore suppose that it increases instantly from zero to the peak concentration at time $t = 0$, and it then decays. As we will see in Case Study 1b, the decay can be well modeled using the exponential decay function

$$c(t) = c_0 e^{-kt} \tag{1}$$

where c_0 is the concentration at $t = 0$ and k is a positive constant. Equation 1 is plotted in Figure 3.

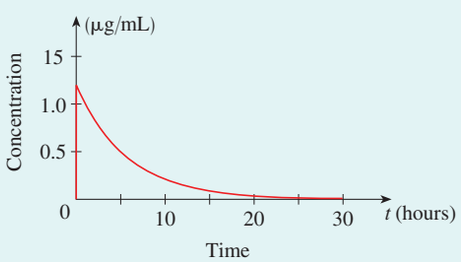

FIGURE 3
Drug concentration profile modeled by the function $c(t) = c_0 e^{-kt}$ with $c_0 = 1.2$ μg/mL and $k = 0.175$

1. Adapted from S. Imre et al., "Validation of an HPLC Method for the Determination of Ciprofloxacin in Human Plasma," *Journal of Pharmaceutical and Biomedical Analysis* 33 (2003): 125–30.

In Figure 2 it looks like the dose response relationship doesn't vary much up to a concentration of around 0.013 μg/mL. It then drops suddenly to a low value and remains relatively constant as the antibiotic concentration increases further. To simplify matters, let's model the dose response relationship by the piecewise defined function

$$r(c) = \begin{cases} r_2 & \text{if } c < MIC \\ r_1 & \text{if } c \geqslant MIC \end{cases}$$

where MIC is a constant that is referred to as the *minimum inhibitory concentration* ($MIC = 0.013$ μg/mL in this case), r_1 and r_2 are constants giving the bacteria population growth rate under high and low antibiotic concentration, respectively, and $r_1 < 0$ and $r_2 > 0$. This function is plotted in Figure 4.[2]

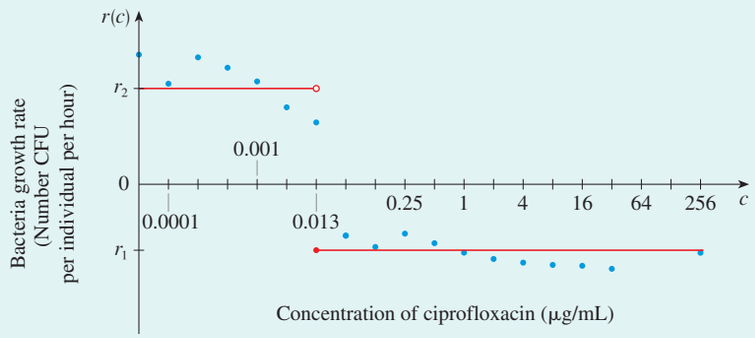

FIGURE 4
Dose response relationship modeled by the piecewise defined function $r(c)$

The functions in Figures 3 and 4 will, together, determine how the bacteria population size changes over time. At $t = 0$ the antibiotic concentration is c_0, and if c_0 is greater than $MIC = 0.013$ μg/mL, then the bacteria population size will decline. At the same time the antibiotic concentration will decay as time passes, eventually reaching a value of $MIC = 0.013$ μg/mL. At this point the growth rate of the bacteria population becomes positive.

In Case Study 1b you will show that, using the functions in Figures 3 and 4, a suitable model for the size of the bacteria population $P(t)$ (in CFU/mL) as a function of time t (in hours) is given by the piecewise defined function

(2a)
$$P(t) = \begin{cases} 6e^{t/3} & \text{if } t < 2.08 \\ 12 & \text{if } t \geqslant 2.08 \end{cases}$$

if $c_0 < 0.013$, and

(2b)
$$P(t) = \begin{cases} 6e^{-t/20} & \text{if } t < a \\ 6Ae^{t/3} & \text{if } a \leqslant t < b \\ 12 & \text{if } t \geqslant b \end{cases}$$

if $c_0 \geqslant 0.013$, where the constants a, b, and A are defined by $a = 5.7 \ln(77c_0)$, $b = 6.6 \ln(77c_0) + 2.08$, and $A = (77c_0)^{-2.2}$.

2. Adapted from W. Bär et al., "Rapid Method for Detection of Minimal Bactericidal Concentration of Antibiotics," *Journal of Microbiological Methods* 77 (2009): 85–89, Figure 1.

1. Plot $P(t)$ as a function of time for each of the concentrations $c_0 = 0$, 0.019, 0.038, 0.075, 0.15, 0.3, 0.6, 1.2. These are the kill curves predicted by the model. Comment on the similarities and differences between the predicted curves and those from the data in Figure 5.[3]

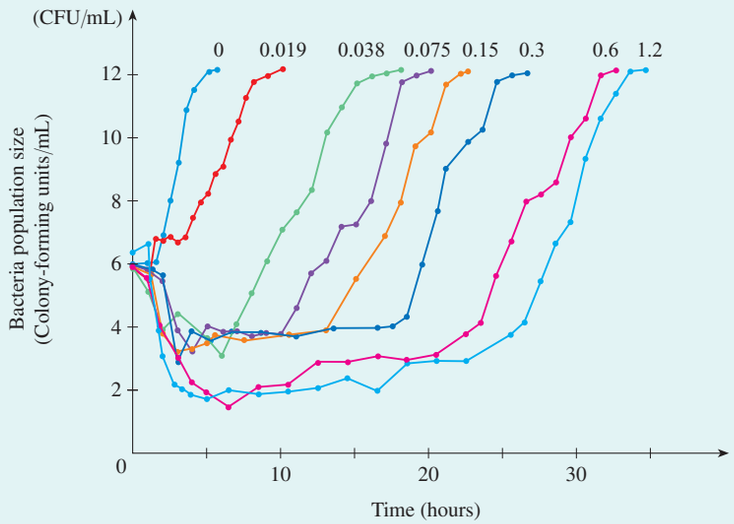

FIGURE 5
The kill curves of ciprofloxacin for *E. coli* when measured in a growth chamber. The concentration of ciprofloxacin at $t = 0$ is indicated above each curve (in $\mu g/mL$).

Our goal is to summarize the model kill curves from Problem 1 in a simpler form in order to see more clearly the relationship between the magnitude of antibiotic treatment and its predicted effectiveness.

To do this, we need to obtain a measure of the magnitude of antibiotic treatment as well as a measure of its effectiveness. We first obtain a measure of the magnitude of antibiotic treatment from the antibiotic concentration profiles that underlie each predicted kill curve. Three measures are commonly used: (1) the peak antibiotic concentration divided by *MIC*, denoted by ρ; (2) the duration of time for which the antibiotic concentration remains above *MIC*, denoted by τ; and (3) the area under the antibiotic concentration profile divided by *MIC*, denoted by α. These measures are illustrated graphically in Figure 6.

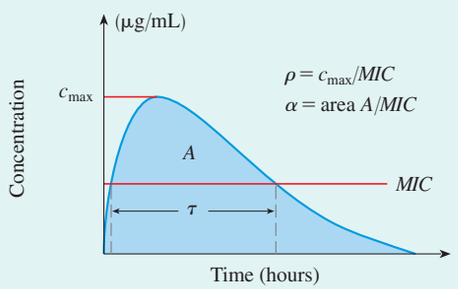

FIGURE 6
Three measures ρ, α, and τ of the magnitude of antibiotic treatment

3. Adapted from A. Firsov et al., "Parameters of Bacterial Killing and Regrowth Kinetics and Antimicrobial Effect Examined in Terms of Area under the Concentration-Time Curve Relationships: Action of Ciprofloxacin against *Escherichia coli* in an In Vitro Dynamic Model." *Antimicrobial Agents and Chemotherapy* 41 (1997): 1281–87.

2. Find expressions for ρ and τ in terms of k, c_0, and MIC, using Equation 1 for the antibiotic concentration profile.

3. In Case Study 1c you will show that $\alpha = \dfrac{1}{k}\dfrac{c_0}{MIC}$. Sketch graphs of ρ, τ, and α as functions of c_0, using the values $k = 0.175$ (1/hours) and $MIC = 0.013$ μg/mL. What are their units?

You will notice from Problem 3 that, for a given antibiotic and bacterial species (in other words, for a given value of k and MIC), all three quantities ρ, τ, and α increase with one another. For example, it is not possible to have a high value of α without also having high values of ρ and τ. Therefore, since these measures are not independent of one another, we need to consider only one of them. We will focus the remainder of our study on α, since it is the most commonly used.

Next we need to quantify the effectiveness of the antibiotic by quantifying different properties of the kill curves. Let's consider two possibilities: (i) the time taken to reduce the bacteria population size to 90% of its initial size, denoted by T, and (ii) the drop in population size before the population rebound occurs, denoted by Δ. Both measures are shown in Figure 7.

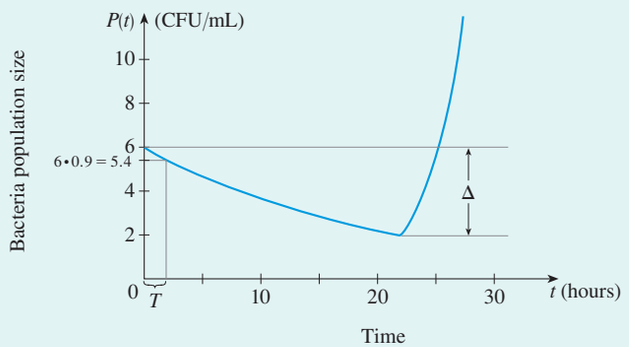

FIGURE 7
Two measures T and Δ of antibiotic effectiveness

4. Find expressions for Δ and T in the modeled populations in terms of c_0.

Our final goal is to use the results from Problem 4, along with the expression for α, to plot Δ against α and to plot T against α as well. This will give us the model's predictions for the plots in Figures 5 and 6 in Case Study 1 on page xlii.

5. Substitute the values $k = 0.175$ and $MIC = 0.013$ into the expression for α. This expression, along with the results from Problem 4, should give you functions of the form $T = f(c_0)$, $\Delta = g(c_0)$, and $\alpha = h(c_0)$ for some functions f, g, and h. [*Note:* Some of the functions might actually be independent of c_0.]

6. Using the concept of inverse functions, explain how to obtain a function that gives Δ as a function of α in terms of g and h^{-1}. Find an explicit expression for this function.

7. What is T as a function of α?

8. Plot the functions obtained in Problems 6 and 7. You should obtain the curves shown in Figures 8 and 9.

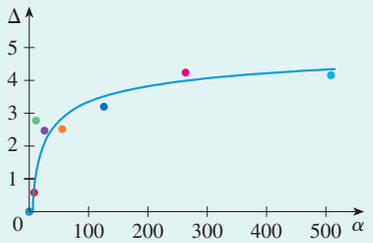

FIGURE 8
Predicted relationship between Δ and α, along with the observations obtained using the kill curve data in Figure 5

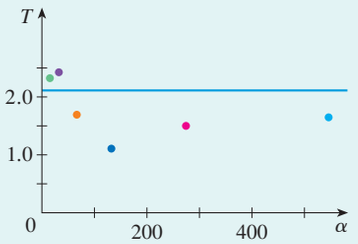

FIGURE 9
Predicted relationship between T and α, along with the observations obtained using the kill curve data in Figure 5

9. From Figures 8 and 9 you can see that this relatively simple model predicts the observed data reasonably well. In particular, T is predicted to be independent of the magnitude of antibiotic treatment, whereas Δ increases with it. Provide a biological explanation in terms of the model for why this occurs. [*Hint:* Relate the fact that T is predicted to be independent of α to the form of the kill curves from Problem 1 for different antibiotic doses.]