A SOLUTION TO THE SPECTROPHOTOMETRY HOMEWORK PROBLEM

1. Preliminary examination of the 5 μl and 50 μl samples from both GEX and GM2 suggests that something in the bacteria preparation is inhibiting or interfering with the assay. (The activity in the 50 μl sample is clearly not 10-fold higher than the 5 μl sample.) A potential problem with the 5 μl sample is that the GM2 sample is not much above the blank. This could introduce a larger error term in the calculations. It is important to use the same volume (i.e., conditions) for both samples. I chose the 50 μl samples, although the 5 μl samples are probably more valid since there appears to be an inhibition of activity associated with increasing numbers of bacteria.

2. Examination of the graph of the 50 μl GEX sample indicates that the activity is not linear. This is the result of the substrate being depleted during the assay and a slowing of the reaction rate. (The initial rate of enzyme activity depends on substrate concentration.) Therefore it is necessary to determine the initial slope (eg., first 0.5 min) of this sample.

3. Calculate the ΔA from the data table (or from the graph):

   ΔA_{blank} per 5 min = 0.026 - 0.005 = **0.021**

   ΔA_{GEX} per 0.5 min = 0.22 - 0.117 = 0.103 (as per step 2)

   ΔA_{GEX} per 5 min = 0.103 x 10 = **1.03**

   ΔA_{GM2} per 5 min = 0.494 - 0.103 = **0.391**

4. Using Beer's law ($A = \varepsilon dc$) and plugging in the known values generates the following equation:

$$\text{activity}^* = \frac{(\Delta A_{sample} - \Delta A_{blank})(0.001 \text{ liter})(10^6 \text{ μmole/mole})}{(9600 \text{ liter/mole·cm})(1 \text{ cm})(5 \text{ min})(0.05 \text{ ml})}$$

OR

$$\text{activity} = (\Delta A_{sample} - 0.021) \times 0.417 \text{ μmole/min·ml}$$

(Note there are many ways to solve this problem and it is not necessary to develop such an equation. If the same assay is done routinely, though, such equations are quite convenient.)

5. Plug in the ΔA values from step 3 into the equation from step 4:

   GEX contains **0.42 units** of activity and

   GM2 contains **0.15 units** of activity

   where a unit of activity equal the μmoles of glutathione transferred per min per ml of bacteria suspension

6. Using the conversion factor, the bacteria suspension contain 5 x 10^8 bacteria/ml. To convert the above activity to units per 10^6 bacteria, divide by 5 x 10^8 and multiply by 10^6 (i.e., divide by 500). The activity is now:

   GEX contains **8.4×10^{-4} units** of activity and

   GM2 contains **3×10^{-4} units** of activity

   where a unit of activity is μmoles of glutathione transferred per min per million bacteria

*Explanation of values in equation # 3:

ΔA_{sample}	is the change in absorption of the samples being measured for enzyme activity
ΔA_{blank}	represents the spontaneous formation of product and needs to be subtracted from all of the samples
0.001 liter	is the volume in the cuvette expressed in same units as extinction coefficient
9600 liter/mole·cm	is the molar extinction coefficient used to convert absorption vales to concentration values
1 cm	is the thickness of the cuvette (standard size and usually ignored in all calculations)
5 min	is the time interval over which the ΔA is measured. This value must match all of the samples and blanks. If the time intervals are not the same for all of the samples and blanks, then a ΔA/min must be determined for each sample before proceeding with the remainder of the calculation.
0.05 ml	is the volume of sample measured. (Typically the equation is also divided by the protein concentration.)