BSC 2010L

ENZYME KINETICS

How to Calculate Reaction Rates

Figures: You will construct four graphs for this exercise, each one drawn separately on a full sheet of graph paper. Follow all of the formatting guidelines in Appendix A of your lab manual. Make at least rough drafts of the first two figures and bring them to lab with you next week for review by your instructor

1. **Asborbance vs. Time**: *Temperature* (similar to example of manual page 41).
2. **Asborbance vs. Time**: *pH*.
3. **Reaction Rate vs. Temperature** (similar to example on manual page 38).
4. **Reaction Rate vs. pH**.

Be meticulous! You may have to redraw each figure more than once to get the best figure possible.

Calculation of Enzyme Kinetics Reaction Rates

1. You can not calculate a reaction rate until you have plotted your raw data and drawn best fit curves for each set of data (Figures 1 and 2).
2. Once you have drawn a best fit curve for each set of data, use **the curve** to calculate the values for the *Reaction Rate Calculation Sheet* – NOT the raw data.
3. Follow this example for a hypothetical set of data from the temperature experiment. Only one temperature is included in this example – you will have to do this for all six temperatures and put them on the same graph (your Figure 1). . You will perform similar calculations with the pH data as well (your Figure 2).

Table 5-3. Absorbance readings over time of reactions at different temperatures.

	TIME (min)										
Temp.(°C)	0	1	2	3	4	5	6	7	8	10	20
30	2.4	2.3	2.2	2.1	1.6	1.5	1.2	1.1	1.0	0.6	0.4

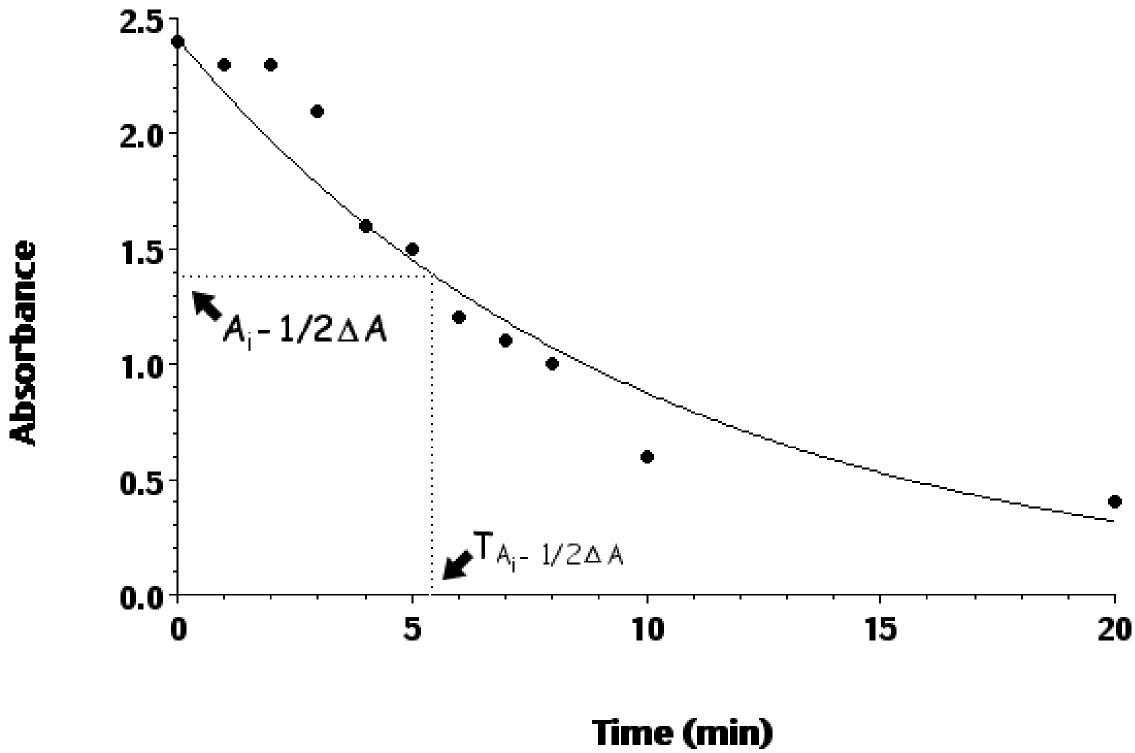
4. Reaction rate is calculated as half of the total change in absorbance divided by the time it took this change to occur, or:

$$\text{Reaction Rate} = \frac{1/2\Delta \text{ Absorbance}}{\text{Time that } A_i\text{-}1/2\Delta \text{ Absorbance Took}}$$

5. Variables used in the reaction rate calculation are as follows:

A_i	your initial absorbance at t = 0 minutes
A_f	your final absorbance at t = 20 minutes
ΔA	$A_i - A_f$
$1/2\ \Delta A$	divide ΔA by 2
$A_i - 1/2\ \Delta A$	perform this subtraction, then find the resulting value on the Y-axis of your graph. Draw a horizontal dotted line to your best-fit line, then drop another dotted line vertically to the X-axis.
$T_{A_i-1/2\Delta A}$	This is the time indicated by the vertical dotted line.

6. Here is what the figure would look like for these data:



7. Using the figure above, you can fill in the table in the *Reaction Rate Calculation Sheet*as follows:

				*		†	
Temp.(°C)	A_i	A_f	ΔA	1/2ΔA	A_i-1/2ΔA	$T_{A_i-1/2\Delta A}$	R.r. (*/†)
30	2.4	0.4	2.0	1.0	1.4	5.3	0.19

Ai = 2.4 Af = 0.4 ΔA = 2.4 – 0.4 = 2.0 1/2 ΔA = 2.0/2 = 1.0

A_i–1/2ΔA = 2.4–1.0 = 1.4 So, find 1.4 on your Y-axis and draw a horizontal line to your best fit line or curve.

$T_{A_i-1/2\Delta A}$ This will be the value on the X-axis indicated by the vertical dotted line.

8. The $T_{A_i-1/2\Delta A}$ for this curve is 5.3. You can no calculate the reaction rate for this data set with the values in the columns marked "*" and "†" on the *Reaction Rate Calculation Sheet*
9. Reaction rate = 1/2ΔA / $T_{A_i-1/2\Delta A}$ = 1.0 / 5.3 min. = 0.19 min^{-1}.

DO THESE CALCULATIONS FOR EVERY TEMPERATURE. YOU WILL HAVE SIX BEST-FIT CURVES ON ONE GRAPH WITH SIX SETS OF CALCULATIONS TO FIND 1/2ΔA AND $T_{A_i-1/2\Delta A}$. Consider using colored pencils so that you can distinguish between each line!

Plot Reaction Rates vs. Tempratures (your Figure 3)

Draw a line through all of the points as on example on p. 38. This is NOT a best-fit curve. Locate the optimum temperature (the highest point on the curve).

Make a similar figure for Reaction rate vs. pH (your Fgure 4).