

**Six: Conclusions are formed based on recorded data, impacts of experimental error inherent in experimental design are identified and discussed, and validity of data is determined...**

**Answer  
(written) data  
analysis  
questions**

Answer the questions. Students can work on this in groups and then discuss:

1. What is the chemical reaction that is catalyzed by the enzyme catalase?
2. Look at the times recorded on the class spreadsheet for one of the peroxide concentrations. Tell which concentration you picked, and describe the data in words. Your description should include a discussion of the means, range and variance of the data.
3. Discuss sources of experimental error that could account for some of the variance. Explain how these could be minimized.
4. Based on an examination of your graph, state (in words) the relationship between concentration of substrate and the time it takes for the discs to rise. Why is the graph a curve rather than a straight line?
5. State the relationship between concentration of substrate and rate of the decomposition reaction. How does this relate to your initial hypothesis?
6. How does this experiment relate to what happens in your body?
7. Theoretically, how does the conformation (structure) of the enzyme catalase affect its function?
8. What factors might change the conformation of the catalase and therefore alter the activity of the catalase?
9. How could this experiment be improved?
10. What questions for further research are raised by this experiment?

## Purification Pathways

Let's use the example of a protein enzyme

<ul style="list-style-type: none"> <li>• Determine a rich source of the enzyme activity based on units of product formed per total protein (depending on the type of catalyst under study). This means you run an enzyme assay and determine the total protein in the sample you assayed.</li> </ul>	
<ul style="list-style-type: none"> <li>• Specify standard conditions of pH, temperature, salt, substrate concentration and measure the product formed AND the total amount of protein per unit volume.</li> </ul>	
<ol style="list-style-type: none"> <li>1. Homogenize tissue in hypotonic buffer (to burst, lyse) the cells.</li> </ol>	
<ol style="list-style-type: none"> <li>2. Add increasing increments of ammonium sulfate salt. Different proteins precipitate out at different concentrations of this salt. At each salt level, centrifuge a portion of the mixture and test the liquid supernatant AND redissolve the precipitate and assay a portion of that fraction. Determine protein concentrations of each fraction to locate the highest concentration of enzyme activity per unit protein.</li> </ol>	
<ol style="list-style-type: none"> <li>3. Repeat the ammonium sulfate fractionation to recover the portion with the best enzyme activity. Redissolve this material in buffer. At this point, the researcher can take one of several paths, to separate the protein based on its charge, its size or specific other properties, like DNA or antibody binding.</li> </ol>	
<ol style="list-style-type: none"> <li>4. Apply the partially purified enzyme preparation to several sizing columns for proteins above 10,000, 50,000, 100,000 MW. Collect fractions and assay each AND determine protein concentration of each. Locate the fractions with the highest enzyme activity per unit protein.</li> </ol>	
<ol style="list-style-type: none"> <li>5. Apply the pooled fractions to a charge (other property) column and assay each fraction.</li> </ol>	
<ol style="list-style-type: none"> <li>6. Examine high activity samples of each step (1-5) by protein gel electrophoresis.</li> </ol>	