INTRODUCTION

Enzymes are of fundamental importance in many of the chemical reactions which take place in living organisms. When digestion occurs, enzymes released into the mouth, stomach, and intestines catalyze or accelerate reactions which result in the breakdown of large food molecules into small 'building block' molecules.

For example:

- salivary amylase: starch --> maltose (a disaccharide)
- gastric pepsin: protein --> smaller peptides
- pancreatic chymotrypsin: protein --> smaller peptides
- pancreatic lipase: fats --> fatty acids

Enzymes are protein molecules. The molecules upon which an enzyme acts are called the substrates. Any environmental conditions which destroy protein molecules will also abolish enzymatic activity. For example, when a chicken egg is cooked the color and consistency of the white and yolk change.

The rate of the reaction (or enzyme activity) can be changed. The most effective way to change enzyme activity is to alter the concentration of substrates, products, or enzymes themselves. The relationship between substrates, enzymes and products can be represented by the equation:

```
ENZYME
SUBSTRATE ------------------> PRODUCT
```

The enzyme promotes conversion of the substrate into the product, but is not used up during the reaction.

**Salivary amylase:** Approximately one liter of saliva is secreted into the human mouth each day by three pairs of salivary glands. Saliva contains many enzymes, including salivary amylase, an enzyme which catalyzes the breakdown of starch (a polysaccharide) into smaller molecules as follows:

```
POLYSACCHARIDE + SALIVARY AMYLASE --->
MALTOSE + SALIVARY AMYLASE + smaller fragments of the polysaccharide
```

The substrate for amylase is starch, a polysaccharide composed of amylose + amylopectin. The product of the amylase reaction is maltose, a disaccharide (made from two glucose molecules).
TEACHER REFERENCE PAGES - AMYLASE ACTIVITY LAB

EXPERIMENTAL GOALS

Each of the following experiments can be done separately to study a different aspect of enzyme activity. In this experiment you will do the following:
A. Establish a method to measure the amount of maltose produced.
B. Determine the effect of different amounts of enzyme on the rate of reaction.
C. Determine the effect of different amounts of substrate on the rate of the reactions.
D. Determine the effect of pH and temperature on the rate of the reaction

EQUIPMENT

2 UV-Vis Spectrophotometer
2 Water bath
4 Vortex mixers
4 Pipet pump dispensers

SUPPLIES (for 2 lab groups each/maximum of eight groups)

PART A-MALTOSE STANDARDS
10 pyrex test tubes (1.5 X 13 cm)
2 test tube racks
2 Styrofoam cups with ice
2 200-1000 μL pipettors with tips
2 50 mL beakers (for standard maltose solutions, on ice)
  maltose solution
  dns solution
  amylopectin solution
  NaK Tartrate solution
  DI Water

PART B-AMYLASE CONCENTRATION
10 pyrex test tubes (1.5 X 13 cm)
2 test tube racks
2 Styrofoam cups with ice
2 200-1000 μL pipettors with tips
1 40-200 μL pipettors with tips
2 50 mL beakers (for amylase solution, on ice)
  amylase solution
  dns solution
  amylopectin solution
  NaK Tartrate solution
  DI Water

June 2011
PART C-AMYLOPECTIN (SUBSTRATE) CONCENTRATION
10 pyrex test tubes (1.5 X 13 cm)
2 test tube racks
2 Styrofoam cups with ice
2 200-1000 µL pipettors with tips
1 40-200 µL pipettors with tips
2 50 mL beakers (for amylpectin solution, on ice)
  amylase solution
  dns solution
  amylpectin solution
  NaK Tartrate solution
  DI Water

PART D-pH, TEMPERATURE EFFECTS
10 pyrex test tubes (1.5 X 13 cm)
2 test tube racks
2 Styrofoam cups with ice
2 200-1000 µL pipettors with tips
3 50 mL beakers
  pH 4 Citrate buffer
  pH 7 Phosphate buffer
  pH 10 Borate buffer
  amylase solution
  dns solution
  amylpectin solution
  NaK Tartrate solution

NOTE: Test tubes will need to be stored in a refrigerator after the first day of the lab.
SOLUTIONS: All solutions will be provided for van visits, ready to use. The following solutions have an extended shelf-life.

To prepare buffers: Weigh out the appropriate amount of the buffer salt. Dissolve in one half the volume of DI water. Titrate to the desired pH with NaOH or HCl. Dilute to final volume with DI water.

Borate buffer: 0.2 M at pH 10.0
38.1 g Na₂B₄O₇·C₁₀H₂O
500 mL DI water
Titrate with NaOH to pH 10.0. Note: This is very near saturation; adjust the pH to dissolve completely.

Citrate buffer: 0.2 M at pH 4.0
29.41 g sodium citrate
500 mL DI water.
Titrate with HCl to pH 4.0

Phosphate buffer: 0.2 M at pH 7.0
17.4 g Potassium Phosphate (K₂HPO₄) in 500 mL DI water
Titrate with H₃PO₄

Phosphate buffer: 0.02 M at pH 7.0
Dilute 0.2 M buffer.

DNS solution
Saturated DNS (3,5-dinitrosalicylic acid)
1L 2.0 M NaOH

Tartrate solution
500g Na-K tartrate
Add DI water to dilute to 1 L. Note: This is near saturation, and endothermic. Allow to warm to room temperature to dissolve completely.

The following solutions may be made in advance and stored in the refrigerator for up to two weeks.

Potato amyllopectin solution
500 mL 0.02 M PO₄ buffer, pH 7.0
0.2g NaCl
1.7g amyllopectin

Maltose solution (1.0 mg/ml)
500mg maltose
500mL double distilled water

Amylase stock solution
1.0g barley amylase
500mL 0.02 M PO₄ buffer, pH 7.0
UV-Vis Spectrophotometer Operating Instructions

A. Turn on computer, load program.
1. Fold down the paper tray on the printer and add paper to it.

2. Turn on the power box switch, UV-Vis spectrophotometer and the printer. The UV-Vis switch is at the back left corner; the printer switch is at the front left. The computer will self boot and run through self tests; the UV-Vis will also power up and run through self tests. Clicking sounds coming from UV-Vis are normal. Wait until busy light on the UV-Vis goes out.

3. When Windows icons appear, double click on the UV-Vis icon.

4. When the password prompt comes up, click on CANCEL.

5. Once the program is running, click on FILE, then on LOAD METHOD then select the appropriate method for your lab and click ok.
   - Greenhouse: grnhouse.m
   - Amylase: amylase.m
   - Equilibrium: equilib.m
   - Full spectrum: sunglass.m

6. To load the sample table, click on FILE, then on LOAD SAMPLE TABLE then select the appropriate sample table for your lab and click ok.
   - Greenhouse: grnhouse.st
   - Amylase: amylase.st
   - Equilibrium: equilib.st
   - Full spectrum: sunglass.st

B. Scanning samples:

7. At the start of the day, or anytime after you have changed the program, you need to scan a blank. To do this either click on MEASURE then on BLANK, or press F4. When you do this, a blank spectrum window is opened. Simply close the window by clicking on the box with the X in it in the upper right hand corner of the blank window.

8. Fill clean cuvette 3/4 full. Do not touch the clear sides. If there are fingerprints on the clear sides, wipe them off with Kimwipes. Insert the cuvette into the sample holder on the UV-Vis with the clear sides oriented to the light beam (ridges facing front and back). Lock into place gently. Remember to unlock the holder before removing sample.

9. To scan, click on MEASURE, then on SAMPLE, or press F5. Next, click on the ANALYZE button. Sample number and absorbance will appear. Note that the sample numbers are consecutive; they may not match the numbers you have recorded for each sample.
10. Repeat Steps 8 & 9 for each of your remaining samples to record data for each one.

11. Record the absorbance for each sample in the table on your data sheet.

12. To print data, click on FILE, then PRINT, then RESULTS.

C. **Prepare for next lab group:**
13. To remove data and start the next group, click on EDIT, then CLEAR, then SAMPLES.

D. **Shutting down:**

14. To shut down the program click on FILE, then on EXIT CHEMSTATION, or click on the box with the X in it in the upper right hand corner. **MAKE SURE THAT NONE OF THE CHECK BOXES ARE CHECKED.**

15. To shut down the computer click on START (bottom left hand corner), then on SHUT DOWN, then select SHUT DOWN COMPUTER, then click on YES.

16. Turn the UV-Vis off using the same switch you used to turn it on.

17. Once the light on the monitor has turned to amber, turn off the power strip.