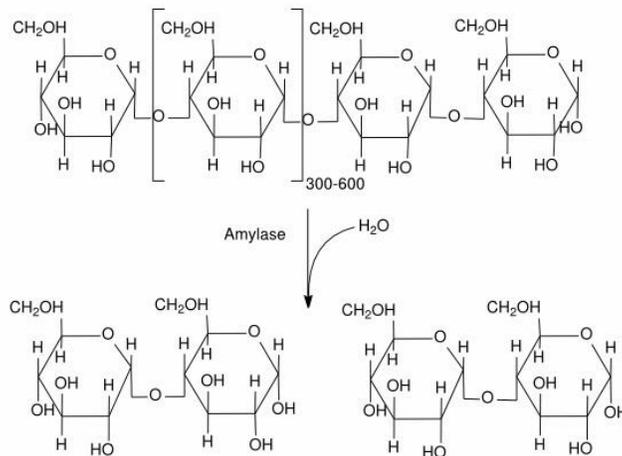


## Effects of Concentration on Enzyme Activity: Amylase

As you recall from reading the section on naming and preparation of enzymes, amylase is an enzyme that breaks down starch to produce smaller molecules called dextrans and an even smaller disaccharide, maltose.



If you haven't read the section on naming and preparing enzymes, please read it now before proceeding with the lab.

When doing enzyme studies researchers can either look for the presence of the enzyme's product or the absence or disappearance of the enzyme's substrate. In this activity we are going to identify the appearance of the product, maltose, a reducing sugar. If you recall from the earlier module on carbohydrates Benedict's reagent can be used to identify the presence of reducing sugars. Benedict's reagent identifies monosaccharides and some disaccharides. The light blue colored reagent is added to the test solution and boiled. If the solution changes color, from light blue to yellow or orange or dark red then reducing sugars are present.

In this activity you will be looking at the effect of enzyme concentration on enzyme activity.

### Materials:

Saltines - crushed  
Test tubes – 5  
Distilled water  
Test tube rack  
Benedict's reagent

Hot plate  
Beaker with boiling beads and water  
Amylase  
Weigh boat  
Balance

1. Pick up 5 test tubes and test tube rack from the supply table.
2. Plug in and turn on hot plate. Half fill beaker with water. Add boiling beads and place on hot plate.
3. Label test tubes, CA, CS, 1, 2, 3, and 4. Label each test tube with the group members' names.
4. Use the balance and weigh boat to weigh 1 gm of crushed saltines. Add the crushed saltines to the test tubes labeled CS. Repeat this procedure and add saltines to test tubes 1, 2, 3 and 4.
5. Add distilled water to test tubes CS, 1, 2, 3 and 4 until the water just covers the surface of the saltines.

6. Add distilled water to the test tube labeled CA to the same level as the level in test tubes CS, 1, 2, 3 and 4.
7. Add 10 drops of amylase to the tube labeled CA and to the test tube labeled 1. Add 20 drops of amylase to tube 2. Add 40 drops of amylase to tube 3 and add 60 drops of amylase to 4.
8. Get a fresh pipette and add 60 drops of water to tube CS and 50 drops of water to tube CA and tube 1. Add 40 drops of water to tube 2 and 20 drops of water to tube 3. All of the liquid levels in the tubes should be approximately the same.
9. Mix the tubes by gently rolling them back and forth in your hands.
10. Allow the tubes to incubate on your desk for 15 minutes.
11. Add 2 mL of Benedict's solution to each tube. Place the tubes in the boiling water bath. Boil for 5 minutes or until color development.

Record the results below.

	Enzyme quantity (drops)	Starch present	Colors with Benedict's Reagent	Reducing Sugar Present?
CA				
CS				
1				
2				
3				
4				
5				

1. What was your independent variable/s?
  
2. What is your dependent variable?
  
3. What are CA and CS? Explain what they tell you about your experiment.
  
4. The positive reaction with Benedicts indicates what has been produced?
  
5. Name the enzyme, substrate and products for this activity.