Amylase Activity

Exploring the Effect of Concentration and Temperature on Reaction Rates

Introduction

Living cells need energy to survive and thrive. Starch is a macromolecule that provides energy for human cells. What role does saliva play in preparing starch for energy use in our cells? How fast does starch decompose in the presence of amylase from saliva?

Concepts

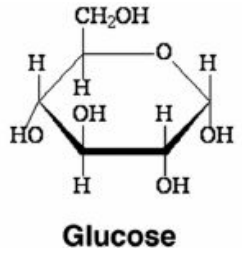
- Kinetics
- Half-life
- Visual Spectroscopy
- Concentration and Dilution
- Structure and Function
- Energy and Matter

Background

Structure and Function of Starch and Iodine:

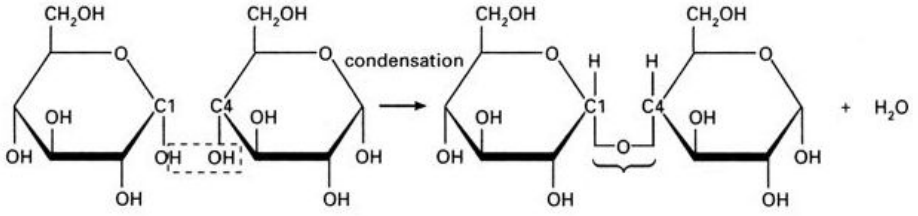
Starch is a long chain of glucose sugars. Glucose is a molecule of carbon, oxygen, and hydrogen which is a product of photosynthesis and the source of energy for nearly all living organisms.

The structure of glucose, $C_6H_{12}O_6$, can be represented with a Lewis structure, including a carbon ring with H and OH groups attached to each carbon atom.

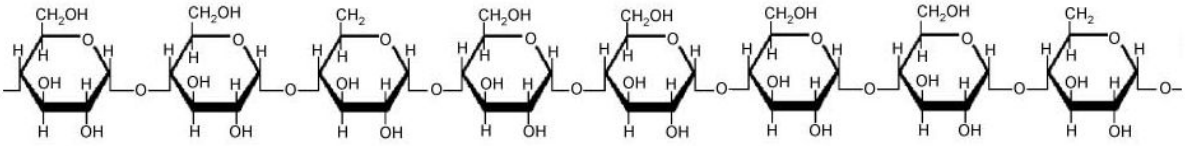


Glucose

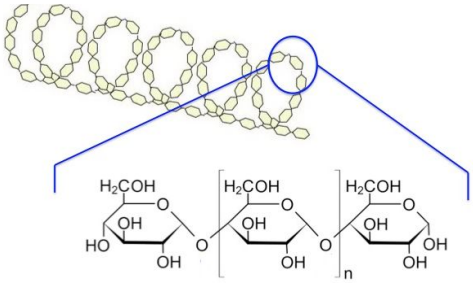
Starch is formed as OH regions of two glucose molecules react, bond, and release water in a condensation reaction as follows:



As the reaction occurs over and over again, a long chain of starch results, making a polymer that is very useful in delivering large amounts of energy to living organisms.

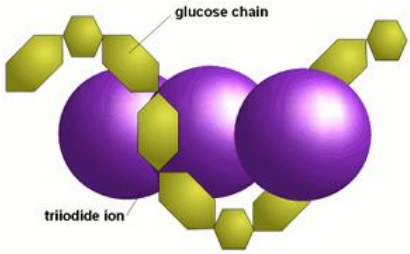


The starch does not remain in a straight line. The polarity of the OH groups found along the chain result in one OH group attracting and attaching to another, forming hydrogen bonds, and the entire structure forms a long helical coil.



When starch is dissolved in water, it does not absorb any visual frequencies of light, so the water appears colorless. In order to measure the starch concentration in water, we use a reaction with triiodide that causes the starch-triiodide complex to absorb visible light..

If iodine molecules and iodide ions interact with the starch, the two atoms of iodine in the I_2 molecule react with the one atom of iodine in the I^- ion to form an ion of three iodine atoms, triiodide, I_3^-. This new linear triiodide is drawn to the helix coil of starch, and slides into the open region in the center.



In the process, the electron structure of the ion complex changes in a way that longer wavelengths of visible red light are absorbed, and the shorter waves of blue visible light are reflected. This visible blue color in the solution makes it possible to measure the amount of starch present.

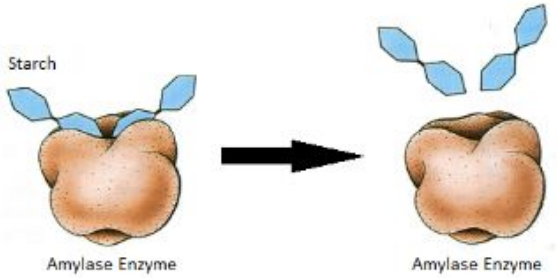
If the glucose chain making up the starch molecule is digested, or decomposed into individual glucose molecules, the interaction of triiodide in starch is lost, and the visible blue color is no longer visually detectable.

The blue color in the starch solution is a guide to measure the amount of starch present. The disappearance of the blue color can be used to measure how quickly the reaction proceeds.

To make these color measurements quantitative, a set of starch solutions of decreasing concentration will be prepared. These solutions will act as a standard, and we can compare the color of the experimental starch solution to the standard to see how the starch concentration decreases over time.

Amylase as Catalyst for Breaking down Starch into Glucose

The decomposition of starch into glucose is a very slow process that can take years to complete. As such, it is necessary to use a catalyst in order to speed up the reaction. There is an enzyme found in saliva, a protein which binds to the starch chain and easily breaks the bonds between glucose molecules.



Just how fast does amylase break down starch, and what factors may affect how much amylase is produced in your saliva?

An Assay for Starch Concentration:

If we set up a standard of solutions of decreasing starch concentrations, we can add the iodine/iodide chemical and visually see how much starch is in solution.

With the standard as our measuring tool, we can add a starch solution with blue indicator to a saliva sample and use the standard solutions to measure how much starch is digested over time, until the solution becomes colorless.

Experimental Overview

In this experiment, you will collect approximately 3 mL of your own saliva. You will use a starch solution, which will be made visible through the addition of iodine, to create a set of reference solutions that you can use to measure changes in starch concentrations over time. From the data you collect, it is possible to determine the activity of the amylase enzyme in units of micromoles glucose formed per minute.

Pre-Lab Questions

1. What atoms in the glucose molecule create the forces of attraction along the starch chain that cause the chain to form a helical coil?

    a. How did the electronegativity of carbon, hydrogen, and oxygen atoms in the structure lead to the attractive forces that created the coil?

    b. Compare the atoms in starch and their electronegativities to the structure of a water molecule. Do you predict that starch would be able to dissolve in water? Why?

2. How did the changes in the structure of the starch coil and the iodide ions lead to our ability to see the presence of starch in solution?

3. Describe how a set of reference solutions of starch concentration can be used to measure the concentration of starch in an unknown solution.

Materials

- Starch Solution, 0.5% Aqueous (Flinn S0151, 500 mL, $9.65)
- Iodine-potassium iodide solution (Flinn I0027, 500 mL, $9.75) dilute to 25%
- 3 mL of saliva collected in a test tube by chewing parafilm
- 24-well Reaction Plate (Flinn AP1447, 1 per lab group, $4.55)
- Graduated Beral-Type Pipet (Flinn AP1721, Pack of 20, $1.70)
- Distilled water
- Test Tubes with Screw Caps, Plastic, Pkg. of 30 (Flinn AP7116, $17.10)
- Parafilm (Flinn AP1501, 4”, 125 ft, $25.50)

Safety Precautions

Iodine solution is irritating to the eyes and skin. Iodine will stain clothing, books, hands, and anything containing starch. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron.

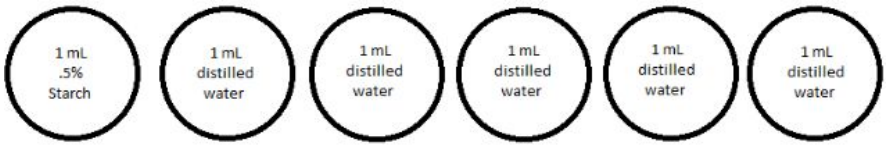
Laboratory Technique

Saliva Collection Protocol

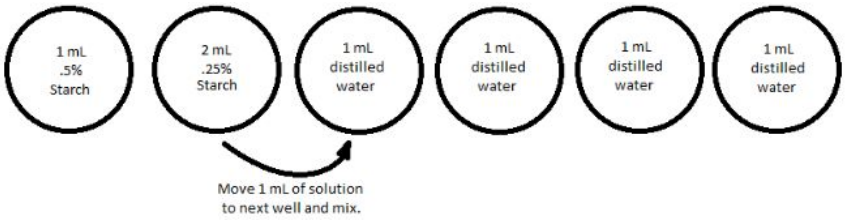
1. Take a square of parafilm, and chew it like gum. The parafilm will stimulate saliva flow.
2. Allow saliva to pool in your mouth, then with your head tilted forward, gently guide the saliva into the graduated test tube.
3. Collect approximately 3-5 mL of saliva. Make sure you do not include any bubbles in your measurement, you'll need 3-5 mL of liquid.

Starch Standard Calibration Curve

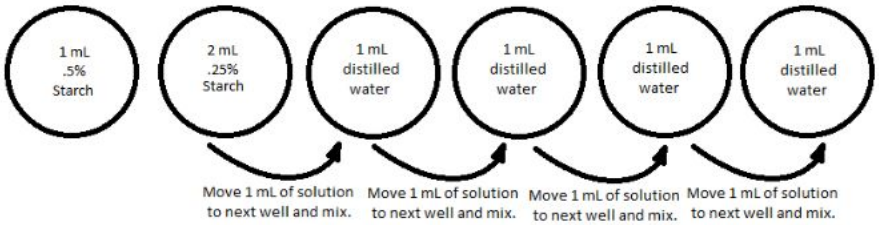
1. Start by adding 1 mL of .5% starch into the first well with the graduated pipette, and 1 mL of distilled water into the next five wells. Make sure you rinse your pipette well by drawing and dispensing water through the pipette two or three times before using the pipette to dispense distilled water. Your six wells should be filled as such:



2. You will now add 1 mL of .5% starch to the second well, and mix, changing the concentration of the second well from .5% to .25% starch. The well now has 2 mL of .25% starch.
3. Now, use the graduated pipette to draw 1 mL of the .25% starch solution from the second well and add it to the 1 mL of distilled water in the third, and mix well. Your third well will now have ½ the concentration of the second.



4. Continue to draw 1 mL from the freshly mixed solution in the second well and dispense into the third, mix, then draw 1 mL from that well, add it to the next and mix, etc. Continue until you have mixed the solution in the final well.



5. Now you'll have 1 mL of solution in each of the first five wells, and 2 mL of solution in the final well. Draw 1 mL of solution from the final well and discard into the waste beaker. You will then have 1 mL of solution in each well.
6. When we test the saliva samples, we will add 1 mL of starch to 1 mL of saliva leaving 2 mL reacting in a well. In order to match the amount of the saliva/starch mixture, we will need to add 1 mL more to each well of our standard, bring the amount in each well to 2 mL. Go ahead and add 1 additional mL of distilled water to each well and swirl the well plate until well mixed.
7. To make the starch in solution visible, add 1 drop of iodine-iodide solution to each well and swirl the well plate until the color in each well is uniform.

If the dilution was done with precision, you should observe that the first well is quite dark and each well progressing to the last becomes more and more transparent until the final well is nearly clear. The black "X" drawn beneath each well will serve as a guide in matching the standards to the wells in which we'll run our experiment.

Saliva Dilution

1. In the second row on the plate, set up four wells of saliva solution in a serial dilution containing 1 mL each. Use the same serial dilution procedure you applied to make the starch standard, beginning with 1mL of full strength saliva in the first well, and 1 mL full strength saliva in the second with 1 mL of water.
2. The saliva solution in the second well will be diluted to 50% after the addition of 1 mL of water. Now that you have 2 mL of 50% saliva in well s, move 1 mL of the solution to well 3 and mix with another 1 mL of water. Dilute each well this way until the fourth well, then discard 1 mL of solution from the third well.

Data Collection for Reaction Between Starch and Amylase in Saliva

1. In row three, place 1 mL of .5% starch solution in each of the wells below your saliva solutions, and add 1 drop of indicator to each well. Your well plate should now have three rows of solutions:
    a. First: Serial dilution of starch, 2 mL in each well, decreasing in concentration made visible with the addition of the iodine/iodide.
    b. Second: Serial dilution of saliva, 1 mL in each well.
    c. Third: 1 mL of starch solution in each well beneath the saliva solutions, with the indicator added.
2. To collect your data, get ready to quickly draw the starch solutions in row three into your piped and squirt the solution into the saliva solutions in row 2. Start with the saliva solution of least concentration.
3. To collect your data, start your timer and quickly begin with the saliva solution of least concentration. Draw all of the blue starch solution from the well below it into your pipet, and dispense it into the saliva solution.
4. Repeat step 3 for each of the saliva solutions in the dilution, and once you've added to the last one, swirl the well plate until the solutions look uniform throughout.
5. Every minute, measure the concentration of the starch in each well by visually comparing the concentration of the reacting solution with the concentrations of the reference solutions in row 1.
6. If you have a cell phone with a camera, it is recommended that you take a photo of the well plate every minute to verify your readings after the data collection is completed.
7. Continue data collection for at least 15 minutes.

Guided-Inquiry Design and Procedure

A. Effect of Concentration on the Rate of Reaction Between Amylase and Starch

Discuss the following questions with your lab group.

1. When you mix the 1 mL of saliva solution with the 1 mL of starch, both the concentration of starch, and the concentration of saliva will be reduced by one half. Upon mixing, saliva and starch concentrations, in rows two and three, determine what the concentration of both saliva and starch will be in the reacting wells. Complete the table.

Row 3	Well 1	Well 2	Well 3	Well 4
Saliva %				
Starch %				

2. In order to determine the factors affecting the rate of the amylase/starch reaction, why was it necessary to dilute the saliva solutions?

3. What other factors, other than the concentration of amylase in saliva, would affect the rate of the reaction? How can you control these factors to ensure that concentration is the only variable affecting the rate?

4. Why is it necessary to collect data for at least 15 minutes? After running the experiment, was the data collected sufficient to determine the rate of the reaction?

5. Is it useful to take photographs of the reaction plate every minute? Why?

Sample Data Table

Each minute record the concentration for each of the three saliva samples. After collecting the data, review the photos you took, and verify that your readings were as accurate as possible. Attached to the end of the lab is a Data Collection Sheet to facilitate reading the concentration of the wells in the starch standard, and taking a photographic record of the concentration every minute.

% Starch over Time

Time	Well 1 Full Strength Saliva	Well 2 Half Strength Saliva	Well 3 Quarter Strength Saliva	Well 4 Eight Strength Saliva
Initial 0 min				
1 min				
2 min				
3 min				
4 min				
5 min				
6 min				
7 min				
8 min				
9 min				
10 min				
11 min				
12 min				
13 min				
14 min				
15 min				
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17 min				
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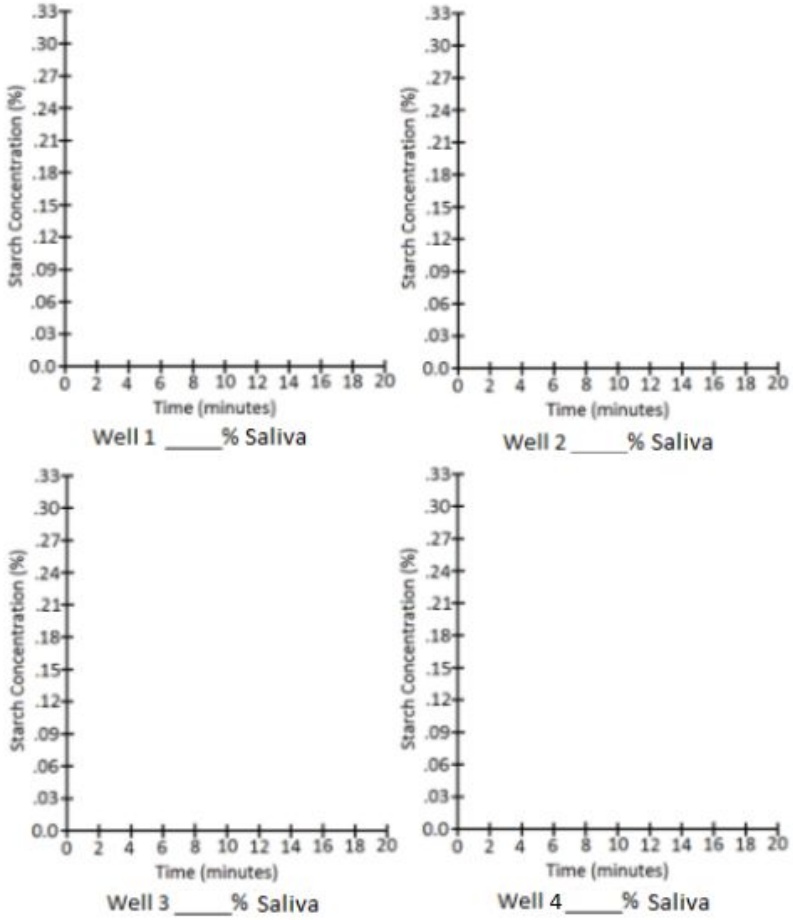
27 min				
28 min				
29 min				
30 min				

Sample Graphs



Determine the half-life of the digestion of starch:

Often when reactions occur in the body, the rate at which a substance is used up is measured in a half-live. A half-life is the amount of time it takes for one half of the amount of a reactant to be used up.

1. After graphing the data from the digestion of starch in each well, draw a best fit curve through of your measured points.
2. Use the best fit curve to determine how long it took for each solution of saliva to digest ½ of the starch present, and record the time at each point at which the starch concentration decreased by ½.

% Saliva	Time 0 Initial Starch Concentration	Time 1 Starch decreased by 1/2	Time 2 Starch decreased by ½ again	Time 3 Starch decreased by ½ again	Time 4 Starch decreased by ½ again

3. Use the data from your graphs and from the table above to calculate the average time it took for each amylase solution to decompose ½ of the starch solution.

Determine the Enzyme Activity for the Amylase in the Saliva:

Enzymatic activity is a measurement of just how fast the amylase enzyme was at digesting the starch. This activity is unique to the environment in which the enzyme is functioning, and will change with changes in environment.

Each saliva sample had a certain number of amylase enzyme proteins in the solution. We will use the rate at which the sample digested the starch to determine how many units of amylase were in each solution.

One enzyme unit is the amount of amylase needed to digest 1 micromole of starch every minute.

Starch is a very complex molecule, but it does break down into much simpler glucose molecules. We are going to measure starch in units of glucose.

To determine the total mass of starch consumed per minute, we first need to determine the amount of starch remaining at the end of our experiment. To do this we'll take the % starch at the beginning, and the % starch at the end.

Let's say our third well consumed the starch in 6 minutes. We started with .25% starch and nothing remained. So we can calculate the mass of starch using the percentage.

There are .5 g of starch in 100 mL of solution, and there was 1.0 mL of solution in our well.

$$\left(\frac{1\ \text{mL Solution}}{1}\right)\left(\frac{0.50\ \text{g Starch}}{100\ \text{mL Solution}}\right) = .0050\ \text{g Starch}$$

Starch is a chain of glucose, so .0025 grams of starch is .0025 grams of glucose.
1 mol of glucose is equal to 180 grams of glucose, and 1 mole is 1000000 micromoles so,

$$\left(\frac{.0050\ \text{g glucose}}{1}\right)\left(\frac{1\ \text{mol glucose}}{180\ \text{g glucose}}\right)\left(\frac{10^6\ \text{umol}}{1\ \text{mol}}\right) = 28\ \text{umol glucose}$$

We measured that it took 6 minutes to digest 14 micromoles of glucose. We calculate the number of micromoles of glucose per minute:

$$\left(\frac{28\ \text{umol glucose}}{6\ \text{minutes}}\right) = 4.6\ \text{umol glucose} \cdot \text{min}^{-1}\left(\frac{1\ \text{Unit of Amylase}}{1.0\ \text{umol glucose} \cdot \text{min}^{-1}}\right) = 4.6\ \text{Units of Amylase}$$

The saliva sample was able to digest 4.6 umol of glucose per minute, so at this temperature and at this concentration, the saliva solution contained 4.6 enzyme units of amylase.

4. Go ahead and use a similar method to calculate the number of units of amylase found in each of the four saliva solutions you measured.

B. Effect of Temperature on the Rate of Reaction Between Amylase and Starch

Discuss the following questions with your lab group.

1. If you intend to discover how temperature will affect the rate of the amylase/starch reaction, what would be the independent variable for the reaction?
2. What other variables would need to be controlled so as not to affect the rate of the reaction?
3. Often in chemistry, in order to modify and control the temperature of a reactant, hot and cold water baths can be used. How could you apply a hot water bath to the procedure you used to measure the effect of concentration on the reaction rate?
4. What would you change about the concentrations of the saliva you used in row 2?
5. Would you need to modify the data you collected over the duration of the reaction?
6. What would you have to modify about the graphs you use to present the data.
7. Given that saliva functions best in a living human being, what do you predict about the ideal temperature for the amylase reaction?

Opportunities for Inquiry

Amylase Production in the Body

The human body is tremendously adaptable. Using the assay procedures described in this lab investigation, how could you explore larger effects on how saliva changes as factors affect a human being. For example, would changes in diet change how the salivary glands generate amylase? Is it possible that amylase production in saliva changes with age, or across gender. Does the amylase production in saliva change in times of fasting, or with high levels of exercise? Make a prediction as to how one of these factors would affect amylase production and design an experiment using the protocols outlined in the lab to collect data related to your prediction.

Review Questions

1. Make a claim, and support it with evidence from the lab, regarding how changes in amylase concentration affect the rate at which starch is digested in your mouth.

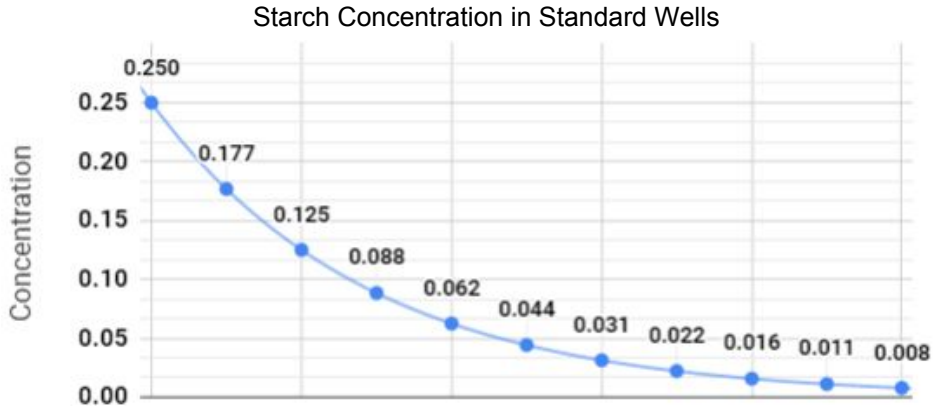
2. How does determining an enzymatic unit of amylase and the half-life of the reaction help simplify the presentation of the data you collected?

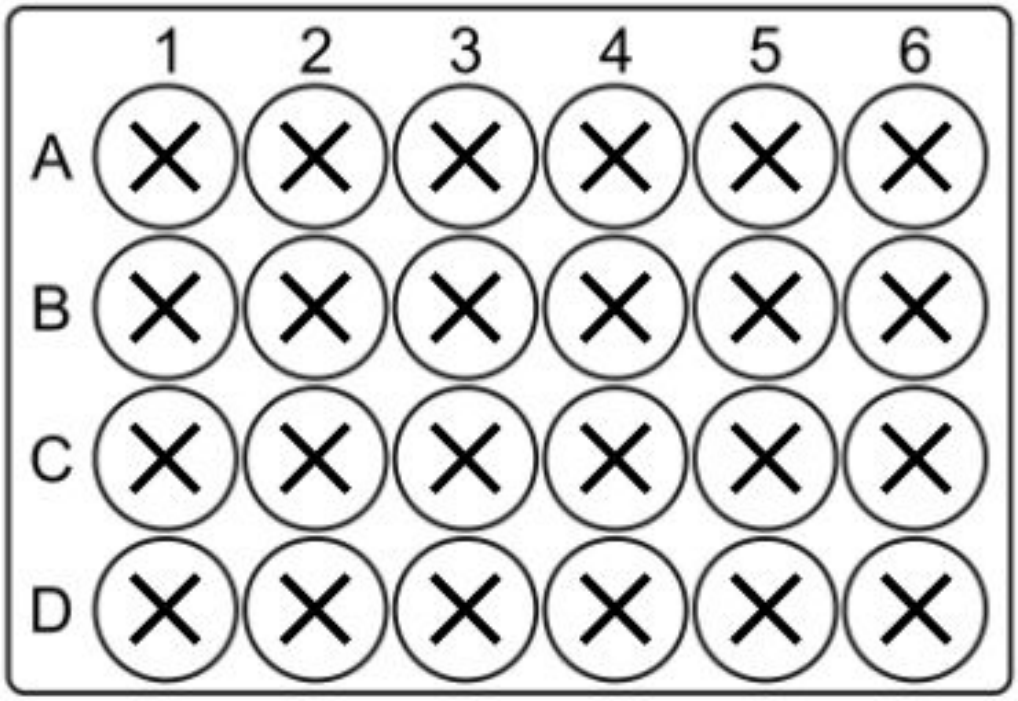
3. Describe why the light absorbed by the starch changed as the starch decomposed into glucose molecules.

4. How effective were the reference solutions of starch in your standard in making the measurements needed to determine the rate of the amylase starch reaction? In what ways could you improve the procedure to make better measurements?

5. What did you notice happened when the concentration of amylase was diluted? Why do you believe this happened?

Data Collection Page





Time(min) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
(After taking a picture, cross off the time.)

Teacher's Notes

Exploring the Effect of Concentration and Temperature on Reaction Rates

Materials From Flinn Scientific

- Starch Solution, 0.5% Aqueous (Flinn S0151, 500 mL, $9.65)
- Iodine-potassium iodide solution (Flinn I0027, 500 mL, $9.75) dilute to 25%
- 3 mL of saliva collected in a test tube by chewing parafilm
- 24-well Reaction Plate (Flinn AP1447, 1 per lab group, $4.55)
- Graduated Beral-Type Pipet (Flinn AP1721, Pack of 20, $1.70)
- Distilled water
- Test Tubes with Screw Caps, Plastic, Pkg. of 30 (Flinn AP7116, $17.10)
- Parafilm (Flinn AP1501, 4", 125 ft, $25.50)

Additional Materials Needed (for each lab group)

- Three beakers,
  - one with distilled water for solution dilution
  - one with distilled water for rinsing the pipet
  - one for disposing of the waste.
- Excel Worksheet Software for data analysis
- Stopwatch
- Cell phone with camera
- Stirring rod
- Thermometer
- Dropper bottles for iodine/iodide

Additional Materials Needed (for *Pre-Lab Preparation*)

- Hot plate, if preparing starch solution.
- Volumetric flask to dilute iodine/iodide solution
- Freezer/fridge to store saliva samples
- solution for each group

Pre-Lab Preparation

Preparation of .5% Starch Solution

1. Place 100mL of distilled or deionized water in a 250-mL beaker and bring to boiling on a hot plate.
2. Make a smooth paste with .5 g of soluble starch and a small volume (several mL or so) of distilled or deionized water.
3. Once the water on the hot plate is boiling, carefully remove the beaker containing the boiling water from the hot plate. Pour the starch paste into the boiling water and stir until all of the starch is dissolved. The resulting solution may be somewhat cloudy.
4. Allow the starch solution to cool to room temperature before use. *Note:* This is especially important if the starch solution is to be used in a kinetics experiment where temperature is a factor.

Tip:

- Starch solutions have a poor shelf life and will deteriorate quickly. Therefore, starch solutions should be kept refrigerated. It is best to prepare a starch solution on the day of the lab.

Dilution of Iodine-Potassium Iodide Solution

1. Add 25 mL of Iodine-potassium iodide solution to a 100 mL volumetric flask.
2. Fill to 100mL mark

Tip:

- Transfer solution to individual dropper bottles to make it easy for students to add to the wells in the well plate.

Saliva Preparation

Fresh saliva may be used in the experiment, however the presence of mucus and particulates often found in saliva may make the full strength sample hard to measure. It is recommended that the saliva is collected prior to the day of experimentation, frozen overnight, rethawed, and either centrifuged at 1000g for 2 minutes and the supernatant collected, or filtered. This will ensure the saliva is clear when used in the experiment.

Safety Precautions

Iodine solution is irritating to the eyes and skin. Iodine will stain clothing, books, hands, and anything containing starch. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Review current Safety Data Sheets for additional safety, handling and disposal information.

Disposal

Starch iodine solutions may be reduced with sodium thiosulfate by adding just enough reducing agent to decolorize the blue color of the starch-iodine complex.

Alignment to NGSS Framework

- HS-PS1-2: Explain simple chemical reactions using electron states, trends, and patterns. HS-PS4-4: Evaluate claims of how different frequencies of electromagnetic radiation are absorbed by matter.
    - Students will explain how changes in structure of starch and iodide result in a new **electron state** able to absorb red wavelengths of light.
- HS-PS1-5: Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.
    - Students will collect rate evidence at varying temperatures and concentrations for the decomposition of starch in the presence of amylase and apply collected evidence support a claim in how concentration and temperature affect the rate.

Lab Hints

- Have students collect, label, and prepare saliva samples prior to the day of experimentation.
- Dilution of the starch solution is best accomplished when students mix the solution in each well prior to moving 1 mL to the next well. One way to mix, is to draw the solution into the pipet and dispense back into the same well two or three times before moving 1 mL to the following well.
- When taking visual spectrophotometric readings of the reactions, it is useful to pick up the well plate a few inches above the x marked paper to allow some light to shine through the bottom of the well plate.
- After taking photos of the solutions reacting, students may adjust the brightness or sharpness of the images to make a better visual comparison of the standard starch solutions.
- Students should rinse the transfer pipet two or three times before moving from the starch solution to the distilled water.
- It is recommended that lab data is analyzed and graphs are generated using the Excel worksheets.
- The full strength saliva reaction usually proceeds quite rapidly, and may be complete before the first minute. You may want to begin the reaction with a 75% or 50% dilution of the saliva.
- If the iodine solution used to test for starch is too concentrated, the excess iodine will remain brownish yellow and change the color present for the solution comparison. It may be necessary to dilute the test solution slightly to have optimal results.

Teaching Tips

- A fun introduction to this lab is to have students place a soda cracker in their mouth and let it dissolve for some time as the starch turns into sugar.
- Discussion of absorbance and energy levels of electrons is useful in helping students understand the visual colorimetric analysis used in the lab.
- After students have collected data regarding the amylase activity in their saliva, it is good to have a discussion of the variance in the activity, and postulate over reasons why those differences may exist. Help students generate questions into how they might investigate biological or environmental factors that affect amylase production in saliva.

Answers to Pre-Lab Questions

1. The $-OH^-$ regions along the ring of the glucose are polar and form the hydrogen bonds that hold the chain in its helical coil.

    a. The electronegativity of oxygen is 3.5 while the electronegativity of hydrogen is only 2.1, which is a great enough difference to create a dipole with significant partial positive and negative charge.

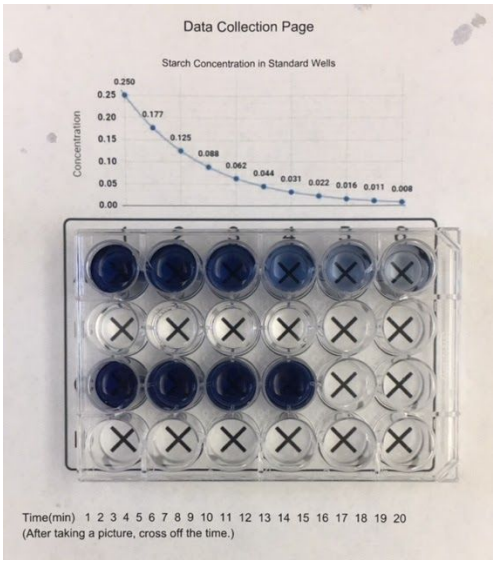
b. The -OH^- groups on the glucose structure may form hydrogen bonds with the OH group on the water molecules, which will enable the glucose to dissolve in water.

2. The iodine attaching to iodide formed an I_3^- ion which was a longer ion. As it interacted with the electrons in the starch coil, the frequency of light that the molecule could absorb changed to the visible range, and as a result we could see the colors reflected back from the molecule.

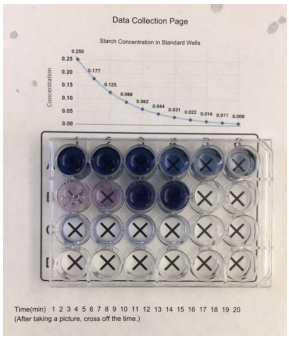
3. The concentration of the blue color in the reference solutions becomes less and less dark as the concentration becomes more dilute. By comparing the depth and darkness of the color in the reaction to the color of each standard well, we could match our reacting well to the known concentration of the standard, and quantify the concentration.

Sample Data, Results, and Analysis

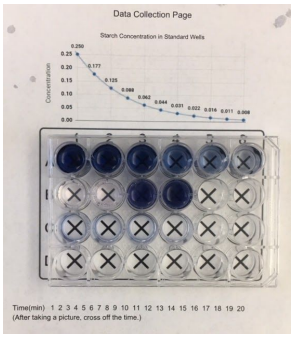
Sample Starch Standard Curve



Sample Photographs for Reaction



30 seconds



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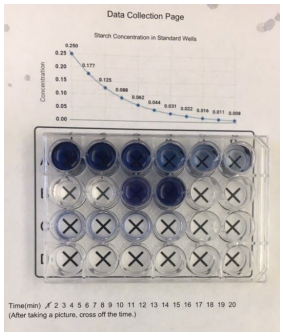

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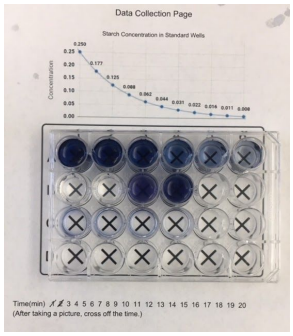

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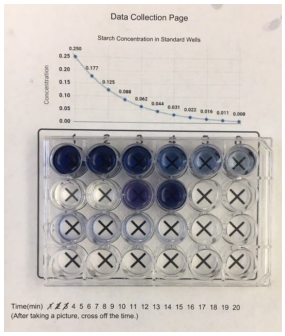

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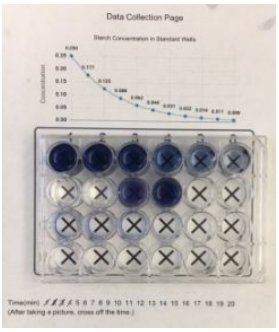

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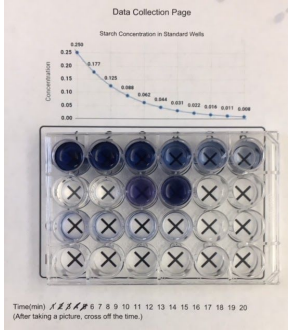

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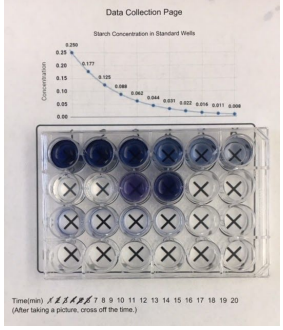

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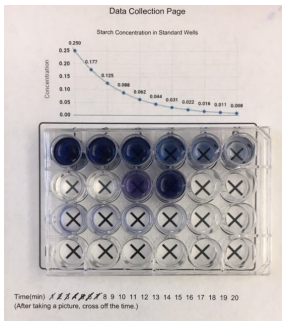

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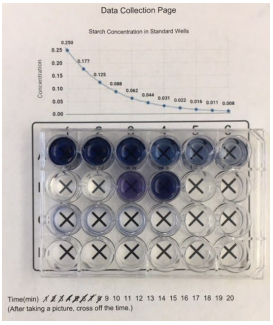

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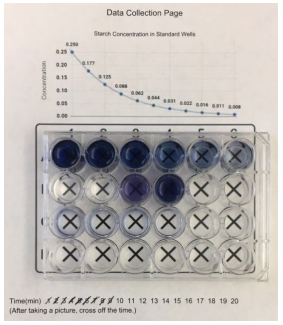

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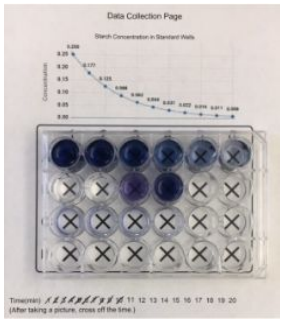

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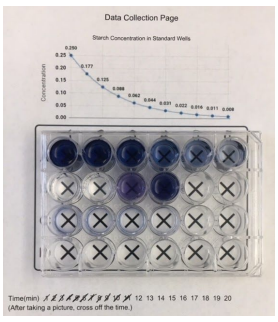

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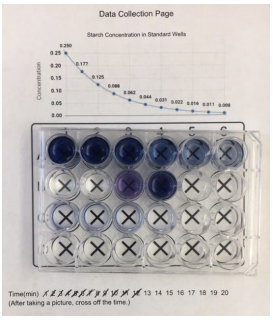

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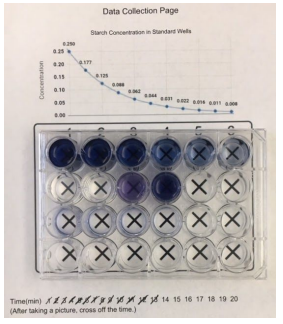

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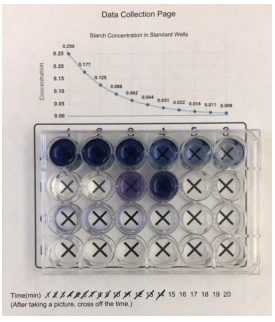

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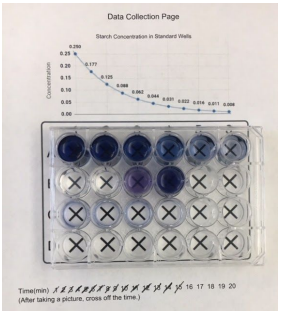

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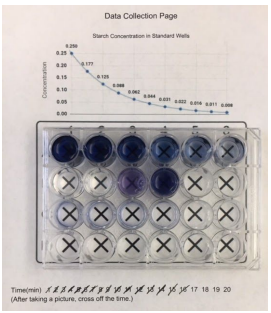

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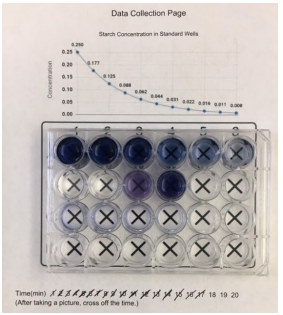

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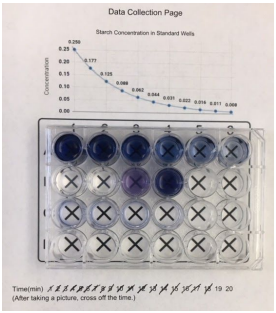

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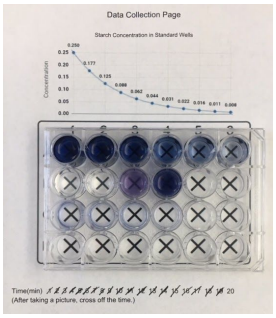


20 minutes

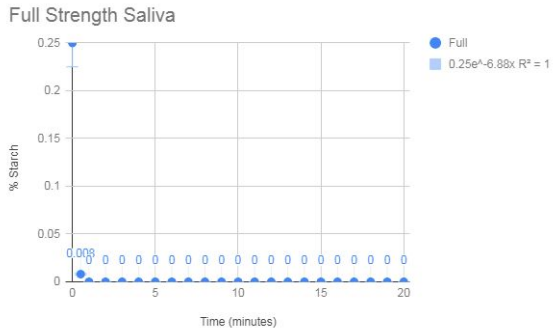
Sample Data Table

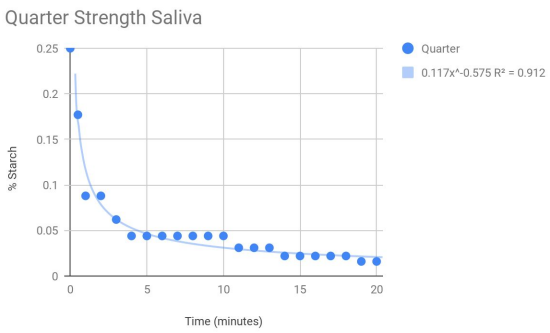
% Starch over Time

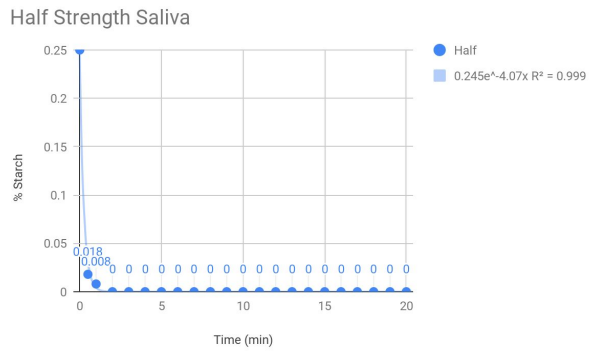
Time	Well 1 Full Strength Saliva	Well 2 Half Strength Saliva	Well 3 Quarter Strength Saliva	Well 4 Eight Strength Saliva
Initial 0 min	.25	.25	.25	.25
.5 min	.008	.016	.177	.177
1 min	0	.008	.088	.177
2 min	0	0	.088	.125

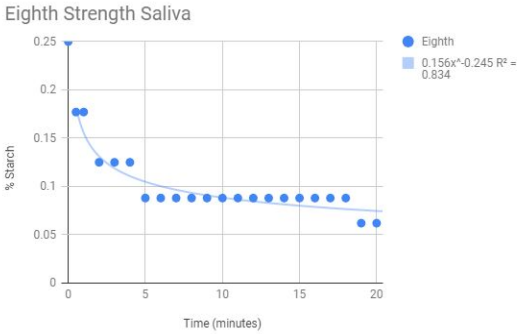
3 min	0	0	.062	.125
4 min	0	0	.044	.125
5 min	0	0	.044	.088
6 min	0	0	.044	.088
7 min	0	0	.044	.088
8 min	0	0	.044	.088
9 min	0	0	.044	.088
10 min	0	0	.044	.088
11 min	0	0	.031	.088
12 min	0	0	.031	.088
13 min	0	0	.031	.088
14 min	0	0	.022	.088
15 min	0	0	.022	.088
16 min	0	0	.022	.088
17 min	0	0	.022	.088
18 min	0	0	.022	.088
19 min	0	0	.016	.062
20 min	0	0	.016	.062

Sample Graphs









Discussion Questions

1.

Row 3	Well 1	Well 2	Well 3	Well 4
Saliva %	50	25	13	6.3
Starch %	25	13	6.3	3.1

2. Diluting the saliva solutions changes the concentration of the amylase found in the reacting solution. With the change in amylase compared to the change in concentration for each diluted sample, it is possible to see how concentration affects the rate of the reaction.
3. Changes in concentration of starch, temperature, volume of saliva, volume of starch solution, etc. could all possibly affect the rate of the reaction. By making sure each variable listed is constant for every trial of the reaction, it is possible to eliminate the effect on rate, and become confident that changing amylase concentration is the only factor that is changing the rate.
4. Collecting data for 15 minutes gives sufficient time for the slower reacting samples to show change. The full strength and half strength saliva samples digested the starch very rapidly, while the quarter and eighth concentration samples required data collection over a longer period of time to get a good picture of how rates were affected.
5. The changes in visual color of the starch concentration were very quick for the full and half samples, so it may have been more useful to take pictures every 10 seconds or so, however with the slower reacting samples at quarter and eighth strength, taking a photo every minute was sufficient to generate data necessary to calculate rates.

Sample Calculations

1. See Graphs Above
2. Use the best fit curve to determine how long it took for each solution of saliva to digest ½ of the starch present, and record the time at each point at which the starch concentration decreased by ½.

% Saliva	**Time 0 Initial Starch Concentration**	**Time 1 Starch decreased by 1/2**	**Time 2 Starch decreased by ½ again**	**Time 3 Starch decreased by ½ again**	**Time 4 Starch decreased by ½ again**
100%	**.25**	**.1 min**	**.1**	**.1**	**.1**
50%	**.25**	**.2**	**.2**	**.2**	**.2**
25%	**.25**	**2**	**2**	**4**	**10**
13%	**.25**	**4**	**20**	**NA**	**NA**

3. Use the data from your graphs and from the table above to calculate the average time it took for each amylase solution to decompose ½ of the starch solution.
    a. 100% .1 minutes
    b. 50% .2 minutes
    c. 25% 4.5 minutes
    d. 13% 14 minutes
4. Go ahead and use a similar method to calculate the number of units of amylase found in each of the four saliva solutions you measured.
    a. 100% 28 umol/min
    b. 50% 14 umol/min
    c. 25% .25% dropped to .016% in 20 minutes, .234/.25 x 100% = 94% of 28umol consumed = 26.3umol/20 min = 1.3 umol/min
    d. 13% .25 dropped to .062 in 20 min, .19 consumed/.25 x 100% = 75% of 28umol consumed = 21 umol/ 20 min = 1.0 umol/min

Answers to Review Questions

1. As the concentration of amylase is decreased the rate of the reaction decreases. According to the data, the amylase in 100% saliva digested the amylase at a rate of 28umol / min. Amylase in 50% saliva digested at 14umol / min, 25% at 1.3umol / min, and 13% at 1.0 umol / min.
2. The data is compiled into an average amount consumed per minute, making the comparison of the four different reactions much more concise.
3. The light absorbed that allowed the blue color was absorbed by the structure of the starch chain wrapped around the I_3^- ion. As the starch molecule was digested and the I_3^- molecule was released, the structure that absorbed the light was no longer there, and the solution lost its color. The starch standard seemed most effective toward the end of the spectrum where the differences in color were most apparent. Measurements could be improved by using a colorimeter to measure the absorbance of the red light by each solution. The colorimeter would be able to measure more precisely than trying to gauge the concentration with the naked eye.
4. The diluted saliva reduced the amount of enzyme available to digest the starch, and with less enzyme interacting with the starch, the rate at which the starch was digested decreased dramatically.

References

Dhar, N.R (1923) The Starch-Iodine Reaction, The Journal of Physical Chemistry, 28 (2), 125-130

Madhu, S, Evans, H, et. al. (2016) Infinite Polyiodide Chains in the Pyrroloperylene-Iodine Complex: Insights into the Starch-Iodine and Perylene-Iodine Complexes, Angew. Chem. Int. Ed, 55, 8032 - 8035

Supporting supplies are available from Flinn Scientific, Inc.

Catalog No.	Description
S0151	Starch Solution, 0.5% Aqueous, 500 mL
I0027	Iodine-potassium iodide solution
AP1447	24-well Reaction Plate
AP1721	Graduated Beral-Type Pipet
AP7116	Test Tubes with Screw Caps, Plastic, Pkg. of 30
AP1501	Parafilm 4”, 125 ft