# Lesson 1: Qualitative Kinetics: Examining the effect of an enzyme on a reaction.

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| **Introduction**  | Chemical kinetics and buffers are two topics that are extremely difficult for students to understand. Combining the two topics will allow for a staggered, repetitive approach to teaching students to understand of how these two topics in chemistry actually work. Students will both qualitatively and quantitatively track the effect and enzyme has on a reaction, calculate the reaction rate and buffer capacity. Students will use a variety of lab techniques including calculations using Beer’s Law and spectrophotometry. |
| **Real Science Application**  | Buffers are used frequently in industry to protect and stabilize certain sensitive reactions. Without buffers, small additions of acids and bases would result is drastic spikes and dips in pH. In the first activity, a buffer is used to slow down pH change caused by the hydration reaction of CO2 so that students can more easily observe the change. This buffer will also slow down the enzyme catalyzed reaction, but the enzyme will still drastically speed up the reaction. Students will then be tasked with identifying an ‘ideal’ concentration based on the degree to which the reaction as sped up as well as the cost of the enzyme. The data collected will be a qualitative measurement of time to end-point.The second activity focuses only on the behavior of the buffer and the indicator. By measuring the absorbance of the solution at 573 nm using a spectrophotometer, students will be able to compare the measurements to the number of protons added to the solution. This technique is used in industry and academia to create equations to relate the absorbance with concentrations of acids and bases.The third activity will use the absorbance calculations developed in the second activity to quantify the reaction rate observed in the first activity. Students will be expected to calculate and determine the order of the rate law empirically, rather that working with prepared data in a textbook. Students will be expected to trouble-shoot problems and identify potential problems with the experiment.The fourth activity will target an understanding of the Henderson-Hasselbach equation. Students will be expected to calculate the concentration of the acid and conjugate base present in the buffer based on the effect a strong acid has on the pH of the solution. Students will then calculate the amount of acid required to change the pH by a specified amount. |

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| **Curriculum Alignment**  | NC Essential Standards

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| Content Area | Grade Level | NC SCS | Lesson 1 |
| Chemistry | 9-12 | CHM.3.1.1 | X |
| Chemistry | 9-12 | CHM.3.2.2 |  |

Next Generation Science Standards

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| NGSS | Lesson 1 |
| HS-PS1-5 | X |

AP Chemistry Concept Outline

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| Essential Knowledge | Lesson 1 |
| 4.A.1 | X |
| 4.D.1 | X |
| 4.D.2 | X |
| 6.C.1 |  |
| 6.C.2 |  |

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| **Community Engagement**  | Prior to the lab activities, a speaker from Novozymes will come to the class to explain how their enzymes are developed, manufactured and used in industry.After the second lab activity, students will go on a field trip to the Novozymes site to take a tour of some on the different labs. |
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| **Learning Outcomes**  | Students will describe and explain the relative effect of an enzyme on a chemical reaction.Students will evaluate the effect enzyme concentration has on a chemical reaction.Students will determine the ‘ideal’ concentration of enzyme based on cost and effect. |
| **Time Required and Location**  | 45-60 minutes in a science lab.45 minutes of prep. |
| **Materials Needed**  | Teacher List:* 1.00 L of 50mM Bicine Buffer w/ Cresol Red
* 1.00 L of saturated CO2 water
* 1.0 mL vials of the following enzyme solutions for every group:
	+ 750 µg/mL (Labeled A)
	+ 500 µg/mL (Labeled B)
	+ 250 µg/mL (Labeled C)
	+ 125 µg/mL (Labeled D)

Student List:* Pre-Lab assignment (1 per student).
* Lab Flow Diagram (1 per student).
* 1 micropipetter (10 µL) for every 2-3 students (1 per class will work, but will make the lab considerably longer).
* 2 50 mL beakers for every 2-3 students.
* 2 graduated cylinders (10 mL capacity) for every 2-3 students.
* 1 gallon size ziplock bag for every 2-3 students.
* Stopwatch.
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| **Safety**  | Safety Concerns:When prepping the enzyme solutions, the teacher should take care when handling the powdered enzyme. Powdered enzymes can easily become aerosolized and may cause irritation or allergic reactions if inhaled.When making the CO2 water, use caution as dry ice is extremely cold and should always be handled with insulated gloves or tongs.

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| PPE for teacher prep:* Lab coat / apron
* Gloves
* Goggles
* Mask
* Insulated gloves
 | PPE for lab activity:* Lab coat / apron
* Goggles
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SDS:* [Bicine Solution](https://drive.google.com/file/d/0B0mR-l2-rJaYOC1EM1BwMXdWTk0/edit?usp=sharing)
* [Cresol Red Powder](http://www.flinnsci.com/Documents/SDS/C/CresolRed.pdf)
* [Cresol Red Solution](http://www.flinnsci.com/Documents/SDS/C/CresolRedIndSol.pdf)
* [Sodium Chloride Crystal](http://www.flinnsci.com/Documents/SDS/S/SodiumChloride.pdf)
* [Sodium Chloride Solution](http://www.flinnsci.com/Documents/SDS/S/SodiumChlorideSol.pdf)
* [Bovine Carbonic Anhydrase Powder](https://drive.google.com/file/d/0B0mR-l2-rJaYa3JTYzhNTVE0dnc/edit?usp=sharing)
* [Solid Carbon Dioxide](http://education.jlab.org/frost/msds/dry_ice.pdf)
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| **Student Prior Knowledge**  | Students are expected to have a basic understanding of the indicators of a reaction, specifically color change. Background knowledge of enzymes will be useful, but not necessary.  |
| **Teacher Preparations**  | Time: 45 minutes**Solution Preparation:**1.00 L of 50mM Bicine Buffer:Dissolve 8.16 g of Bicine powder and 15.555 g of Sodium Chloride into approximately 800 mL of distilled water in a 1.00 L beaker/flask.Add 0.50 g of cresol red indicator powder.Using a pH meter and a Sodium Hydroxide solution, titrate the solution to a pH of 8.65.Pour the solution into a 1.00 L volumetric flask.Bring the volume up to the calibration mark by adding distilled water.This solution should be stored in a dark storage cabinet to prevent UV exposure. This solution has a 7 day shelf-life.1. L of CO2 water:

Using a large flask or beaker, measure out approximately 1.0 L of distilled water.Using caution, drop several large pieces of dry ice into the solution.Allow the dry ice to sublime and repeat.This solution should be made at the beginning of the class in which it is being used.Enzyme Solutions:Solution A:Dissolve 0.00750 g of Bovine Carbonic Anhydrase into 10 mL of distilled water.Pipette 1.0 mL of each solution into a 1.5 mL vial or centrifuge tube labeled A.Solution B:Repeat the procedure for Solution A using 0.00500 g of the enzyme.Solution C:Repeat the procedure for Solution A using 0.00250 g of the enzyme.Solution D:Repeat the procedure for Solution A using 0.00125 g of the enzyme.Store in freezer when not in use. When stored in freezer, solutions have a 3 year shelf-life. |
| **Activities**  | Have students discuss briefly what the lab activity will be about. Call on students to explain each step outlined on the pre-lab activity.Distribute the lab flow diagram to students.Demonstration:As a demonstration, prep two 200 mL beakers of the buffer. (You may want to dilute this down as to not waste too much buffer)Add a small amount of enzyme solution A to one of the beakers.Describe to the class what you have added and have students predict what will occur.Add a small amount of leftover dry ice to each beaker and observe.The beaker without the enzyme should take considerably longer to experience a color change than the beaker with the enzyme.Place students in groups of 2-3 and instruct them to gather their materials and PPE.Once each group has its materials, the students may begin to work.Monitor the classroom to ensure that students are using appropriate techniques. After students have completed part 1 of the activity, they will need to have their work approved before they can continue working on parts 2-5.Once all lab work is complete, have students clean up their lab area and return to their seats to work on the analysis questions individually.  |
| **Assessment**  | Student lab work will be graded using the laboratory activities rubric. |
| **Critical Vocabulary**  | Enzyme: a protein that acts as a catalyst for specific substrates in specific reactions.Catalyst: a chemical or biochemical that acts to lower the activation energy of a reaction, allowing the reaction to proceed more rapidly.Substrate: the chemical or compound that an enzyme targets.Buffer: a combination of a weak acid and its conjugate base that resists changes in pH. |
| **Chemical Disposal** | Please review all federal, state and local regulations that may apply before proceeding.Pipette tips should be disposed of by each group in ziplock bags and sealed before being thrown away. Student-made solutions and extra Bicine solution should be neutralized with acetic acid before being disposed of. Recommended disposal follows Flinn Disposal Method #26b. |
| **Extension Activities**  | Part 1 of this activity can be done with CO2 water at a range of temperatures to show the difference in the solubility of CO2 with respect to temperature. High temperature CO2 water will not proceed to the end point as there will be relatively little CO2 dissolved. Low temperature CO2 water will proceed to the end point faster than room temperature CO2 water as more CO2 is dissolved.  |

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| PreLab: Qualitative Kinetics |
| Introduction: | Chemical kinetics is the study of the rate at which a reaction takes place. Many factors can influence this rate including concentration, temperature, surface area and the presence of a catalyst. In this lab, we will examine the qualitative effect that the presence of an enzyme (a biological catalyst) has on a reaction. |
| Mechanics: | The Enzyme:We will be working with Carbonic Anhydrase in this lab. Carbonic anhydrase is an enzyme found in many different organisms and catalyzes the hydration and dehydration reaction of carbon dioxide. In this lab, we will be looking at how carbon dioxide is hydrated.The Hydration Reaction:CO2 (aq) + H2O(l) 🡪 HCO3­­-(aq) + H+(aq) |
| Outline of the Lab: | Reaction 1)You will be mixing 10 mL of buffer with 10 mL of CO2 water and timing the reaction. This will be repeated three times.Reaction 2) You will be mixing 10 mL of buffer doped with 10 µL of enzyme solution D with 10 mL of CO2 water and timing the reaction. This will be repeated three times.Reaction 3)You will be mixing 10 mL of buffer doped with 10 µL of enzyme solution C with 10 mL of CO2 water and timing the reaction. This will be repeated three times.Reaction 4)You will be mixing 10 mL of buffer doped with 10 µL of enzyme solution B with 10 mL of CO2 water and timing the reaction. This will be repeated three times.Reaction 5)You will be mixing 10 mL of buffer doped with 10 µL of enzyme solution A with 10 mL of CO2 water and timing the reaction. This will be repeated three times. |
| Safety Considerations: | Enzyme:When you are done using the micropipetter, be sure to dispose of the tips in the ziplock bag |

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| Lab Flow Diagram: Qualitative Kinetics. |
| Introduction: | Chemical kinetics is the study of the rate at which a reaction takes place. Many factors can influence this rate including concentration, temperature, surface area and the presence of a catalyst. In this lab, we will examine the qualitative effect that the presence of an enzyme (a biological catalyst) has on a reaction. |
| Materials: | 1 micropipetter (10 µL) 2 50 mL beakers 2 graduated cylinders (10 mL capacity) 1 gallon size ziplock bag 1 stopwatch |
| Reaction 1: | Using the graduated Cylinder, obtain 10 mL of both the buffer and CO2 water and pour them into separate beakers.Pour the CO2 water into the buffer and time how long it take for the color change to occur.Pour the mixture down the drain, rinse your beakers with distilled water and repeat two times. |
| Reaction 2: | Using the graduated Cylinder, obtain 10 mL of both the buffer and CO2 water and pour them into separate beakers. Add 10 µL of enzyme solution D to the buffer.Pour the CO2 water into the buffer and time how long it take for the color change to occur.Pour the mixture down the drain, rinse your beakers with distilled water and repeat two times. |
| Reaction 3: | Using the graduated Cylinder, obtain 10 mL of both the buffer and CO2 water and pour them into separate beakers. Add 10 µL of enzyme solution C to the buffer.Pour the CO2 water into the buffer and time how long it take for the color change to occur.Pour the mixture down the drain, rinse your beakers with distilled water and repeat two times. |
| Reaction 4: | Using the graduated Cylinder, obtain 10 mL of both the buffer and CO2 water and pour them into separate beakers. Add 10 µL of enzyme solution B to the buffer.Pour the CO2 water into the buffer and time how long it take for the color change to occur.Pour the mixture down the drain, rinse your beakers with distilled water and repeat two times. |
| Reaction 5: | Using the graduated Cylinder, obtain 10 mL of both the buffer and CO2 water and pour them into separate beakers. Add 10 µL of enzyme solution A to the buffer.Pour the CO2 water into the buffer and time how long it take for the color change to occur.Pour the mixture down the drain, rinse your beakers with distilled water and repeat two times. |
| Results: | Copy the following data table into your lab notebook and complete the table with your observed times.

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| Enzyme | Trial | Time (s) |
| None | 1 |  |
| None | 2 |  |
| None | 3 |  |
| D | 1 |  |
| D | 2 |  |
| D | 3 |  |
| C | 1 |  |
| C | 2 |  |
| C | 3 |  |
| B | 1 |  |
| B | 2 |  |
| B | 3 |  |
| A | 1 |  |
| A | 2 |  |
| A | 3 |  |

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| Conclusion: | Answer the following questions in your lab notebook:Was there any inconsistency in your data? How might you control for this variation in the future?What other variables might this experiment be altered to examine?What enzyme sample was the “best” for speeding up this reaction? Keep in mind cost of enzyme and explain your reasoning. |

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| Lab Activities Rubric |
| Criteria | 4 | 3 | 2 | 1 |
| Participation:How well did the student participate in the lab activity? Did the student actively participate, or were they observing? Did the student have a role within the group (data keeper, lab flow monitor etc.) |  |  |  |  |
| Lab Notebook: Explanation:Is the student’s description of the lab and mythology consistent with the pre-lab and lab flow diagram? Is the writing sufficient to replicate the experiment? |  |  |  |  |
| Lab Notebook: Results:Are the student’s results displayed well in the lab notebook? Are they clear and labeled appropriately? Are all graphs requested present? |  |  |  |  |
| Lab Notebook: Conclusion:Is the student’s conclusion well written and thought out? Is the student specific in ways to improve the lab? Are the student’s responses thoughtful? |  |  |  |  |