# Lesson 2: Absorbance Curves: Using spectrophotometers to quantize the effects of a strong acid on a buffer.

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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   |  |  |  |  | | --- | --- | --- | --- | | Content Area | Grade Level | NC SCS | Lesson 2 | | Chemistry | 9-12 | CHM.3.1.1 |  | | Chemistry | 9-12 | CHM.3.2.2 | X |   Next Generation Science Standards   |  |  | | --- | --- | | NGSS | Lesson 2 | | HS-PS1-5 |  |   AP Chemistry Concept Outline   |  |  | | --- | --- | | Essential Knowledge | Lesson 2 | | 4.A.1 |  | | 4.D.1 |  | | 4.D.2 |  | | 6.C.1 | X | | 6.C.2 | X | |
| **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 construct absorbance curved for a 50mM Bicine solution.  Students will become familiar with the use and operation of a spectrophotometer.  Students will analyze the experimental method for flaws and test hypotheses on how the experiment can be refined. |
| **Time Required and Location** | 90 minutes in a science lab.  30 minutes of prep. |
| **Materials Needed** | Teacher List:   * 500 mL of 50mM Bicine Buffer w/ Cresol Red * 500 mL of 50mM Hydrochloric Acid * 500 mL of distilled water.   Student List:   * Pre-Lab assignment (1 per student). * Lab Flow Diagram (1 per student). * 21 cuvettes for every 2-3 students. * 4 50 mL beakers for every 2-3 students. * 4 graduated cylinders (5 mL capacity) for every 2-3 students. |
| **Safety** | Safety Concerns:   |  |  | | --- | --- | | PPE for teacher prep:   * Lab coat / apron * Gloves * Goggles | PPE for lab activity:   * Lab coat / apron * Goggles |   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) * [Hydrochloric Acid Solution](http://www.flinnsci.com/Documents/SDS/H/HydrochloricAcidSol0.1M-Less.pdf) |
| **Student Prior Knowledge** | This activity is designed to support student understanding of acid/base buffers and should be used either as a pre-learning activity or as a post learning activity. |
| **Teacher Preparations** | Time: 30 minutes  **Solution Preparation:**  500 mL of 50mM Bicine Buffer:  Dissolve 4.08 g of Bicine powder and 7.777 g of Sodium Chloride into approximately 400 mL of distilled water in a 600+ mL beaker.  Add 0.025 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 500 mL 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.  500 mL of 50mM Hydrochloric Acid  Add 250 mL of 0.10 M HCl to 250mL of distilled water. |
| **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.  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 their first trial of the activity, they will need to have their work approved before they can continue collecting data for the second, third and fourth trials.  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 (Required)** | Buffer: a combination of a weak acid and its conjugate base that resists changes in pH.  Absorbance: a measure of how light of a specific wavelength is absorbed by a colorimetric indicator. |

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| PreLab: Absorbance Curves | |
| Introduction: | Colorimetric indicators are used not only for qualitative, but also quantitative chemistry. By analyzing the amount of light that passes through a sample at certain wavelengths, the concentration of the acid and base versions of the indicator can be measured directly. |
| Mechanics: | The Spectrophotometer:  A Spectrophotometer sends light of a very specific wavelength at a sample and measures that amount of light that is transmitted through the sample as well as the amount that is absorbed. By measuring these two values, a Beer’s Law plot can be made to relate the absorbance to the concentration of hydronium ions and therefore the pH.  Beer’s Law  a = εbc  Where a = absorbance, ε = molar absorptivity constant, b = path length of the sample and c = concentration.  The ε and the b will remain constant throughout the experiment, so put more simply, beers law is a = kc where k is a constant. By determining this constant, we can calculate the relationship between absorbance and concentration. |
| Outline of the Lab: | During the course of this lab, you will be mixing various concentrations of hydrochloric acid with the buffer. You will run three different absorbance curves, each with seven samples.  Curve 1:  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.4 mL of distilled water and 0.1 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 2.3 mL of distilled water and 0.2 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 2.2 mL of distilled water and 0.3 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 2.1 mL of distilled water and 0.4 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 2.0 mL of distilled water and 0.5 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid.  Curve 2:  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.3 mL of distilled water and 0.2 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 2.1 mL of distilled water and 0.4 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 1.7 mL of distilled water and 0.8 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 1.5 mL of distilled water and 1.0 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 1.3 mL of distilled water and 1.2 mL of acid.  Curve 3:  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.2 mL of distilled water and 0.3 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 1.6 mL of distilled water and 0.9 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 1.3 mL of distilled water and 1.2 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 1.0 mL of distilled water and 1.5 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 0.7 mL of distilled water and 1.8 mL of acid. |
| Safety Considerations: | Hydrochloric Acid  We are working with a strong acid. While the concentration is not very high, you should still be cautious. Any spills need to be sprinkled with baking soda. |

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| Lab Flow Diagram: Absorbance Curves | |
| Introduction: | Colorimetric indicators are used not only for qualitative, but also quantitative chemistry. By analyzing the amount of light that passes through a sample at certain wavelengths, the concentration of the acid and base versions of the indicator can be measured directly. |
| Materials: | 21 cuvettes  4 50 mL beakers  4 graduated cylinders (5 mL capacity) |
| Curve 1: | Obtain some distilled water, hydrochloric acid and buffer in three separate, labeled beakers.  For each sample, measure out the required volumes of the solutions into your remaining beaker using separate cylinders for each solution. Then measure out 1.0 mL of the mixture in your fourth cylinder and pour it into a cuvette.  Place cuvette in the spectrophotometer and record the absorbance.  Flush all solutions down the drain with excess water.  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.4 mL of distilled water and 0.1 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 2.3 mL of distilled water and 0.2 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 2.2 mL of distilled water and 0.3 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 2.1 mL of distilled water and 0.4 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 2.0 mL of distilled water and 0.5 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid. |
| Curve 2: | Obtain some distilled water, hydrochloric acid and buffer in three separate, labeled beakers.  For each sample, measure out the required volumes of the solutions into your remaining beaker using separate cylinders for each solution. Then measure out 1.0 mL of the mixture in your fourth cylinder and pour it into a cuvette.  Place cuvette in the spectrophotometer and record the absorbance.  Flush all solutions down the drain with excess water.  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.3 mL of distilled water and 0.2 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 2.1 mL of distilled water and 0.4 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 1.7 mL of distilled water and 0.8 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 1.5 mL of distilled water and 1.0 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 1.3 mL of distilled water and 1.2 mL of acid. |
| Curve 3: | Obtain some distilled water, hydrochloric acid and buffer in three separate, labeled beakers.  For each sample, measure out the required volumes of the solutions into your remaining beaker using separate cylinders for each solution. Then measure out 1.0 mL of the mixture in your fourth cylinder and pour it into a cuvette.  Place cuvette in the spectrophotometer and record the absorbance.  Flush all solutions down the drain with excess water.  Sample 1: mix a solution using 2.5 mL of buffer and 2.5 mL of distilled water.  Sample 2: mix a solution of 2.5 mL of buffer with 2.2 mL of distilled water and 0.3 mL of acid.  Sample 3: mix a solution of 2.5 mL of buffer with 1.9 mL of distilled water and 0.6 mL of acid.  Sample 4: mix a solution of 2.5 mL of buffer with 1.6 mL of distilled water and 0.9 mL of acid.  Sample 5: mix a solution of 2.5 mL of buffer with 1.3 mL of distilled water and 1.2 mL of acid.  Sample 6: mix a solution of 2.5 mL of buffer with 1.0 mL of distilled water and 1.5 mL of acid.  Sample 7: mix a solution of 2.5 mL of buffer with 0.7 mL of distilled water and 1.8 mL of acid. |
| Results: | Copy the following data table into your lab notebook and complete the table with your observed times.   |  |  |  |  | | --- | --- | --- | --- | | Curve | Sample | Absorbance | Concentration of HCl (mM) | | 1 | 1 |  | 0 | | 1 | 2 |  | 1 | | 1 | 3 |  | 2 | | 1 | 4 |  | 3 | | 1 | 5 |  | 4 | | 1 | 6 |  | 5 | | 1 | 7 |  | 6 | | 2 | 1 |  | 0 | | 2 | 2 |  | 2 | | 2 | 3 |  | 4 | | 2 | 4 |  | 6 | | 2 | 5 |  | 8 | | 2 | 6 |  | 10 | | 2 | 7 |  | 12 | | 3 | 1 |  | 0 | | 3 | 2 |  | 3 | | 3 | 3 |  | 6 | | 3 | 4 |  | 9 | | 3 | 5 |  | 12 | | 3 | 6 |  | 15 | | 3 | 7 |  | 18 |   Also, plot a graph for each curve with concentration in the x axis and absorbance in the y axis. |
| 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 is the experimentally determined value for k?  Did you k value from all three curves agree? |

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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? |  |  |  |  |