**pH and Buffers**This lab is designed to help us:  
understand the meaning of pH values.  
understand the importance of pH regulation in biological systems such as the effects of pH on proteins  
understand the function of buffers.  
  
**Water** is one of the most abundant and important molecules on earth. Water’s structure - two hydrogen atoms linked to a single oxygen atom via polar covalent bonds – seems simple. Electrons are shared unevenly between atoms which causes water to be a polar molecule. Water can dissociate to form H+ ions, also called protons, and OH- or hydroxide ions.   
Other molecules, when dissolved in water, may dissociate to produce either H+ or OH- ions.   
**Acids:** Substances that produce H+ ions when dissolved in water are acids such as hydrogen chloride (HCl) which becomes hydrochloric acid in aqueous solution. Its dissociation produces H+ and Cl-.   
**Bases:** Substances that produce OH- ions when dissolved in water are bases. For example, the dissociation of a base, sodium hydroxide (NaOH), produces Na+ and OH-   
We use the term pH when discussing acids and bases.  
 **pH reflects the amount of H+ in a solution.**   
When pure water dissociates, an equal number of H+ and OH- ions are produced. In pure water, the concentration of H+ ions and of OH- ions is the same and = 1.0 x 10-7 moles per liter of water.  
Moles are written as M. M means molarity. (Molarity (M) is just a mathematical unit of concentration. 1.0 x 10-7 moles of H ions per liter of water is the same as writing 1/10,000,000 moles of H ions per liter of water.   
The pH of a solution is equal to the negative log of the hydrogen ion concentration given in moles/liter. Whew….  
Here’s how it works…  
Pure water has a pH of 7. That number comes from the calculation -log [1.00 x 10-7 H+ ] = 7.00  
pH 7 is said to be neutral because the concentrations of H+ and OH- ions are equal.  
  
The pH scale extends from 0 to 14. Solutions with greater than 1 x 10-7 M H+ ions are acidic and their pH   
values are below 7. Basic, or alkaline solutions, have fewer than 1 x 10-7 M H+ ions, and so have pH values over 7.   
**Acids have a pH below 7  
Bases have a pH above 7**   
Since pH is based on a logarithmic scale, a solution with a pH 5 has ten times the concentration of H+ ions than a solution with a pH 6.   
pH plays a particularly important role in biology. Most biological processes will work only within certain pH ranges. This is primarily due to the profound effect pH has on proteins. Proteins are large molecules that perform a variety of functions in all organisms. Many of them are enzymes (biological catalysts) and their activity is directly related to pH. When the pH becomes much higher or lower than the optimum pH for a particular protein, the protein loses its shape and function, and is said to be denatured. When an acid and a base are mixed in an aqueous solution, there will be a reaction known as neutralization. The H+ ions released from the acid and the OH- ions released from the base will combine to form water.   
 An example of the neutralization reaction is HCl + NaOH = NaCl + H2O   
 **Regulation of pH with Buffers** A **buffer** is a chemical that absorbs excess H+ when pH is too low or releases H+ when pH is too high.  
  
Because pH plays such a significant role in protein function, many biological systems are naturally buffered to maintain pH within a relatively narrow range. For example, the pH of human blood is normally held to a value between 7.35 and 7.45. If blood pH moves beyond this range its disastrous.  
The pH of the blood is maintained in the proper range by a buffering system.   
  
Our blood transports many important substances, among them carbon dioxide. When CO2 dissolves in blood, it reacts with water as shown below to form carbonic acid that can then dissociate into H+ and OH- ions. This reaction is reversible, allowing for the amount of H+ to be raised or lowered as needed. This mechanism maintains blood pH by adjusting the amount of H+ in solution.  
 CO2 + H2O 🡪 H2CO3 🡪 H+ + HCO3   
   
 **Using a pH probe**In this laboratory exercise, we will be using a pH meter to measure pH values. One student in each group should assume the responsibility for handling the pH meter. You will need a beaker for waste solutions and a squeeze bottle of deionized water to rinse the pH probe between measurements. To measure the pH of solutions, first rinse the end of the probe with deionized water (over a waste beaker) then place the probe in the solution to be measured. Wait until the reading is stable, then record that measurement. Be sure to rinse the probe after each reading. Do not allow the end of the probe to become dry. If you must remove the probe from a solution, rinse it, and return it to the pH 7.00 buffer.   
  
**Exercise 1: Determining pH  
Procedure:**You have tasted some of these types of compounds in your food.   
Citric acid gives lemons their characteristic sour taste. Alkaline compounds have a bitter taste.  
We describe the degree of acidity or alkalinity according to the following terms:   
very acidic (0-3)  
slightly acidic (4-6)  
neutral (7)  
slightly basic (8-10)  
very basic (11-14)  
The purpose of this exercise is to determine the pH of various household solutions. To do this, we will use two methods to measure pH (pH paper and pH probe).   
1. Obtain a clean, plastic, 12 well spot plate. Fill each well ¾ full with one of the following solutions:  
Aspirin  
Lemon Juice  
Vinegar  
Nair  
Solution X  
Coca Cola  
Tea/Coffee   
Bleach  
2. Tear off a short strip of pH paper, dip it into the well to saturate one end, and remove.  
3. Compare color with the pH color key on the package. Record the pH.   
4. Measure the pH of each solution using the pH probe (refer to above guidance) and record the pH in the table below. After measuring pH with the pH probe, remember to rinse the pH probe thoroughly with deionized water and blot off the excess water with a paper towel. Be sure the pH probe doesn’t become dry. When the final solution has been measured, rinse the probe very well and return it to the pH 7.00 buffer.   
5. Determine whether the solution is an acid or base.  
Which solution is the strongest acid?  
Which is the strongest base?  
6. Why do you think you obtained, in some cases, different measurements depending on the method you used?   
  
**Exercise 2: Examining the effects of extreme pH on biological molecules.   
Procedure:**  
1. Obtain a 12 well plastic spot plate from the supply bench.  
Refer to the picture on the board to determine the number of each well.   
Using a plastic pipette, fill wells 1, 2, and 3 half full with egg white.  
 fill wells 4, 5, and 6 half full with egg yolk.  
 fill wells 7, 8 and 9 half full with milk.   
Leave wells 10, 11, and 12 empty at this point  
2. Take the filled plate, a small dropper bottle of 1M HCl, a small dropper bottle of 1M NaOH, and a small dropper bottle of deionized water back to your bench.  
3. Using the droppers, fill wells 1, 4, 7, and 10 with 1.0 M HCl  
 fill wells 2, 5, 8, and 11 with 1.0 M NaOH  
 fill wells 3, 6, 9, and 12 with deionized water.  
4. Wells 10, 11, and 12 should contain only acid, base, or water.   
Use the pH probe to measure the pH of the solutions in these wells only.   
5. With a toothpick, carefully mix the contents of each of the other 9 wells. Use a new toothpick for each sample. Let them stand until the end of the lab period.  
6. Describe the appearance of the material treated by HCl, NaOH, or H2O.  
  
**Exercise 3: Observing the results of a neutralization reaction.**  
Our stomachs contain very acidic fluid that aids breakdown of food. Stomach acid may be as concentrated as 0.1 M HCl (pH range 1-3). Occasionally some of this acid escapes into the esophagus which causes a burning sensation commonly called “heartburn.” When discomfort from heartburn occurs, it helps to neutralize some of the escaped stomach acid by consuming antacids.   
Some antacids contain hydroxide ions. Others contain baking soda or other ions such as bicarbonate ions that act as bases in solution. They are all basic and able to neutralize stomach acid by combining with the free H+ released from stomach acid. Under ideal conditions, cells are able to regulate pH without these antacid medications.   
**Procedure:**1. Use the mortar/pestle to grind 1 tablet of TUMS. Pour into a beaker. Add 25 mL of water. Set aside.  
2. Use a graduated cylinder to measure 25 mL 0.1 M HCl (hydrochloric acid).  
 Pour the acid into a beaker. Into this beaker, place a large magnetic stir bar and then put the beaker   
 on a stir plate. Set the stir plate to a medium setting.   
4. Add 10 drops of methyl orange to the acid.   
 Methyl Orange is a pH indicator that is pinkish red in solutions with pH 3.1 or lower.  
 As the pH increases, methyl orange gradually turns from pinkish red to orange.  
 At pH 4.4 or higher, methyl orange is orange.  
 5. Using a 10 mL pipette, add 1 mL of the TUMS solution. Wait 60 seconds. Repeat until the solution   
 stays yellow for approximately for 30 seconds. Record the amount of TUMS that must be added to   
 change the color of the solution to orange.   
6. Repeat procedures 1-5 using 1 g baking soda in 25 mL of tap water.  
 Record the amount of baking soda that must be added to change the color of the solution to orange.  
7. Which might be a better antacid, TUMS or baking soda  
  
**Exercise 4: Demonstrating the capacity of a buffer to maintain the pH of a solution.**Remember, buffers are solutions that have the capacity to absorb or release H+ ions to maintain a specific pH. Eventually, a buffer will reach a saturation point after which the pH will change dramatically. \*\*It is crucial to avoid cross contamination of solutions for this exercise. For this reason, use the proper graduated cylinders (labeled at the supply bench) to measure and dispense into your beakers. Measure and dispense the solutions at the supply bench – please do NOT carry them back to your own lab bench. **Procedure:**   
1. Use a graduated cylinder to measure 25 mL tap water. Pour the water into a 250 mL beaker.   
2. Put a small magnetic stir bar in the water and place the beaker on a stir plate. Set the stirring apparatus to a low setting.   
3. Label a 50 mL beaker “acid” and take it to the supply bench. Pour approximately 20 mL of 0.1M HCl from the stock bottle into the labeled beaker and take the beaker back to your work area.   
4. One student should hold the pH probe so that the tip is submerged in the beaker of solution on the stir plate but not in danger of being hit by the stir bar. This student will continue to hold the probe in this position for the remainder of the procedure. Do not remove the probe but allow it to continuously record the pH. Do not lean the probe against the side of the beaker. Measure and record the pH of the water in the beaker on the stir plate.   
5. A second student will be responsible for adding the acid. This student should fill a 5 mL pipette with acid, and then add 1 mL 0.1M HCl to the beaker on the stir plate. Allow the solution to mix and record the pH when the reading has stabilized. You may have to wait a moment for the reading to become constant.   
6. Continue adding the HCl, 1 mL at a time, until you have added a total of 3 mL. Record the pH after each addition.  
7. Using the magnetic wand, remove the stir bar from the 250 mL beaker and pour the contents into the waste container. Rinse the beaker and stir bar very well with tap water.   
8. Use the provided, labeled graduated cylinder to measure 25 mL of pH 7.00 buffer at the supply bench. Pour the buffer into the 250 mL beaker you just washed. Add the stir bar and place on the stir plate as before.  
9. Measure and record the initial pH of the buffer.   
10. Using the 5mL pipette, add 1mL 0.1M HCl to the beaker. Continue adding HCl 1mL at a time until you have added a total of 15 mL, recording the pH on the table after each addition.   
11. Using the magnetic wand, remove the stir bar from the beaker and pour the contents of your beaker into the waste container. Rinse the beaker and stir bar very well with tap water.   
  
12. Now repeat the experiment steps 1-11 (above), this time using 0.1 M NaOH (a base) instead of 0.1 M HCl. For step 3, you will need to label a 50 mL beaker “base” and pour in approximately 20 mL of 0.1 M NaOH at the supply bench. Take the beaker of base back to your lab bench.  
13. You now have recorded four sets of data that will enable you to plot four lines on a graph. Prepare a graph of this data showing the data you’ve collected.  
 The x axis should be labeled “volume of NaOH” or volume of HCl” added.   
 Use the darker lines on the graph paper to represent 1 mL intervals.  
   
 The Y axis should be labeled “pH.”   
 Use the darker lines on the graph paper to represent intervals of 1 on the pH scale.   
  
So, the volume added values are used for your x-coordinates, and the “pH” values for your y-coordinates. Use a different color or symbol to represent each of the four sets of points. You will connect the points to draw and LABEL four lines on the graph:  
Beaker 1 - Water plus acid   
Beaker 2 - Buffer plus acid  
Beaker 3 - Water plus base  
Beaker 4 - Buffer plus base  
  
**Please answer these questions:**1. What is an acid?  
 What is a base?  
 What does it mean to say that a solution is neutral?  
  
 2. What is a buffer?  
  
  
 3. Why is it important to regulate pH in biological systems like blood?  
  
  
 4. Saliva has a pH range of 5.5 to 6.9. Is it an acidic or basic solution?  
  
  
 5. Which is more acidic, a solution with pH 3 or one with pH 6?  
  
  
6. When an acid is added to water, should the pH of the mixture go up or down?  
  
  
7. What type of reaction occurs when an acid reacts with a base?  
  
8. Referring to part I, which solution had the greatest H+ concentration?  
  
 Which solution had the lowest H+ concentration?  
  
9. Referring to part II, what was the purpose of adding water to one set of wells?  
  
  
10. What happens to proteins exposed to pH extremes?   
  
  
11. Refer to the graph you prepared for part IV.  
 a) What was the pH of water before acid or base was added?   
  
 How much did the pH change when 1mL of acid was added? How much did the pH change when   
 1mL of base was added?  
  
 b) What was the pH of the buffered solution before acid or base was added?  
  
 How much did the pH change when 1mL of acid was added? How much did the pH change when   
 1mL of base was added?   
  
 c) How many milliliters of HCl must be added to pH7 buffer to change the pH to the same pH as   
 water plus 3 milliliters of acid?   
  
 How many milliliters of NaOH must be added to pH7 buffer to change the pH to the same pH as   
 water plus 3 milliliters of base?  
  
 d) What can you conclude from this graph about the function of a buffer?  
  
  
 e) Give an example of why it might be important to have a solution buffered.