Laboratory Investigation: BIOLOGICAL BUFFERS
How do Biological Materials Respond to Acids and Bases?

Even small changes in pH can cause distress to organisms. The changes in hydrogen ion concentration in cells can change the rates of some chemical reactions. For example, the pH of human blood is normally about 7.4 and must be in the range of 7.0-7.8 for survival. If the pH is in the 7.0-7.3 range the person will feel tired, have trouble breathing, and may even be disoriented. If the pH of the blood is in the 7.5-7.8 range, the person will feel dizzy and rather agitated.

It is very important for organisms to be able to maintain a fairly constant internal environment (homeostasis). To prevent the hydrogen ion concentration of the cytoplasm from changing too much, cells have chemical compounds called "buffers" that will bind with hydrogen ions when their concentration increases too much. Buffers can also release bound hydrogen ions when their concentration in the solution decreases too much. In our blood stream, carbonic acid (H_2CO_3) acts as the buffer that maintains our blood pH within a normal range.

$$H_2CO_3 \text{ <----------> } (HCO_3)^- + H^+$$

Products such as Alka Seltzer take up hydrogen ions and reduce the acidity of the stomach fluids. They consist of sodium bicarbonate, citric acid, and salicylate analgesic. When placed in water, sodium citrate and carbon dioxide are produced. Sodium citrate is a strong buffer.

In this laboratory, you will investigate how several materials respond to the addition of an acid and a base to determine whether living materials have buffering capacity.

You will follow a generic procedure using a variety of biological materials and other substances, then try to figure out the explanation for the results. Typically, when acid is added to water, the pH will drop quickly and then level out. Conversely, when base is added to water, the pH will rise quickly and then level out. When acid or base is added to buffer, the pH barely changes.

Before you start, hypothesize about the buffering ability of each substance to be tested. List (on your paper, as **Hypotheses**) the substances that will have the best capacity to act as buffers, moderate buffering capacity, and poor or no buffering ability

Materials

- pH probe & LabPro setup
- dH_2O in wash bottle
- 50 mL container
- *30 mL graduated syringe*
- 0.1 M NaOH in dropper bottle
- 0.1 M HCl in dropper bottle
- *pH 4 buffer solution*
- various homogenates
- Alka Seltzer solution

Procedure

You will measure the changes in pH resulting from adding acid and base to plain tap water, each of the homogenates, the pH 4 buffer solution, and the Alka Seltzer solution. Make sure you record all of your pH measurements in your data charts.

1. Pour 25 mL of test substance into the 50 mL beaker. Measure the pH of the solution following directions for using the LabPro with pH probe. Record in Table 1.
2. Add 0.1 M HCl one drop at a time, swirling after each drop. After you have added 5 drops, measure the pH again. Record in Table 1.
3. *Repeat step 2, recording the pH after each 5 drops, until a total of 30 drops have been added.*
4. Rinse the beaker well and add another 25 mL of test substance. Rinse the pH probe with distilled H_2O.
5. Follow steps 2-3 again, but this time use 0.1 M NaOH and record in Table 2.
6. Repeat the whole procedure (steps 1-5) for each of the test solutions.
7. Graph your results using Microsoft Excel. Use separate graphs for the response to HCl and the response to NaOH.

Data Charts

Table 1: The pH of various substances after adding HCl

Test Substance	0 drops HCl	5 drops HCl	10 drops HCl	15 drops HCl	20 drops HCl	25 drops HCl	30 drops HCl	Overall Change
Tap water								
Buffer pH 4								
Alka Seltzer								
Potato								
Egg White								
Yeast								
Broccoli								
Chicken Liver								
Milk								

Table 2: The pH of various substances after adding NaOH

Test Substance	0 drops NaOH	5 drops NaOH	10 drops NaOH	15 drops NaOH	20 drops NaOH	25 drops NaOH	30 drops NaOH	Overall Change
Tap Water								
Buffer pH 4								
Alka Seltzer								
Potato								
Egg White								
Yeast								
Broccoli								
Chicken Liver								
Milk								

Calculate the average change of the acid and base trials combined. You do not need to graph this data.

Substance	Tap Water	Buffer pH 4	Alka Seltzer	Potato	Egg White	Yeast	Broccoli	Chicken Liver	Milk
Average Change									

Analysis Questions – <u>Type up</u> your responses and hand in with your printed graph.

1. Examine your graphs. Are the responses of the biological homogenates to the addition of acid and base more similar to the responses of the tap water or to the responses of the buffer solutions? Explain your observations.

2. Which of the biological homogenates (not tap water or pH 4 buffer) has the most buffering capacity? How did you determine this? Why do you think this substance buffers so well?

3. Which of the biological homogenates (not tap water or pH 4 buffer) has the worst buffering capacity? How did you determine this? Why might this substance have such a poor buffering ability compared to the other biological substances?

4. What did you learn about the ability of living systems to regulate pH?

5. Explain why buffers are important to living systems. Give examples to support your ideas.