

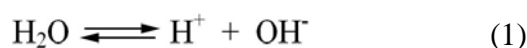
## Biology 3A Laboratory: Acids, Bases and Buffers

### Objectives

- To understand the concept of pH
- To be able to calculate pH from acid molar concentration
- To measure pH using instrumentation and indicators
- To understanding the effect of a buffer on pH

### Introduction

This lab assumes a knowledge of the chemical concept of molar concentration. An important application of molarity, or molar concentration, involves hydrogen ions in solution. The concentration of free hydrogen ions in pure water is  $1.0 \times 10^{-7}$  M. This occurs because water can dissociate as shown in this equation



In pure water, the concentration of  $\text{OH}^-$  and  $\text{H}^+$  ions are equal (both are  $1 \times 10^{-7}$  M). If we increase the concentration of hydrogen ions the solution becomes acidic; if we decrease the concentration of hydrogen ions the solution will become basic.

Chemists use the pH scale to indicate acidity. In this scale, pH is equal to the negative logarithm of the hydrogen ion concentration

$$\text{pH} = -\log [\text{H}^+] \quad (2)$$

The pH scale generally ranges from 0.0 to 14.0. Pure water has a pH of 7.0. If we consider 1.0 M HCl, it has a hydrogen ion concentration of  $1 \times 10^0$ . Thus it has a pH of 0.0. If we were to have a 10.0M HCl solution, its pH would be -1.0!

In this lab we will look the problem of measuring pH using indicators and instruments, and then consider ways to control the fluctuation of pH.

In this lab we will use the notation M to indicate molar solutions (number of moles per liter) and mM to indicate millimolar solutions (number of millimoles per liter). If a solution has 1 mole per liter, that is also 1000 millimoles per liter; therefore  $1 \text{ M} = 1000 \text{ mM}$ .

### pH Indicators

An indicator is a chemical that changes predictably under changing condition. There are several well-known pH indicators that change color at specific levels of hydrogen ion concentration. Five of the well-known pH indicators are shown in Table 1.

**Table 1.** Some common pH indicator chemicals and their respective colors in acidic and basic solutions

Indicator	pH	Color in Acid	Color in Base
Phenolphthalein	8.3 – 10.0	Colorless	Red - Pink
Litmus	4.5 – 8.3	Red	Blue
Bromocresol Purple	5.2 – 6.8	Yellow	Purple
Bromophenol Blue	3.0 – 4.6	Yellow	Blue
Methyl Violet	0.2 – 3.0	Yellow	Blue - Violet

### **Litmus, a common biological pH indicator**

In Table 1, litmus is a pH indicator that seems to broadly cover the neutral range of pH (both sides of 7.0). Litmus is a naturally occurring compound found in many plants. If you are familiar with the Hydrangea, you may know that it can occur in both a blue and a pink form. Actually, it's the same plant and same flowers; it's just the pH of the soil that alters the color! Grow it in acidic soil and it's pink; grow it in basic soil and it's blue. Another source for litmus is red cabbage (*or perhaps blue cabbage, depending on the pH*). We can use red cabbage to create a litmus solution, which we can then use to observe pH changes.

#### **Procedure 1. Make and use Litmus from red cabbage**

1. Cut some red cabbage leaves. Using the blender and a small amount of tap water, shred the leaves.
2. Strain about 30 ml of the solution from the leaves into a beaker. This is the litmus solution. What color is it right now? What is the current pH?
3. Add about 80 ml tap water. What can you conclude about the pH tap water here Saddleback College?
4. Add 0.1 M HCl drop by drop until you see a color change. Describe the color change. What is the current pH of the solution?

If you were going to make a cabbage salad and you wanted the red cabbage to be bright red, what dressing could you use? (If this question is unclear, answer it after you complete the following section.)

#### **Procedure 2. Measuring the pH of common solutions**

Some indicators have a much broader pH range. Hydrion® paper is an example of a broad range indicator conveniently applied to a paper used for pH testing. Using Hydrion pH paper, measure and record pH of some common solutions provided in lab. Enter the measured pH values into Table 2.

#### **Procedure 3. Using a pH meter**

A pH meter can be used to directly measure the hydrogen ion concentration. **Please watch your instructor's demonstration of the pH meter.** Always wash the electrode with deionized water after use. Always place the washed electrode into deionized water after washing. In procedure 2 you measured the pH of three HCl solutions. Use the meter to measure the pH of each of these solutions again. Enter your measurements into Table 2.

#### **Procedure 4. Compare Calculated pH with pH paper and pH meter**

Let's calculate the actual free hydrogen ion concentration in an acid solution of known concentration. In the earlier exercise you measured the pH of a 0.01 M HCl solution. In an aqueous solution HCl completely dissociates, or in other words, the H<sup>+</sup> and the Cl<sup>-</sup> ion part company totally. Thus if we know the concentration of HCl, that is equal to the free hydrogen ion concentration or [H<sup>+</sup>].

So in our 0.01 M HCl we have a free hydrogen ion concentration of  $1.0 \times 10^{-2}$  moles per liter.  $\text{pH} = -\log [\text{H}^+] = -\log [1.0 \times 10^{-2}] = 2$ . So the pH of 0.01 M HCl should be 2.0

Calculate the expected pH for 0.001 M HCl and 0.00001 M HCl. Enter these expected values into Table 2. Compare your measured values using pH paper and the pH meter to the calculated values. Please discuss any variability in your results from each of the three measurements.

### Procedure 5. How effective are antacids?

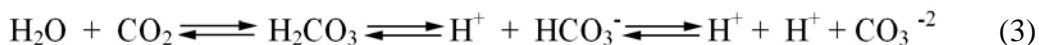
Typical over-the-counter (OTC) antacids claim to “neutralize excess stomach acid.” You’ve seen the commercials and advertisements. Since the acid produced by your stomach is HCl, we can easily find out how well they work. The typical pH of the acid in your stomach is around 2.0. In lab you will find a selection of OTC antacids. Test each of these in the following manner. In addition, test 1/4 teaspoon of baking soda (sodium bicarbonate, NaHCO<sub>3</sub>) in the same fashion. Proceed as follows:

1. Using a mortar and pestle, crush one tablet and add it to 100 ml of deionized water in a beaker.
2. Using a stirring rod, mix until the powder is dissolved. It will remain milky.
3. Add 6 drops of Bromcresol Purple indicator to the beaker. Table One shows the pH range of various pH indicator chemicals. What’s the pH range for Bromcresol Purple? Is this an appropriate indicator to use for this work?
4. Add 0.1 M HCl drop by drop until the solution turns to yellow. (What does this color change indicate?)
5. Record the number of drops in Table 3
6. Repeat this procedure for each of the antacids and the NaHCO<sub>3</sub>.

Which antacid neutralizes the greatest amount of acid? Which one neutralizes the least? What can you conclude regarding the advertisements you have seen for these products? How did the baking soda behave in this test? Could you use a weak solution of baking soda instead of an OTC antacid for your “sour stomach?”

### Buffers

Small changes in pH can alter the function of biologically important molecules such as enzymes. For this reason, in most organisms pH is very closely regulated. pH can be kept relatively constant by buffers, chemical which absorb or release hydrogen ions to maintain fairly constant pH. A very common example of this is found when carbonate ions are dissolved in water. The system is shown below:



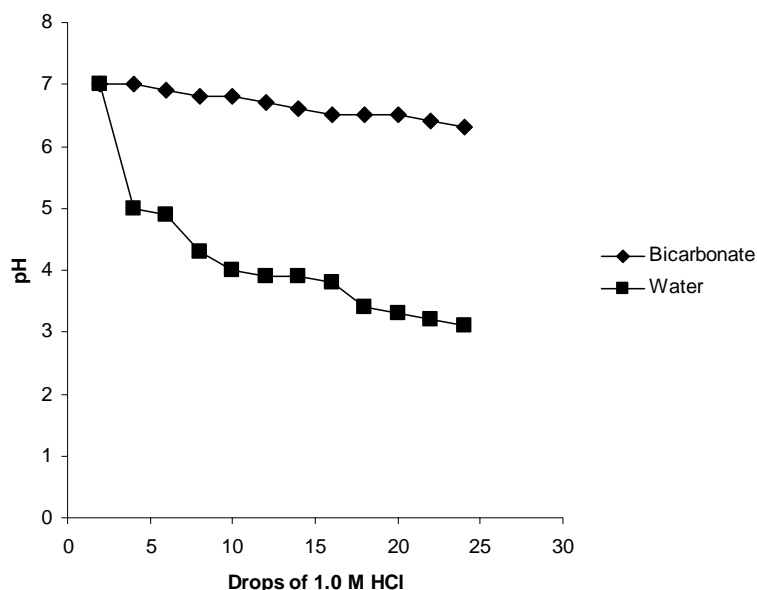
In this set of equations, carbon dioxide combines with water to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>). This weak acid can dissociate to form free hydrogen ions and bicarbonate ion (HCO<sub>3</sub><sup>-</sup>). Finally the bicarbonate can lose another hydrogen to form carbonate ion(CO<sub>3</sub><sup>-2</sup>). In most vertebrate animals, this system maintains blood pH very close to 7.4.

### Procedure 6. Measuring the effect of a buffer on pH change

1. Place 100 ml of deionized water into each of two 200 ml beakers.
2. To one beaker add 1/8 tsp of NaH<sub>2</sub>CO<sub>3</sub>
3. Using the pH meter, record the change in pH for each beaker after each addition of 2 drops of 0.1M HCl
4. Record your data in Table 4.

Explain any difference between the pH change of the water and the bicarbonate solution when acid is added. *Please create a graph of these data in Excel.*

Here's an example of what your graph should look like:



### Procedure 7. Effects of CO<sub>2</sub> on pH

When you observe equation 3, it becomes clear that an excess of CO<sub>2</sub> molecules would push the equilibrium to the right. If this were to occur, what would happen to the pH? Would the free hydrogen ion concentration increase?

1. Place 100 ml of distilled water into a 200 ml beaker and add 1.0 ml 0.2 M NaOH
2. Add four drops of phenolphthalein indicator
3. Using a soda straw placed blow bubbles of exhaled air into this solution
4. Observe and record the time for a color change to take place

What does this color change mean?

### Procedure 8. Effect of exercise on rate of CO<sub>2</sub> production

Does the concentration of CO<sub>2</sub> in exhaled air increase after exercise? Let's use the last protocol to test this hypothesis.

1. In this protocol you will use the same subject as you did in Procedure 7.
2. This person should run up and down the stairs next to the lab 10 times
3. While your lab partner runs, prepare a beaker following steps 1 and 2 in Procedure 7.
4. The runner should return immediately to the lab, and begin exhaling (carefully!) through the straw into the solution.
7. Record the time for a color change to take place.

What can you conclude regarding our initial hypothesis?

**Procedure 1. Litmus pH indicator**

What can you conclude about the pH tap water here Saddleback College?

As you add acid, describe the color change.

What is the current pH of the solution?

If you were going to make a cabbage salad and you wanted the red cabbage to be bright red, what dressing could you use? (you may want to use information from the following section)

**Procedure 2, 3 and 4. pH of common solutions, pH meter and pH calculation**

**Table 2.** pH of some common solutions measured by paper, meter and calculated

<b>Solution</b>	<b>pH paper</b>	<b>pH meter</b>	<b>pH calculate</b>
vinegar		XXXXXXXXXX	XXXXXXXXXX
household ammonia		XXXXXXXXXX	XXXXXXXXXX
lemon juice		XXXXXXXXXX	XXXXXXXXXX
cola		XXXXXXXXXX	XXXXXXXXXX
black coffee		XXXXXXXXXX	XXXXXXXXXX
household bleach		XXXXXXXXXX	XXXXXXXXXX
nonfat milk		XXXXXXXXXX	XXXXXXXXXX
HCl 0.01M			
HCl 0.001M			
HCl 0.0001M			

Comment on the measured and calculated values for the HCl solutions:

### Procedure 5. Antacid effectiveness

**Table 3.** Effectiveness of some common antacids (one dose dissolved in 100 ml water) measured by dropwise neutralization of 0.1ml HCL

<b>Antacid Product</b>	<b>Number of drops of acid</b>

Which antacid neutralizes the greatest amount of acid? Which one neutralizes the least?

What can you conclude regarding the advertisements for these products?

How did the baking soda behave in this test? Could you use baking soda instead of an OTC antacid for your “sour stomach?”

### Procedure 6. Measuring the effect of a buffer on pH

**Table 4.** pH change after acid addition to water and bicarbonate solution

<b>No. of drops of 0.1M HCl</b>	<b>Pure Water</b>	<b>Bicarbonate Sample</b>
0		
2		
4		
6		
8		
10		
12		
14		
16		
18		
20		
22		
24		
<b>26</b>		

Explain any difference between the pH change of the water and the bicarbonate solution when acid is added.

*Please create a graph of these data in Excel. Use a line graph, with one line indicating pure water pH and one line indicating bicarbonate pH.*

### **Procedures 7 and 8. CO<sub>2</sub> production and pH**

How much time did it take for the color to change in the resting subject?

What does this color change mean?

How much time did it take for the color to change in the subject after exercise?

Explain any difference in the time for color change to take place between the resting and exercise data.

Our initial hypothesis stated that CO<sub>2</sub> production would increase after exercise. What can you conclude?