LAB TOPIC 2

pH and Buffers

Introduction

Molecules that are dissolved in water may separate (ionize) into charged fragments. pH is a measure of the concentration of one of those charged fragments, hydrogen ions (H^+), in solution. A substance that has a high concentration of H^+ is acidic. A substance that has a low concentration of H^+ is basic (alkaline).

The pH scale ranges from 0 (most acidic) to 14 (most basic). There is a tenfold difference between pH units. For example, a solution with a pH value of 6 has a ten-times-greater concentration of hydrogen ions than a solution with a pH value of 7. Some examples are shown in Table 2.1.

Outline

Exercise 2.1: Introduction to Acids, Bases, and pH

Exercise 2.2: Using Red Cabbage Indicator to Measure pH

  Activity A: Making a Set of Standards

  Activity B: Comparing pH of Beverages and Stomach Medicines

Exercise 2.3: Using the pH Meter to Determine Buffering Capacity

Exercise 2.4: Designing an Experiment

Exercise 2.5: Performing the Experiment and Interpreting the Results

EXERCISE 2.1

Introduction to Acids, Bases, and pH

Objectives

After completing this introductory exercise, you should be able to

1. Explain what makes a solution acidic or basic.
2. Explain the pH scale.
3. Describe the phenol red test for pH.

An **acid** is a substance that releases or causes the release of H^+ into solution. Solutions that have pH values lower than 7 are considered to be acids. Some common acids are hydrochloric acid, acetic acid, carbonic acid, and sulfuric acid. All of these compounds contain hydrogen. When

Table 2.1
pH Scale

pH	Relative strength	Examples
0	Strong acid	
		Battery acid
1		
2		Gastric fluid
3	Moderate acid	Orange juice
4		Tomato juice
5	Weak acid	
		Rainwater
6		Milk
7	Neutral	Pure water
		Blood
8		
		Baking soda
9	Weak base	Milk of Magnesia
10		
11	Moderate base	Household ammonia
12		
		Hair remover
13	Strong base	
		Oven cleaner
14		

the compound is dissolved in water, hydrogen ions are released, and the pH of the solution is low. Your instructor will use an indicator called **phenol red** to demonstrate the acidity of hydrochloric acid. Phenol red is red when the solution is basic and turns yellow in acidic solution.

What color is the phenol red solution initially?

What happens when hydrochloric acid is added?

A compound does not have to contain hydrogen ions itself in order to be an acid. Carbon dioxide (CO_2), for example, can combine with water to generate H^+. Your instructor will use phenol red again to demonstrate that CO_2 is an acid. Briefly describe this demonstration.

The reaction of SO_2 (sulfur dioxide) is similar to that of CO_2. The presence of SO_2 in the atmosphere is partially responsible for acid rain.

A **base** is a substance that can remove H^+ from solution, thus lowering the concentration of H^+. Many bases ionize to produce hydroxyl ions (OH^-), which combine with H^+ to make water (H_2O). Some common bases are sodium hydroxide (NaOH), magnesium hydroxide ($Mg(OH)_2$), and potassium hydroxide (KOH).

Mixing an acid with a base can produce a neutral solution by combining the H^+ with the OH^- to make water (H_2O). Pure water, which ionizes to produce equal numbers of H^+ and OH^-, is neutral (pH 7). Don't expect to get a pH of 7 when you measure water in the lab, though. Tap water contains impurities, and its pH varies a great deal. Distilled water is weakly acidic; its pH is usually around 6.

It is important for organisms to maintain a constant internal pH. As you will learn in later laboratories, biological molecules, especially proteins, are sensitive to pH, and they may not function correctly when the pH is changed.

In the following exercises you will make an indicator solution to measure pH and also learn how to use a pH meter. You will determine the pH values of some common substances and investigate how buffer systems work to maintain a constant pH.

Wear safety glasses while performing these exercises. Strong acids and strong bases are corrosive. Inform your instructor immediately if any solution is spilled or comes in contact with your skin or clothing.

EXERCISE 2.2
Using Red Cabbage Indicator to Measure pH

Objectives

After completing this exercise, you should be able to

1. Explain what a pH indicator is used for.
2. Describe how to measure pH using red cabbage indicator.

Several methods are available for determining pH. Many of these methods rely on the ability of certain chemicals called **indicators** to change color, depending on the pH of the surrounding solution. Papers saturated with indicators, such as litmus paper and alkacid test paper, can also be used.

An indicator can easily be made from a solution of anthocyanins, the pigments responsible for red, blue, and purple colors in flowers, fruits, and autumn leaves. These pigments change color as the pH changes. Red cabbage is loaded with anthocyanins, so we can make a pH indicator by boiling red cabbage to extract the pigments. Your instructor will make the extract at the beginning of class.

The use of standards, a set of known quantities, is an important technique in biological research. By comparing unknowns with the standards, we can determine what we want to know about the unknowns. The color of cabbage extract depends on the pH of the solution it is in. Your set of standards will show the color of the cabbage extract at pH 2, 4, 6, 7, 8, 10, and 12. You will then determine the pH values of various substances by mixing each substance with cabbage extract and comparing its color to the standards.

Activity A: Making a Set of Standards

Your lab team should make a set of standards using the cabbage extract and solutions of known pH according to the following procedure.

Procedure

1. Put seven clean test tubes in a rack and label them 2, 4, 6, 7, 8, 10, and 12.
2. Pipet 5 mL of the appropriate buffer into each tube (pH 2 buffer into the tube labeled 2, and so on).
3. Get a dropping bottle of cabbage extract from your instructor. Add 3 mL of cabbage extract to each tube.
4. Cover the tubes with Parafilm and mix well.
5. Record the color in each tube in Table 2.2.

Save this set of standards for use throughout the lab period.

Table 2.2
Color of Standard Solutions for Red Cabbage Indicator

pH	Color
2	
4	
6	
7	
8	
10	
12	
Record both the initial and final colors at pH 12. The pigments are not stable at this pH.	

Activity B: Comparing pH of Beverages and Stomach Medicines

Look at Table 2.3 to see what aspect of pH is being investigated in this experiment, and answer the following questions.

Table 2.3
pH Values of Beverages and Medicines

Beverages	pH	Medicines	pH
White grape juice		Milk of Magnesia ($Mg(OH)_2$)	
7-Up		Sodium bicarbonate ($NaHCO_3$)	
White wine		Maalox	
Seltzer water			

What hypothesis could be tested with this experiment?

What is the independent variable in this experiment?

What is the dependent variable?

What substance could be used as a control for this experiment?

Predict the outcome of the experiment in terms of your hypothesis. What results will support the hypothesis? What results will prove the hypothesis false?

Use the cabbage indicator method to measure the pH of the substances listed in Table 2.3.

Procedure

1. Put 2 droppersful of the solution to be tested in a clean test tube.
2. Add 1 dropperful of cabbage extract.
3. Swirl the tube gently to mix.
4. Compare the color of the solution to the colors of your cabbage indicator standards.
5. Record the pH value for each substance in Table 2.3.
6. Measure and record the pH of your control.

   Control: __________

   pH of control: __________

Was your hypothesis proven false or supported by the results? Use data to support your answer.

What components of the beverages you tested might be responsible for the pH values of the beverages?

What components of the medicines you tested might be responsible for the pH values of the medicines?

Explain why stomach medicines should have pH values that are much higher than normal stomach pH, which is around 2. (Hint: Why do people take these medicines?)

EXERCISE 2.3

Using the pH Meter to Determine Buffering Capacity

Objectives

After completing this exercise, you should be able to

1. Define buffer, and explain why buffers are important to organisms.
2. Describe how to use a pH meter.
3. Interpret a titration curve (graph of pH versus milliliters of HCl and NaOH added) to determine whether a solution has buffering capacity and, if so, over what pH range.
4. Explain why some solutions have buffering capacity and others don't.

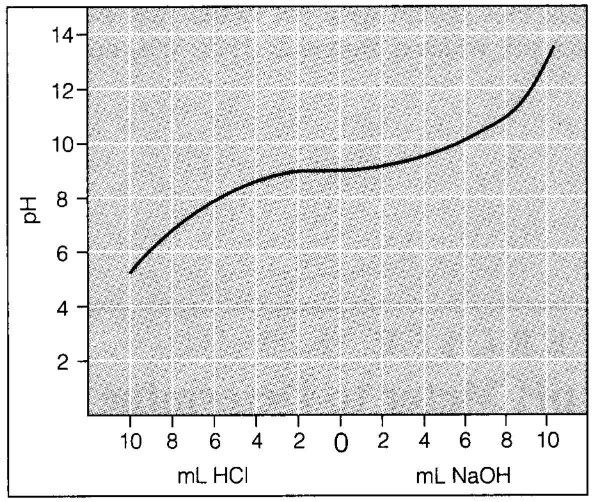
In order for normal physiological processes to occur, pH must remain relatively constant. An excess of H^+ or OH^- can interfere with the functioning of biological molecules, especially proteins. In our bodies, for example, blood pH is usually maintained between 7.3 and 7.5. However, blood returning to the heart contains CO_2 picked up from the tissues, which lowers the blood pH. Metabolic reactions in cells may contribute an excess of hydrogen ions. Our diets may also affect blood pH. Several buffering systems keep the pH constant.

A **buffer** is a solution whose pH resists change on addition of small amounts of either an acid or a base. To be a good buffer, a solution should have a component that acts as a base (takes H^+ out of solution) and a component that acts as an acid (puts more H^+ into solution when there is an excess of OH^-).

The buffering capacity of a solution is tested by adding small amounts of acid (for example, HCl) and base (for example, NaOH) and checking the pH after each addition. If the pH changes only slightly, the solution is a good buffer. Eventually its buffering capacity will be exhausted, however, and the pH will change dramatically.

A buffer operates in a specific pH range. The buffering systems in our blood, for example, buffer at around pH 7.4. That is, they maintain the pH at or very close to 7.4. The solutions you used to make up your standards for red cabbage indicator maintain each buffer at a certain pH. The pH 2 buffer maintains pH at 2, the pH 4 buffer maintains pH at 4, and so on. Notice that the purpose of a buffer is *not* to make the pH neutral (7).

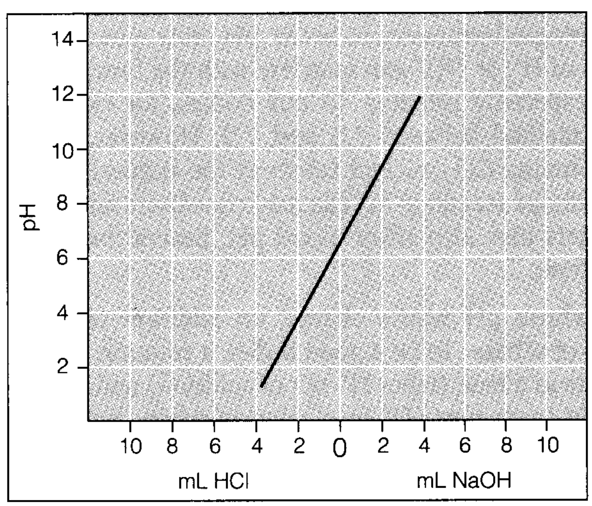
Figure 2.1.
Titration curve for an unknown solution.



Look at Figure 2.1. Is this solution a good buffer? Explain how you know.

At what pH does the solution buffer?

Figure 2.2.
Titration curve for an unknown solution.



Look at Figure 2.2. Is this solution a good buffer? Explain how you know.

You will use a pH meter to test buffering capacity in this exercise. The pH meter has a sensitive electrode that measures the H^+ concentration in solution. It can measure in tenths of pH units; some models measure hundredths of pH units.

Most of the control knobs are used only to calibrate the machine (a buffer of known pH is used to standardize the pH meter). Figure 2.3 illustrates

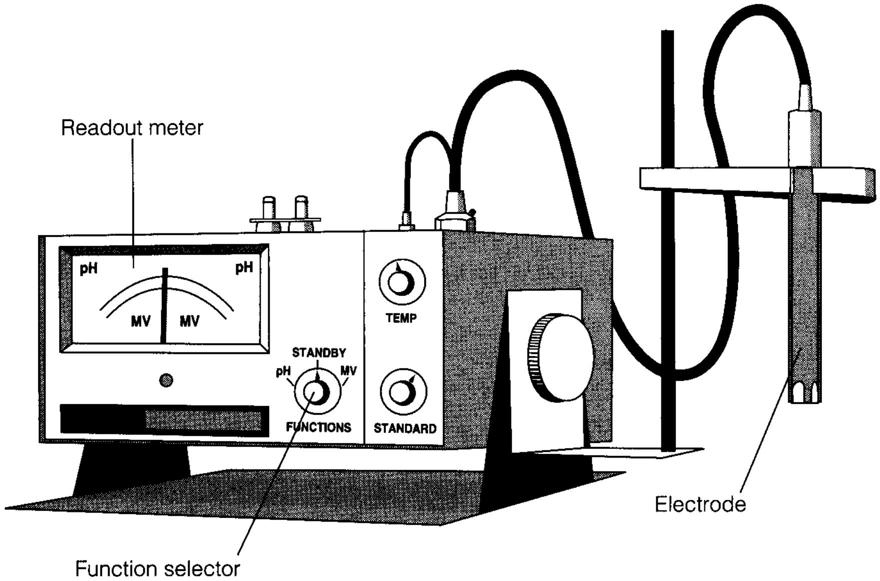


Figure 2.3a.
Analog pH meter.

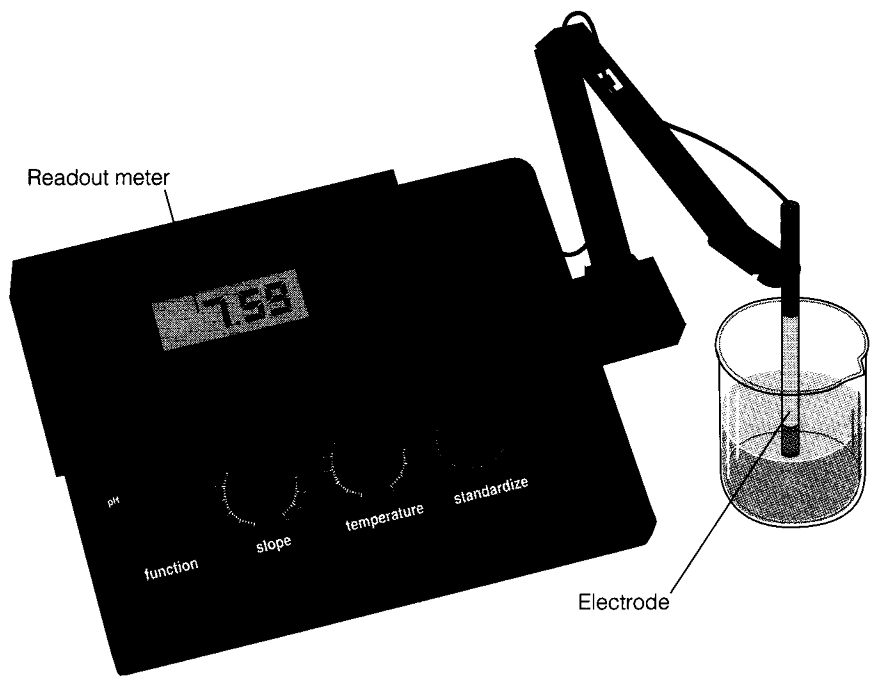


Figure 2.3b.
Digital pH meter.

two commonly used types of pH meters: digital and analog. Your instructor can help you identify the parts you will need to use for the model of pH meter available in your laboratory.

Familiarize yourself with the pH meter in your laboratory by locating the following parts.

Controls

Readout meter: Shows the pH of the solution. On an analog meter, there are usually two scales. One shows pH and the other shows millivolts. You will use the pH scale. (If you are using a digital pH meter, only the number representing the solution's pH will be displayed.)

Function selector (pH/standby switch): Use the pH position only when the electrode is immersed in the solution you want to measure. (Some digital models do not have this switch.)

Standardization knob: Used to calibrate the machine.

Temperature control: Temperature affects pH measurement, so adjusting the temperature control should be part of the calibration procedure.

Electrode: The delicate glass electrode is generally protected by a plastic sleeve. Even so, be careful not to bang the electrode on the glassware or stir bar.

When you are measuring the pH of a solution, swirl it gently to assure good mixing and proper sampling by the electrode. A magnetic stir plate is a convenient way to make sure the solutions are well mixed for your experiment on buffering capacity. To use the stir plate, put a small stir bar in the beaker, and set the beaker in the middle of the stir plate. Turn the knob slowly, and the stir bar will begin to revolve in the beaker. Let it stir gently. If the bar starts to jump around, turn the knob off and then back on again more slowly.

Your instructor will assign your team one of the buffering solutions used to make the cabbage indicator standards in Exercise 2.2. You will test the buffering capacity of that solution and of water.

Buffering solution assigned to your team:

What hypothesis is being tested?

What is the independent variable in this experiment?

What is the dependent variable?

On the axes of Figure 2.4, sketch the curve you expect to see for Solution X if your hypothesis is supported. On the same axes, sketch the curve you expect to see if your hypothesis is proven false.

Why should you determine the buffering capacity of water as part of this experiment?

Procedure

1. Pour 40 mL of your assigned solution into a 100-mL beaker. Put a stir bar in the beaker and put it on the magnetic stirrer.

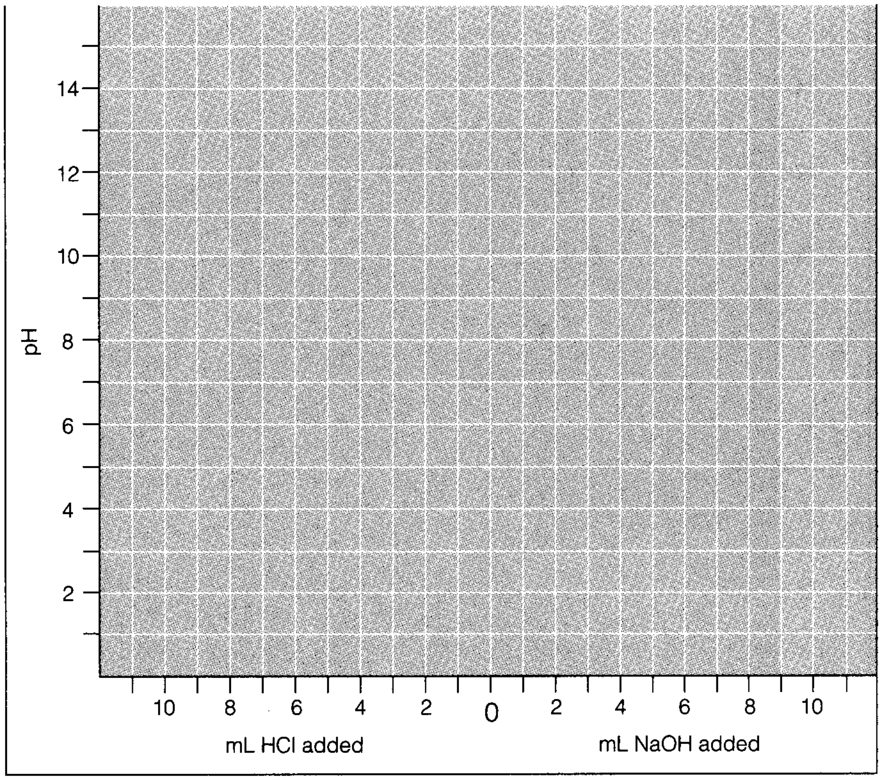


Figure 2.4.
Predicted results of buffering capacity experiment.

2. Determine the pH of the solution by following steps a–d below.
   a. Raise the electrode out of the soak beaker and rinse it with distilled water from the wash bottle.
   b. Immerse the electrode in the solution you want to measure. Swirl the beaker gently. If you are using a magnetic stir plate, make sure the bar clears the electrode before you turn it on.
   c. If your pH meter has a function switch, change it from standby to pH.
   d. Read the pH value on the readout meter or digital display.
3. Record the pH value at 0 on the x-axis of Figure 2.5 on the next page.
4. Add 1.0 mL of 0.1*N* HCl. *N* stands for normal, a measure of concentration. (If you're using a magnetic stirrer, you can leave it on with the electrode immersed throughout the procedure. If you must take the electrode out of the beaker to mix, turn the function switch to standby first.)
5. Record the new pH at "1 mL HCl added" on the x-axis of Figure 2.5.
6. Add another 1 mL of HCl and record the new pH at "2 mL HCl added" on the x-axis of Figure 2.5.
7. Continue to add 1 mL of HCl at a time and record the pH until you have added 10 mL *or* there is a significant decrease in pH, whichever comes first.
8. If the pH meter has a function switch, turn it back to standby.
9. Raise the electrode out of the solution.

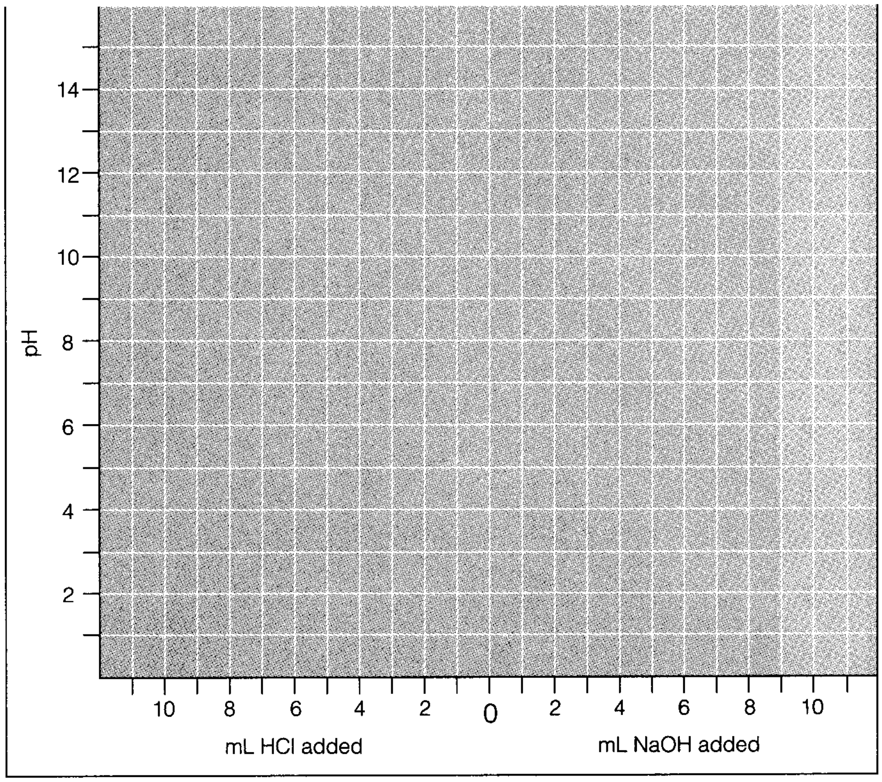


Figure 2.5.
Results of buffering capacity experiment.

10. Rinse the electrode with distilled water, and wipe it with a cleaning tissue.

Always rinse the electrode with distilled water after use to avoid contamination of solutions.

11. Dispose of your solution, and rinse and dry the beaker and stir bar.
12. Put another 40 mL of the same solution into the beaker and repeat the procedure using 0.1*N* NaOH instead of HCl until you have added 10 mL *or* there is a significant increase in pH, whichever comes first. Record the pH values on the "mL NaOH added" side of the x-axis of Figure 2.5.
13. When you are finished determining the buffering capacity of the solution you were assigned, repeat steps 1–12 to determine the buffering capacity of water. Use the axes in Figure 2.5 to graph the results.

When you're done with this procedure, leave the electrode immersed in some solution (water or a buffer). It should never be allowed to dry out.

Write a descriptive title for Figure 2.5.

Review your prediction (Figure 2.4) about the buffering capacity of the solution you were assigned. Was your prediction correct?

If the solution is a buffer, at what pH does it buffer?

Evaluate your hypothesis. Is it supported or proven false by the results?

Describe the buffering capacity (or lack of it) of this solution.

Describe the buffering capacity (or lack of it) of water.

EXERCISE 2.4

Designing an Experiment

Objective

After completing this exercise, you should be able to

1. Design an original experiment to investigate some aspect of pH or buffering capacity.

In Exercises 2.2 and 2.3 you learned a method of measuring pH and a method of determining buffering capacity. In Exercises 2.4 and 2.5, your lab team will design an experiment using one of these methods, perform your experiment, and present and interpret your results.

The following materials will be supplied for your group.

For pH Measurement Using Red Cabbage Indicator

You will already have the standards made up from Exercise 2.2.

extra test tubes

pasteur pipets and rubber bulbs

For Determining Buffering Capacity

You will use the same materials used in Exercise 2.3. Your instructor will be able to tell you what additional materials will be available.

Proposed Experiment

If you are considering an experiment using red cabbage indicator, you might want to review Table 2.1 for ideas. Also, try to think of substances whose function may be pH dependent. For example, you may recall hearing the terms pH, acid, or base used in advertisements. If you are planning an investigation of buffering capacity, consider what substances might be expected to be good buffers.

Describe your experiment below.

Question or Hypothesis

Dependent Variable

Independent Variable

Explain why you think this independent variable will affect pH or buffering capacity.

Control Treatment(s)

Replication

Brief Explanation of Experiment

Predictions (What results would support your hypothesis? What results would prove your hypothesis false?)

Method

Design a Table to Collect Your Data

List Any Additional Materials You Will Require

EXERCISE 2.5

Performing the Experiment and Interpreting the Results

Objectives

After completing this exercise, you should be able to

1. Perform the experiment your lab team designed.
2. Present and interpret the results of your experiment.

Before you do the experiment, be sure that everyone on your lab team understands the techniques that will be used. You may want to divide up the tasks before you begin work.

Be thorough in collecting data. Don't just write down numbers; record what they mean as well. Don't rely on your memory for information that you need when reporting on your experiment later! If you have any questions, doubts, or problems during the experiment, be sure to write them down, too.

Results

Before you begin to prepare your results for presentation, decide on the best format to use. Remember, you want to give the reader a clear, concise picture of what your experiment showed. Refer to the data presentation section of Appendix A (Tools for Scientific Inquiry) for help. If you are drawing graphs, use graph paper. Complete your tables and/or graphs before attempting to interpret your results.

Write a few sentences *describing* the results (don't explain why you got these results or draw conclusions yet).

Discussion

Look back at the hypothesis or question you posed in this experiment. Look at the graphs or tables of your data. Do your results support your hypothesis or prove it false? Explain your answer, using your data for support.

Did your results correspond to the prediction you made? If not, explain how your results are different from your expectations and why this might have occurred.

Describe how your data are supported by information from other sources (for example, textbooks or other lab teams working on the same problem).

If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.

If you had an opportunity to repeat and extend this experiment to make your results more convincing, what would you do?

Summarize the conclusion you have drawn from your results.

Questions for Review

1. You have blown air from your lungs into a solution of phenol red and changed its color from red to yellow. Suggest a way to turn the color back to red.

2. Give an example of two substances that, when mixed together, will produce a neutral solution.

3. You measure the pH of your garden soil and find that it is 6. You measure the pH of peat moss and find that it is 4. How much greater is the concentration of hydrogen ions in peat moss than in the garden soil?

4. What's one ingredient that could make soft drinks acidic?

5. Aspirin has a pH of 3. Some people who take large amounts of aspirin (for example, for arthritis) take a pill that combines aspirin with Maalox. What's the purpose of this combination?

6. If you want to do an experiment to measure buffering capacity, why is red cabbage indicator not a good choice of methods?

7. On the graph in Figure 2.6, which compound is the best buffer? Explain why. Over what pH range does the compound buffer?

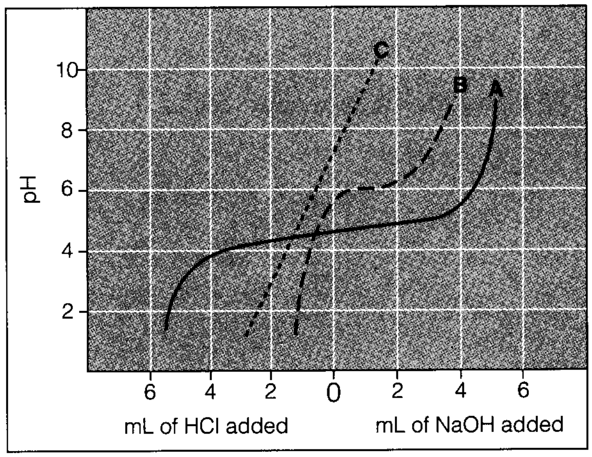


Figure 2.6.
Graph of buffering activity of three different compounds.

8. Considering that CO_2 is produced as a by-product of cellular metabolism, why is it important for our blood to contain buffers?