LABORATORY 3

pH and Buffers

OVERVIEW

The concentration of hydrogen ions in solution, expressed as pH, is of great importance to living systems. Because both cell structure and function can be affected by even small changes in pH, maintaining pH within a narrow range is a major goal of cellular homeostasis.

During this laboratory, you will learn how the concentration of hydrogen ions in a solution can be changed or maintained. You will also learn how to prepare various types of solutions and how to use several methods of determining pH.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency. After reading Exercise A, Understanding pH, you should complete the problems.

✓ EXERCISE A Understanding pH

Molecules that are dissolved in water may separate (dissociate or ionize) into charged fragments or ions. Often one of these fragments is a hydrogen ion (H^+). The pH of a solution is a measure of the concentration of hydrogen ions (written as $[H^+]$, where [ ] means "concentration of") in that solution, and pH is a measure of the **alkalinity** (*basicity*) or **acidity** of a solution.

Objectives

- ☐ Define pH.
- ☐ Define acid and base.
- ☐ Define neutralization.
- ☐ Given the molarity of a solution of an acid or base, calculate its pH.
- ☐ Given the pH of H^+ concentration of a solution, calculate its pOH or OH^- concentration.

Procedure

Water ionizes when a hydrogen atom that is covalently bound to the oxygen of one water molecule leaves its electron behind and, as a hydrogen ion (H^+), joins a different water molecule. Two ions are produced by this reaction: a hydroxide ion (OH^-) and a hydronium ion (H_3O^+). We can express this reaction as follows:

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

Convention, however, allows us to express the ionization of water more simply as

$$H_2O \rightleftharpoons H^+ + OH^-$$

In any given volume of pure water, or in any solution, a small but constant number of water molecules are ionized. In pure water, the number of H^+ ions exactly equals the number of OH^- ions, since one cannot be formed without the other being formed. In pure water, $[H^+] = 1 \times 10^{-7}$ M and $[OH^-] = 1 \times 10^{-7}$ M (where M stands for molar concentration or moles per liter).* The product of the molar concentrations of the two ions, $[H^+][OH^-]$, in pure water is always 1×10^{-14}, a number known as the **ion product of water,** and this represents a dissociation constant (K_w) for pure water. (Note that the brackets indicate molar concentration for the substance they enclose.) So, for pure water,

$$[H^+][OH^-] = 1 \times 10^{-14}$$

The concentrations of OH^- and H^+ ions can be written in terms of the number 10 with an exponent. Recall that in a number such as 1×10^{-7}, 10 is the base and $^{-7}$ is the exponent (the power to which 10 is raised). Remember that to find the product of two numbers with exponents, you add the exponents. So, again, for pure water,

$$\mathbf{[H^+][OH^-] = 1 \times 10^{-14}} \qquad \text{or}$$
$$[1 \times 10^{-7}][1 \times 10^{-7}] = 1 \times 10^{-14}$$

Numbers such as these can more easily be expressed as logarithms (base 10). A logarithm is the power to which a base, in this case 10, must be raised to give the desired number. Thus the log of 1×10^{-7} is -7, since this is the power to which 10 must be raised to give the number 0.0000001. pH is defined as the negative logarithm ($\log_{10}$) of the molar hydrogen ion concentration in a solution:

$$\mathbf{pH = -\log_{10} [H^+]} \quad \text{or} \quad \text{pH} = \log_{10} \frac{1}{[H^+]}$$

We use the negative logarithm in working with pH so that the numbers, and the pH scale, are positive—note that $-\log_{10} (1 \times 10^{-7}) = +7$, or simply, 7.

For pure water, we can also determine the pOH, based on the concentration of OH^- ions in a solution:

$$\mathbf{pOH = -\log_{10} [OH^-]} = \log_{10} \frac{1}{[OH^-]}$$

We can establish a relationship between the pH and the pOH of a solution by using the expression for the ion product of water. In logarithmic form, this can be written as

$$\log_{10} [H^+] + \log_{10} [OH^-] = \log_{10} (1 \times 10^{-14})$$

Since pH is expressed in terms of the *negative* logarithm of the H^+ concentration in solution, we will express the above equation in *negative* logarithmic form:

$$-\log_{10} [H^+] + (-\log_{10} [OH^-] = -\log_{10} (1 \times 10^{-14})$$

Since the letter "p" in pH stands for "negative logarithm of," we can write this equation as

$$p[H^+] + p[OH^-] = -\log (1 \times 10^{-14}) \qquad \text{or}$$
$$\mathbf{pH + pOH = 14}$$

**In a liter of water, there are 3.34×10^{25} water molecules—55.5 moles × Avogadro's number (6.02×10^{23}). Of these, 1×10^{-7} mole per liter is ionized, so a liter of water contains 6.02×10^{16} H^+ ions. If you divide the number of H^+ ions in a liter by the total number of water molecules in a liter ($6.02 \times 10^{16}/3.34 \times 10^{25}$), you will discover that only 2×10^{-9} or 2 ten-millionths of a percent of all water molecules present in 1 l of water are ionized. That is a very small amount!*

Since for pure water, $[H^+] = 1 \times 10^{-7}$ M and $[OH^-] = 1 \times 10^{-7}$ M, then pH = 7 and pOH = 7, so pH + pOH = 14.

It is important to realize that in any solution, as is the case for pure water, the *product* of $[H^+]$ and $[OH^-]$ is *constant*. For pure water, the molar amounts of H^+ and OH^- are equal. If an ionic or polar substance is dissolved in water, it may change the relative amounts of H^+ and OH^- but the product of the two concentrations is always 1×10^{-14} because water is a source of H^+ and OH^- ions and the ion product (which reflects the tendency of water to ionize) remains constant. For instance, if a substance added to a volume of water ionizes to produce sufficient H^+ ions that the H^+ concentration of the solution increases to 1×10^{-5} M (expressed as $[H^+] = 1 \times 10^{-5}$ M), then the OH^- concentration decreases to $[OH^-] = 1 \times 10^{-9}$ M. The ion product of water for the solution will always equal 1×10^{-14} (*remember:* to find the product of two numbers written in logarithmic form, add the logarithms according to the rules for exponents):

$$[H^+][OH^-] = 1 \times 10^{-14}$$
$$[1 \times 10^{-5}][1 \times 10^{-9}] = 1 \times 10^{-14}$$

Likewise, if a substance added to a volume of water increases the $[OH^-]$, the $[H^+]$ will necessarily decrease. It is important to note, however, that adding H^+ to a solution not only increases the number of H^+ ions but *also* decreases the number of OH^- ions, since some of the added H^+ will combine with OH^- to make water. Adding OH^- to a solution can also decrease the H^+ concentration for the same reason—H^+ and OH^- combine to form H_2O! But, no matter what is added, the product $[H^+][OH^-]$ must always be 1×10^{-14}, so if we know the concentration of one ion, we always know the concentration of the other.

We now can use the expression pH + pOH = 14 to generate the pH scale. If we know the pH we can calculate the pOH (pOH = 14 − pH), and if we know the pOH we can calculate the pH (pH = 14 − pOH). The sum of the negative logarithms of $[H^+]$ and $[OH^-]$ is always 14 and the product of the molar ion concentrations, $[H^+][OH^-]$, is always 1×10^{-14}. To be sure that you understand this relationship, examine Table 3A-1 carefully.

A solution of pH = 7 is **neutral,** at the midpoint of the pH scale. Solutions with a pH value lower than 7 are said to be **acidic.** In an acidic solution, the number of H^+ ions exceeds the number of OH^- ions. Solutions with a pH above 7 are **basic** or alkaline: the number of OH^- ions exceeds the number of H^+ ions. Expressed another way, the more acidic a solution, the higher the number of H^+ ions and the lower the number of OH^- ions. The more basic a solution, the lower the number of H^+ ions and the higher the number of OH^- ions. Check Table 3A-1 to make sure this is true. Also, remember that the pH scale is logarithmic, not arithmetic: if two solutions differ by 1 pH unit, then one solution has *ten* times the hydrogen ion concentration of the other.

An **acid** is a substance that causes an increase in the number of H^+ ions and a decrease in the number of OH^- ions in solution. This increase is most often the result of an ionization that produces H^+. Some common acids and their ionization products are

Hydrochloric acid	$HCl \longrightarrow H^+ + Cl^-$
Acetic acid	$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$
Carbonic acid	$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
Phosphoric acid	$H_3PO_4 \longrightarrow H^+ + H_2PO_4^- \longrightarrow H^+ + HPO_4^{2-}$

The more completely the acid ionizes, the more H^+ is released, and the stronger the acid is. For example, if the concentration of an HCl solution is 0.1 mole/l and the HCl ionizes completely, we have 0.1 mole/l of H^+. This could also be expressed as $[H^+] = 1 \times 10^{-1}$ M.

Of the acids listed above, HCl and H_3PO_4 are considered *strong acids* since they ionize completely. CH_3COOH, H_2CO_3, and $H_2PO_4^-$ are relatively *weak acids*. A weak acid might, for example, ionize only 10%, so a 1×10^{-2} M solution would contain only 1×10^{-3} mole/l of H^+. Once $[H^+]$ is known, pH can be calculated.

Table 3A-1 The pH Scale

$[H^+]$ M	pH	$[OH^-]$	MpOH	
10^0 (1.0)	0	10^{-14}	14	
10^{-1} (0.1)	1	10^{-13}	13	
10^{-2} (0.01)	2	10^{-12}	12	
10^{-3} (0.001)	3	10^{-11}	11	**acidic**
10^{-4} (0.0001)	4	10^{-10}	10	
10^{-5} (0.00001)	5	10^{-9}	9	
10^{-6} (0.000001)	6	10^{-8}	8	
10^{-7}	7	10^{-7}	7	**neutral**
10^{-8}	8	10^{-6} (0.000001)	6	
10^{-9}	9	10^{-5} (0.00001)	5	
10^{-10}	10	10^{-4} (0.0001)	4	
10^{-11}	11	10^{-3} (0.001)	3	**basic**
10^{-12}	12	10^{-2} (0.01)	2	
10^{-13}	13	10^{-1} (0.1)	1	
10^{-14}	14	10^0 (1.0)	0	

Example A solution with $[H^+] = 1 \times 10^{-2}$ M ionizes 10%.

$$[H^+] = 1 \times 10^{-2}\text{ M}$$

$$10\% = 0.10 = 1 \times 10^{-1}$$

$$(1 \times 10^{-2}) \times 10\% = (1 \times 10^{-2})(1 \times 10^{-1}) = 1 \times 10^{-3}$$

$$[H^+] = 1 \times 10^{-3}\text{ M}$$

$$\text{pH} = \log_{10}\frac{1}{[H^+]} = \log_{10}\frac{1}{(1 \times 10^{-3})} = 3$$

1. Fill in Table 3A-2 by calculating the pH for each acid.

Table 3A-2 Calculating pH for Acids

Molarity of Acid	Degree of Ionization	$[H^+]$ M	pH
1×10^{-3}	100%		
1×10^{-3}	10%		
1×10^{-3}	1%		
1×10^{-4}	100%		
1×10^{-2}	100%		
1×10^{-1}	100%		

A substance need not give up hydrogen ions itself to cause an increase in the $[H^+]$ in a solution. For instance, an important molecule in biological systems is CO_2 (carbon dioxide) which combines with water to form carbonic acid (H_2CO_3), which ionizes to produce bicarbonate ion (HCO_3^-):

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

The reaction of SO_2 (sulfur dioxide) with atmospheric water is, in part, responsible for acid rain. It dissolves in water to form sulfurous acid (H_2SO_3):

$$SO_2 + H_2O \longleftrightarrow H_2SO_3 \longleftrightarrow H^+ + HSO_3^-$$

A **base** is a substance that causes a decrease in the number of H^+ in solution and an increase in OH^-. In many cases this is achieved by the ionization of the molecule to produce OH^- (hydroxyl ion), which not only adds to the OH^- in solution but also *removes* H^+ from solution by combining with it to form water, thus raising the pH.

$$KOH + HCl \longleftrightarrow K^+ + OH^- + H^+ + Cl^- \longleftrightarrow KCl + H_2O$$

Thus, **neutralization** of an acid by a base produces a **salt** (an ionic compound composed of a negative ion from an acid and a positive ion from a base, such as KCl) and **water.**

Some common bases that ionize to produce OH^- are

Sodium hydroxide	$NaOH \longrightarrow Na^+ + OH^-$
Magnesium hydroxide	$Mg(OH)_2 \longrightarrow Mg^{2+} + 2\,OH^-$
Potassium hydroxide	$KOH \longrightarrow K^+ + OH^-$

These are all *strong bases* because they ionize completely in solution.

Ammonia (NH_3), dissolved in water, is also basic. It does not produce OH^- but it can remove H^+ from solution:

$$NH_3 + H_2O \longrightarrow NH_4^+ + OH^-$$

The bicarbonate ion (HCO_3^-) is also basic. It, too, can accept H^+:

$$H^+ + HCO_3^- \longleftrightarrow H_2CO_3$$

2. Fill in Table 3A-3. Remember the relationship between pH and pOH discussed earlier.

Table 3A-3 Calculating pH for Bases

Molarity of Base	Degree of Ionization	$[OH^-]$ M	$[H^+]$ M	pH
1×10^{-3}	100%			
1×10^{-3}	10%			
1×10^{-2}	100%			
1×10^{-5}	100%			
1	100%			

3. Keeping in mind that $[H^+][OH^-] = 1 \times 10^{-14}$, give the following for a *neutral* solution:

$[H^+] =$ ______

$[OH^-] =$ ______

pH = ______

✓ EXERCISE B Using Indicators to Measure pH

Indicators are chemicals that change color depending on the pH of the solution and are often used to determine the pH of a solution colorimetrically.

Objectives

- ☐ Use a colorimetric pH indicator.
- ☐ Use an indicator solution or alkacid test paper to determine the pH of some common solutions.
- ☐ Prepare and use a series of standards for qualitative measurements.

✓ PART I Making a pH Indicator

A solution of certain **anthocyanins,** the plant pigments responsible for red, blue, and purple colors in flowers, fruits, and autumn leaves, can be used as a pH indicator. At low pH, solutions of anthocyanins turn red; at high pH, they turn blue. In some flowers, however, soil pH affects the uptake of certain metals that can complex with the anthocyanin pigment and prevent its normal color expression. For instance, in hydrangeas, low soil pH promotes the uptake of aluminum, which then complexes with the anthocyanins and prevents the production of "normal" pink color. Instead, the flowers appear blue. Raising the pH of the soil prevents aluminum uptake and the pink color can be expressed. At more neutral pH values, the flowers appear intermediate in color.

A solution of anthocyanins extracted from red cabbage will be used as a pH indicator in this laboratory. At the beginning of class, your instructor will make an extract of anthocyanins by boiling red cabbage in water for 3 minutes and then filtering the cabbage extract through cheesecloth.

To use an indicator to determine the pH of solutions of unknown pH, you must first determine the color changes that occur when the indicator is used with substances whose pH is known. The color changes that occur in the cabbage extract indicator when mixed with substances of pH 2, 4, 6, 7, 8, 10, 12, and 14 will be used as a set of **standards.** You can then determine the pH of various substances by mixing each with cabbage extract and comparing the resulting colors with the colors of the standards.

Procedure

1. Work in pairs. Put eight clean test tubes in a rack and label them 2, 4, 6, 7, 8, 10, 12, and 14. Use a pipette to measure 5 ml of the appropriate buffer into each test tube (i.e., place the pH 2 buffer in the tube labeled "2," and so on).
2. Use a pipette to measure 3 ml of cabbage extract and add it to each test tube. Cover with Parafilm and invert to mix well.
3. Record the color of each tube below. *Note:* Record both initial and final colors at pH 12 and 14. The pigments are not stable at these pH values.

pH	Color
2	
4	
6	
7	
8	
10	
12	
14	

✓ PART 2 Measuring pH with Cabbage Indicator

Procedure

1. Obtain two clean test tubes and label them A and B.
2. In tube A, mix 2 ml of solution A with 1 ml of cabbage extract.
3. Compare the color in tube A with the colors of the standards. The approximate pH of the unknown solution is the pH of the standard whose color most closely matches the color in tube A.

   pH of A ______ $[H^+]$ ______

4. Use the same method (steps 2 and 3) to measure the pH of solution B.

   pH of B ______ $[H^+]$ ______

   a. Which solution is more acidic? ______

✓ PART 3 Using Alkacid Test Paper

The cabbage indicator method can be used only with colorless or white solutions. Alkacid test papers, which are impregnated with indicators, are another means of estimating pH and can be used with colored solutions.

Procedure

1. Hold a test paper with forceps. Use a clean stirring rod to apply a drop of solution A to the test paper.
2. While the paper is still wet, compare its color with the standard pH color scale on the label of the alkacid paper's container.

   pH of A ______

3. Measure and record the pH values of solutions B, C, and D.

   pH of B ______ pH of C ______ pH of D ______

   a. Do the results obtained with the alkacid papers match those obtained using the cabbage indicator method? ______

   b. Explain any discrepancies (alkacid paper doesn't have a standard for pH 7, so a neutral solution will be closer to either 6 or 8). ______________________________

   ____

EXERCISE C Determining the pH of Some Common Solutions (*Optional*)

Cabbage indicator and alkacid test paper can be used to determine the pH of common beverages, medicines, and cleaning solutions.

Objectives

☐ Understand the relationship of pH to the taste of common beverages and to the activity of medicines and cleaning solutions.

PART 1 pH of Beverages

Many beverages differ in their acidity or alkalinity, often due to CO_2 content (if carbonated) or organic acids.

Formulate a hypothesis that predicts the relative differences in pH values for the beverages listed in the Procedure below.

HYPOTHESIS:

NULL HYPOTHESIS:

Which beverage do you **predict** will have the lowest pH?

What is the **independent variable**?

What is the **dependent variable**?

Procedure

1. Work in pairs. Use the cabbage indicator method (see Exercise B, Part 2) to determine pH values for 7-Up and wine. Use alkacid paper (see Exercise B, Part 3) for determining the pH of coffee and apple juice. Record the values below.

Beverage	pH
Apple juice	
Coffee (black)	
7-Up	
White wine	

2. Arrange these solutions in order of increasing $[H^+]$:
   1. ______________
   2. ______________
   3. ______________
   4. ______________

Do your results support your hypothesis? ______ *Your null hypothesis?* ______

Was your prediction correct? ______

a. Did you discover anything about the beverages that surprised you? ______________________________

b. Based on your results, can you think of reasons to drink or not drink any of these beverages in excess?

____

PART 2 pH and Activity of Some Common Medicines

Many medicines, especially those used to guard against acidity in the digestive tract, are fairly alkaline, while other medicines tend to be acidic.

Formulate a hypothesis that predicts the relative differences in pH values for the medicines listed in the Procedure below.

HYPOTHESIS:

NULL HYPOTHESIS:

Which medicine do you **predict** will have the highest pH?

What is the **independent variable**?

What is the **dependent variable**?

Procedure

Work in pairs. Use the cabbage indicator method to determine pH values for the following medicines.

Medicine	pH
Aspirin	
Milk of Magnesia [$Mg(OH)_2$]	
Sodium bicarbonate (Alka-Seltzer) ($NaHCO_3$)	
Maalox	

Do your results support your hypothesis? ______ *Your null hypothesis?* ______

Was your prediction correct? ______

a. *It is often recommended that aspirin be taken with a large glass of milk or water. Based on your results above, why might this be important?* ______

b. *Would apple juice or citrus juice be a good accompaniment to aspirin?* ______ *Explain why or why not.*

Enzymes function best at particular pH values. In the normal human stomach, a pH of 2.0 to 3.0 provides the environment required for the proper functioning of the digestive enzymes found there. The last three medicines in the list above are often used for treatment of "acid indigestion" of the stomach, a condition in which a reduction in pH interferes with efficient enzyme action and thus with digestion.

c. *Based on your results, how would you explain the action of these medicines?* ______

d. *What might happen if an excess of any of these medicines were used?* ______

PART 3 pH and the Action of Some Cleaning Solutions

Cleaning solutions vary in their chemical composition. This, in turn, determines what materials they mix with for cleaning purposes.

Based on your experience with cleaning your clothes, dishes, body, and even your sink, formulate a hypothesis that predicts the relative differences in pH values for the cleaning solutions listed in the following Procedure.

HYPOTHESIS:

NULL HYPOTHESIS:

Which cleaning solution do you **predict** will have the highest pH?

What is the **independent variable**?

What is the **dependent variable**?

Procedure

Work in pairs. Use the cabbage indicator method to determine the pH values of the cleaning solutions listed below.

Solution	pH
Drano	
Ivory Liquid	
Cascade	
Tide	

Do your results support your hypothesis? ______ *Your null hypothesis?* ______

Was your prediction correct? ______

a. What do all these solutions have in common? ______

EXERCISE D Soil pH and Plant Growth (*Optional*)

Since plants obtain most of their nutrients in the form of ions, the kinds and amounts of nutrient ions available from the soil affect plant growth and health. They also have a significant effect on the types of plants that can be grown in soils of various compositions. The chemical composition of soil varies greatly from region to region, due to differences in geology, topography, climate, and plant and animal life. In any soil, the complex equilibria among soil particles, water, and nutrient ions are influenced by soil pH. For example, phosphorus (P), an element essential for plant growth, is taken up in the form of phosphate ions, either as $H_2PO_4^-$ or HPO_4^{2-}. If the soil is acidic, the $H_2PO_4^-$ form is most abundant. HPO_4^{2-} is prevalent under neutral conditions, and PO_4^{3-} exists in alkaline soils. Plants absorb the $H_2PO_4^-$ form most readily, so phosphorus is most available in acidic soils. If the pH is too low, $H_2PO_4^-$ combines with other ions and is precipitated out of the soil. Thus phosphorus is most available at pH 5.5 to 6.5. A similar effect is seen with aluminum, as described in Exercise B, Part 1.

There are at least 13 elements plants must obtain from the soil, and each type of plant thrives within a more or less narrow range of chemical balance. There is no single soil pH that provides the perfect environment for all plants. Instead, plants have adapted to various soil chemistries, and each species has its own range of pH tolerance (Table 3D-1).

Table 3D-1 pH Preference of Selected Species

Plant	pH Range
Eastern hemlock, azalea, rhododendron, gardenia, cranberry, blueberry	4.5 to 6.0
Coleus, iris, tomato, squash, strawberry, tobacco	5 to 7
Gladiolus, cherry, pear, sugar maple, alfalfa, asparagus, yellow poplar	6 to 8

Objectives

- ☐ Relate soil pH to plant nutrition.
- ☐ Measure the pH of soil samples.

Procedure

In this exercise you will determine the pH of several soil samples. Water has been added to these samples to help release some of the ions into solution so the pH can easily be determined.

Based on your experience with gardening and your knowledge of soil and plants common to different environments, formulate a hypothesis that predicts the relative differences in pH values for the soils listed below.

HYPOTHESIS:

NULL HYPOTHESIS:

What type of soil do you **predict** would have the lowest pH?

What is the **independent variable**?

What is the **dependent variable**?

Work in pairs. Use alkacid test paper to measure the pH of each of the following soil samples.

Sample	pH
Potting mix	
Clay	
Sand	
Lime	
Peat moss	

Do your results support your hypothesis? ______ *Your null hypothesis?* ______

Was your prediction correct? ______

a. *Suppose that a soil sample from your garden has a pH of 4. You want to grow* Coleus, *which requires a pH range of 5 to 7. Which of the substances tested could be used to adjust the pH to one suitable for growing* Coleus *plants?* ______________ *Why?* ______________

b. *Suppose that you want to grow azaleas, which require a pH range of 4.5 to 6.0. The soil in your garden has a pH of 7. Which of the substances tested could be used to adjust the pH to one suitable for growing azaleas?*

______________ *Why?* ______________

____

c. *Your hydrangea bush produces beautiful pink flowers year after year. One spring you decide you would like to have blue hydrangea flowers instead. [Recall that certain pigments called anthocyanins are often complexed with metals abundant in the soil, and are responsible for pink, purple, and blue color in many flowers (see Exercise B, Part 1).] What should you do?* ______________

____

✓ EXERCISE E The pH Meter

In Exercises B, C, and D, you learned how the pH of a solution can be estimated by comparing experimental indicator colors with known standards. In many cases, however, higher accuracy and greater reliability are necessary. Electrometric methods of pH determination, using pH meters, give more precise results.

The standard laboratory pH meter has a **glass electrode** that is sensitive to hydrogen ion activity ("activity" is defined as the effective concentration of an ionic species in solution; it is usually expressed in moles per liter) and a **reference electrode** which completes the electrical circuit. On some pH meters, a combination electrode performs both functions. Other parts of the pH meter include the following:

Readout meter An analog meter with a pH scale for pH determinations and a millivolt (mV) scale for millivolt measurements.

Mechanical meter zero An adjustment that mechanically zeros the meter pointer.

Function selector A switch that maintains the meter on standby when measurements are not being taken and that selects the measuring mode, either pH or millivolts.

Standardize control A control that allows the meter to be set to the pH of the buffer solution used to standardize the instrument.

Temperature control A control that compensates for the temperature of the solution being measured (it is active only when the function selector is in the pH mode).

Objectives

- ☐ Use the pH meter to measure the pH of a solution.

Procedure

1. Work in pairs. Locate each of the parts (listed above) on the pH meter available in your laboratory. You instructor will calibrate and standardize the pH meters in your lab by adjusting the pH reading on the analog scale of the meter when its electrodes are placed in a buffer solution of known value (the standard). To obtain valid results when measuring the pH of a solution, the standardization buffer should have a pH close to that of the sample to be tested. In other words, if you are working with acidic solutions, you should standardize the pH meter with a known buffer solution of pH 4, *not* pH 13.

   Because of the range in pH values for the solutions being tested in this exercise, you will need to select a pH meter that has been standardized with a buffer that has a pH close to the estimated or expected pH of the solution you are testing (the cabbage extract indicator or alkacid paper has given you an estimate of pH for each solution). Each pH meter in your laboratory is labeled to indicate the pH of the buffer with which it has been standardized.

2. At one of the pH meters, you will find a 50-ml beaker containing 20 ml of apple juice and a small magnetic stirring bar. Check the approximate pH from your previous results in Exercise C, Part 1, and select the proper pH meter. Record your estimated pH values in Table 3E-1.
3. Set the beaker on the magnetic stirring plate next to the pH meter and turn on the stirrer so that the magnetic bar revolves slowly in the solution.
4. Check to be sure that the function selector is on "standby." Raise the pH electrode out of its storage beaker. Over a waste beaker, rinse the electrode with distilled water from a wash bottle.
5. While taking care that the stirring bar does *not* hit the electrode, immerse the electrode in the beaker of apple juice.
6. Change the function selector from "standby" to "pH."
7. Record the pH in Table 3E-1.
8. Turn the function switch back to "standby."
9. Raise the electrode out of the solution. Over a waste beaker, rinse the electrode with distilled water and place it into its own storage beaker.
10. Repeat this procedure for the remaining solutions listed in Table 3E-1.

Table 3E-1 pH of Common Solutions

Solution	Estimated pH	Measured pH	$[H^+]$ M	$[OH^-]$ M
Apple juice				
7-Up				
Maalox				
Tide				
Ivory Liquid				

11. Now that you can obtain a more accurate measure of pH (to tenths of pH units), you can calculate $[H^+]$ or $[OH^-]$ by using your calculator. Since pH is expressed as a negative logarithm of $[H^+]$, you need to determine the antilogarithm ("antilog") of the pH expressed as a negative number.

Example A solution has a pH of 3.2. What is its hydrogen ion concentration? Using a Texas Instruments calculator or other scientific calculator (see your calculator's directions if it uses reverse Polish notation—RPN),

ENTER 3.2

Change it to -3.2 (use the $+/-$ key)

Press (INV)(LOG) $=$ 0.000631, or 6.31×10^{-4}

The hydrogen ion concentration is 6.31×10^{-4} M.

a. What is the hydroxide ion $[OH^-]$ concentration of this solution? ______________

12. Given $[H^+]$, you can also determine the pH.

Example A solution has an $[H^+]$ of 1.95×10^{-7} M. What is its pH? Using a scientific calculator, enter the number 1.95×10^{-7} into your calculator in scientific notation form:

ENTER 1.95

Press the exponent entry key (EE) to indicate to the calculator that the next numbers entered represent the exponent.

Enter 7 and press the +/− key to convert to −7

Press LOG and the +/− key: $-\log 1.95 \times 10^{-7} = 6.71$

The pH is 6.71.

13. Practice on the following.

Solution	$[H^+]$ M	pH
Urine	5.89×10^{-5}	
Pancreatic juice	7.95×10^{-8}	

Solution	pH	$[H^+]$ M
Lemon juice	2.8	
Milk	6.4	

14. Refer to Appendix III, Preparing Solutions, and then prepare 100 ml of the solutions listed in the table below. Work in pairs.

Solution	Estimated pH	Measured pH	$[H^+]$ M	$[OH^-]$ M
0.1 M NaOH				
0.1 *N* $Ca(OH)_2$				
0.2 M KH_2PO_4				
5% NaCl				

15. Use alkacid test paper to estimate the pH of each solution. Then use the pH meter to measure the pH of each solution. Record your results in the table.

16. Now that you know that pH + pOH = 14 and that $[H^+][OH^-] = 10^{-14}$, and you know how to use logarithms to convert from ion concentration to pH or antilogarithms to convert from (−)pH to ion concentration, you can convert from any of the following quantities to the other (see next page):

Example If the pH of a solution is 6, what is its OH^- concentration? First, find the antilog of −6. This will give you the H^+ concentration of the solution. Divide 1×10^{-14} by $[H^+]$ to give $[OH^-]$. Calculate this value. ______________

b. Given that a solution has on OH^- concentration of 1×10^{-4} M, what is its pH? ________

c. Given that a solution has $[H^+] = 1 \times 10^{-6}$ M, what is its $[OH^-]$? ______________

$$\begin{tikzcd}[column sep=huge, row sep=huge]
\text{pH} \arrow[r, leftrightarrow, "\text{antilog }(-)\text{pH}", "-\log_{10}[\text{H}^+]"'] \arrow[d, leftrightarrow, "\text{pOH} = 14 - \text{pH}"', "\text{pH} = 14 - \text{pOH}"] & {[\text{H}^+]} \arrow[d, leftrightarrow, "{[\text{OH}] = \frac{1 + 10^{-14}}{[\text{H}^+]}}"', "{[\text{H}^+] = \frac{1 + 10^{-14}}{[\text{OH}^+]}}"] \\
\text{pOH} \arrow[r, leftrightarrow, "\text{antilog }(-)\text{pOH}", "-\log_{10}[\text{OH}^+]"'] & {[\text{OH}^+]}
\end{tikzcd}$$

EXERCISE F Buffers

Physiological processes require that pH remain relatively constant. The pH of blood in our bodies, for example, is usually maintained between 7.3 and 7.5. However, blood returning to the heart contains CO_2 picked up from the tissues (recall that CO_2 combines with water to form carbonic acid), and our diets, as well as the normal metabolic reactions in cells, may contribute an excess of hydrogen ions. The pH must be kept constant by several *buffer systems.*

A **buffer** is defined as a solution that *resists change* in pH when small amounts of acid or base are added. Bicarbonate, phosphate, and protein buffer systems maintain our blood pH. We will use the phosphate buffer system as an illustration.

A buffer is made by mixing a weak acid with its salt in order to have in solution something that can act as an acid (give up hydrogen ions) *and* something that can act as a base (accept hydrogen ions). In a potassium phosphate buffer, the weak acid, $H_2PO_4^-$, is supplied as KH_2PO_4 (monobasic potassium phosphate) and its salt as K_2HPO_4 (dibasic potassium phosphate).

At equilibrium, these substances are ionized to some degree:

$$KH_2PO_4 \rightleftharpoons K^+ + H^+ + HPO_4^{2-}$$

$$K_2HPO_4 \rightleftharpoons 2K^+ + HPO_4^{2-}$$

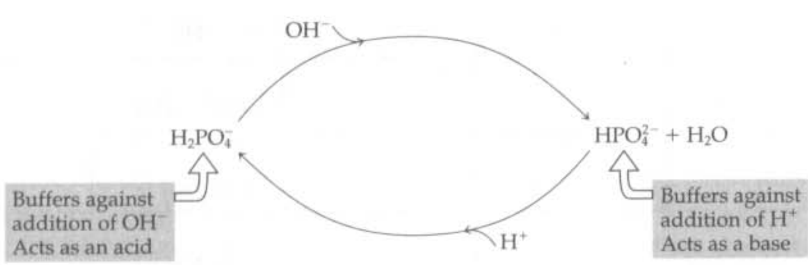
If hydrogen ions are added to the solution, they can be picked up by HPO_4^{2-}, which acts as a base:

$$H^+ + HPO_4^{2-} \longleftrightarrow H_2PO_4^-$$

If hydroxyl (OH^-) ions are added to the solution, they can be picked up by H^+:

$$OH^- + H^+ \longleftrightarrow H_2O$$

In summary,



Objectives

- ☐ Define buffer and discuss why buffers are important to organisms.
- ☐ Use a buffer system to show how buffers work.
- ☐ Explain why some substances have buffering capacity and others do not.

Procedure

In this exercise, you will use colorimetric indicators to determine which of four unknown solutions act as buffers. A change in one of the indicators, congo red or thymolphthalein, indicates a change in pH.

The four solutions, A, B, C, and D, were prepared using K_2HPO_4 or KH_2PO_4, or a combination of these, dissolved in distilled water.

1. Locate the four solutions marked A, B, C, and D on your laboratory table.
2. Pour approximately 40 ml of one of the unknown solutions into a 100-ml beaker. Insert a stirring bar and place the beaker on a stirring plate.
3. Add 1 dropperful of congo red. Record the color of the solution in Table 3F-1.
4. Measure the pH using a pH meter and record this value.
5. Using a pipette, add 2 ml of 0.1 *N* HCl. Record the color of the solution and its pH.
6. Dispose of this solution, and wash and dry the beaker and stirring bar thoroughly.
7. Pour another 40 ml of the same unknown solution into the beaker, insert the stirring bar, and return it to the stirring plate.
8. Add 1 dropperful of thymolphthalein and again measure the color and pH and record in Table 3F-1.
9. Now use a pipette to add 2 ml of 0.1 *N* NaOH. Record the color and pH.
10. Dispose of the solution and wash and dry the beaker and stirring bar thoroughly.
11. Repeat these steps for all three of the other unknown solutions, to complete Table 3F-1.

Table 3F-1 Determination of Changes in pH and Color

Unknown Solution	Initial Color	Initial pH	Solution Added	Final Color	Final pH
A Congo red			0.1 *N* HCl		
Thymolphthalein			0.1 *N* NaOH		
B Congo red			0.1 *N* HCl		
Thymolphthalein			0.1 *N* NaOH		
C Congo red			0.1 *N* HCl		
Thymolphthalein			0.1 *N* NaOH		
D Congo red			0.1 *N* HCl		
Thymolphthalein			0.1 *N* NaOH		

12. Answer the following questions based on your observations of color changes that occurred when either acid or base was added.

a. Which unknown solution buffered against both acids and bases? ______ *What did this solution contain?* ______________________

b. Which unknown solution buffered only against the addition of acid? ______ *What did this solution contain?* ______________________

Did the solution act as an acid or base? ____________ *Explain.*

____

c. Which unknown solution buffered only against the addition of base? ______ *What did this solution contain?* ______________________

Did the solution act as an acid or base? ____________ *Explain.*

____

d. Which solution did not buffer against addition of either acid or base? ______ *What did this solution contain?* ______________________

e. Which indicator would be the best to use to determine the presence of an acid?

______________ *What color would it appear at pH = 2?* ____________

pH = 7? ____________ *pH = 11?* ____________

f. Which indicator would be the best to use to determine the presence of base?

______________ *What color would it appear at pH = 2?* ____________

pH = 7? ____________ *pH = 11?* ____________

Use of indicators gives qualitative evidence of pH change. Qualitative observations can be further supported quantitatively by considering the actual H^+ and OH^- concentration changes.

When adding 2 ml of a 0.1 *N* acid or base, you are adding the equivalent of 5×10^{-3} M H^+ ions or OH^- ions.

$$\text{Increase in } H^+ \text{ or } OH^- = \frac{\text{volume of acid or base added}}{\text{volume of solution added into}} \times \text{normality of solution added}$$

Note: When dealing with acids and bases it is convenient to use equivalents to indicate the concentration of reacting particles. For example, a solution is 1 *N* when it contains one gram equivalent weight of reacting particles. (See Appendix III, Normal Solutions.)

In our example,

$$\text{Increase in } H^+ = \frac{0.002\ \text{l}}{0.04\ \text{l}} \times 0.1 \text{ equivalents/liter}$$

where 0.002 l = 2 ml of HCl or NaOH added to 0.04 l or 40 ml of solution. This gives

$$\text{Increase in } H^+ = 0.005 \text{ M (or } 5 \times 10^{-3} \text{ M)}$$

Therefore, if the solution you are testing does not buffer against an acid or a base, you will expect a 5×10^{-3} M increase in its H^+ or OH^- concentration. However, if your solution does buffer, the change in H^+ or OH^- concentrations will not be as dramatic.

13. Calculate the changes in H^+ and OH^- that you observed in your four unknown solutions. To calculate the change in H^+, subtract the initial H^+ concentration from the final H^+ concentration. To calculate the change in OH^-, first convert the pH into pOH. Then, subtract the initial OH^- concentration from the final OH^- concentration. Record these values in Table 3F-2.

Table 3F-2 Calculated Changes in H^+ and OH^- Concentrations

Unknown Solution	Initial $[H^+]$	Final $[H^+]$	Change in $[H^+]$	Initial $[OH^-]$	Final $[OH^-]$	Change in $[OH^-]$
A						
B						
C						
D						

g. From these results, did the indicators identify the buffering abilities of the unknown solutions accurately? ______________________

____

Laboratory Review Questions and Problems

1. HCl ionizes completely in water. What is the $[H^+]$ of a 0.01 M solution of HCl? What is the pH?

2. Is the hydrogen ion concentration of a pH 3.8 solution higher or lower than that of a solution with a pH of 6.2?

3. If one solution has 100 times as many hydrogen ions as another solution, what is the difference, in pH units, between the two solutions?

4. If solution A contains 1×10^{-6} M H^+ ions and solution B contains 1×10^{-8} M H^+ ions, which solution contains *more* H^+ ions?

5. Make a statement relating hydrogen ion concentration to the acidity and basicity of solutions.

6. HA is an acid that ionizes 10% in solution. What is the $[H^+]$ of a 0.01 M solution of HA? What is its pH?

7. Write the equation for the neutralization of NaOH by HCl.

8. What is the $[H^+]$ of a solution whose pH is 8? What is the $[OH^-]$?

9. Complete the following table.

$[H^+]$	$[OH^-]$	pH
	1×10^{-6}	
		4
1×10^{-3}		

10. You have made the following solutions:

Solution A Hydrogen ion concentration $= 1 \times 10^{-6}$ M

Solution B pH = 5

State whether each of the following is true or false. If false, explain why.

Solution A contains more H^+ ions than solution B.

Solution A contains 1×10^{-8} M OH^- ions.

Solution B contains 1×10^{5} M H^+ ions.

Solution B is acidic and solution A is basic when measured using the pH scale of 0–14.

Solution A is less acidic than solution B.

If the pH of solution A is to be raised by one pH unit, you would want to increase the OH^- concentration to 10^{-7} by adding base.

The two solutions differ in hydrogen ion concentration by a factor of 10 (i.e., one solution has 10 times the hydrogen ion concentration of the other).

11. Assuming complete ionization, what are the $[OH^-]$, $[H^+]$, and pH of a 0.01 M solution of NaOH?

12. Assuming complete ionization, what are the $[OH^-]$, $[H^+]$, and pH of a 0.1 M solution of KOH?

13. Calculate the pH of the listed solutions.

Solution		pH
Maalox	$[H^+] = 3.1 \times 10^{-9}$ M	
Saliva	$[H^+] = 1.95 \times 10^{-7}$ M	
Vinegar	$[OH^-] = 2.4 \times 10^{-12}$ M	

14. Calculate the H^+ and OH^- concentrations of the listed solutions.

Solution	pH	$[H^+]$ M	$[OH^-]$ M
Tomato juice	4.2		
Blood plasma	7.4		
Seawater	8.2		

15. State two reasons why pH maintenance is important to biological systems, and give examples of how pH may affect biological reactions.

16. You have four 1,000-ml beakers filled with four different clear solutions:

0.1 M NaH_2PO_4

0.1 M Na_2HPO_4

0.1 M phosphate buffer, pH 7.2

Distilled water

Oops! You forgot to label them and they all look alike. You get the congo red and thymolphthalein from the lab and test a sample of each solution, labeling the beakers randomly as A, B, C, and D. You use congo red when HCl is added to the sample, and thymolphthalein when NaOH is added. You get the following results.

	Color Before Addition	Add	Color After Addition
A	Red	HCl	Red
A	Colorless	NaOH	Blue
B	Red	HCl	Blue
B	Colorless	NaOH	Blue
C	Red	HCl	Red
C	Colorless	NaOH	Colorless
D	Red	HCl	Blue
D	Colorless	NaOH	Colorless

What is the identity of solutions A, B, C, and D?

A

B

C

D

14. Calculate the H^+ and OH^- concentrations of the listed solutions.

Solution	pH	$[H^+]$ M	$[OH^-]$ M
Tomato juice	4.2		
Blood plasma	7.4		
Seawater	8.2		

15. State two reasons why pH maintenance is important to biological systems, and give examples of how pH may affect biological reactions.

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	Color Before Addition	Add	Color After Addition
A	Red	HCl	Red
A	Colorless	NaOH	Blue
B	Red	HCl	Blue
B	Colorless	NaOH	Blue
C	Red	HCl	Red
C	Colorless	NaOH	Colorless
D	Red	HCl	Blue
D	Colorless	NaOH	Colorless

What is the identity of solutions A, B, C, and D?

A

B

C

D

17. You have buffers of pH 2, 4, 6, 8, and 10, but you need a pH 7 buffer for your experiment. Describe how you will make the pH 7 buffer.

18. A gardener planted pink hydrangeas in his yard last year, but this year when the flowers bloomed they were blue. He doesn't understand what happened. He took good care of the plants and mulched them with pine straw, just like the gardening encyclopedia said. What happened to his plants? How could he make them produce pink blooms again?