INTRODUCTION

PART 1: ACIDS, BASES, AND THE pH SCALE

The nature of acids and bases has been known for guite some time. Chemically speaking, acids are interesting compounds. But one of the best reasons for studying acids is that a large number of common household substances are acids or acidic solutions. For example, vinegar contains ethanoic or acetic acid, cranberries contains malic acid, sauerkraut (fermented cabbage) and yogurt (fermented milk) contain lactic acid, lemons contain citric acid. Acids cause foods to have a sour taste. On the other hand, many common household substances are bases (i.e., alkaline). For example, milk of magnesia contains the base magnesium hydroxide, and household ammonia is a common basic cleaning agent. Bases have a smooth or slippery feel between the fingertips. NOTE: Do not taste or feel compounds unless you are sure of what they are due to their caustic nature. Indicator dyes, in addition to litmus, turn various colors according to the strength of an acid or base that is applied to it.

An acid in water solution contains more hydrogen ions than hydroxide ions. Pure water, which is neutral, exists mostly as H_2O molecules. To a very slight extent, it does break into H^+ and OH^- ions.

$$HOH \rightarrow H^+$$
 (aq) + OH^- (aq)

This reaction forms to the extent of 0.0000001 moles of H^+ (aq) per liter of water. In scientific notation, it is written as 1 x 10⁻⁷ moles of H^+ per liter of solution.

The pH scale was devised to measure the concentration of hydrogen ion in a solution. The term pH refers to the "power of hydrogen", the concentration of hydrogen ion in solution. In a neutral solution, the concentration of H⁺ (aq) is 1 x 10^{-7} moles per liter and has a pH of 7. If the concentration is H⁺ (aq) is 1 x 10^{-5} moles per liter, its pH is 5; if the concentration is H⁺ (aq) is 1 x 10^{-12} moles per liter and its pH is 12. Please note that the pH scale is a logarithmic scale and each whole number change of the pH represents a change in hydrogen ion concentration of 10 times. A pH of 8 has 10 times the concentration of hydrogen ions as a pH of 9 and 100 times that of a pH 10.

Aqueous systems are seldom pure water and other substances affect the pH of the solution. If a substance *increases* the concentration of hydrogen ion in a solution, we call it an **acid**. Acids cause the pH of a solution to decrease, so the number becomes smaller than 7. If the substance *decreases* the concentration of hydrogen ion or increases the hydroxide concentration, we call it a **base**. Bases increase the pH of the solution, raising it above a pH of 7.

Methods of Measuring pH

Color Indicators: An important method of determining pH values involves the use of "indicators". These are certain organic substances that have the property of changing color in dilute solutions when the hydrogen-ion concentration of the solution reaches a definite value. For example, phenolphthalein is a colorless substance in any aqueous solution of which the hydrogen-ion concentration if greater than 10⁻⁹ M, or the pH is less than 9. In solutions for which the hydrogen-ion concentration is less than 10⁻⁹, the phenolphthalein imparts a red or pink color to the solution. Substances like phenolphthalein are called acid-base indicators and they often are used for determining the approximate pH of solutions. Electrical measurements can determine the pH more precisely.

The pH meter: Where rapid and accurate pH measurements are required, we use an instrument known as a pH meter. The pH meter is essentially a voltmeter designed to measure the voltage difference between a reference electrode and a sensory electrode. The reference electrode usually contains silver chloride solution of known concentration. The sensory electrode is in contact with the solution to be tested. The pH meter is calibrated so that a certain difference between the voltages of silver chloride and the test solution reads a certain pH value. To obtain accurate pH readings, we must standardize the electrode using buffers of known pH before measuring an unknown. Static electricity can affect pH readings, so always blot the end of the electrode, do not wipe it off, when washing the electrode and moving it to the next solution. Temperature also affects pH, so make sure that your calibration buffer is at the same temperature as the test solution. Occasionally, the pH meter has a temperature compensation adjustment with an internal temperature sensor.

pH METHODS

NOTE: In all activities, replace the cap on the bottles and **DO NOT** touch the tip of the bottle to the CHEMPLATE or to the solution in the cavities. This could contaminate the reagents for the remainder of the students. When placing the droplets, hold the bottle vertically and squeeze gently, dropping only one drop per well.

Activity 1 – Determining the effect of pH on indicator dyes

Purpose: To determine the color change for each of 3 indicators: methyl orange, brom thymol blue and phenolphthalein.

Procedure:

- 1. Place the CHEMPLATE on a sheet of white paper.
- 2. Place 1 drop of methyl orange in cavities #1 and #2.
- 3. Place 1 drop of brom thymol blue in cavities #5 and #6.
- 4. Place 1 drop of phenylphthalein in cavities #9 and #10.
- 5. Carefully add 1 drop of pH 1 to cavities #1, #5 and #9.
- 6. Carefully add 1 drop of pH 13 to cavities #2, #6 and #10.
- 7. Record your observations on your data sheet for this exercise.
- 8. Rinse the CHEMPLATE with tap water in the sink and dry with a paper towel.

RESULTS: Activity 1– Determining the effect of pH on indicator dyes

Methyl orange solution is what color?		
Methyl orange changes to		in an acid (pH1) solution.
	(color)	
Methyl orange changes to		in a base (pH13) solution.
	(color)	
Brom thymol solution is what color?		
Drom thread shares to		in an acid (nU1) solution
Brom mymor changes to	(color)	in an acid (pH1) solution.
Brom thymol changes to		in a base (pH13) solution.
, , , , , , , , , , , , , , , , , , , ,	(color)	
Dhanalnhthalain solution is what calor?		
Phenoiphunaletti solution is what color?		
Phenolphthalein changes to		in an acid (pH1) solution.
	(color)	
Phenolphthalein changes to		in a base (pH13) solution.
- •	(color)	· · · ·

Activity 2 – Determining the pH range with indicator dyes

Purpose: To establish the specific pH range in which the color changes for each indicator dye.

Procedure:

Methyl Orange

- 1. Place 1 drop of methyl orange in each cavity numbered 1-7.
- 2. Carefully add 1 drop of pH1 to cavity #1, 1 drop of pH3 to cavity #2, 1 drop of pH5 to cavity #3, 1 drop of pH7 to cavity #4, 1 drop of pH9 to cavity #5, 1 drop of pH11 to cavity #6 and 1 drop of pH13 to cavity #7.
- 3. Record the color change for methyl orange and the pH on your data sheet.
- 4. Rinse the CHEMPLATE with tap water in the sink and dry with a paper towel.

Brom Thymol Blue

- 1. Place 1 drop of brom thymol blue in each cavity numbered 1-7.
- Carefully add 1 drop of pH1 to cavity #1, 1 drop of pH3 to cavity #2, 1 drop of pH5 to cavity #3, 1 drop of pH7 to cavity #4, 1 drop of pH9 to cavity #5, 1 drop of pH11 to cavity #6 and 1 drop of pH13 to cavity #7.
- 3. Record the color change for brom thymol blue and the pH on your data sheet.
- 4. Rinse the CHEMPLATE with tap water in the sink and dry with a paper towel.

Phenolphthalein

- 1. Place 1 drop of phenolphthalein in each cavity numbered 1-7.
- Carefully add 1 drop of pH1 to cavity #1, 1 drop of pH3 to cavity #2, 1 drop of pH5 to cavity #3, 1 drop of pH7 to cavity #4, 1 drop of pH9 to cavity #5, 1 drop of pH11 to cavity #6 and 1 drop of pH13 to cavity #7.
- 3. Record the color change for phenolphthalein and the pH on your data sheet.
- 4. Rinse the CHEMPLATE with tap water in the sink and dry with a paper towel.

RESULTS: Activity 2 – Determining the pH range with indicator dyes

Methyl orange changes to		at	pH.
	(color)		(number)
Brom thymol changes to		at	рН.
	(color)		(number)
Phenolphthalein changes to		at	pH.
	(color)		(number)

Activity 3 – Determining a color standard using a universal indicator dye

Purpose: To determine a color standard for universal indicator dyes to determine the pH of unknown samples.

Procedure:

- 1. Place 1 drop of universal indicator in each cavity numbered 1-7.
- 2. Carefully add 1 drop of pH1 to cavity #1, 1 drop of pH3 to cavity #2, 1 drop of pH5 to cavity #3, 1 drop of pH7 to cavity #4, 1 drop of pH9 to cavity #5, 1 drop of pH11 to cavity #6 and 1 drop of pH13 to cavity #7.
- 3. Record the color of the indicator for each pH on your data sheet.
- 4. DO NOT rinse the CHEMPLATE or mix the reactions in the cavities.

RESULTS: Activity 3 – Determining a color standard using a universal indicator dye

#1 (pH 1) ______ #2 (pH 3) ______ #3 (pH 5) ______ #4 (pH 7) #5 (pH 9) ______ #6 (pH 11) ______ #7 (pH 13) _____

DO NOT RINSE OUT YOUR PLATE

Activity 4 – Determining the pH of unknown solutions

Purpose: To identify the pH of an unknown solution by using the universal indicator.

Procedure:

- 1. Place 1 drop of universal indicator in cavities #10, #11 and #12.
- 2. Place 1 drop of unknown I in cavity #10.
- 3. Place 1 drop of unknown II in cavity #11.
- 4. Place 1 drop of unknown III in cavity #12.
- 5. Compare the colors in cavities #10, #11 and #12 with the colors in cavities numbered 1-7 from Activity 3.
- 6. Record the pH for the unknown samples on your data sheet.
- 7. Rinse the CHEMPLATE with tap water in the sink and dry with a paper towel.

RESULTS: Activity 4 – Determining the pH of unknown solutions

The pH of Unknown I is _	
The pH of Unknown II is	
The pH of Unknown III is	

Activity 5 – Determination of the pH of common household products

Purpose: To use the pH meter to determine the pH of common solutions.

THE INSTRUCTOR WILL DEMONSTRATE THE PROPER USE OF THE pH METER, WHICH WILL NEED TO BE CALIBRATED USING THREE SOLUTIONS OF KNOWN pH (i.e., pH 4, pH 5, and pH 10). THE INSTRUCTOR WILL ALSO SHOW YOU HOW TO CLEAN THE ELECTRODE AND STORE IT SO THAT THE CHANCE OF DAMAGE TO THIS INSTRUMENT IS MINIMIZED DURING USAGE.

Procedure:

- 1. Select any 4 beakers of common household solutions from the bench at the front of class.
- 2. Record the name of your selections on the group worksheet.
- 3. Insert the probe of the pH meter into each solution and record the pH on the data sheet.
- 4. List the products in the order of increasing acidity.

RESULTS: Activity 5 – Determination of the pH of common household products

Product	рН

List the household products in order of increasing acidity:

PART 2: ACIDS, BASES, AND BUFFERS IN LIVING SYSTEMS

Cells "live" in, and contain aqueous, or water-based, solutions. Therefore, **water plays an important role in cellular reactions**. Remember that water is neither acidic nor basic, but is neutral, with a pH of 7.

Acids and bases are damaging to the molecules in a cell, causing dysfunction or even cell death. It is important to the health of cells that they maintain a pH as close to neutral (or a value of 7) as possible. If pH changes by even 1 pH unit, the proteins in a cell can denature, leading to changes in cell structure and, more importantly, to catalysis of cellular reactions. (Review: what does the term "denature" mean?)

рΗ

You will remember that water dissociates into hydrogen ion (H+) and hydroxyl ion (OH-). This reaction can be written: $H_2O \Leftrightarrow H+ + OH-$

Like all reactions, the dissociation of water is an equilibrium reaction, for which an equilibrium constant (Keq) can be written. The Keq is a measure of the extent of the reaction at thermodynamic equilibrium. That is, at equilibrium, the value of Keq tells how much product is formed relative to how much reactant remains. This is calculated by multiplying the concentrations of products, and dividing that value by the concentrations of reactants. So, for the dissociation of water, the Keq can be written:

$$Keq = ([H+][OH-])/[H_2O]$$

The dissociation of water, and its Keq value, is the basis for pH measurement. It is known that the concentration of pure water ([H₂O]) is 55.5 moles/L, and experiments have shown that the Keq of water is 1.8×10^{-16} moles/L. Rearranging the Keq equation and using these values, we find:

Keq x $[H_2O] = [H+][OH-]$ (1.8x10⁻¹⁶) x 55.5 = [H+][OH-] 1.0 x 10⁻¹⁴ = [H+][OH-]

In a neutral solution (neither acidic nor basic), [H+] = [OH-], so

 $1.0 \times 10^{-7} = [H+], and$

1.0 x 10⁻⁷ = [OH-]

Let's consider the hydrogen ion concentration ([H+]): we have just shown that in neutral solution [H+] is 1.0×10^{-7} . Writing concentrations in the form of negative exponents is cumbersome, so scientists have developed a more convenient method of measurement, the "p" notation. First, they use logarithms in place of exponents. This would convert the value 1.0×10^{-7} to the whole number -7. To do away with the negative sign, they multiply these values by -1 (so, -7 x -1 = 7). Now the [H+] concentrations can be written as whole, positive numbers (such as 7) instead of as negative exponents (1.0×10^{-7}). This gives us the pH scale.

Acids and Bases

If a solution has a large number of H+ ions (for example 1.0×10^{-2}), it is considered an acid, and, in this example, has a pH of 2.

An acid is a molecule that releases a hydrogen ion (H+). An acid can be either strong or weak, depending on how easily the H+ is released from the rest of the molecule. Strong acids easily release H+, tending to be fully "dissociated." An example of a strong acid is HCI. In water, most HCI molecules exist as H+ and CI-, with little of the hydrochloric acid (HCI) remaining in the solution. The HCI dissociation reaction can be written: HCI \Leftrightarrow H+ + CI-

(Here, CI- is called the "conjugate base" of the original acid. (Note the conjugate base holds a negative charge.)) A strong acid solution would be expected to have a high concentration of H+.

The HCl dissociation reaction at equilibrium has a characteristic Keq value. The Keq for this reaction can be written:

$$Keq = ([H+] [CI-])/[HCI]$$

In the case of acids, the Keq is called the acid dissociation constant, or Ka, rather than Keq. (Remember that if Ka has a large value, the concentrations of the products of the reaction are much higher than the concentrations of the

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reactants. So this tells you that at equilibrium, the reaction has gone far to the right (or much product has been formed, and little reactant remains)). A large Ka is expected for a strong acid such as HCI, which easily dissociates and goes mostly to product (individual ions) at equilibrium.

<u>Most biological acids are not as strong as HCI</u>. That is, they don't give up their H+ as easily as does HCI. The weaker the acid, the less likely it is to dissociate into hydrogen ion and the conjugate base of the acid. The strength of an acid depends on its chemical and electronic structure, which cause it to want to either "hold on" to or release its H+. A solution of a very weak acid would have some H+ but most of the acid molecules would remain associated with its original H.

An example of a weak acid is acetic acid (CH₃COOH). The acetic acid dissociation reaction can be written:

 $CH_3COOH \Leftrightarrow H+ + CH_3COO-$

(Here, the conjugate base is called acetate ion.)

The Ka for acetic acid would be written:

 $Ka = ([H+] [CH_3COO-])/ [CH_3COOH]$

Because acetic acid does not give up its H+ as easily as does HCl, you would not expect the numerator in the Ka equation to be high, while the denominator in the Ka equation would be large; overall the Ka for acetic acid is low, which is true of all weak acids. Therefore, a solution of acetic acid would not be expected to have as high a concentration of H+ as would a strong acid like HCl, and the pH of an acetic acid solution would be higher than the pH of an equimolar HCl solution.

рКа

The strength or weakness of an acid is a chemical characteristic of that acid, and can be determined mathematically using the Ka equation. Using acetic acid as an example:

	$Ka = ([H+] [CH_3COO-])/ [CH_3COOH]$
Rearranging:	[H+] = (Ka x [CH ₃ COOH])/[CH ₃ COO-]
Taking negative log:	- log [H+] = -log Ka – log ([CH ₃ COOH]/[CH ₃ COO-])
	- log [H+] = -log Ka + log ([CH ₃ COO-]/[CH ₃ COOH])
Use "p" notation:	$pH = pKa + log ([CH_3COO-]/CH_3COOH])$

This is the <u>Henderson-Hasselbach equation</u>, <u>which relates the pKa of a solution</u> (a measure of how easily an acid gives up its H+) to the pH of a solution of that acid. Note, however, that the initial concentrations of the weak acid and its conjugate base are also important to the equation.

Because the Ka is now in "p" notation, a high value denotes a weak acid (one which doesn't easily give up its H+; its Ka would be small, but its pKa would be large.) Consider an example of a very strong acid, like HCI. Its Ka would be large (approximately 1.0×10^{-2} [H+] in solution); in "p" notation, pKa=2. For a very weak acid, with a Ka that is small (say 1.0×10^{-6} [H+] in solution), the pKa would be 6.

The Henderson Hasselbach equation allows experimental determination of pKa. pKa can be determined by measuring the pH of a solution where the concentration of weak acid = concentration of conjugate base:

For acetic acid:	$pH = pKa + log ([CH_3COO-]/CH_3COOH])$
When [acid]=[conj base]	pH = pKa + log 1
	pH = pKa + 0
	pH = pKa

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Buffer Systems

Buffers are composed of a weak acid and its conjugate base. Buffers have the ability to resist a change in pH when either H+ or OH- ions are introduced.

The weak base in a buffer solution is available to react with any added acid (H+), thus neutralizing the acid and keeping the pH from changing to a great extent. (Would you expect the addition of added H+ to cause the pH to decrease or increase, if a buffer were not present?) Likewise, if base (OH-) were added to a buffer solution, the weak acid available could give up its H+ to react with, and neutralize, the added base (making H₂O), again keeping the pH from changing significantly. However, buffer systems have limits, and their neutralizing effects can be destroyed by the introduction of an excessive amount of either H+ or OH- ion.

Acetic acid and its conjugate base (acetate ion) can form acetic acid buffer. The Henderson-Hasselbach equation can be used to determine how to prepare the buffer, and can calculate the effect of adding additional acid and base to the existing buffer, by means of a titration experiment. The scientist starts with a solution of acetic acid of known concentration, and measures its pH using a pH meter. Hydroxide ion (in the form of 1 M sodium hydroxide solution) is added in increments, and the pH of the solution is measured after each addition. It is expected that as OH- is added, the acetic acid will give up its H+, forming H₂O with the OH-. As more and more H+ is dissociated and forms neutral water, producing more and more acetate ion, the pH will increase (fewer H+ ions will be free in the solution and more acetate will be available). A plot of amount of NaOH added (x axis) vs. pH (y axis) will yield a curve. Weak acids that can act as buffers will have a flattened region, at which a relatively large addition of added base will result in a relatively small pH change in the region of the acid's pKa. As shown above, when pH=pKa, the concentration of weak acid = concentration of its conjugate base. This is the area of highest buffering ability, since there are as many acid molecules available (to react with any base added to the solution) as there are conjugate base molecules (to react with any acid added to the solution).

Cells, and all the water in living organisms, maintain active buffer solutions to guard against extensive changes in pH, should acids or bases be encountered. This allows the cells' proteins to maintain their conformation, and their function.

In today's experiment, students will perform a titration experiment, and will make acetic acid buffer solutions of varied concentrations. The pH of these solutions will be measured experimentally, as well as calculated using the Henderson-Hasselbach equation. A buffer will be "tested" by measuring pH after adding acid or base, and the results of this "test" compared to pH changes in pure water with the addition of acid and base.

PROCEDURES

Standardizing Your pH Meter

Obtaining accurate reading with a pH meter depends on effective standardization, the degree of static charge, and the temperature, as well as other factors. Particularly important to the proper use of a pH meter is accurate standardization, as glass electrodes must be carefully calibrated using buffers of known pH. Generally, it is desirable to calibrate the meter with a standard buffer of a pH as close as possible to the pH of the solution to be measured. The problem of static charge on the electrode can be minimized by blotting (not rubbing) the surface of the electrode dry when transferring from one solution to another. Temperature also affects pH to a certain extent, but the problem can be easily eliminated by keeping all solutions at the same temperature.

Standardize the meter at your table for pH 4, since your experiments will be centered on acetic acid, which has a relatively low pH.

The Titration of Acetic Acid

You will use 0.2 M acetic acid and 1.0 M NaOH for this experiment.

1. Add 100 mL of 0.2 M acetic acid to a 250 mL beaker or container. One person in each group should gently swirl the solution throughout the experiment.

- 2. Lower the pH electrode into the solution.
- 3. With constant swirling, add NaOH in 1-mL increments, recording the resulting pH after every addition of base on the data sheet.
- 4. Continue the titration until the pH is approximately 8.0.
- 5. Follow the directions on the data sheet regarding plotting your data and finding conclusions.

How to Make an Acetate Buffer

You will use 0.2 M acetic acid and 0.2 M sodium acetate solutions for this experiment.

1. Obtain 5 vials, and label them 0, 1, 2, 3 and 4. Each number represents the volume of acetic acid you will place in the vial; the total volume in each vial will be 5 mL.

2. In vial 0, measure 5 mL sodium acetate.

3. In vial 1, measure 1 mL acetic acid + 4 mL sodium acetate.

4. In vial 2, measure 2 mL acetic acid + 3 mL sodium acetate.

5. In vial 3, measure 3 mL acetic acid + 2 mL sodium acetate.

6. In vial 4, measure 4 mL acetic acid + 1 mL sodium acetate.

7. Measure the pH of each vial, and record the data on your data sheet. Follow the directions on your data sheet regarding calculations with your results.

Effect of Added Acid and Base on Buffers

You will use pure water, 0.2 M acetic acid and 0.2 M sodium acetate solutions for this experiment. You will also need **0.5 M HCI** and **0.5 M NaOH** solutions.

- 1. Obtain 4 vials. Label 2 "buffer" and 2 "water.
- 2. In the vials labeled "buffer," add 2.5 mL acetic acid and 2.5 mL sodium acetate in each.
- 3. In the vials labeled "water," add 5 mL pure water in each.
- 4. Measure the pH of one of the buffer vials and record this value on your data sheet.
- 5. To this vial, with constant swirling, add 0.2 mL HCl. Record the pH.
- 6. Add another 0.2 mL acid and test the pH again.
- 7. Continue this process of adding acid at 0.2 mL increments until 1.0 mL acid has been added.
- 8. Repeat this procedure with a distilled water vial.
- 9. Use the other buffer and water vials in a similar manner, except add 0.5 M NaOH.
- 10. Follow the directions on your data sheet regarding calculations with your results.

Titration of Acetic Acid

pH, Acids, Bases, and Buffers

NaOH Added (mL)	Hq	NaOH Added (mL)	<u>pH</u>
the pKa of ace the point when the chemical for at the beginn the chemical for at the end of	(Draw a box around it) etic acid e [CH ₃ COO-] = [CH ₃ COOH] ormula of acetic acid that predom ning of the titration ormula of acetic acid that predom f the titration	inates	
How to Make an Acet	ate Buffer		
Vial Number	[CH3COO-]/[CH3COOH]	pH (exper)	pH (calc'd)
_acetic acid			

* This value can be obtained from the first reading of the titration experiment, when 0 mL NaOH was added. Use the Henderson-Hasselbach equation to calculate the pH of each buffer solution.

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Effect of Added Acid and Base on Buffers

Additions	pH of Buffer	pH of Water
None		
0.2 mL HCl		
0.4 mL HCl		
0.6 mL HCl		
0.8 mL HCl		
1.0 mL HCl		
None		
0.2 mL NaOH		
0.4 mL NaOH		
0.6 mL NaOH		
0.8 mL NaOH		
1.0 mL NaOH		

Draw two graphs: one of mL HCl added (x axis) vs. pH (y axis), and one of mL NaOH added (x axis) vs. pH (y axis).

What conclusions can you draw about the effects of a buffer on pH with added acid and base, compared to adding acid or base to water?

Use your graphs to determine the pKa of acetic acid buffer, when [CH₃COO-]=[CH₃COOH].