

EXERCISE 3

Name _____

A. Understanding pH and buffers

B. Introduction to the brightfield microscope

Objectives

After completing this exercise, you should be able to:

- ◆ Define and correctly use the following terms: dissociation, acid, base, pH, buffer, buffering range, and buffering capacity.
- ◆ Explain how solutions with different pH compare with respect to their concentrations of H^+ and OH^-
- ◆ Calibrate and use a pH meter to measure and adjust the pH of a solution.
- ◆ Prepare buffered solutions and determine the pH range at which a solution acts as a buffer.
- ◆ Plot and interpret a pH titration curve for a solution.
- ◆ Identify the various parts of the brightfield microscope and describe the correct way to use and care for it.
- ◆ Describe the particular features of the brightfield microscopes available in your lab.
- ◆ Determine the power of magnification when viewing objects with the brightfield microscope.
- ◆ View both wet mounts and prepared slides using the brightfield microscope.

Prelab

Before you come to lab, read this entire exercise. You must also answer all questions and complete all assignments on the first 12 pages of this exercise. Your instructor will give you directions on when and where to turn in your work.

What is dissociation?

The chemistry of life is based largely on the chemistry of water. When ionic or polar solutes are mixed with water, the water molecules (which are polar) attract them and may pull them apart into oppositely charged ions. This process is called **dissociation**. In fact, water molecules may even pull other water molecules apart to produce oppositely charged **hydrogen ions (H^+)** and **hydroxyl ions (OH^-)**:



The double-headed arrow in the equation above indicates that this is a **reversible reaction**. This means it can occur in both directions. Because each water molecule that dissociates produces one H^+ and one OH^- , pure water always has the same concentration of H^+ and OH^- , which turns out to be $1 \times 10^{-7} M$:

$$\text{In pure water, } [H^+] = [OH^-] = 1 \times 10^{-7} M$$

⇒ **NOTE: The brackets indicate “the concentration of” whatever is inside the brackets.**

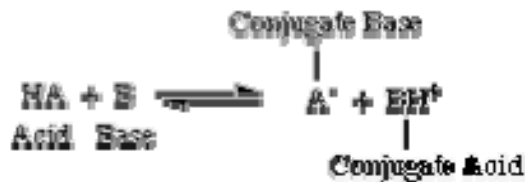
When solutes are dissolved in pure water, the concentrations of $[H^+]$ and $[OH^-]$ may be altered. However, in any aqueous solution the product of $[H^+]$ and $[OH^-]$ is **always** $1 \times 10^{-14} M$:

$$\text{In all aqueous solutions, } [H^+] \times [OH^-] = 1 \times 10^{-14} M$$

Because of this relationship, for any solution it is possible to determine the concentration of OH^- if one knows the concentration of H^+ (or vice versa). Furthermore, it means that as $[H^+]$ increases, $[OH^-]$ must decrease and, of course, the opposite is true as well.

What are acids and bases?

Although acids and bases have been defined in several different ways, in this class we will define an **acid** as a proton donor, and a **base** as a proton acceptor. Remember, a proton is just another name for a hydrogen ion (H^+). These definitions mean that acids transfer protons to bases. When an acid transfers a proton to a base, the acid becomes a **conjugate base** (since it is now a proton acceptor) and the base becomes a **conjugate acid** (since it is now a proton donor):



Because acids are proton donors, they act to **increase** $[\text{H}^+]$ when dissolved in water. For example, when hydrochloric acid (HCl) is dissolved in water, it dissociates to form H^+ and Cl^- . The release of H^+ ions increases $[\text{H}^+]$ in the solution. Also remember that in any solution, as $[\text{H}^+]$ increases $[\text{OH}^-]$ will decrease.

Bases, on the other hand, are proton acceptors. Therefore, they act to decrease the $[\text{H}^+]$ in an aqueous solution. Bases can decrease the $[\text{H}^+]$ in two ways:

- 1) They can dissociate to form OH^- . For example, sodium hydroxide (NaOH) is a strong base that dissociates to form Na^+ and OH^- . As $[\text{OH}^-]$ increases, $[\text{H}^+]$ decreases, because some of the H^+ ions that were present in the water before the NaOH was added will combine with the extra OH^- to form water.
- 2) They can combine directly with H^+ ions, thereby removing “free” H^+ ions from the solution. As the number of H^+ ions declines, more water molecules dissociate, increasing the number of OH^- ions. For example, ammonia (NH_3) combines with H^+ ions in water to form ammonium ions (NH_4^+), thereby decreasing the concentration of free H^+ ions.

In sum, when an **acid** is added to an aqueous solution, $[\text{H}^+]$ increases and $[\text{OH}^-]$ decreases. When a **base** is added to an aqueous solution, $[\text{OH}^-]$ increases and $[\text{H}^+]$ decreases.

Acids and bases can vary in the extent to which they alter $[\text{H}^+]$ and $[\text{OH}^-]$. Strong acids, such as hydrochloric acid (HCl) and sulfuric acid (H_2SO_4), almost completely dissociate in water. Therefore, they produce a relatively large increase in $[\text{H}^+]$ and a correspondingly large decrease in $[\text{OH}^-]$. On the other hand, weak acids, such as acetic acid and citric acid, have a much lower level of dissociation. Therefore, they produce a relatively small increase in $[\text{H}^+]$ and a correspondingly small decrease in $[\text{OH}^-]$. Similarly, strong bases, such as sodium hydroxide (NaOH) and potassium hydroxide (KOH), produce a large increase in $[\text{OH}^-]$ when dissolved in water, while weak bases such as ammonia (NH_3) and Trizma® base produce a much smaller increase in $[\text{OH}^-]$. Most biomolecules have properties of weak acids and/or bases.

What is pH?

The pH scale was developed to measure how acidic or basic (alkaline) a solution is. The **pH** of a solution is defined as the negative logarithm, to the base 10, of the hydrogen ion concentration of the solution:

$$\text{pH} = - \text{logarithm (to base 10)} [\text{H}^+]$$

⇒ **NOTE: Like absorbance, pH is the logarithm of a number and therefore has no units.**

A logarithm (or log) is the power to which a base, in this case 10, must be raised to give the desired number. For example, the log of 100 is 2 (to get 100, we must raise 10 to the power of 2) and the log of 1,000 is 3 (to get 1,000, we must raise 10 to the power of 3). A neutral solution has a hydrogen ion concentration of 1×10^{-7} M and a hydroxyl ion concentration of 1×10^{-7} M. The logarithm of 1×10^{-7} is -7, so the negative logarithm of 1×10^{-7} is 7. Therefore, the pH of a neutral solution is 7.

Your Turn

Fill in the pH for each of the following solutions:

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

$[H^+] = 1 \times 10^{-8.7} \text{ M}$ _____

$[OH^-] = 1 \times 10^{-3} \text{ M}$ _____

$[OH^-] = 1 \times 10^{-8.7} \text{ M}$ _____

How do solutions of various pHs compare?

Remember that in a neutral solution, $[H^+] = 1 \times 10^{-7} \text{ M}$. In addition, an acidic solution has a **higher** $[H^+]$ than a neutral solution, and a basic solution has a **lower** $[H^+]$ than a neutral solution. Remember too, that as the absolute value of a negative exponent gets larger (-1, -2, -3, -4...), the number it represents gets smaller. For example, 1×10^{-7} , or 0.0000001 is **ten times smaller** than 1×10^{-6} or 0.000001. The following table may help you remember how numbers with different negative exponents compare:

Comparing numbers with negative exponents

Scientific notation	1×10^{-2}	1×10^{-1}
Decimal	0.01	0.1
Fraction	1/100	1/10
Showing both with a common denominator	1/100	10/100
How many <i>hundredths</i> does each represent?	one	ten
Logarithm	- 2	- 1
- log	2	1

Your Turn

Fill in the $[H^+]$ for each of the following solutions. Make sure that the exponents of your answers use only integer powers (e.g., 10^{-7} or 10^{-2}). Use a calculator to convert non-integer exponents to integers (e.g. $10^{-2.6}$ becomes 2.5×10^{-3} .)

pH 5 _____ M

pH 2 _____ M

pH 9 _____ M

pH 12 _____ M

pH 6 _____ M

pH 7 _____ M

pH 3.2 _____ M

pH 8.7 _____ M

Your Turn

Determine which pHs in the previous box are acidic. List the **acidic** pHs below, in order from most acidic to least acidic:

Most acidic pH _____ [H⁺] _____ M
(Highest conc. H⁺'s)
acidic pH _____ [H⁺] _____ M
acidic pH _____ [H⁺] _____ M
Least acidic pH _____ [H⁺] _____ M
(Lowest conc. H⁺'s)

Next, determine which pHs in the previous box are basic. List the **basic** pHs below, in order from most basic to least basic.

Most basic pH _____ [H⁺] _____ M
(Lowest conc. H⁺'s)
basic pH _____ [H⁺] _____ M
Least basic pH _____ [H⁺] _____ M
(Highest conc. H⁺'s)

How do solutions of different pH compare?

A solution of pH 5 has a _____ times larger concentration of H⁺ than a solution of pH 6. Therefore a pH 5 solution is _____ times more acidic than a pH 6 solution.

A solution of pH 2 has a _____ times larger concentration of H⁺ than a solution of pH 6. Therefore a pH 2 solution is _____ times more acidic than a pH 6 solution.

A solution of pH 9 has a _____ times lower concentration of H⁺ than a solution of pH 7. Therefore a pH 9 solution is _____ times more basic than a pH 7 solution.

A solution of pH 12 has a _____ times lower concentration of H⁺ than a solution of pH 9. Therefore a pH 12 solution is _____ times more basic than a pH 9 solution.

What is a buffer?

A **buffer** is a mixture of molecules that release or bind H^+ in order to maintain a relatively constant pH. Most buffers consist of a weak acid (which releases H^+) and a weak base (which binds H^+ .) If additional base is added to a buffered solution, the acid part of the buffer releases H^+ ions which combine with the extra OH^- ions to form water. Therefore, the solution does not become as basic as it would have without the buffer. On the other hand, if additional acid is added to a buffered solution, the base component of the buffer combines with the H^+ ions that are released by the acid. This reduces the concentration of free H^+ ions in the solution, so the solution doesn't become as acidic as it would have if the buffer had not been present.

Note that the function of a buffer is **NOT** to keep a solution neutral (at pH 7); its function is to minimize the change in pH when base or acid is added to the solution. Also note that there are many different buffers, and each one will stabilize the pH of a solution only within a specific pH range. One buffer may be effective within a range of pH 2 to pH 6, while another may be effective within a range of pH 10 to pH 12. Beyond its **buffering range**, a buffer no longer acts to stabilize the pH of the solution.

Buffers are extremely important to living organisms because most biochemical processes proceed normally only when the pH remains within a fairly narrow range. An excess of H^+ or OH^- can interfere with the structure and activity of many biomolecules, especially proteins. Therefore, buffers are commonly used in living organisms to help maintain a relatively stable pH. In humans, for example, buffers act to maintain blood pH between 7.35 and 7.45 even though acids and bases are continually being added to and removed from the blood as it travels through the body. The 3 main buffer systems in our bodies are the bicarbonate buffer system, the phosphate buffer system, and the protein buffer system.

In the laboratory, molecular and cellular biologists make extensive use of buffers to stabilize the pH of aqueous solutions. When studying biomolecules in a test tube, the biomolecules may be altered or may behave in ways that are uncharacteristic of their natural behavior if they are in a solution with a pH that is significantly different from the pH of their natural environment. As another example, growing cells or tissues in the lab often requires a growth medium that is buffered to a specific pH. This is because living cells release waste products that can change the pH of the medium they are growing in. Because cells are very sensitive to changes in pH, the cells could not be cultured for long before they died from pH changes caused by their own metabolism. In this situation, adding a buffer to the growth medium can extend the time that the cells can survive.

How can I make buffered solutions with specific solute concentrations and pH?

A buffered solution generally contains several different solutes. At a minimum, it must contain both the acid and base components of the buffer. In addition, buffered solutions often contain other solutes such as nutrients, electrolytes, and/or various biomolecules. To prepare a buffer with specific concentrations of several different solutes, do a separate calculation to determine the amount of each solute needed (using the procedures you learned in exercise #2), mix all of the solutes together, and then add enough solvent to bring the solution to its final volume.

In addition to controlling solute concentrations, making a buffer also involves controlling pH. The pH of a buffer is determined by the **relative** concentrations of the acid and base components. For example, in lab you will be preparing an acetate buffer. In this buffer, the acid component is acetic acid and the base component is the acetate ion. As long as the concentrations of these 2 components are equal, the pH of the acetate buffer will be 4.6. Therefore, if you prepare one acetate buffer where the concentration of each component is 50 mM and a second acetate buffer where the concentration of each component is 900 mM, both buffers will have a pH of 4.6. On the other hand, even though both buffers will have the same pH, the second buffer will have a much higher **buffering capacity**, meaning it will have a much greater ability to resist changes in pH as additional acid or base is added to the buffer.

It is also important to realize that as the **relative** concentration of the acid component of a buffer increases, the pH of the buffer will decrease. For example, an acetate buffer containing 88 mM acetic acid and 16 mM sodium acetate (a source of acetate ions) will have a pH of 3.8 (compared to a pH of 4.6 when both components have the same concentration.) On the other hand, as the relative concentration of the acid component of a buffer decreases, the pH of the buffer will increase. For example, an acetate buffer containing 21 mM acetic acid and 79 mM sodium acetate will have a pH of 5.2.

There are several ways to control the pH of a buffer. One method is to start with one buffer component (either acid or base) and then gradually add the second component until the desired pH is reached (the pH can be measured with a pH meter.) A second method is to use an equation to calculate the concentrations of acid and base components needed to produce a buffer with a specific pH. A third method is to consult a reference to determine the concentrations of acid and base components needed to produce a buffer with a specific pH. In this lab you will make two buffered solutions using the third method.

Your Turn

In lab, the following stock solutions will be available:

- 0.1 M sucrose
- 0.2 M acetic acid
- 0.2 M sodium acetate
- 0.2 M anhydrous sodium carbonate
- 0.2 M sodium bicarbonate

- A. Calculate the amount of 0.1 M sucrose solution and the amount of dH₂O needed to prepare 100 mL of unbuffered 20 mM sucrose. Write your answers in the spaces below **and in the spaces provided on page 13 of the Lab Procedures**. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of sucrose stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of dH₂O needed _____

- B. The **acetate buffer** consists of acetic acid (**CH₃COOH**) and acetate ions (**CH₃COO⁻**). A chemistry reference indicates that a solution containing 40 mM acetic acid and 60 mM sodium acetate (a source of acetate ions) will produce an acetate buffer with a pH of 4.8. Calculate the amount of each stock solution and the amount of dH₂O needed to prepare 100 mL of an acetate buffer that contains 20 mM sucrose, 40 mM acetic acid, and 60 mM sodium acetate. Write your answers in the spaces below **and in the spaces provided on page 13 of the Lab Procedures**. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of each stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of 0.2 M acetic acid solution needed _____

Amount of 0.2 M sodium acetate solution needed _____

Amount of dH₂O needed _____

- C. The **bicarbonate buffer** consists of carbonate ions (**CO₃⁻²**) and bicarbonate ions (**HCO₃⁻**). A chemistry reference indicates that a solution containing 22 mM anhydrous sodium carbonate (a source of carbonate ions) and 28 mM sodium bicarbonate (a source of bicarbonate ions) will produce a bicarbonate buffer with a pH of 9.8. Calculate the amount of each stock solution and the amount of dH₂O needed to prepare 100 mL of a bicarbonate buffer that contains 20 mM sucrose, 22 mM anhydrous sodium carbonate, and 28 mM sodium bicarbonate. Write your answers in the spaces below **and in the spaces provided on page 13 of the Lab Procedures**. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of each stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of 0.2 M anhydrous sodium carbonate solution needed _____

Amount of 0.2 M sodium bicarbonate solution needed _____

Amount of dH₂O needed _____

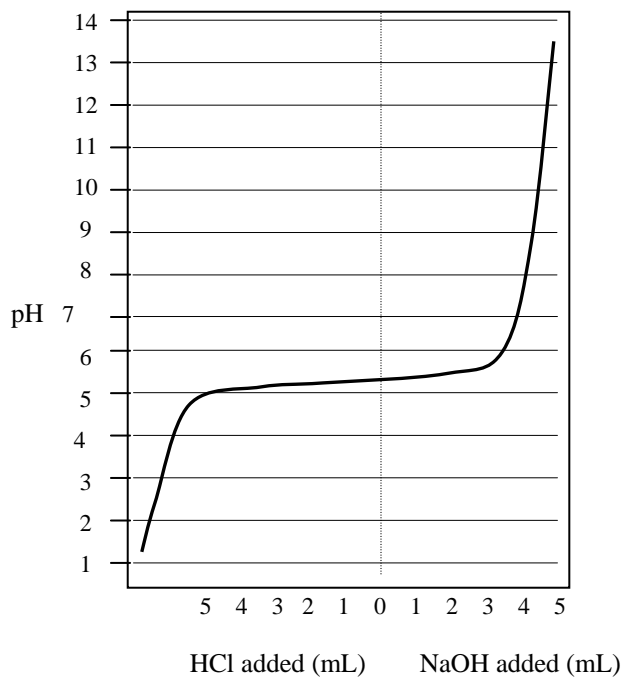
program such as Excel is to use positive values to represent the volume of base added (e.g. let 1, 2, and 3 represent 1, 2, and 3 mLs of base added) and use negative numbers to represent the volume of acid added (e.g. let -1, -2, and -3 represent 1, 2, and 3 mLs of acid added.) If you use this approach, make sure it is clearly indicated on your graph.

After you have labeled the axes of your graph, plot the data points and draw a smooth curve as close as possible to all of the points. The resulting plot is referred to as a titration curve. The titration curve serves to profile the unknown solution. The shape of the curve reveals much chemistry including information about the buffering range and buffering capacity of your unknown solution.

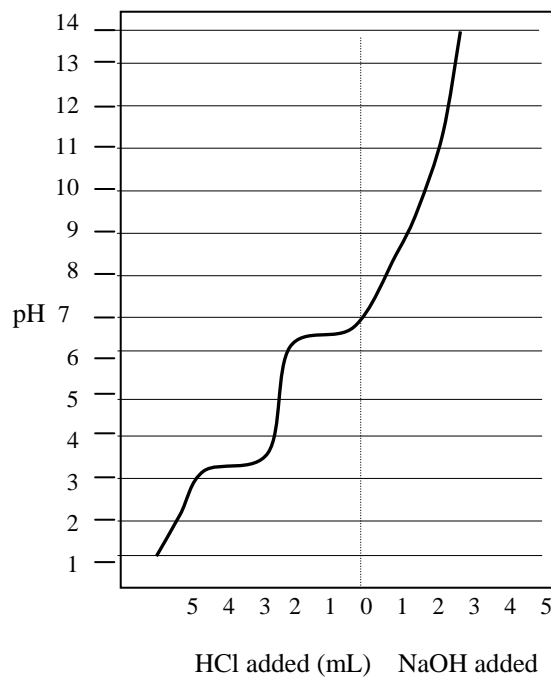
Your Turn

The following graphs show the titration curves of two unknown solutions:

Solution A



Solution B



What is the buffering range of solution A? _____

What are the buffering ranges of solution B? _____

Which solution has the greater buffering capacity? _____

Would either solution be a good buffer for studying enzymes or other biomolecules found in human blood?
Explain your answer.

Why is the brightfield microscope so important for the study of cell structure and function?

Cytology is the study of cell structure and function. Because most cells are very small, they can only be seen when magnified with an instrument such as a microscope. Microscopes not only provide important information about cell structure, they also provide clues about how the cell works.

There are 2 basic types of microscopes: the light microscope (creates an image using a beam of light), and the electron microscope (creates an image using a beam of electrons.) The most common type of light microscope, and the one you will use in this lab, is the **brightfield microscope**. The brightfield microscope is an example of a **compound microscope**. That means light from the object you are viewing passes through two lenses before it reaches your eye.

Microscopes not only **magnify** the object you are viewing, they also provide increased **resolution**, which is the ability to distinguish two points as separate points. The better the resolution, the sharper and crisper the image. The resolving power of the naked eye is approximately 0.2 mm, meaning that our eyes can distinguish two points that are 0.1 mm apart. A light microscope can improve resolution as much as 1000-fold. In addition, discernment of cellular detail can be improved with the use of dyes that add color and contrast to subcellular structures.

In this exercise, you will learn about the different parts of the brightfield microscope, how to care for the microscope, and the correct procedure for viewing specimens. You will then practice using the microscope to view both a wet mount and a prepared slide of the eukaryotic organism *Euglena*.

What are the parts of the brightfield microscope?

Study the location and the function of each of the following parts of a brightfield microscope:

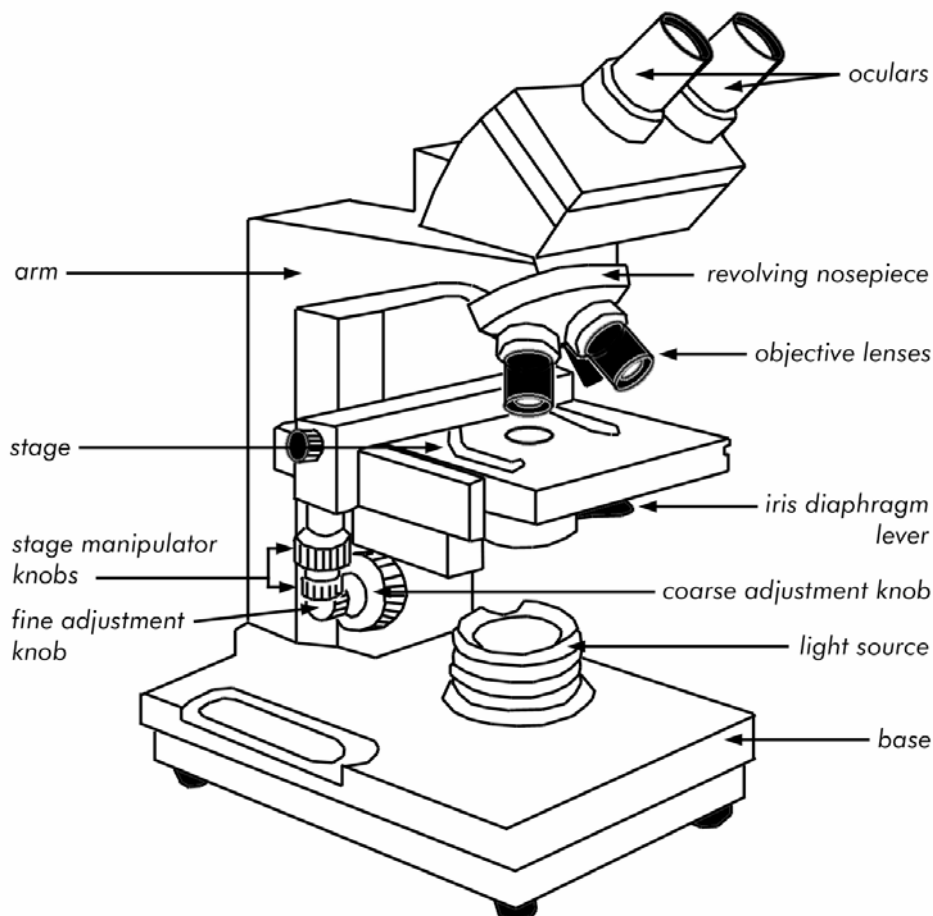


Figure 3.1 Parts of a brightfield microscope.

1. **Base** - The part of the microscope that rests on the table is called the **base**.
2. **Light Source** - The **illuminator** for your microscope is built into the base and is controlled by an on/off switch.
3. **Arm** - The **arm** is a vertical support that connects the base of the microscope to the body tube.
4. **Body Tube** - The **body tube** is the part of the microscope that connects the oculars with the revolving nosepiece. Some of the microscopes in lab have a small knob that sticks out from the front of the body tube. This knob can be used to move a pointer that is visible when you look through the microscope.
5. **Ocular lenses** - The **oculars** are the lenses you look through. Some microscopes are *monocular*--that is, there is only one eyepiece to look through. Other microscopes are *dual-viewing*; this means there are two oculars--one pointing upwards and one pointing backwards. These microscopes allow two people to view the same specimen at the same time--each looking through a single eyepiece. Yet other microscopes are *binocular*--that is, there are two eyepieces side-by-side and you look through both of the eyepieces, as you would a pair of binoculars. Most ocular lenses have a 10X magnification.
6. **Revolving Nosepiece and Objective Lenses** - Attached to the **revolving nosepiece** (which is attached to the bottom of the body tube) are several lenses called **objectives**. Most light microscopes have objective lenses of 4 magnifications: scanning small (small), low power lens (medium), high-power lens (large), and oil immersion lens (largest).
7. **Stage** - The surface on which you place your slide is called the **stage**. It contains a hole in the center called the **stage aperture**. Two **stage clips** or a **slide clamp** are used to hold the slide in place.
8. **Substage** - The area under the stage, called the **substage**, contains the diaphragm. In addition, some microscopes also have a condenser located under the stage.
 - a. **Condenser** - The **condenser** contains a series of lenses that focus light onto the specimen.
 - b. **Diaphragm** - The **diaphragm** is an adjustable light barrier built into the condenser that regulates the amount of light passing through the specimen. It is very important that the diaphragm be correctly adjusted in order to get the best possible image. Use the smallest opening that does not interfere with the field of view. The condenser and diaphragm assembly may be adjusted vertically with a knob projecting to one side. Proper adjustment often yields a greatly improved view of the specimen. This adjustment has been made for you, and you should consult your instructor if you think that it must be readjusted.
9. **Focus Knobs** - You can focus your microscope by using the **coarse and fine adjustment knobs** located on the sides of the arm. Note that the coarse adjustment knob is larger than the fine adjustment knob. The coarse adjustment is used for initial focusing and should be used **ONLY** when the scanning (4X) or low power (10X) objective is in alignment. The fine adjustment makes very slight changes, allowing precision focusing on low or high power.

What is the correct way to care for the brightfield microscope?

The microscope is a very delicate and expensive instrument. In order to get the best possible results, while minimizing the chances of damaging the microscopes, the following rules should **ALWAYS** be observed:

1. Carry the microscope in an upright position, using one hand to grasp the arm and the other to support the base.
2. Place the microscope down gently. Do not swing it around or bang it into any other objects.
3. Keep the microscope level on the table. Do NOT tilt it to look through the oculars.
4. Never place the microscope close to the edge of the lab table or counter.

5. Place the power cord out of the way so you cannot trip on it or drag the microscope off the table.
6. Clean the oculars and objectives with special **lens paper** before each use. Never touch the lens with anything except the special lens paper provided. You may use paper towels to clean and dry the microscope slides.
7. Keep all parts of the microscope dry. Immediately wipe off water or other damaging chemicals.
8. Because of the length of the high power and oil immersion objectives, it is possible to hit the slide with these objectives when they are in the viewing position. Therefore, you should always observe the objectives and slide from the side when rotating either of these objectives into viewing position. Always rotate the nosepiece slowly, and stop if it appears the objective will hit any portion of the slide or coverslip.
9. Always begin your observation of each slide with the low power or scanning objectives. This will make it **EASIER** to locate and focus the specimen and will reduce the chances of damaging the microscope.
10. **NEVER** move the coarse adjustment knob when the high power or oil immersion objectives are in viewing position.
11. When you are finished with your microscope, clean it off (use **ONLY** lens paper to clean the lenses), rotate the low power objective into viewing position, lower the stage, and return the microscope to the cabinet.

Lab Procedures

- ⇒ **CAUTION:** You will be using acids and bases during this lab. You must wear approved safety eyewear and gloves during the first 3 parts of this lab, avoid direct contact with all solutions, and notify your instructor immediately if any spills occur.

I. Prepare unbuffered and buffered solutions of 20 mM sucrose

- ⇒ **NOTE:** The following calculations should have been completed as part of your Prelab.

In lab, the following stock solutions will be available:

0.1 M sucrose
0.2 M acetic acid
0.2 M sodium acetate
0.2 M anhydrous sodium carbonate
0.2 M sodium bicarbonate

1. Calculate the amount of 0.1 M sucrose solution and the amount of dH₂O needed to prepare 100 mL of unbuffered 20 mM sucrose. Write your answers in the spaces below. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of sucrose stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of dH₂O needed _____

2. The **acetate buffer** consists of acetic acid (**CH₃COOH**) and acetate ions (**CH₃COO⁻**). A chemistry reference indicates that a solution containing 40 mM acetic acid and 60 mM sodium acetate (a source of acetate ions) will produce an acetate buffer with a pH of 4.8. Calculate the amount of each stock solution and the amount of dH₂O needed to prepare 100 mL of an acetate buffer that contains 20 mM sucrose, 40 mM acetic acid, and 60 mM sodium acetate. Write your answers in the spaces below. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of each stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of 0.2 M acetic acid solution needed _____

Amount of 0.2 M sodium acetate solution needed _____

Amount of dH₂O needed _____

3. The **bicarbonate buffer** consists of carbonate ions (**CO₃⁻²**) and bicarbonate ions (**HCO₃⁻**). A chemistry reference indicates that a solution containing 22 mM anhydrous sodium carbonate (a source of carbonate ions) and 28 mM sodium bicarbonate (a source of bicarbonate ions) will produce a bicarbonate buffer with a pH of 9.8. Calculate the amount of each stock solution and the amount of dH₂O needed to prepare 100 mL of a bicarbonate buffer that contains 20 mM sucrose, 22 mM anhydrous sodium carbonate, and 28 mM sodium bicarbonate. Write your answers in the spaces below. [Hint: use the formula $c_1v_1 = c_2v_2$ to calculate the amount of each stock solution needed.]

Amount of 0.1 M sucrose solution needed _____

Amount of 0.2 M anhydrous sodium carbonate solution needed _____

Amount of 0.2 M sodium bicarbonate solution needed _____

Amount of dH₂O needed _____

4. **Have your instructor check your calculations before you proceed.**

5. Using the information from your calculations, prepare **and clearly label** the following solutions:

100 mL of unbuffered 20 mM sucrose

100 mL of 20 mM sucrose in an acetate buffer with a pH of 4.8

100 mL of 20 mM sucrose in a bicarbonate buffer with a pH of 9.8

6. Save these 3 solutions for the next 2 sections of today's lab.

II. Titrate the unbuffered sucrose solution

1. You will be adding 0.4 M HCl to your unbuffered sucrose solution, one mL at a time, and then recording the pH of the solution after each addition. **Before you begin**, prepare a clearly labeled table in your lab notebook to record your results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of HCl has been added.
2. Pour half (50 mLs) of your unbuffered 20 mM sucrose solution into a beaker and save the rest for later use. Add a stir bar to the beaker, and place the beaker on a stir-plate next to a pH meter. Switch on the stir-plate and adjust the stirring rate so mixing is moderately fast, but the solution does not splash.
3. Consult your instructor and/or the handouts provided to learn the correct procedure for using your pH meter.
 - ⇒ **IMPORTANT:** Always return the pH electrode to the storage solution when you are finished using it. If you allow the electrode to dry out, this can damage the pH meter.
 - ⇒ **IMPORTANT:** To avoid contamination, always rinse the electrode with dH₂O when transferring it from one solution to another.
 - ⇒ **IMPORTANT:** Never turn the pH electrode upside down. Notice that it is filled with a solution, and this liquid can drain out of the air hole at the top of the electrode if it is turned upside down.
4. Remove the pH electrode from the storage solution and rinse it with some dH₂O from a squeeze bottle. Allow excess water to drip from the electrode into the rinse beaker provided. Calibrate the pH meter with TWO standard buffers following the instructions provided. (If no instructions are provided, follow the instructions in Appendix C at the end of this Manual.)
5. Slowly lower the electrode into the unbuffered 20 mM sucrose solution. Rest the electrode in the bottom of the beaker so it is stable without holding it, but be careful to position the electrode so that the spinning stir-bar will not hit it.
 - ⇒ **IMPORTANT:** The pH electrode has a delicate glass membrane at its tip; take care not to shatter it by knocking against something or allowing the stir bar to knock against it.
6. Once the reading in the display window has stabilized, record the pH of the sucrose solution in your data table (after 0 mL of HCl has been added.)
7. Using an automatic pipettor, add 1 mL of 0.4 M HCl to the sucrose solution. Wait for the HCl to thoroughly mix into the solution and for the reading on the pH meter to stabilize, and then record the pH of the solution in your data table (after 1 mL of HCl has been added.)
 - ⇒ **CAUTION:** 0.4 M HCl is a strong acid. Take special care to not splash it or make contact with your skin. Report any spills immediately to your instructor.
8. Using the automatic pipettor, add another 1 mL of HCl, wait for the reading in the display to stabilize, and then record the pH of the solution in your data table (after 2 mL of HCl has been added.)

- Continue to add HCl to the sucrose solution, 1 mL at a time, and record the pH after each addition, until the pH drops below 3.
- Switch the pH meter to standby, remove the electrode from the beaker, rinse it with dH₂O, and place it in the storage solution.
- Discard the unbuffered sucrose solution that you just titrated with HCl. Thoroughly rinse the beaker with dH₂O and then place the remaining 50 mLs of unbuffered sucrose solution in the beaker with a stir bar.
- For the second part of this titration, you will be adding 0.4 M NaOH to your unbuffered sucrose solution, one mL at a time, and then recording the pH of the solution after each addition. **Before you begin**, prepare a clearly labeled table in your lab notebook to record your results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of NaOH has been added.
- Titrate the remaining unbuffered sucrose solution with 0.4 M NaOH. Follow the same procedure you used to titrate your unbuffered sucrose solution with HCl. Record the pH after 0 mL of NaOH has been added, and after each 1 mL addition. Continue to add NaOH, 1 mL at a time, until the pH rises above 11.

⇒ **CAUTION:** 0.4 M NaOH is a strong base. Take special care to not splash it or make contact with your skin. Report any spills immediately to your instructor.
- When you are finished with the titration, switch the pH meter to standby, remove the electrode from the beaker, rinse it with dH₂O, and place it in the storage solution. Discard the unbuffered sucrose solution that you just titrated with NaOH, and thoroughly rinse the beaker with dH₂O.

III. Titrate the two buffered sucrose solutions

- Check the calibration of your pH meter using the pH 7 and pH 4 buffers.
- Next, you will be using HCl to titrate your solution of 20 mM sucrose in acetate buffer. **Before you begin**, prepare a data table in your lab notebook to record the results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of HCl has been added.
- Pour half (50 mLs) of your solution of 20 mM sucrose in acetate buffer into a beaker. Following the procedure that you used in the previous section, measure the pH of this solution and record it in your data table (after 0 mL of HCl has been added.)
- Titrate the solution of 20 mM sucrose in acetate buffer with 0.4 M HCl. Add 1mL at a time, and record the pH after each addition, until the pH drops below 3. When you are finished, discard your solution.
- Next, you will be using NaOH to titrate your solution of 20 mM sucrose in acetate buffer. **Before you begin**, prepare a data table in your lab notebook to record the results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of NaOH has been added.
- Pour the remaining 50 mLs of your solution of 20 mM sucrose in acetate buffer into a beaker. Following the procedure that you used in the previous section, measure the pH of this solution and record it in your data table (after 0 mL of NaOH has been added.)
- Titrate the solution of 20 mM sucrose in acetate buffer with 0.4M NaOH. Add 1mL at a time, and record the pH after each addition, until the pH rises above 11. When you are finished, discard your solution.
- Check the calibration of your pH meter using the pH 7 and pH 10 buffers.
- Next, you will be using HCl to titrate your solution of 20 mM sucrose in bicarbonate buffer. **Before you begin**, prepare a data table in your lab notebook to record the results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of HCl has been added.

10. Pour half (50 mLs) of your solution of 20 mM sucrose in bicarbonate buffer into a beaker. Measure the pH of this solution and record it in your data table (after 0 mL of HCl has been added.)
11. Titrate the solution of 20 mM sucrose in bicarbonate buffer with 0.4 M HCl. Add 1mL at a time, and record the pH after each addition, until the pH drops below 3. When you are finished, discard your solution.
12. Next, you will be using NaOH to titrate your solution of 20 mM sucrose in bicarbonate buffer. Before you begin, prepare a data table in your lab notebook to record the results. Give the table a descriptive title, and be sure to provide a space where you can record the pH of the solution after 0 mLs of NaOH has been added.
13. Pour the remaining 50 mLs of your solution of 20 mM sucrose in bicarbonate buffer into a beaker. Measure the pH of this solution and record it in your data table (after 0 mL of NaOH has been added.)
14. Titrate the solution of 20 mM sucrose in bicarbonate buffer with 0.4 M NaOH. Add 1mL at a time, and record the pH after each addition, until the pH rises above 11. When you are finished, discard your solution.

Clean up

When you are finished with your titrations, ask your instructor to check your results. After your instructor has checked your results, remove label tape and any marks made with a marking pen from all glassware. Wash and rinse all used glassware, give it a final rinse with dH₂O, and leave it inverted at your work area in order to drain.

IV. Examine the features of the microscope you will be using

Students should work alone or in groups of 2 for all exercises that involve use of a microscope. If necessary, remove a microscope from the cabinet and bring it back to your work station. **Always carry the microscope upright using two hands, and be careful not to bang it against anything.**

The microscopes available for student use are not all identical. Before you view any specimens, explore the features and characteristics of the microscope you will be using.

Plug in your microscope and turn on the illuminator.

Learn about the ocular lens(es) on your microscope:

Is your microscope a monocular, binocular, or dual-viewing microscope? _____

Oculars may be permanently attached to the body tube, or they can slide out and be replaced with oculars of different magnification (5X and 10X are most common).

What is the magnification of the ocular lens(es) on your microscope? _____

Oculars may have a **pointer** that can be seen when you look through the ocular. You can move a specimen around until the part you wish to refer to is at the tip of the pointer. (In binocular microscopes, usually only one ocular contains a pointer.)

Is there a pointer in the ocular lens? _____

An ocular may have a **reticle**, which resembles a ruler. The reticle may be part of the pointer. (In binocular microscopes, usually only one ocular contains a reticle.)

Is a reticle present? _____

Learn about the objective lenses on your microscope:

Each objective lens has several numbers engraved on its side. Usually, the first number indicates the **magnification** of the objective while the second number indicates its **numerical aperture (NA)**. In the following Table, indicate which objectives are found on your microscope.

Type of Objective Lens	Magnification	Does this microscope have this type of objective?
Scanning power	4X	
Low power	10X	
High power	40X or 43X	
Oil immersion	93X or 100X	

What happens to the length of the objectives as the magnification increases? _____

When you are viewing a fairly large specimen (easily visible with the naked eye) you can begin with the scanning objective; otherwise, you should begin viewing each new slide with the low power objective.

⇒ **NEVER BEGIN VIEWING A SLIDE WITH THE HIGH POWER OR OIL IMMERSION OBJECTIVES!**

Slowly rotate the nosepiece until you feel the low power objective click into place. The **total magnification** of your microscope is calculated by multiplying the magnification of the ocular by the magnification of the objective.

Ocular lens magnification X Objective lens magnification = Total Magnification

Calculate the total magnification for each ocular/objective combination on your microscope:

Magnification of ocular lens	Magnification of objective lens	Total magnification when using this objective

Learn about the microscope stage on your microscope:

Place a clean microscope slide on the stage and fasten it securely between the stage clips or clamps. If your microscope has a mechanical stage, movement of the slide is controlled by 2 knobs located on the side or bottom of the stage. Try rotating each **stage manipulator knob** and note the direction the slide moves. Using the stage manipulator knobs, move the slide until the center is directly above the stage aperture.

If your microscope does not have a mechanical stage, you will have to move the slide by hand until the center is directly above the stage aperture.

Does your microscope have a mechanical stage? _____

Examine the power switch on your microscope:

Is the switch to turn on the illuminator a rheostat--that is, can you use it increase and decrease the brightness of the light--or is it a simple on/off switch?

Explore the features found in the substage area of your microscope:

Directly beneath the stage you will find the diaphragm, which is used to adjust the amount of light passing from the illuminator through the slide on the stage. Find the diaphragm on your microscope.

An **iris diaphragm** consists of a circle of overlapping metal plates. The size of the opening is regulated by a lever that projects from the side of the iris diaphragm. If your microscope has an iris diaphragm, carefully move the lever back and forth and note how the amount of light passing through the stage aperture changes. Leave the diaphragm set halfway between the largest and smallest setting.

An **annular diaphragm** consists of a round plate with holes of different diameters. You can regulate the amount of light passing through your specimen by rotating the plate to position the various holes in the light path. As you rotate the plate you should feel it "click" into place when one of the holes is directly below the stage aperture. You should also note a number (from 1 to 5) on the plate where it projects from beneath the stage.

What type of diaphragm does your microscope have? _____

If your microscope has an annular diaphragm, slowly rotate the plate as you feel the holes "click" into position. As the exposed number on the plate increases, what happens to the amount of light passing through the stage aperture?

The **condenser** contains a series of lenses that focus light onto the specimen. It is moved up or down by a knob that projects from its side.

Does your microscope have a condenser? _____

The condenser should be accurately adjusted for optimum resolution, contrast, and clarity of the image. However, for our purposes, you can get satisfactory results by always making sure the **condenser focus knob** is rotated to raise the condenser to its highest position. If your microscope has a condenser, check it to make sure it is raised to its highest position.

Observe the action of the coarse focus and fine focus knobs

With the low power objective in place, view the microscope from the side (do NOT look through the ocular) and slowly rotate the coarse adjustment knob, being careful not to hit the slide with the objective.

Depending on the type of microscope you are using, the focus knobs will raise and lower either the stage or the body tube. Which part of the microscope moves when you rotate the coarse adjustment?

Now rotate the fine adjustment knob. How does the movement when rotating the fine focus knob compare to the movement you observed when using the coarse focus knob?

With the dual-viewing microscopes, the person using the SIDE ocular should focus the image using the coarse and fine adjustment knobs. Once these adjustments are made, the person using the TOP ocular can make additional fine adjustments for his/her own eyesight by rotating the tube that supports the top ocular.

V. Prepare and View a Wet Mount

Euglena are unicellular, photosynthetic, algae that live in freshwater ponds and lakes. They have a long flagellum for swimming, and a contractile vacuole that pumps excess water out of the cell. *Euglena* lack a cell wall, but have green chloroplasts and a reddish **eye spot**. The eyespot appears as a swelling near the base of the flagellum, and is used to detect light, which is needed for photosynthesis. In this exercise, you will prepare a wet mount of *Euglena* and observe it with the brightfield microscope.

1. Make sure the low power (10X) objective is in viewing position. While viewing the microscope from the side, use the coarse adjustment to move the objectives as far from the stage as possible and then clean the oculars and objectives with lens paper. When you are finished, make sure the low power objective (10X) is in viewing position.
2. Obtain a blank microscope slide from the box provided. Even if your slide looks CLEAN, it is a good idea to polish it with a paper towel to remove any dust or fingerprints.
3. Use the dropper labeled "Euglena" to place a single drop of the *Euglena* culture in the center of your slide. Try to take your sample from an area of the culture that looks bright green. Place a coverslip over the drop on the slide as follows: First lower one edge of the coverslip onto the slide a few millimeters away from the drop and drag that edge toward the drop until it just touches the drop. Then gently lower the remainder of the coverslip onto the drop of liquid. Do not press down on the coverslip after it is set in place.

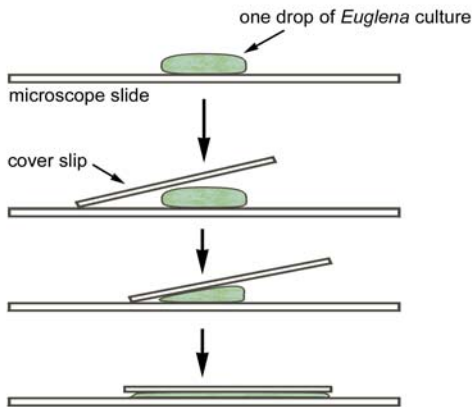


Figure 3.2 Correct Procedure for Preparing a Wet Mount

4. There should be a uniform layer of liquid under the coverslip with no air bubbles present. If any liquid has been forced out between the slide and coverslip, or if the layer of liquid is so thick that the coverslip appears to vibrate above the liquid, blot off the excess liquid with the torn edge of a paper towel. But do not remove so much liquid from under the coverslip that the coverslip adheres tightly to the glass slide.
5. Rotate the stage manipulator knobs to move the slide until the cover slip is directly below the viewing objective. While viewing the microscope from the **SIDE**, use the coarse adjustment knob to move the objective as close to the slide as possible without hitting it. **Whenever** you move the objective towards the slide, you should view it from the **SIDE** to make sure the objective does not hit the slide.
6. Now look through the ocular and use the coarse adjustment knob to **SLOWLY** move the objective **AWAY** from the slide until the slide comes into focus. It helps to slowly move the slide back and forth with the stage manipulator knobs before and while you rotate the coarse adjustment knob. When you see something that moves as you move the slide, then you know you are focusing on an object on the slide and not on dust or smudges on one of the external glass surfaces.
7. *Euglena* are small green elongated cells. You should see some using their flagella to swim around in the drop of water. Use the fine adjustment knob for more precision focusing.

8. Use the diaphragm to adjust the light intensity.
9. Look at the slide from the side and move the slide slightly to the left. Now look through the ocular and move the slide in the same way that you just did. What direction does the **IMAGE** of the slide move as you move the slide to the left?

to the right? _____

towards you? _____

away from you? _____

10. Examine your slide and try to find some stationary *Euglena*. Once you have located some stationary *Euglena* you may switch to the high power (40X or 43X) objective, following these instructions:

Never attempt to use high power until you have located and focused the object on low power first.

Do NOT attempt to use the oil immersion (93X or 100X) objective without your instructor's help.

Before you switch to high power, make sure your specimen is in the very CENTER of the field of view because this is the only area you will see when you switch to the high power objective.

Viewing the microscope from the side, CAREFULLY rotate the high power (40X or 43X) objective into place. **MAKE SURE IT WILL NOT HIT THE SLIDE.** If the objective is too long to avoid hitting the slide, you will not be able to view this slide on high power.

Your microscope is **parfocal** meaning that an image in focus when using one objective lens is very nearly in focus with the next higher power objective lens. Because of this, when changing to a higher power objective lens, you should be able to refocus the image using the **FINE FOCUS** only!

Using the coarse focus with high power or oil immersion objectives is the number one cause of slide breakage. Don't let this happen to you!

If you "lose" your specimen when switching from the low to high power objectives, most likely your specimen was not in the exact center of your field of view before you switched, or you did not focus clearly enough at low power first.

Never use the coarse focus adjustment when the high power objective is in viewing position. To avoid damaging the microscope or your slide, you must switch back to lower power, relocate your specimen, center and focus it precisely, and then switch back to high power.

If you still have trouble, ask your instructor to help. The problem may be a lack of experience on your part, or there may actually be a defect in your microscope.

Readjust the light using the diaphragm. Does the specimen require more or less light on high power? _____

11. Like the human eye, the lenses of a microscope have limited **depth of field**. For any given lens setting, there is only one distance from the lens where the object is exactly in focus; objects that are closer or farther away from the lens will not be in focus. However, because focus falls off gradually in front of and behind that distance, there is a region where the subject is in reasonable focus. The size of this region is called the depth of field.

While viewing *Euglena* on high power, slowly move the fine focus knob back and forth. Notice how different layers of the cell come in and out of focus. Is depth of field greater on high or low power? _____

12. When you have finished observing your wet mount, discard the coverslip in the receptacle provided, wash off the slide in the sink, dry it with a paper towel, and return it to the slide box.

VI. View a Prepared Slide

In this exercise you will look at a slide of *Euglena* that has been commercially prepared. Note that specimens on commercially prepared slides have been killed, preserved, mounted, and stained - THEY ARE NO LONGER ALIVE. These slides are long lasting, but the specimens may look quite different from the living organism.

1. With the low power objective in place, secure a commercially prepared slide of *Euglena* on the stage using the stage clips.
2. Following the same procedure described for viewing a wet mount, examine *Euglena* using low power first and then high power. Note: The *Euglena* should be brightly stained (probably green); if the object you are looking at is black or gray, it is probably a piece of dust or debris.
3. How does the commercially prepared slide of *Euglena* compare with the wet mount of *Euglena*? Describe the similarities and differences in your lab notebook.
4. While viewing *Euglena* with the high power objective, draw a diagram of this organism in your lab notebook.
5. Remove the slide from your microscope and return it to its original location. Turn off your microscope, unplug it, wrap the power cord around the base, rotate the scanning objective into viewing position, and return the microscope to the microscope cabinet.

Clean up

Clean your glassware and place all equipment and solutions back where you found them. Leave your work area in the same order that you found it in.

All disposable glassware goes into the special glass disposal receptacle.

Wipe off your work space with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.

Postlab

A. pH and Buffers

1. Following the procedure described in the Prelab, plot a titration curve for each of the following solutions:

20 mM sucrose in dH₂O

20 mM sucrose in an acetate buffer with a pH of 4.8

20 mM sucrose in a bicarbonate buffer with a pH of 9.8

Plot each curve on a separate sheet of paper, and use the entire page for each graph. Use the Checklist on the bottom of p.3 and the top of p.4 of Appendix E to make sure you have included all necessary information on your graph.

2. How does the titration curve of the unbuffered sucrose solution compare with the titration curves of the 2 buffered solutions?
3. What is the **buffering range** of the acetate buffer? **Explain your answer.**
4. What are the **buffering ranges** of the bicarbonate buffer? **Explain your answer.**
5. Which solution, if any, seems to have the greater **buffering capacity**? **Explain your answer.**
6. Would either the acetate buffer or the bicarbonate buffer be a good choice for studying enzymes or other biomolecules found in human blood? **Explain your answer.**
7. Explain how the pH scale is used to describe the H^+ concentration of a solution.
8. Describe what a buffer is and explain why buffers are important to living organisms.
9. You wish to study a protein found in cow's milk called *α -lactalbumin*. You measure the pH of cow's milk and find that it is 6.0. You then check a biochemistry reference manual to identify a suitable buffer for use during purification and study of this enzyme. Your research indicates that phosphate buffer has a buffering range of pH 5.7 to pH 8.0. Further, to prepare a phosphate buffer with a pH of 6.0, you need a solution that contains 87.7 mM monobasic sodium phosphate (FW = 137.99) and 12.3 mM dibasic sodium phosphate (FW = 141.96). You also want your buffer to contain 0.02% thimerosal, a biocide that will prevent the growth of bacteria, mold, and other organisms in the buffer. Write a short paragraph describing **exactly** how you would prepare 900 mL of this buffer. Describe your actions—exactly what you would **do**—step-by-step—when preparing this buffer. Also show all calculations. Be sure all amounts include units of measurement.

B. Introduction to the Brightfield Microscope

1. Answer each of the following questions regarding the brightfield microscope:
 - a. What is the diaphragm used for?
 - b. What is the condenser used for?
 - c. What are the magnifications of your objective lenses, and what is the total magnification of the microscope when using each lens?
 - d. What does parfocal mean?
 - e. Why is it important to focus your specimen on low power first, before switching to high power?
2. What is meant by **field of view**? Explain what happens to the field of view as you increase the magnification of the objective lens.
3. What is meant by **depth of field**? Explain what happens to the depth of field as you increase the magnification of the objective lens.
4. Explain why *Euglena* seem to move so much faster when viewed at high magnification than when viewed at low power.
5. Living *Euglena* are quite visible by brightfield microscopy without staining or other special preparation. Give a possible reason for their visibility.
6. Based on your observations, list at least one advantage that a permanent dry mount has over a wet mount for examining *Euglena* by brightfield microscopy.
7. Based on your observations, list at least one advantage that a wet mount has over a permanent dry mount for examining *Euglena* by brightfield microscopy.