EXERCISE 3 - 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 dH_2O 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 dH_2O needed _____

2. The **acetate buffer** consists of acetic acid (**CH_3COOH**) and acetate ions (**CH_3COO^-**)**.** 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 dH_2O 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 dH_2O needed _____

3. The **bicarbonate buffer** consists of carbonate ions (**CO_3^{-2}**) and bicarbonate ions (**HCO_3^-**)**.** 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 dH_2O 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 dH_2O 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 dH_2O 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 dH_2O 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.)

9. 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.

10. Switch the pH meter to standby, remove the electrode from the beaker, rinse it with dH_2O, and place it in the storage solution.

11. Discard the unbuffered sucrose solution that you just titrated with HCl. Thoroughly rinse the beaker with dH_2O and then place the remaining 50 mLs of unbuffered sucrose solution in the beaker with a stir bar.

12. 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.

13. 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.

14. When you are finished with the titration, switch the pH meter to standby, remove the electrode from the beaker, rinse it with dH_2O, and place it in the storage solution. Discard the unbuffered sucrose solution that you just titrated with NaOH, and thoroughly rinse the beaker with dH_2O.

III. Titrate the two buffered sucrose solutions

1. Check the calibration of your pH meter using the pH 7 and pH 4 buffers.

2. 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.

3. 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.)

4. 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.

5. 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.

6. 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.)

7. 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.

8. Check the calibration of your pH meter using the pH 7 and pH 10 buffers.

9. 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 dH_2O, 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.

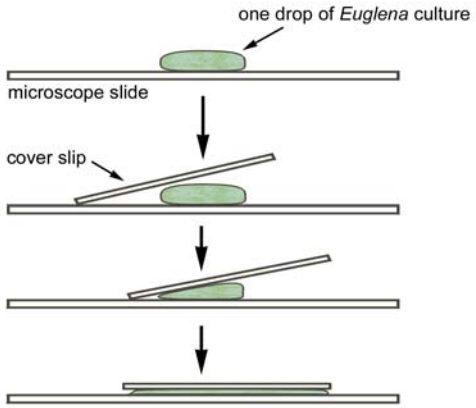

Figure 3.2 Correct Procedure for Preparing a Wet Mount

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.