

Experiment GB-2: Membrane Permeability

Background

Cells are the basic units of life. Cells are aligned with other cells to form tissues, tissues align to form organs, and organs align to form organisms. But, the life of an organism is always dependent on the biochemical reactions taking place in the one, or hundreds, or millions of cells that constitute an organism.

The biochemical reactions are able to take place in a cell because the enzymes, substrates, and products of these reactions are contained within the cell by a structure known as the cell membrane. Some reactions, like the ones that produce cellular energy, take place in organelles that are separated from the cytoplasm by membranes.

The cell membrane is more than just a barrier that separates the contents of the cell from the surrounding environment. The cell membrane regulates the movement of molecules, that could be nutrients or waste products, into or out of a cell. Since some, but not all, particles are allowed to move across cell membranes, membranes are considered to be **selectively permeable**.

The processes by which molecules move across cell membranes, and the rates at which they do it, are dependent on the type of molecule and its size, electrical charge, and concentration on either side of the membrane. Processes that consume energy are classified as **active transport**, and those that do not require energy are considered to be forms of **passive transport**.

In this experiment, students will examine some of the properties of passive transport across a model of a membrane created with dialysis tubing. Each sac created with dialysis tubing will be filled with one of three different solutions, each containing a different set of ions. When each dialysis sac is placed in a beaker of deionized water, ions will move across the membrane. The movement of ions between the dialysis sac and the surrounding water will be indicated by a change in the pH of the water surrounding the dialysis sac.

Equipment Required

PC Computer
IWX/214 data acquisition unit
USB cable
IWX/214 power supply
ISE-100 combination pH electrode
Magnetic stirrer
Stir bar
Ringstand
Utility clamps (2)
100 ml beakers (2)
250 ml beakers (6)
1000 ml beaker
pH 4 and pH 7 buffer solutions
1.0M HCl solution
1.0M NaOH solution
1.0M Na Acetate solution
2 cm wide dialysis tubing
String
Binder clips (3)
Deionized water

IWX/214 Setup

- 1 Place the IWX/214 on the bench, close to the computer.
- 2 Check Figure 1-1 in Chapter 1 for the location of the USB port and the power socket on the IWX/214.
- 3 Use the USB cable to connect the computer to the USB port on the rear panel of the IWX/214.
- 4 Plug the power supply for the IWX/214 into the electrical outlet. Insert the plug on the end of the power supply cable into the labeled socket on the rear of the IWX/214. Use the power switch to turn on the unit. Confirm that the red power light is on.

Start the Software

- 1 Click the Windows **Start menu**, move the cursor to **Programs** and then to the **iWorx** folder and select **LabScribe**; or click on the LabScribe icon on the Desktop
- 2 When the program opens, select **Load Group** from the **Settings menu**.
- 3 From the dialog box, select **AddedLabs.iws**. Click **Load**.
- 4 Click on the **Settings menu** again and select the **MembranePermeability** settings file.
- 5 After a short time, LabScribe will appear on the computer screen as configured by the **MembranePermeability** settings. Open the **View menu** and select **Voltmeter** to display the current pH level to appear on the **Main window**.

pH Electrode Setup

Plug the DIN connector on the end of the cable of the ISE-100 pH electrode into Channel 4 on the iWorx unit (Figure GB-2-1 on page 2).

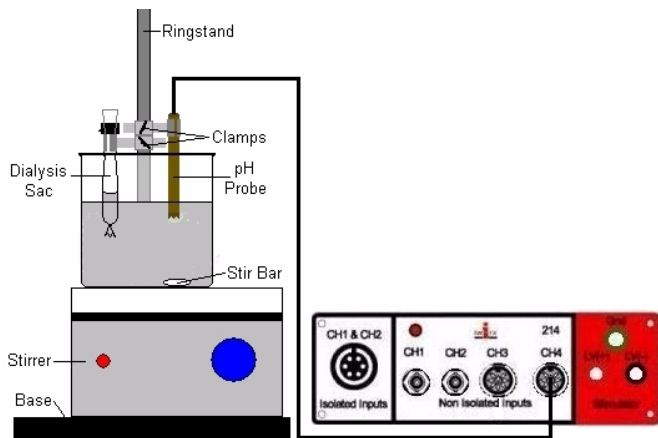


Figure GB-2-1: The arrangement of the stirrer, electrode, and beaker for measuring the changes in the pH in model of a membrane.

Calibration of the pH Electrode

- 1 If the pH electrode is still stored in its bottle of buffer, remove the electrode from the bottle. Rinse the electrode with deionized water while holding the electrode over a 1000 ml beaker used for the collection of waste liquids.
- 2 Place the tip of the pH electrode in a 250 ml beaker containing enough room temperature deionized water to submerge the tip. Keep the electrode in deionized water for at least ten minutes.
- 3 Prepare two 100 ml beakers filled with the pH buffers used for calibrating the pH electrode. The buffers should be a room temperature. One beaker is filled with pH 7 buffer; and the other is filled with pH 4 buffer. Each beaker should be filled with enough buffer to cover the tip of the pH electrode, and also allow the stir bar in the beaker to spin without touching the pH electrode.
- 4 Place the beaker containing the pH 7 buffer on the magnetic stirrer (Figure GB-2-1 on page 2). Carefully place a stir bar in the beaker. Remove the pH electrode from the deionized water and blot any drops of water from the electrode. Position the tip of the electrode in the beaker of pH 7 buffer so that the tip is away from the stir bar. Adjust the speed of the stirrer so the stir bar is rotating evenly at a moderate speed.
- 5 Click **Start** on the **LabScribe Main window** to begin recording. The trace will eventually reach a stable baseline toward the top of the recording channel. Type the words **Calibration - pH 7** on the comment line to the right of the **Mark button**. Press the **Enter key** on the keyboard to mark the stable baseline of the recording. This comment marks the output of the pH electrode in pH 7 buffer at room temperature. Continue recording while changing the beakers of buffers.
- 6 Turn off the stirrer and remove the pH electrode from the

beaker of pH 7 buffer. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode.

- 7 Remove the beaker of pH 7 buffer from the stirrer and place the beaker of pH 4 buffer on the stirrer. Carefully place a stir bar in the beaker. Position the tip of the pH electrode in the beaker of pH 7 buffer so that the tip of the electrode is away from the stir bar. Adjust the speed of the stirrer so the stir bar is rotating evenly at a moderate speed.
- 8 As you continue to record, the trace will reach a stable baseline toward the bottom of the recording channel. Type the words **Calibration - pH 4** on the comment line to the right of the **Mark button**. Press the **Enter key** on the keyboard to mark the stable baseline of the recording. This comment marks the output of the pH electrode in room temperature pH 4 buffer. Click **Stop** to halt the recording.
- 9 Select **Save As** in the **File menu**, type a name for the file. Choose a destination on the computer in which to save the file (e.g., a class folder). Click the **Save button** to save the file (as an *.iwd file).
- 10 Turn off the stirrer. Remove the pH electrode from the beaker of pH 4 buffer. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode and place it in a beaker of deionized water.

Units Conversion

- 1 Locate the section of the recording where output of the pH electrode was measured in pH 4 and pH 7 buffers. To view this section of the recording in its entirety on the same window, it may be necessary to click either of the **Display Time icons** in the **LabScribe toolbar** (Figure GB-2-2 on page 2).

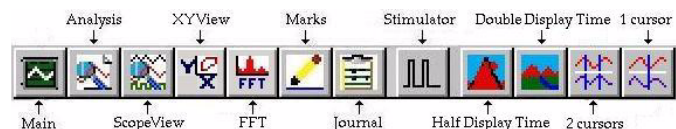


Figure GB-2-2: The **LabScribe toolbar**.

- 2 Click the **2-Cursor icon** (Figure GB-2-2 on page 2) so that two blue vertical lines appear over the recording window. Place one cursor on the plateau recorded while the pH probe was in pH 7 buffer. Place the other cursor on the plateau recorded while the pH probe was in pH 4 buffer.
- 3 **Right-click** on the Channel 4 window to open the **right-click menu**. Select **Units** from the **right-click menu**. Note that the voltage values for the positions of **Cursors 1** and **2** are already entered in the **units conversion window**.
 - Next to the voltage value for **Cursor1**, enter **7**.
 - Next to the voltage value for **Cursor2**, enter **4**.
 - Next to the **unit name**, enter **pH**.
 - Click **OK**. The units on the Y-axis are equal to **pH units**.

Preparation of the Dialysis Tubes

- 1 Obtain three pieces of two centimeter-wide dialysis tubing that are fifteen centimeters long. Soak the tubing in deionized water to soften them.
- 2 Tie one end of each dialysis tubing with string to form a sac.
- 3 Fill one of the sacs about two-thirds full with 1.0M HCl. Fold over the top of sac and place a binder clip over it.
- 4 Rinse the outside of the dialysis sac containing the 1.0M HCl with tap water to remove any acid that may have gotten on the outside.
- 5 Stand the completed dialysis sac with 1.0M HCl in a clean, dry 250 ml beaker to keep the sac clean
- 6 Repeat Steps 3, 4, and 5 to make a sac filled with 1.0M NaOH and a sac filled with 1.0M Na Acetate. Put each dialysis sac in its own clean, dry 250 ml beaker.

Exercise 1: Movement of Small Positive Ions Across a Membrane

Aim: To determine if small, positively charged, hydrogen ions can move across a membrane from a region of higher concentration (1.0M HCl) to a region of lower concentration (deionized water).

Procedure

- 1 Place a magnetic stirrer on or next to the base of a ringstand. Place 100 mL of deionized water, at room temperature, in a clean 250 ml beaker. Add a stir bar to the beaker and place the beaker on the magnetic stirrer. Turn on the stirrer and position the stir bar to one side of the beaker bottom.
- 2 Remove the pH electrode from the beaker of deionized water. Blot the drops of deionized water from each device. Mount the electrode in a clamp on the ringstand. Position the pH electrode over the beaker of deionized water. Then, lower the tip of the electrode into the water.
- 3 Turn on the stirrer so that the stir bar rotates evenly and moderately.
- 4 Click **Start** on the **LabScribe Main window** to begin recording. When the recording on the channel reaches a stable baseline, type the words **Deionized Water** on the comment line to the right of the **Mark button**. Press the **Enter key** on the keyboard to mark the recording.
- 5 After recording at least fifteen seconds of stable baseline, type the words **Add Dialysis Tube w/ 1.0M HCl** on the comment line.
- 6 Suspend the dialysis tube containing 1.0M HCl over the beaker of deionized water. As you lower the dialysis tube into the deionized water, press the **Enter key** on the keyboard to mark the recording. Tighten the clamp holding the dialysis tube so that about half the solution in the tube is below the surface of the deionized water. Make sure the bottom of the dialysis tube is not touching the stir bar.
- 7 Record the pH of the deionized water for five minutes. At the end of five minutes, click **Stop** to halt the recording.

- 8 Select **Save** in the **File menu**.
- 9 Turn off the magnetic stirrer. Remove the dialysis tube with the 1.0M HCl from the beaker and discard the 1.0M HCl as directed.
- 10 Remove the pH electrode from the beaker. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode and place it in a beaker of deionized water.
- 11 Remove the stir bar from the beaker of deionized water and rinse it with deionized water from a wash bottle. Discard the deionized water.

Data Analysis

- 1 Scroll to the section of data recorded during Exercise 1. Use the **Display Time icons** on the **LabScribe toolbar** (Figure GB-2-2 on page 2) to complete recording from Exercise 1 on the **Main window**.
- 2 Click on the **2-Cursor icon** in the **LabScribe toolbar** (Figure GB-2-2 on page 2), so that two blue cursors appear over the **Main window**.
- 3 Place a cursor on the stable baseline recorded just before the dialysis tubing containing 1.0M HCl was lowered into the beaker of deionized water. Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the water
- 4 Click the **Analysis icon** on the **LabScribe toolbar** (Figure GB-2-2 on page 2) to transfer the data between the cursors to the **Analysis window**.
- 5 In the table across the top of the data display on the **Analysis window**, the parameters, **Title**, **Value1**, **Value2**, **V2-V1**, and **T2-T1** should appear. Any functions that does not appear in the table can be selected from the list of functions on the left side of the **Analysis window** by holding down the **Control key** on the keyboard as the name of the function is clicked (**Control-Click**).
- 6 Place a cursor at the point in the recording when the dialysis tubing with 1.0M HCl was lowered into the deionized water (**Time = 0**). Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the water (**Time = 5**). Select **pH** on the **Value from Ch menu** in the upper left corner of the **Analysis window**.
- 7 Measure the values for the following parameters from the **pH channel** for the region of data selected:
 - **pH at Time = 0**, which is **Value1** on the **pH channel**.
 - **pH at Time = 5**, which is **Value2** on the **pH channel**.
 - **Change (Δ) in pH after 5 Minutes**, which is **V2-V1** on the **pH channel**.
- 8 Record the names and values of the parameters, **Title**, **Value1**, **Value2**, **V2-V1**, and **T2-T1** for the data of interest in the on-line notebook of **LabScribe** that is known as the **Journal**. The information can be entered by typing the names and values directly into the **Journal**. The measure-

ments can also be entered into the **Journal** using the functions in the **right-click menu** of the **Analysis window**:

- Place the cursors in the appropriate locations for making measurements.
 - Select **Add Title to Journal**, from the **right-click menu**, to add the names of the parameters measured to a table in the **Journal**.
 - Select **Add Data to Journal**, from the **right-click menu**, to add the values of the parameters to the **Journal**.
- 9 Record the values for these parameters in Table GB-2-1 on page 5.
 - 10 Click **Save** in the **File menu**.

Exercise 2: Movement of Small Negative Ions Across a Membrane

Aim: To determine if small, negatively charged, hydroxide ions can move across a membrane from a region of higher concentration (1.0M NaOH) to a region of lower concentration (deionized water).

Procedure

- 1 Place 100 mL of deionized water, at room temperature, in a clean 250 ml beaker. Add a stir bar to the beaker and place the beaker on the magnetic stirrer. Turn on the stirrer and position the stir bar to one side of the beaker bottom.
- 2 Remove the pH electrode from the beaker of deionized water. Blot the drops of deionized water from each device. Mount the electrode in a clamp on the ringstand. Position the pH electrode over the beaker of deionized water. Then, lower the tip of the electrode into the water.
- 3 Turn on the stirrer so that the stir bar rotates evenly and moderately.
- 4 Click **Start** on the **LabScribe Main window** to begin recording. When the recording on the channel reaches a stable baseline, type the words **Deionized Water** on the comment line to the right of the **Mark button**. Press the **Enter key** on the keyboard to mark the recording.
- 5 After recording at least fifteen seconds of stable baseline, type the words **Add Dialysis Tube w/ 1.0M NaOH** on the comment line.
- 6 Suspend the dialysis tube containing 1.0M NaOH over the beaker of deionized water. As you lower the dialysis tube into the deionized water, press the **Enter key** on the keyboard to mark the recording. Tighten the clamp holding the dialysis tube so that about half the solution in the tube is below the surface of the deionized water. Make sure the bottom of the dialysis tube is not touching the stir bar.
- 7 Record the pH of the deionized water for five minutes. At the end of five minutes, click **Stop** to halt the recording.
- 8 Select **Save** in the **File menu**.
- 9 Turn off the magnetic stirrer. Remove the dialysis tube with

the 1.0M NaOH from the beaker and discard the 1.0M NaOH as directed.

- 10 Remove the pH electrode from the beaker. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode and place it in a beaker of deionized water.
- 11 Remove the stir bar from the beaker of deionized water and rinse it with deionized water from a wash bottle. Discard the deionized water.

Data Analysis

- 1 Scroll to the section of data recorded during Exercise 2. Display the complete recording from Exercise 2 on the **Main window**.
- 2 Place a cursor on the stable baseline recorded just before the dialysis tubing containing 1.0M NaOH was lowered into the beaker of deionized water. Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was lowered into the deionized water.
- 3 Transfer the data between the cursors to the **Analysis window**.
- 4 Make sure the same parameters used in Exercise 1 appear in the table across the top of the data display on the **Analysis window**.
- 5 Place a cursor at the point in the recording when the dialysis tubing containing 1.0M NaOH was lowered into the deionized water (**Time = 0**). Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the deionized water. (**Time = 5**). Select **pH** on the **Value from Ch menu** in the upper left corner of the **Analysis window**.
- 6 Place a cursor at the point in the recording when the dialysis tubing with 1.0M NaOH was placed in the beaker of deionized water (**Time = 0**). Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the water (**Time = 5**). Select **pH** on the **Value from Ch menu** in the upper left corner of the **Analysis window**.
- 7 Measure the values for the following parameters from the **pH channel** for the region of data selected:
 - **pH at Time = 0**, which is **Value1** on the **pH channel**.
 - **pH at Time = 5**, which is **Value2** on the **pH channel**.
 - **Change (Δ) in pH after 5 Minutes**, which is **V2-V1** on the **pH channel**.
- 8 Use the one of the techniques described in Step 8 of Exercise 1 to record the values of the parameters in the **Journal**.
- 9 Record the values for these parameters in Table GB-2-1 on page 5.
- 10 Click **Save** in the **File menu**.

Exercise 3: Movement of Large Ions Across a Membrane

Aim: To determine if large, negatively charged, acetate ions can move across a membrane from a region of higher concentration (1.0M Na Acetate) to a region of lower concentration.

Procedure

- Place 100 mL of deionized water, at room temperature, in a clean 250 ml beaker. Add a stir bar to the beaker and place the beaker on the magnetic stirrer. Turn on the stirrer and position the stir bar to one side of the beaker bottom.
- Remove the pH electrode from the beaker of deionized water. Blot the drops of deionized water from each device. Mount the electrode in a clamp on the ringstand. Position the pH electrode over the beaker of deionized water. Then, lower the tip of the electrode into the water.
- Turn on the stirrer so that the stir bar rotates evenly and moderately.
- Click **Start** on the LabScribe **Main window** to begin recording. When the recording on the channel reaches a stable baseline, type the words **Deionized Water** on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to mark the recording.
- After recording at least fifteen seconds of stable baseline, type the words **Add Dialysis Tube w/ 1.0M Na Acetate** on the comment line.
- Suspend the dialysis tube containing 1.0M Na Acetate over the beaker of deionized water. As you lower the dialysis tube into the deionized water, press the **Enter** key on the keyboard to mark the recording. Tighten the clamp holding the dialysis tube so that about half the solution in the tube is below the surface of the deionized water. Make sure the bottom of the dialysis tube is not touching the stir bar.
- Record the pH of the deionized water for five minutes. At the end of five minutes, click **Stop** to halt the recording.
- Select **Save** in the **File** menu.
- Turn off the magnetic stirrer. Remove the dialysis tube with the 1.0M Na Acetate from the beaker and discard the 1.0M Na Acetate as directed.
- Remove the pH electrode from the beaker. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode and place it in a beaker of deionized water.
- Remove the stir bar from the beaker of deionized water and rinse it with deionized water from a wash bottle. Discard the deionized water.

Data Analysis

- Scroll to the section of data recorded during Exercise 3. Display the complete recording from Exercise 3 on the **Main window**.

- Place a cursor on the stable baseline recorded just before the dialysis tubing containing 1.0M Na Acetate was lowered into the beaker of deionized water. Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was lowered into the deionized water.
- Transfer the data between the cursors to the **Analysis window**.
- Make sure the same parameters used in Exercises 1 and 2 appear in the table across the top of the data display on the **Analysis window**.
- Place a cursor at the point in the recording when the dialysis tubing containing 1.0M Na Acetate was lowered into the deionized water (**Time = 0**). Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the deionized water. (**Time = 5**). Select **pH** on the **Value from Ch** menu in the upper left corner of the **Analysis window**.
- Place a cursor at the point in the recording when the dialysis tubing with 1.0M Na Acetate was placed in the beaker of deionized water (**Time = 0**). Place the second cursor at the point in the recording that is five minutes after the dialysis tubing was placed in the water (**Time = 5**). Select **pH** on the **Value from Ch** menu in the upper left corner of the **Analysis window**.
- Measure the values for the following parameters from the **pH channel** for the region of data selected:
 - pH at Time = 0**, which is **Value1** on the **pH channel**.
 - pH at Time = 5**, which is **Value2** on the **pH channel**.
 - Change (Δ) in pH after 5 Minutes**, which is **V2-V1** on the **pH channel**.
- Use the one of the techniques described in Step 8 of Exercise 1 to record the values of the parameters in the **Journal**.
- Record the values for these parameters in Table GB-2-1 on page 5.
- Click Save in the File menu.

Table GB-2-1: Changes in pH during Movement of Ions.

Solution in Dialysis Tubing	pH Level		
	T=0 Mins	T=5 Mins	Change (Δ)
1.0M HCl			
1.0M NaOH			
1.0M Na Acetate			

Questions

- During the five minutes that the dialysis sac containing 1.0M HCl was placed in the beaker of deionized water, what happened to the pH of the water? What caused the result that you recorded?

- 2 During the five minutes that the dialysis sac containing 1.0M NaOH was placed in the beaker of deionized water, what happened to the pH of the water? What caused the result that you recorded?
- 3 During the five minutes that the dialysis sac containing 1.0M Na Acetate was placed in the beaker of deionized water, what happened to the pH of the water? What caused the result that you recorded?
- 4 If any of the ions diffused across the membrane, which one diffused more quickly?
- 5 What would increase the rate of diffusion of an ion across a membrane?