Experiment GB-1: Biological Buffers

Background

To survive in changing environments, organisms must be able to maintain steady internal environments in which cellular metabolism can take place.

One of the factors that must be maintained at a steady level in the internal environment is the pH of the fluids in which biochemical reactions take place. If the pH level is not within a proper range, the chemical reactions necessary for life will cease and the organism will die.

One of the ways used to maintain the pH of the internal environment is the use of naturally occurring buffers, which are formed from mixtures of chemicals in and around cells. These buffers maintain pH levels within a narrow range, despite large changes in the amount of acids or bases introduced into the internal environment. Carbonic acid and bicarbonate in the blood are good examples of a pair of chemicals that work as a buffer.

In this experiment, students will determine the buffering capabilities of a variety of solutions by measuring the pH of the solutions when they are treated with either a weak acid or a weak base. Each group of students will measure the pH changes that occur in deionized (DI) water, a buffered physiological saline, and another solution from a list provided.

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 clamp

100 ml beakers (5)

500 ml beaker

1000 ml beaker

Pasteur pipettes (6)

Test tubes (6)

Test tube rack

pH 4 and pH 7 buffer solutions

0.1M HCl solution

0.1M NaOH solution

Various solutions to test as buffers.

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 **BiologicalBuffers** settings file.
5. After a short time, LabScribe will appear on the computer screen as configured by the **BiologicalBuffers** 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-1-1 on page 1).

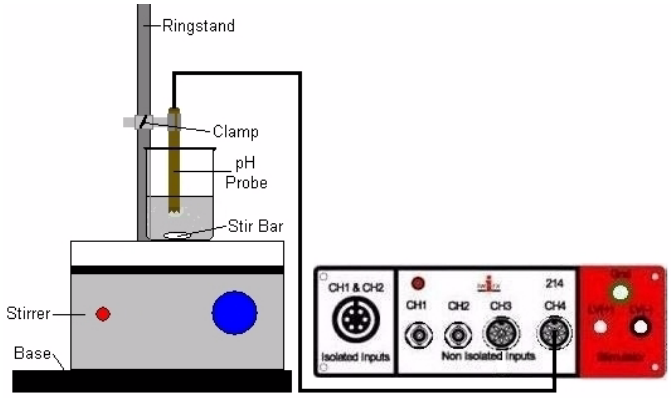

Figure GB-1-1: The arrangement of the stirrer, electrode, and beaker for measuring the changes in the pH of buffers.

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 100 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, each filled with 50 ml of 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-1-1 on page 1). 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-1-2 on page 2).

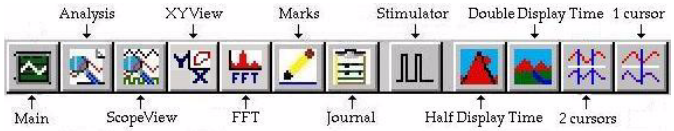

Figure GB-1-2: The LabScribe toolbar.

2 Click the **2-Cursor icon** (Figure GB-1-2 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.

Exercise 1: The Effect of Adding 0.1M HCl to Water

Aim: To determine the changes in pH that take place in deionized water treated with a weak acid.

Procedure

1 Place a magnetic stirrer on or next to the base of a ringstand. Place 50 mL of deionized (DI) water, at room temperature, in a clean 100 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 DI 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 DI 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 **DI 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 **0.1M HCl-10 Drops** on the comment line.

6 As you add ten drops of 0.1M HCl to the beaker of deionized water, press the **Enter key** on the keyboard to mark the recording. Continue recording.

7 Type **Add10 Drops** on the comment line. When the pH of the water in the beaker has reached a new stable level, add ten more drops of 0.1M HCl to the DI water. As you add the drops, press the **Enter key** on the keyboard to mark the recording. Continue recording

8 Repeat Step 7 until a total of 60 drops of 0.1M HCl has been added to the DI water in the beaker.

9 Select **Save** in the **File menu**.

10 Turn off the magnetic stirrer. 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 DI water from the electrode and place the electrode in a clean, empty beaker. the electrode will soon be used in the next section of the exercise.

11 Remove the stir bar from the beaker of acidified water and rinse it with deionized water from a wash bottle. Discard the acidified water, and clean and rinse the beaker.

Data Analysis

1 Scroll to the section of data file in which the pH changes of deionized water treated with 0.1M HCl were recorded.

2 Use the **Display Time icons** on the LabScribe **toolbar** (Figure GB-1-2 on page 2) to position the complete recording on the **Main window**.

3 Click on the **2-Cursor icon** in the LabScribe **toolbar** (Figure GB-1-2 on page 2), so that two blue cursors appear over the **Main window**.

4 Place a cursor on the stable pH level recorded from pure deionized water. Place the second cursor on the stable pH level recorded after a total of 60 drops of 0.1M HCl were added to the deionized water Click the **Analysis icon** on the LabScribe **toolbar** (Figure GB-1-2 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**, and **V2-V1**, 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 Once the cursors have been placed in the proper locations for taking measurements, the names and values of these parameters from the data can be recorded 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 measurements 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.**

7 Place one cursor on the stable pH level of the pure deionized water. Place the second cursor on the stable pH level that occurs after the initial ten drops of 0.1M HCl were added to the deionized water. Select **pH** on the **Value from Ch menu** in the upper left corner of the **Analysis window**.

8 Measure the values for the following parameters from the **pH channel** for the region of data selected:

• **pH-DI Water**, which is **Value1** on the **pH channel**.

• **pH-DI Water & 10 Drops of 0.1M HCl**, which is **Value2** on the **pH channel.**

9 Record the values for these parameters in the **Journal** using one of the procedures described in Step 6, and in Table GB-1-2 on page 5.

10 Move the second cursor from the pH level of DI water containing ten drops of 0.1M HCl to the stable pH level of DI water containing 20 drops of 0.1M HCl, and repeat Steps 8 and 9 to measure and record the pH of the water after a total of twenty drops of 0.1M HCl were added to the water.

11 Move the second cursor to each of the stable pH levels of DI water containing 30, 40, 50, and 60 drops of 0.1M HCl, and repeat Steps 8 and 9 to measure and record the pH of the water after each addition of 0.1M HCl.

12 While the second cursor is on the pH level of DI water containing sixty drops of 0.1M HCl, measure the **Overall Change (Δ) in pH** using the parameter, **V2-V1**, from the **Function Table** in the **Analysis window**.

13 Divide the Overall Change

14 Click **Save** in the **File menu**.

Exercise 2: The Effect of Adding 0.1M HCl to a Physiological Buffer

Aim: To determine the changes in pH that take place in a physiological buffer treated with a weak acid.Procedure

1 Repeat Exercise 1 using a physiological buffer in place of deionized water.

2 Mark the recording with appropriate labels to indicate the number of drops of 0.1M HCl added to the buffer.

3 Select **Save** in the **File menu** to add this data to the existing data file.

Analysis

1. Use the same techniques used in Exercise 1 to measure the pH levels of the buffer after different amounts of 0.1M HCl were added.
2. Use the same techniques explained in Exercise 1 to record the values of the pH levels in the **Journal**, and in Table GB-1-2 on page 5.

Exercise 3: The Effect of Adding 0.1M HCl to Common Solutions

Aim: To determine the changes in pH that take place in different solutions treated with a weak acid. Solutions that are better buffers will have smaller changes in pH.

Procedure

1. Repeat Exercise 1 using a solution which is assigned to your lab group, in place of deionized water. The groups in your lab session will be assigned one of the following solutions to test as a buffer:

- Apple juice
- 2% milk
- Sports drink
- Clear soft drink

2. Mark the recording with appropriate labels to indicate the type of solution used and the number of drops of 0.1M HCl added to the solution.
3. Select **Save** in the **File menu** to add this data to the existing data file.

Analysis

1. Use the same techniques used in Exercise 1 to measure the pH levels of the solutions after different amounts of 0.1M HCl were added.
2. Use the same techniques explained in Exercise 1 to record the values of the pH levels in the Journal, and in Table GB-1-2 on page 5.

Exercise 4: The Effect of Adding 0.1M NaOH to Water

Aim: To determine the changes in pH that take place in deionized water treated with a weak base.

Procedure

1. Repeat Exercise 1 using 0.1M NaOH in place of 0.1M HCl.
2. Mark the recording with appropriate labels to indicate the number of drops of 0.1M NaOH added to the water.
3. Select **Save** in the **File menu** to add this data to the existing data file.

Analysis

1. Use the same techniques used in Exercise 1 to measure the pH levels of the DI water after different amounts of 0.1M NaOH have been added.
2. Use the same techniques explained in Exercise 1 to record the values of the pH levels in the **Journal**, and in Table GB-1-1 on page 5.

Exercise 5: The Effect of Adding 0.1M NaOH to a Physiological Buffer

Aim: To determine the changes in pH that take place in a physiological buffer treated with a weak base.

Procedure

1. Repeat Exercise 1 using a physiological buffer in place of deionized water.
2. Mark the recording with appropriate labels to indicate the number of drops of 0.1M NaOH added to the buffer.
3. Select **Save** in the **File menu** to add this data to the existing data file.

Analysis

1. Use the same techniques used in Exercise 1 to measure the pH levels of the buffer after different amounts of 0.1M NaOH were added.
2. Use the same techniques explained in Exercise 1 to record the values of the pH levels in the **Journal**, and in Table GB-1-1 on page 5.

Exercise 6: The Effect of Adding 0.1M NaOH to Common Solutions

Aim: To determine the changes in pH that take place in different solutions treated with a weak base. Solutions that are better buffers will have smaller changes in pH.

Procedure

1. Repeat Exercise 1 using a solution, that is assigned to your lab group, in place of deionized water. The groups in your lab session will be assigned one of the following solutions to test as a buffer:

- Apple juice
- 2% milk
- Sports drink
- Clear soft drink

2. Mark the recording with appropriate labels to indicate the type of solution used and the number of drops of 0.1M NaOH added to the solution.
3. Select **Save** in the **File menu** to add this data to the existing data file.

Analysis

1 Use the same techniques used in Exercise 1 to measure the pH levels of the solutions after different amounts of 0.1M NaOH were added.

2 Use the same techniques explained in Exercise 1 to record the values of the pH levels in the Journal, and in Table GB-1-1 on page 5.

Table GB-1-1: Changes in pH in Deionized Water, Apple Juice, 2% Milk, and Other Solutions Treated with 0.1M NaOH.

0.1M HCl	Deionized Water		Apple Juice		2% Milk		Sports Drink		Colorless Soft Drink		Buffered Phys. Saline	
	pH	Overall ΔpH	pH	Overal lΔpH	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH
Solution Only												
Solution & 10 Drops												
Solution & 20 Drops												
Solution & 30 Drops												
Solution & 40 Drops												
Solution & 50 Drops												
Solution & 60 Drops												
Percent Change												

Table GB-1-2: Changes in pH in Deionized Water, Apple Juice, 2% Milk, and Other Solutions Treated with 0.1M HCl.

0.1M HCl	Deionized Water		Apple Juice		2% Milk		Sports Drink		Colorless Soft Drink		Buffered Saline	
	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH	pH	Overall ΔpH
Solution Only												
Solution & 10 Drops												
Solution & 20 Drops												
Solution & 30 Drops												
Solution & 40 Drops												
Solution & 50 Drops												
Solution & 60 Drops												
Percent Change												

Questions

1 Besides the buffered physiological saline, which solution demonstrated the lowest percent change in its pH when treated with a weak acid?

2 Besides the buffered physiological saline, which solution demonstrated the lowest percent change in its pH when treated with weak base? Is it the same solution as the one that responds with the lowest percent change in pH when treated with a weak acid?

3 Which solution demonstrated the highest percent change in its pH when treated with a weak acid?

4 Which solution demonstrated the highest percent change in its pH when treated with weak base? Is it the same solution as the one that responds with the highest percent change in pH when treated with a weak acid?

5 Besides the buffered saline, which solutions would be the good buffers?

6 Why would each solution that you selected be a good buffer?