Experiment ADS-CEK-4: Biological Buffers

Background

To survive in changing environments, organisms must be able to maintain steady internal environments in which cellular metabolism can take place. This is also known as homeostasis.

One of the homeostatic mechanisms 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. All biological systems have these buffering systems in place to maintain pH homeostasis.

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.

Experiment ADS-CEK-4: Biological Buffers

Equipment Required

PC or Mac Computer

ADS or ADS MAX Board, USB cable, Power Supply Cable

PHN-100 pH electrode

DCN-100 drop counter

Magnetic stirrer and Stir bar

Ringstand and Utility clamp

100 ml beakers (5)

500 ml beaker

1000 ml beaker

Graduated cylinders

pH 4 and pH 7 buffer solutions

0.1M HCl solution

0.1M NaOH solution

Various solutions to test as buffers

Note: To ease setup, connect the sensor to the A-ADS-IA adapter *before* plugging the adapter into the board. To disconnect, unplug the adapter from the board *first*, then remove the sensor.

Drop Counter Setup

1. Locate the DCN-100 drop counter and plug the connector into Channel 1 on the A-ADS-IA.
2. Position the sensor so that drops fall straight through the center of the opening, approximately 1 inch above the optical slot. Each time a drop passes through the slot, it will generate a pulse in the recording, representing one drop.

Figure ADS-CEK-4-S1: DCN-100 drop counter.

pH Electrode Setup

1. Locate the PHN-100 pH electrode and plug the connector into Channel 2 on the A-ADS-IA.

Figure ADS-CEK-4-S2: PHN-100 pH electrode.

Setup (ADS)

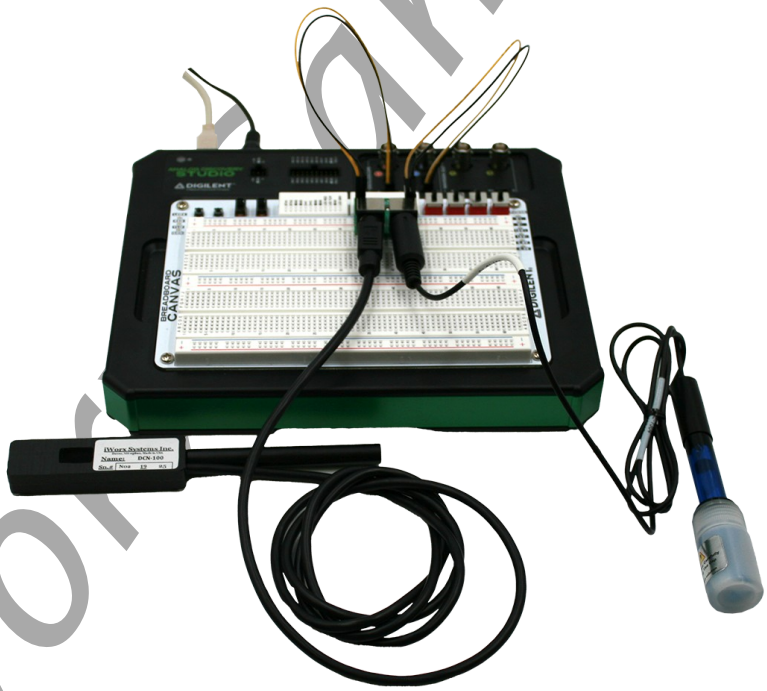

Figure ADS-CEK-4-S3: DCN-100 and PHN-100 setup.

Setup (ADS MAX)

If using the ADS MAX, setup instructions will be the same. Channel connections are labeled on the A-ADS-IA board, as shown below.

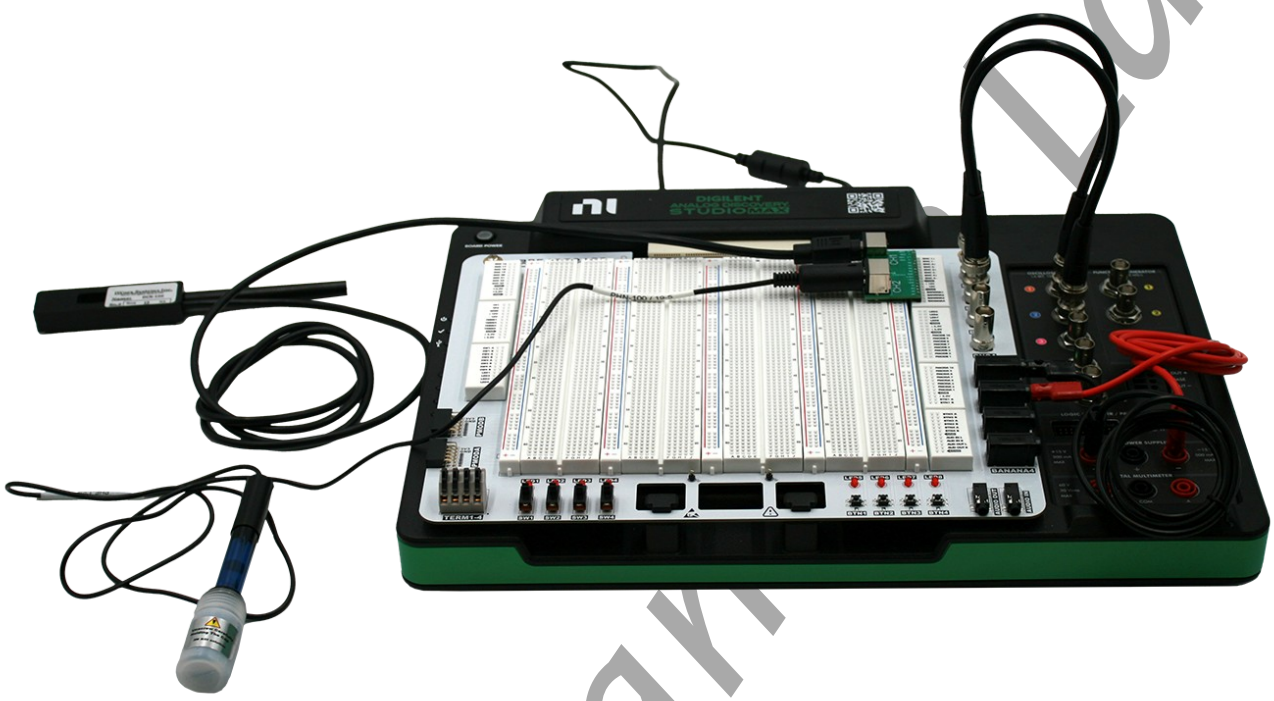

Figure ADS-CEK-4-S4: DCN-100 and PHN-100 setup with ADS MAX Board.

Calibration of the pH Electrode

1. If the PHN-100 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 PHN-100 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 at room temperature. Fill one beaker with pH 7 buffer; and the other with pH 4 buffer. Each beaker should be filled with enough buffer to cover the tip of the PHN-100 pH electrode, and also allow the stir bar in the beaker to spin without touching the electrode.
4. Place the beaker containing the pH 7 buffer on the magnetic stirrer. Carefully place a stir bar in

the beaker. Remove the electrode from the deionized water and blot any extra drops of water. 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 slow speed.

5. Click Record on the LabScribe Main window to begin recording. After a few seconds, the trace will reach a stable baseline toward the top of the recording channel. Type the words **Calibration - pH 7** in the Mark box. Click the mark button to mark the stable baseline of the recording. This will mark the output of the PHN-100 pH electrode in pH 7 buffer. Continue recording while changing the beakers of buffers.
6. Turn off the stirrer and remove the PHN-100 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. Blot any extra drops of water.
7. Place the beaker of pH 4 buffer on the stirrer. Carefully place a stir bar in the beaker. Position the tip of the electrode in the beaker of pH 4 buffer so that it is away from the stir bar. Adjust the speed of the stirrer so the stir bar is rotating evenly at a slow 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** in the Mark box. Click the mark button to mark the stable baseline of the recording. This marks the output of the PHN-100 pH electrode in pH 4 buffer. Click Stop to halt the recording.
9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
10. Turn off the stirrer. Remove the 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 extra drops of water and place the electrode in a beaker of deionized water.

Units Conversion

1. Scroll to the beginning of the calibration data for the PHN-100 pH electrode.
2. Use the Display Time icons to adjust the Display Time of the Main window to show the data collected at pH 7 and pH 4 on the Main window at the same time.
3. Click the 2-Cursor icon so that two cursors appear on the Main window. Place one cursor on the flat section of data collected when the PHN-100 pH electrode was in the pH 7 buffer and the second cursor on the flat section of data collected when the electrode was in the pH 4 buffer.

Figure ADS-CEK-4-S2: The LabScribe toolbar.

4. To convert the voltages at the positions of the cursors to pH values, use the Simple Units Conversion dialogue window. Click on V2-V1 to the right of the pH channel select Simple.
5. On the units conversion window, make sure 2 point calibration is selected in the pull-down menu in the upper-left corner of the window. Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations. Enter the two buffers used in the calibration recording in the corresponding boxes on the right side of the conversion equations. Enter the name of the units, pH, in the box below the buffer values. Click OK.

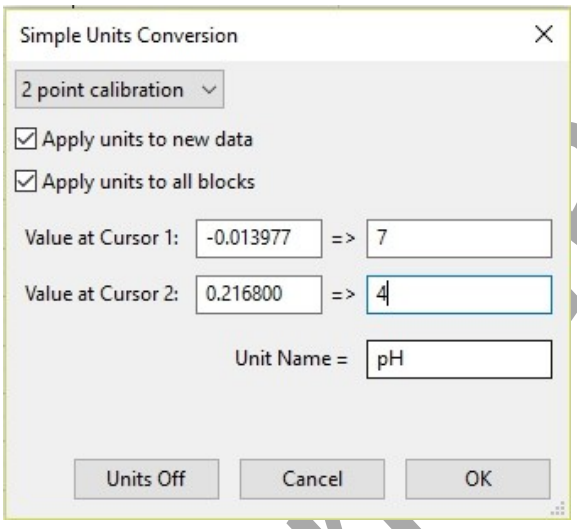

Figure ADS-CEK-4-S3: Simple units conversion dialog window showing the pH conversion.

Experiment ADS-CEK-4: Biological Buffers

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.

Approximate Time: 15

Procedure

1. Using the equipment from the calibration exercise, place 50 mL of room temperature deionized (DI) water 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 ISE-100 pH electrode from the beaker of deionized water used at the end of calibration. Blot the drops of DI water from the electrode. Mount the electrode in a clamp on the ringstand and position it over the new beaker of deionized water. Carefully lower the tip of the electrode into the beaker.
3. Turn on the stirrer so that the stir bar rotates slowly and evenly.
4. Click Record. When the recording on the channel reaches a stable baseline, type **DI Water** in the Mark box. Click the mark button to mark the recording.
5. After recording for at least fifteen seconds of stable baseline, type **0.1M HCl-10 Drops** on the Mark box.
6. As you add ten drops of 0.1M HCl to the beaker of deionized water, click the mark button to mark the recording. Continue recording.
7. Type **Add 10 Drops** in the Mark box. 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, click the mark button to mark the recording. Continue recording.
8. Repeat Step 7 until a total of 60 drops of 0.1M HCl have 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 extra drops of DI water from the electrode and place it in a clean, empty beaker. The electrode will be used in the next 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 in the proper manner, and clean and rinse the beaker.

Data Analysis

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

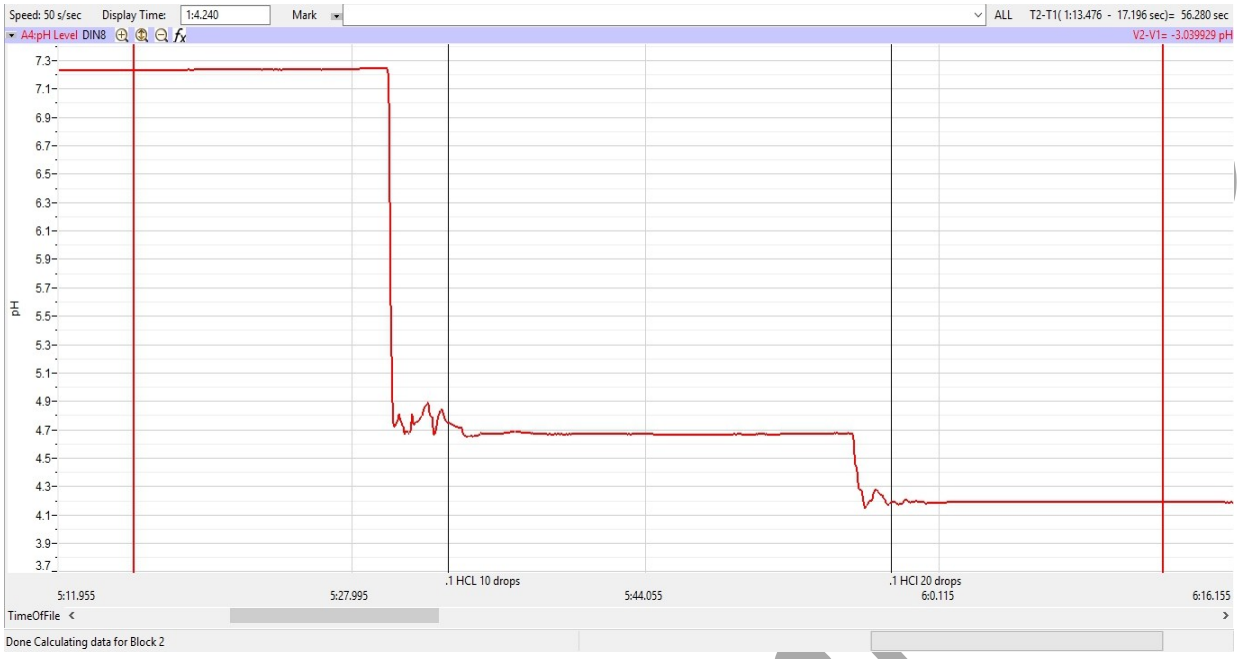

Figure ADS-CEK-4-L1: Recording of the change in pH of the water when HCL is added.

2. Use the Display Time icons on the LabScribe toolbar to position the complete recording on the Main window. The required data can also be selected by:
    - Placing the cursors on either side of the section of data needed. Place one 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
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window.
3. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions that are listed should include Title, Value1, Value2, and V2-V1. The values for these parameters from each channel are seen in the table across the top margin of each channel.
5. Once the cursors are placed in the correct positions for determining the pH, the values for pH can be recorded in the on-line notebook of LabScribe by typing the names and values directly into the Journal.

Figure ADS-CEK-4-L2: pH recording in the Analysis window showing the pH values for the addition of 0.1M HCl to the deionized water.

6. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the pH from the pH channel.
    - Transfer the name of the mathematical function used to determine the pH to the Journal using the Add Title to Journal function in the pH channel pull-down menu.
    - Transfer the value for the pH to the Journal using the Add Ch. Data to Journal function in the pH channel pull-down menu.
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.
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-L1.
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 in pH using the parameter, V2-V1, from the Function Table in the Analysis window.
13. Divide the Overall Change in pH by the initial pH of the DI water, then multiply by 100 to determine the percent change in pH.
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.

Approximate Time: 15 minutes

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.

Data 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 1.

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.

Approximate Time: 15 minutes per solution

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 a solution to test as a buffer. These may include:
    - Apple juice, 2% milk, sports drink, clear soft drink, or any other solution your instructor deems appropriate.
2. Mark the recording with corresponding 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.

Data 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 the data table.

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.

Approximate Time: 15 minutes

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.

Data 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 2.

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.

Approximate Time: 15 minutes

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.

Data 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 2.

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.

Approximate Time: 15 minutes per solution

Note: *Some solutions will be better at buffering an acid than a base and vice versa. Note this while observing your results and those of the other lab groups.*

Procedure

1. Repeat Exercise 1 using the same solution that was assigned to your lab group when testing an acid, in place of deionized water.
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.

Data 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 the data table.

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 good buffers? Are all these solutions biological?
6. Why would each solution that you selected be a good buffer?

Table GB-1-L12: 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 Phys. 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												

Table GB-1-L2: Changes in pH in Deionized Water, Apple Juice, 2% Milk, and Other Solutions Treated with 0.1M NaOH.

0.1M NaOH	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												