Acids, Bases and Buffers

In this experiment, you will explore the principles of acids, bases, and buffers by performing titration analysis.

Written by staff of ADInstruments.

Background

Scientists quantify the "acidity" of an aqueous solution by expressing its molar concentration of hydronium ions (H_3O^+) on a logarithmic scale called the pH scale. The pH of an aqueous solution is calculated using the following equation:

$$pH = -\log[H_3O^+]$$

Acidic solutions have a pH value of less than seven. Solutions with pH values greater than seven are described as basic, or alkaline. We use the pH of water as our definition of neutrality. Water is actually a mixture of molecular water (H_2O), and ionized water (H_3O^+ and OH^-). In pure water, the concentrations of H_3O^+ and OH^- are in equilibrium at 10^{-7}M. Therefore, pure water has a pH of 7.0. Solutions with high concentrations of hydronium ions have low pH values, while solutions with low hydronium ion concentrations have high pH values. It is important to note the intimate relationship of hydronium and hydroxyl ions. As one species becomes more prevalent, the other decreases in concentration. The pH values of several common substances are shown on the scale in Figure 1.

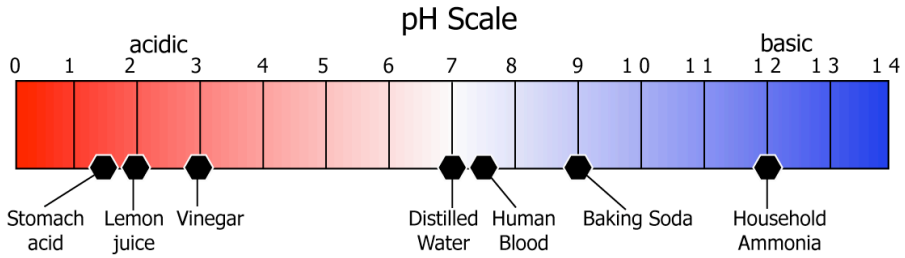

Figure 1. The pH scale, shown with the values for some common substances.

How do scientists define a substance as an acid or a base? The most commonly accepted definitions of acids and bases come from the Brønsted-Lowry theory. The Brønsted-Lowry theory of acids and bases defines an acid as any molecule that can donate a proton (H^+) to a solution, and a base as any molecule that can accept a proton from a solution.

Buffers are in general weak acids or bases. In solution they exist as a mixture of the undissociated acid or base with its conjugate salt. They either add or remove protons in response to the addition of hydroxyl or hydronium ions. Therefore buffered solutions resist changes in pH, even when excess hydronium or hydroxyl ions are added to them. For example, carbonic acid (H_2CO_3) and its conjugate base, bicarbonate ion (HCO_3-), form an important biological buffer system. Bicarbonate ions can take up excess protons from the solution, while carbonic acid can donate protons to the solution. The bicarbonate buffer system is important in the blood.

In this experiment, you will determine the acid-base properties of solutions by performing a titration. Titration is a powerful analytical technique that can be used to determine concentration of unknown solutions. By adding a known volume of an acid or base to an unknown solution and measuring the change in pH, you can determine the molar concentration of that solution. This analysis is possible because hydronium and hydroxyl ions will neutralize each other. When the amount of hydronium ion in solution is exactly matched by the amount of hydroxyl, the pH of the solution will be 7.0. If you know how much acid or base is required to neutralize an unknown, then you can calculate the concentration of that unknown. You can also use a titration to examine the buffering properties of molecules by making a titration curve. A titration curve is a graph of the pH of a solution versus the quantity of titrant added. From a titration curve, you can determine the equilibrium constant, K_a, of an acid.

Required Equipment

A computer system
PowerLab 4/25T
Chart, version 5.0.1 or later
pH Pod
pH Electrode
Reflective drop counter
Ring-stand
Ring clamp
Buret clamp
Buret with Teflon stopcock
250mL beakers
Magnetic stirring motor
Teflon stir bar
Electronic balance
Weighing boats
Graduated cylinders
100mL volumetric flask
10mL serological pipettes (disposable)
Wash bottle
Pipette bulb
Plastic funnel
Plastic drinking straw

Reagents
pH standard solutions: pH 7.0, 4.0, and 10.0
Hydrochloric acid, 1.0N standard solution
Sodium hydroxide, 1.0N standard solution
L-histidine, hydrochloride salt
Sodium bicarbonate
Distilled water
Household vinegar

Procedures

Safety Warning: Always wear gloves and appropriate eye protection when handling reagents.

Part 1. Set up and calibration of equipment

1. Clamp the Drop Counter to your ring stand and position it so it is above the stir motor (Figure 2).
2. Place a 100mL beaker containing pH 7.0 buffer or distilled water on the stir motor.
3. Place the pH electrode into the holder on the Drop Counter so the tip is immersed in the buffer.
4. Connect the cable from the Drop Counter into the Input 1 Pod Port on the front of your PowerLab.
5. Connect the cable from the pH Pod into the Input 2 Pod Port on the front of the PowerLab.
6. Attach the BNC connector on the pH Electrode to the socket on the rear panel of the pH Pod.
7. Mount a 25 mL buret in the buret clamp on your ring stand (Figure 2).
8. Adjust the position of the buret tip so that it is aligned with the alignment marks on the Drop Counter (Figure 3). The buret tip should be positioned 3-5mm above the opening in the drop counter.
9. Close the stopcock on the buret.

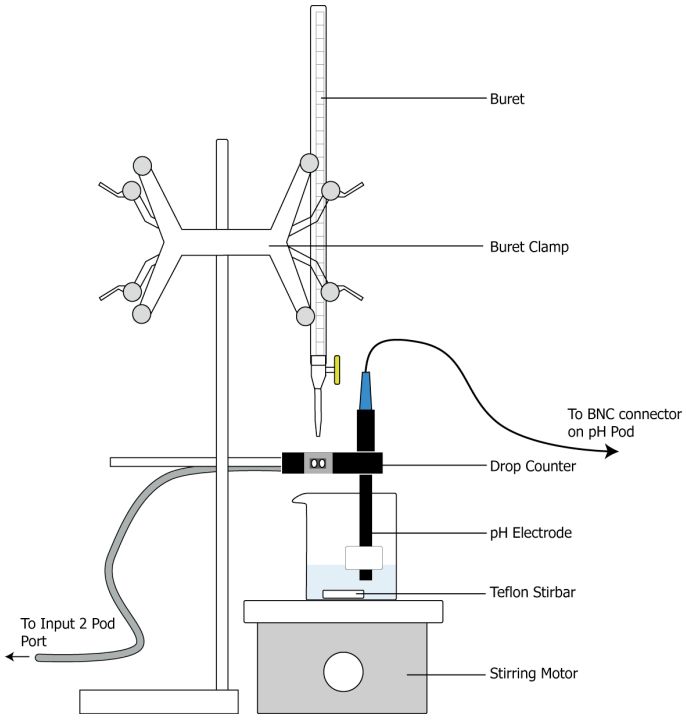

Figure 2. Glassware set up to perform the exercises in this experiment.

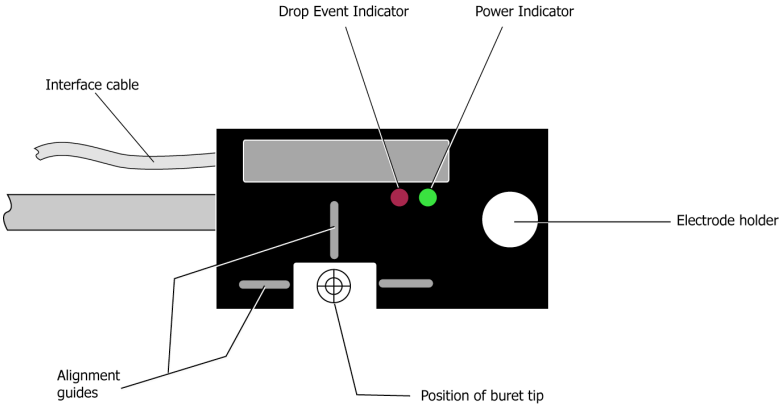

Figure 3. Align the buret tip using the guides on the top of the drop counter.

Calibrating the Drop Counter

Before you begin the exercises, you must calibrate the drop counter so that the Volume channel in Chart reads in milliliters.

1. Make sure your computer is turned on, and that the PowerLab is connected to it via its USB cable.
2. Launch Chart, and from the Experiments Gallery, open the file called "Titration Settings".
3. After a few seconds, a blank Chart file will appear with two channels.
4. Fill your buret completely with distilled water.
5. Place a beaker under the drop counter and open the stopcock on the buret to allow water to fill the tip. Slowly run about 5mL of water through your buret to remove any air bubbles and then close the stopcock.
6. Remove the beaker and refill your buret. You are now ready to calibrate the Drop Counter.
7. Weigh a clean, dry 125mL Erlenmeyer flask and record the mass in Table 1 of your Data Notebook. Next, place the 125mL Erlenmeyer flask beneath the buret and drop counter. Leave the pH electrode in its beaker of buffer, and out of the way of the buret tip, for now.
8. In Chart, click **Start**.
9. **Slowly** open the stopcock so that a slow but steady stream of *individual drops* exit the buret. You should try to achieve a drop rate of one or two drops per second. **Note:** If the water exits the buret in a continuous stream, stop and repeat the calibration procedure.
10. Allow the buret to completely empty into your flask.
11. In Chart, click **Stop**.
12. Weigh the flask with the water. Record the weight in Table 1 of your Data Notebook.

13. Determine the weight of the water in the flask by subtracting the weight of the empty flask from the weight of the full flask. This weight is equal to the volume of water, in milliliters. Record your results in Table 1 of your Data Notebook.

14. In Chart, **select** the data in the Volume channel that has the endpoint of your calibration (Figure 4).

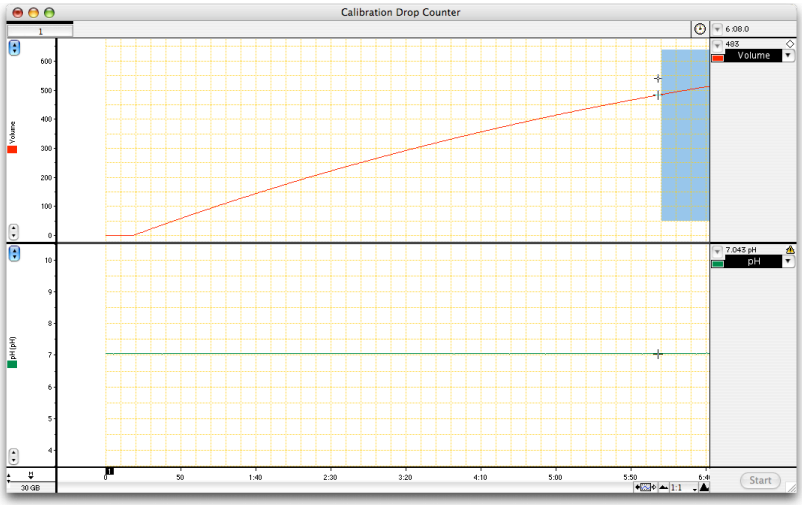

Figure 4. Select the last few seconds of data in the volume channel before proceeding to the Units Conversion dialog.

15. From the Volume channel function pop-up menu, choose **Units Conversion**. A dialog box will appear (Figure 5).

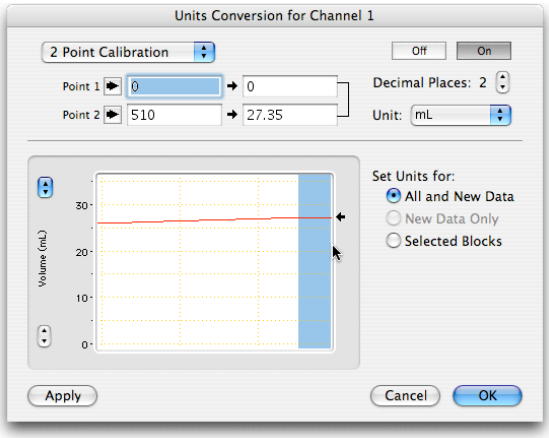

Figure 5. The Units Conversion dialog box is used to calibrate the Drop Counter.

16. Click the part of the trace in the left-hand side of the dialog box that corresponds to the final drop value, and then click the arrow next to Point 2. Point 1 should read zero; leave it as is. When you are done, click OK to return to the Chart view. The Volume channel should now display in milliliters.

Calibrating the pH Electrode

1. Obtain a beaker to use as a wastewater container.
2. Place 30-40mL of pH 4.01 buffer in a 100mL beaker.
3. Place 30-40mL of pH 10.01 buffer in another 100mL beaker.
4. Remove the pH electrode tip from its beaker, and rinse the tip with distilled water, using your "waste" beaker to catch the drips.
5. Place the pH electrode into the beaker of pH 4.01 buffer.
6. In Chart, click **Start**.
7. Record for 20 seconds.
8. Remove the pH electrode from the buffer, rinse the tip into your waste beaker with distilled water, and then replace the tip into the pH 10.01 buffer.
9. Record for 30 seconds.
10. Click Stop.
11. Rinse the pH electrode and return it to its beaker of pH 7.0 buffer.
12. Make a **selection** of your data in the pH channel.
13. From the pH channel function pop-up menu, choose, "**pH**". A dialog box will appear (Figure 6).
14. **Select** the data in the left-hand window that corresponds to pH 4.01, and click the arrow next to Point 1.
15. Select the data corresponding to pH 10.01, and click the arrow next to Point 2.

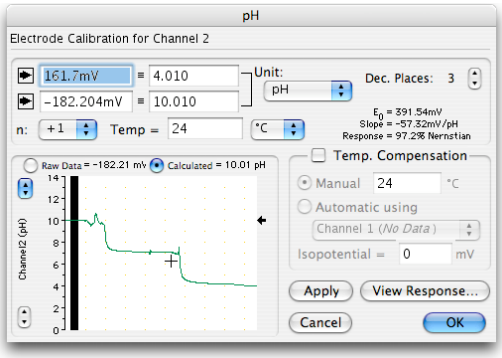

Figure 6. The pH dialog box is similar to the Units Conversion dialog box. The pH extension must be installed in your Chart Extensions folder to access this software feature.

16. When you are done, click OK to return to the Chart view.

Preparing a standard NaOH solution

1. Obtain a clean, dry 100mL volumetric flask.
2. Use a volumetric pipette to add 10mL of 1N NaOH standard to the flask.
3. Fill the flask to the fill line with distilled water.
4. Cap the volumetric flask, and invert it several times to mix the contents fully3.

Exercise 1: A simple acid-base titration

In this exercise, you will titrate sodium hydroxide with hydrochloric acid to determine the equivalence point.

1. Make sure the stopcock on your buret is closed.
2. Fill the buret with 3mL of 0.1M NaOH standard that you prepared. Use the plastic funnel when filling your buret. Then partially drain the buret into a waste beaker marked "alkali waste" so that there are no bubbles in the buret tip. Close the stopcock when no air bubbles remain in the tip. **Note:** Label *all* your beakers so you do not inadvertently mix acids and bases.
3. Fill the buret to the top mark with 0.1N NaOH standard. Make sure there are no air bubbles in the buret tip; drain a little of the NaOH if you have to.
4. Obtain a clean, dry 250mL beaker. Place a Teflon stir bar into the beaker.
5. Add 50mL of distilled water to the beaker.
6. Pipette 1mL of 1N HCl standard into your beaker, and then place the beaker on the stir motor underneath the buret.
7. Rinse the pH electrode and place it into the beaker, making sure that the tip is immersed and not in contact with the stir bar.
8. Turn on the stirring motor, and set it to a slow speed.
9. In Chart, click **Start**.
10. Enter a **comment** called "Part 1" into your data trace.
11. Slowly open the stopcock on your buret until you get a drop rate of 1-2 drops per second.
12. Continue recording until the buret is completely empty.
13. When the buret is empty, click Stop.
14. Close the stopcock on your buret.
15. Save your data with an appropriate filename, such as "Acid-base titration".
16. Follow the procedures in the Analysis section for determining the equivalence point of your titration.

Exercise 2: Determining the concentration of acetic acid in household vinegar

Commercial vinegar contains dilute acetic acid. In this exercise, you will determine the concentration of acetic acid in vinegar by analytical titration.

1. Click the "New Chart Document" button in the Chart Toolbar Menu. If prompted, choose "Use the current settings" and make sure you have saved any previous data.
2. Pipette 5mL of household vinegar into a 100mL volumetric flask.
3. Fill the flask to the fill line with distilled water. This represents a 1:20 dilution of the vinegar.
4. Fill your buret with 0.1M NaOH standard.
5. Make sure there are no air bubbles in the buret tip; use your waste beaker if necessary to drain air bubbles from the tip.
6. Place a Teflon stir bar into a clean 250mL beaker, and pour 50mL of diluted vinegar into it.
7. Place the beaker under the buret; rinse the pH electrode and place it into the vinegar.
8. In Chart, click Start.
9. Enter a **comment** called "vinegar" into your data trace.
10. Slowly open the stopcock on your buret until you get a drop rate of 1-2 drops per second.
11. Continue recording until the buret is completely empty.
12. When the buret is empty, click **Stop**.
13. Close the buret stopcock.
14. Save your date with an appropriate filename, such as "Vinegar Titration".
15. Follow the procedures in the Analysis section to determine the concentration of acetic acid in vinegar.

Exercise 3: The bicarbonate buffer system

Bicarbonate ions help regulate the pH of biological fluids, especially blood. In this exercise, you will determine the pK_a of bicarbonate, and then examine how bicarbonate helps regulate blood pH when CO_2 is added to solution.

Determining the pKa of bicarbonate

1. Click the "New Chart Document" button in the Chart Toolbar Menu. If prompted, choose "Use the current settings" and make sure you have saved any previous data.
2. Place 100mL of distilled water into a clean 250mL beaker and add a Teflon stir bar.

3. Weigh out 0.1g of sodium bicarbonate, and add it to the beaker. Make sure the solution is mixed well and the sodium bicarbonate is completely dissolved.

4. Pipette 1.3 mL of 1.0N HCl into your beaker.

5. Fill your buret with 0.1N NaOH standard, and make sure there is no air in the buret tip.

6. Make sure the stopcock on the buret is closed, and place the beaker on the stirring motor beneath the buret.

7. Rinse the pH electrode with distilled water and position it in your beaker.

8. Turn on the stirring motor to a slow speed.

9. Click **Start** to begin recording.

10. After ten seconds, slowly open the stopcock until you get a drop rate of 1-2 drops per second.

11. Continue recording until your buret is empty.

12. Close the buret stopcock and click **Stop** to end your data collection.

13. Remove the pH electrode from the beaker, rinse it, and return it to the beaker of pH 7 buffer.

Examining the carbonic acid/ bicarbonate buffer system

1. Remove the buret from its holder.

2. Add 100mL of distilled water to a clean, dry 250mL beaker, and add a Teflon stir bar.

3. Place the beaker on the stir motor, rinse the pH electrode tip and place the electrode into the beaker of water.

4. Turn the stirring motor on and set it to a slow speed.

5. Click **Start** to begin recording. Add a **comment** to your trace called "DI water".

6. Have one member of your group exhale into the beaker through a drinking straw. Try to exhale for 10-15 seconds. Enter a comment called "CO_2" to your recording.

7. Click **Stop**, and observe your data.

8. After you observe your data, click **Start** again.

9. Add 0.1g of sodium bicarbonate to your water, and enter a **comment** called "bicarbonate".

10. After the sodium bicarbonate is fully dissolved, perform the exhalation procedure by blowing into the beaker with a straw for 10 seconds. Enter a comment into your data trace called "CO_2".

11. Repeat the bubbling procedure three times, entering a comment each time.

12. Click Stop to end your recording.

13. Save the data with an appropriate filename, such as "Bicarbonate data".

14. Refer to the analysis section for the analysis procedures.

Exercise 4: The titration curve of an amino acid

In this exercise, you will make a complete titration curve of the amino acid histidine, and use your results to calculate its pKa values.

1. Click the "New Chart Document" button in the Chart Toolbar Menu. If prompted, choose "Use the current settings" and make sure you have saved any previous data.

2. Weigh out 0.1g of histidine HCl in a weigh boat using your electronic balance. Record the exact mass you used in your data notebook.

3. Add a Teflon stir bar to a clean, dry 250mL beaker.

4. Add the histidine HCl to your beaker.

5. Add 50mL of distilled water and mix the solution thoroughly.

6. Use a serological pipette to transfer 3.0mL of 1N HCl to the beaker.

7. Rinse your buret with 0.1M NaOH, and then fill the buret. Make sure there are no air bubbles in the buret tip. Make sure that you have at least 50mL of NaOH standard available for the titration.

8. Place the histidine solution underneath the drop counter; rinse the tip of the pH electrode with distilled water and place it into the beaker.

9. Turn on the stir motor to a slow speed. Do not proceed to the next step until you are sure the histidine is fully dissolved.

10. In Chart, click **Start**.

11. Enter a **comment** to your recording called "Histidine".

12. Record for five seconds, and then slowly open the stopcock on your buret until you get a drop rate of 1-2 drops per second.

13. Continue recording until you reach a pH of at least 11.5. If you are using a 25mL buret, you may need to fill it with additional NaOH standard during your titration.

14. When the pH of your solution is 11.5, close the stopcock on your buret.

15. Save your data with an appropriate filename, such as "Histidine".

16. Follow the procedures in the Analysis section to determine the pKa values of histidine.

Analysis

Exercise 1: A simple acid-base titration

1. Make a selection of your entire titration data set by clicking and dragging the mouse in the time axis at the bottom of the Chart view window.

2. Click the X-Y Plot button in the Chart toolbar.

3. In the X-Y window, click the number 1 box on the x-axis and the number 2 box on the y-axis (Figure). This will display pH versus volume.

4. Using the mouse, move the waveform cursor until you find the point on the curve where the pH is 7.0.

5. Record the volume of NaOH added to reach the equivalence point.

6. Calculate the number of moles of NaOH added to neutralize the acid and then calculate the molar concentration of acid using the equations below.

$$\text{moles NaOH} = V_{NaOH} \times M_{NaOH}$$

$$M_{acid} = \frac{\text{moles NaOH}}{V_{acid}}$$

Where V= volume, in mL
M= molarity, in moles per liter

Exercise 2: Determining the concentration of acetic acid in vinegar

1. Examine your recording from Exercise 2.

2. Using the mouse, select the entire data trace in both channels by clicking and dragging the time axis.

3. Click the X-Y Plot button in the Chart Toolbar.

4. In the X-Y window, click the number 1 box on the x-axis and the number 2 box on the y-axis. This will display pH versus volume.

5. Using the mouse, move the waveform cursor until you find the point on the curve where the pH is 7.0.

6. Record the volume of NaOH added to reach the equivalence point.

7. Calculate the number of moles of NaOH required to neutralize the acid, and then calculate the concentration of acetic acid (Hac) in vinegar using the equation below. Record your results in Table 3 of your Data Notebook.

$$M_{Hac} = \frac{\text{moles NaOH}}{V_{Hac}} \times 20$$

Exercise 3: Properties of a buffer solution

1. Examine your data for Exercise 3.

2. Select the titration curve of sodium bicarbonate by clicking and dragging the mouse in the time axis.

3. Click the X-Y plot button in the Chart Toolbar.

4. In the X-Y window, select Channel 1 for the x-axis and Channel 1 for the y-axis.

5. Examine the titration curve. You may wish to use the "Print" function to print the X-Y window.

6. The titration curve should have one or more distinct "plateau" phases, where pH does not change rapidly. The pH value at the mid-point of each plateau corresponds to the pK_a value. This is the pH at which the concentrations of conjugate base and undissociated acid are equal.

7. You can estimate the midpoint of the plateau phase as follows:

   - In Chart, choose: Setup→Channel Settings. The Channel Settings dialog box will open (Figure 7).
   - In the box that reads "number of channels", enter "4", and click OK to return to the Chart view.
   - Two new channels should now be visible in your Chart view. Click the Autoscale button  in the Chart toolbar menu to scale the data in all channels. Channel 3 (pH rate) shows the rate of change of the pH data in Channel 2 with the units of pH sec^{-1}. Channel 4 (Smoothed) is the same data as in Channel 3, but with smoothing applied to reduce noise. You should observe two prominent peaks in Channel 4 (Figure 8).
   - Place the mouse cursor on the first large peak in Channel 4, and click the mouse button once to set the active point. Choose Command→ Add to Data Pad. Open the **Data Pad** by clicking the Data Pad button in the Chart toolbar. You should see two numbers entered: Volume of NaOH and pH. Enter the volume in Table 4b of your Data Notebook as V1.
   - Repeat the procedure in the previous step for the second major peak in Channel 4. Record the volume NaOH in Table 4b as V2.
   - Subtract V2 from V1 to get V3.
   - Divide V3 by 2 to get V4.
   - Add V4 to V1. This will be the midpoint estimate.
   - Move the waveform cursor along your data in the NaOH channel until the number displayed is as close as possible to the midpoint estimate you calculated. Click the mouse button once to set the active point, and then choose Command→ Add to Data Pad. The pH value displayed in the data pad for this point is the estimated pK_a value for bicarbonate.

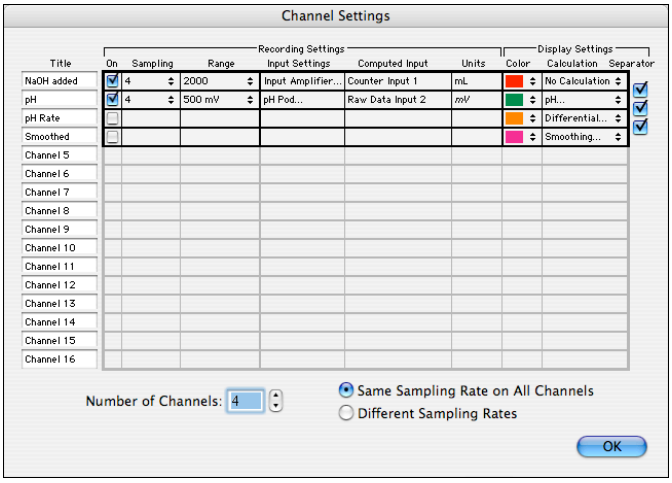

Figure 7. Use the Channel Settings dialog box to add two new channels to your titration file.

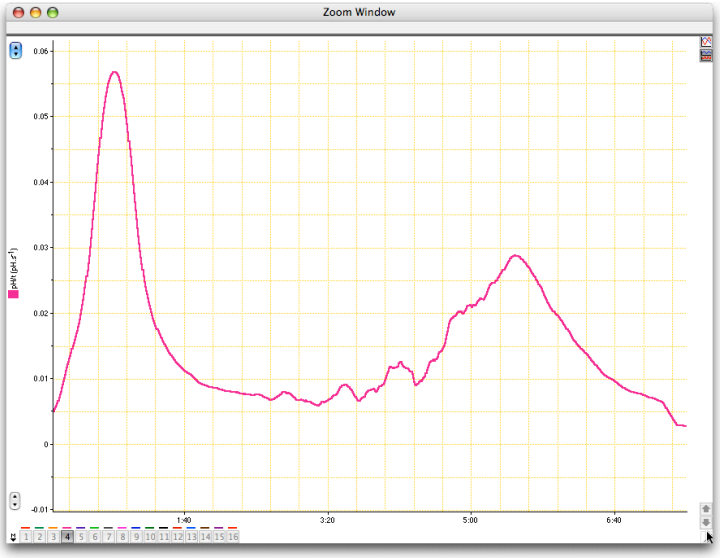

Figure 8. The Zoom view for Channel 4 shows the change in pH over time (∂pH/∂t). The two large peaks in the differential curve can be used to indicate V1 and V2 for determining pK_a.

8. Use the waveform cursor to complete Table 4b in your Data Notebook by recording the appropriate pH values from your breathing experiment.

Exercise 4: The titration curve of an amino acid

1. Examine your data from Exercise 4.

2. Determine the pKa value(s) for histidine by following the procedures in Exercise 3, above, with the following changes:

   - Determine V1, V2 and V3 in Channel 4, as you did in Exercise 3, above.

   - Estimate pka1 as the pH at 1/2 V1.

- Determine the midpoint volumes for the curves between V1 and V2, and between V2 and V3. Estimate pKa_2 and pKa_3 in the same manner as you did for the bicarbonate buffer in Exercise 3.

3. Record the pKa value(s) in Table 5 of your Data Notebook.

Data Notebook

Table 1. Drop Counter Calibration

Mass of empty flask (g)	
Mass of full flask (g)	
Mass of water in flask (g)	
Volume of water (mL)	
Number of drops	
Drops per mL	

Table 2. Results of a simple acid-base titration

Concentration NaOH (M)		Volume HCl (mL)	
Volume NaOH added (mL)		Moles HCl in solution	
Moles NaOH added			
		Concentration HCl (M)	

Table 3. The concentration of acetic acid (Hac) in household vinegar

Concentration NaOH (M)		Volume Hac (mL)	
Volume NaOH added (mL)		Moles Hac	
Moles NaOH added		Concentration Hac in beaker (M)	
		Concentration Hac in vinegar (M)	

Table 4a. The determination of the pKa value of bicarbonate

V1 (mL)	
V2 (mL)	
V3 (mL)	
V4 (mL)	
Estimated midpoint volume (mL)	
Bicarbonate pK_a	

Table 4b. The effect of adding CO_2 to aqueous solutions

Solution	pH before CO_2 added	pH after CO_2 added	Change in pH	Change in $[H_3O^+]$ (M)
Distilled Water				
Bicarbonate buffer solution				

Table 5. pK_a values for histidine

pKa1	
pKa2	
pKa3	

Study Questions

1. A solution has a hydronium ion concentration of 1.8×10^{-3}M. Calculate the pH of this solution.

2. A solution has a hydroxyl concentration of 3.4 x 10-8M. What is the solution's hydronium ion concentration? What is the solution's pH?

3. If pure water is left exposed to air, over time its pH decreases. What explanation can you give for this occurrence? Hint: Recall your results from Exercise 3.

4. What compound is formed when you exhale into water?

5. Compare the change in pH when you exhaled into water with when you exhaled into the bicarbonate solution. How can you explain your results?

6. Why do you suppose bicarbonate is a good buffer for blood?

7. How many pKa values did histidine have? At what pH value(s) could histidine be used as a buffer?

8. Draw the structure of histidine as it appears at:

pH 0.0 pH 4.0 pH 7.5 pH 10.5