Potentiometry: The pH Electrode and Potentiometric Titrations

In this experiment you will investigate the Nernstian response of a pH electrode, perform potentiometric titrations, investigate the acid-base properties of the bicarbonate buffer system, and determine the pKa values of L-histidine.

1. Aims

1.1 Investigate the Nernstian response of a pH electrode by comparing a standard two point calibration of a pH electrode with a wide pH range multipoint calibration of the same electrode.

1.2 Perform potentiometric titrations to determine the concentration of an unknown HCl solution and the concentration of acetic acid in a household vinegar sample.

1.3 Determine the pKa of a bicarbonate solution and examine the carbonic/bicarbonate buffer system.

1.4 Collect a potentiometric titration curve for L-histidine and determine the pKa of its ionising groups.

2. Background

2.1 Acids and Bases

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:

$$pH = -\log\left[H_3O^+\right] \qquad (2.1)$$

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 ionised 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 2.1**.

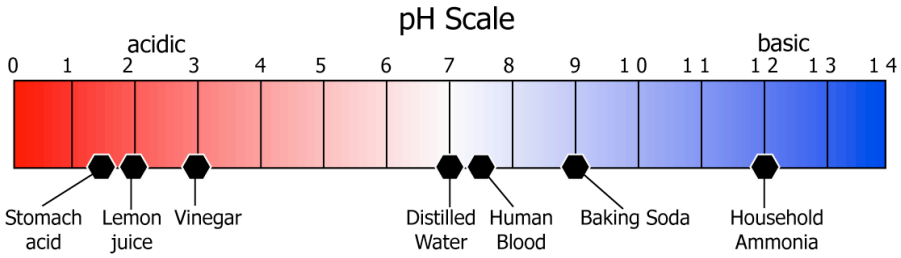


Figure 2.1. The pH scale, shown with the pH values for some common substances.

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.

2.2 The pH Electrode

The cell for measuring pH consists of a indicator electrode and a silver/silver chloride or saturated calomel reference electrode immersed in the solution whose pH is to be determined. Combination pH electrodes combine the reference electrode and indicator electrode in the one probe.

The indicator electrode consists of a tube with a thin pH-sensitive glass membrane at its tip. The tube is filled with a small volume of dilute hydrochloric acid saturated with silver chloride. A silver wire in this solution forms an internal silver/silver chloride reference electrode, which is connected to one of the terminals of a potential measuring device. The other terminal is connected to the external reference electrode in contact with the test solution.

A typical electrode system for measuring pH is shown in **Figure 2.2**.

Figure 2.3 shows the four potentials that develop when pH is being determined with a glass membrane electrode. Two of these, $E_{ref\ 1}$ and $E_{ref\ 2}$ are the external and internal reference electrode potentials. The third is the junction potential E_j across the glass frit that separates the reference electrode from the analyte solution. The fourth and most important potential is the boundary potential (E_b) that forms across the glass membrane, which varies with the pH of the external solution. The two reference electrode simply provide electrical contacts with the solution so that changes in boundary potential can be measured.

There is often a 5th asymmetry potential (E_{asy}) that is not shown in **Figure 2.3** which is found in most membrane electrodes. This potential slowly changes with time and its source is obscure, however calibrating a pH electrode regularly corrects for this potential.

The boundary potential (E_b) is established because at the surface of the glass membrane, hydrogen ions are selectively exchanged with both the external and internal solutions. When equilibrium is established, each surface has a potential E_1 and E_2 which depends on the relative hydronium ion activity between the two solutions. The boundary potential is simply the difference between the potential at the internal and external surface, and from thermodynamic considerations, this can be shown to be equal to **Equation 2.2**.

$$E_b = E_1 - E_2 = 2.303 \frac{RT}{nF} \log \frac{a_1}{a_2} \qquad (2.2)$$

where:

- R is the universal gas constant, 8.314 $JK^{-1}mol^{-1}$;
- T is the temperature of the solution in Kelvin;
- n is the charge of the ion being transferred at the membrane (+1 for H^+);
- F is the Faraday constant, 96487 $Cmol^{-1}$.
- a_1 and a_2 are the hydronium ion activities in the external and internal solutions respectively.

More information on the structure of pH sensitive membranes and the formation of the boundary potential can be found in Skoog *et al* (1998), or any other good textbook on instrumental chemical analysis.

For glass pH electrodes the hydrogen ion activity of the internal solution a_2 is held constant so **Equation 2.2** simplifies to **Equation 2.3** and the boundary potential is simply a measure of the hydronium ion activity of the external solution.

$$E_b = L' + 2.303\frac{RT}{nF}\log a_1 = L' - 2.303\frac{RT}{nF}\text{pH} \qquad (2.3)$$

where:

$$L' = -2.303\frac{RT}{nF}\log a_2$$

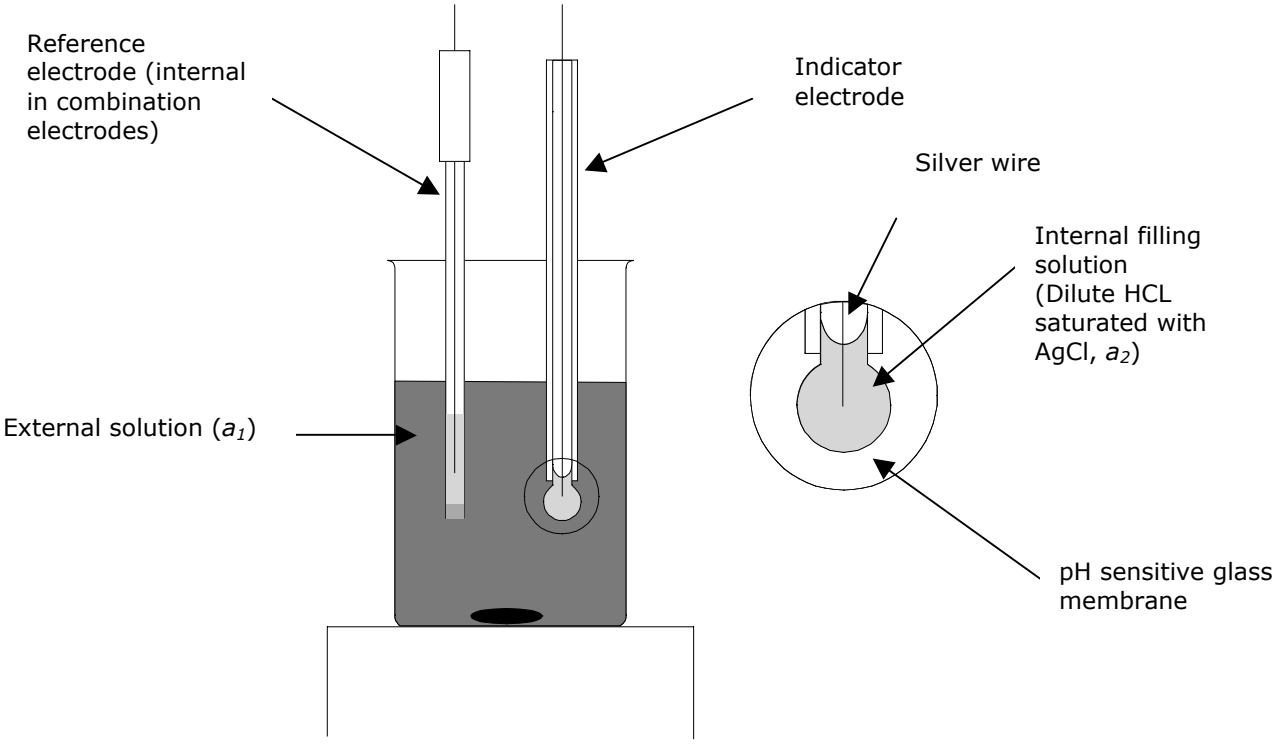


Figure 2.2 Typical electrode system for measuring pH.

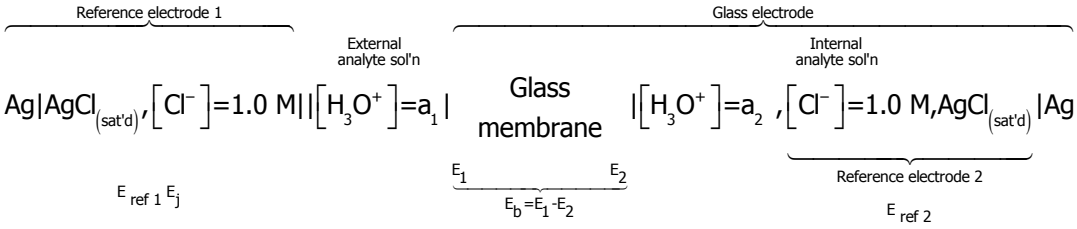


Figure 2.3 Diagram of cell for measurement of pH.

The potential of the glass indicator electrode (E_{ind}) is equal to the sum of the boundary potential E_b, the potential of the internal reference electrode ($E_{ref\ 2}$) and the asymmetry potential (E_{asy}) In equation form,

$$E_{\text{ind}} = E_{\text{b}} + E_{\text{ref 2}} + E_{asy} \qquad (2.5)$$

Substitution of **Equation 2.3** gives

$$E_{ind} = E_0 + 2.303\frac{RT}{F}\log a_1 = E_0 - 2.303\frac{RT}{F}\text{pH} \qquad (2.6)$$

where E_0 is a combination of the three constant terms and is the potential of the indicator electrode at zero pH.

$$E_0 = L' + E_{\text{ref 2}} + E_{\text{asy}}$$

Equation 2.6 has the form of the Nernst equation, and the ideal voltage output of all potentiometric electrodes follow the Nernst equation.

If the response of two or more solutions with known pH are measured, then the values of the potential, E_{ind}, can be used to construct a graph of a straight line to determine E_0 and the 'slope' of the response. An ideal pH electrode will have a slope of $-2.303(RT/nF)$ and the percentage Nernstian response can be obtained using **Equation 2.7**.

$$response = 100\% \times \frac{slope_{obs}}{slope_{calc}} \qquad (2.7)$$

where:

- $slope_{obs}$ is the observed slope in $V.pH^{-1}$
- $slope_{calc} = -2.303(RT/nF)$

New, high quality pH electrodes will have a response in the range 95–102 %, but older electrodes may be well under this range. Other potentiometric electrode, such as ion selective electrodes, will exhibit a wider variation.

3. Equipment Required

- computer with Chart installed (pH and Multipoint Calibration Chart extensions installed)
- e-corder
- EP303 pH Pod
- ET5733 Tuff Tip pH Electrode
- ET226 Drop Counter
- retort stand
- bosshead and clamp
- burette clamp
- 50 mL burette with Teflon stopcock
- a 250 mL beaker
- a 100 mL beaker
- a 20 mL beaker
- magnetic stirrer
- Teflon stir bar
- plastic funnel
- wash bottle filled with distilled water
- a drinking straw
- lint free tissue
- electronic balance
- a thermometer

4. Reagents Required

- 7 pH buffer solutions listed in Table 1, Appendix A.
- 1 M and 0.1 M HCl standards
- 1 M and 0.1 M NaOH standards
- HCl solution with unknown concentration (between 0.01 and and 0.1 M)
- L-histidine, hydrochloride salt
- sodium bicarbonate
- distilled water

- household vinegar

5. Equipment Setup

5.1 Clamp the Drop Counter to your retort stand and position it so it is above the Magnetic stirrer (**Figure 5.1**).

5.2 Place a 100 mL beaker containing distilled water on the magnetic stirrer.

5.3 Place the pH electrode into the holder on the Drop Counter so the tip is immersed in the distilled water.

5.4 Connect the cable from the Drop Counter into the Input 1 Pod Port on the front of your e-corder.

5.5 Connect the cable from the pH Pod into the Input 2 Pod Port on the front of your e-corder.

5.6 Attach the BNC connector on the pH Electrode to the socket on the rear panel of the pH Pod.

5.7 Mount a 50 mL burette in the burette clamp on your ring stand (**Figure 5.1**).

5.8 Adjust the position of the burette tip so that it is aligned with the alignment marks on the Drop Counter (**Figure 5.2**). The burette tip should be positioned 3-5 mm above the opening in the drop counter.

5.9 Close the stopcock on the burette.

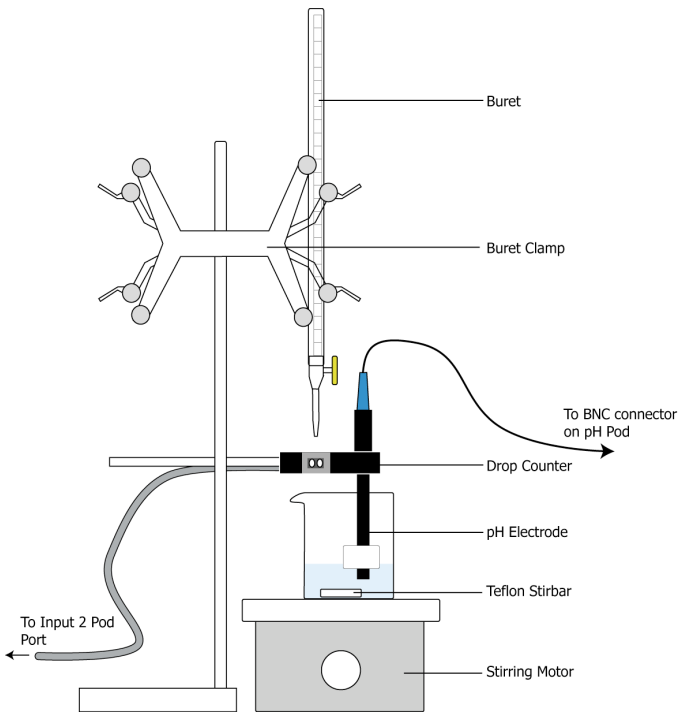


Figure 5.1 Equipment setup to perform the exercises in this experiment.

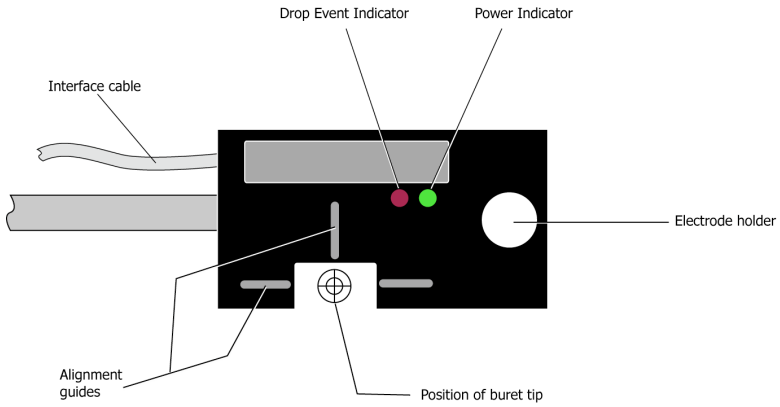


Figure 5.2 Align the burette tip using the guides on the top of the drop counter.

6. Procedure

6.1 Initial Calibration Steps

6.1.1 Remove the burette from the burette clamp.

6.1.2 Record the ambient temperature of the laboratory. For the best results, all reagents should be equilibrated to the laboratory temperature and the temperature in the laboratory should be held as constant as possible.

6.1.3 Select two Channels in the **Channel Settings** under the **Setup** menu. Set the Chart input range to 500 mV on Channel 2, which should be connected to the pH Pod. (This is usually sufficient for readings in the range pH 0–14). Select a 1 Hz low pass filter in the Input Amplifier of the pH Pod.

6.1.4 Set the sampling rate to 4 /s.

6.1.5 Click on **Trigger** under the **Setup** menu. Select a fixed duration block of 2 minutes, triggered by the user. This time should be sufficient for the pH electrode to equilibrate with the solution and obtain a steady signal.

6.2 Two Point pH Electrode Calibration

Two point pH calibrations can be performed with any two standard pH buffers, however buffers of pH 4 and 9 (or 10) are commonly used.

6.2.1 Pour approximately 10 mL of either the Phthalate buffer or the Borax buffer into the 20 mL beaker. Use **Table 2** in **Appendix A** to determine the pH of the solution at the temperature nearest to the laboratory temperature recorded in **6.1.2**.

6.2.2 Rinse the tip of the pH electrode thoroughly with distilled water and dry with lint free tissue.

6.2.3 Immerse the tip of the pH electrode in the buffer contained within the beaker.

6.2.4 Click on the Start button in Chart to begin recording a 2 minute block of data.

6.2.5 After recording the 2 minute block, select the block and use the **Add comment...** command under the **Command** menu and note the type of buffer and the pH.

6.2.6 Remove the electrode from the solution and dispose of the buffer. Rinse the beaker thoroughly and dry with lint free tissue.

6.2.7 Repeat **6.2.1** to **6.2.6** with the other pH buffer.

6.2.8 In the main Chart window, make a selection of data covering the two pH regions of interest (**Figure 6.1**), then choose the **pH...** command from the Channel Function pop-up menu. The pH dialog box will appear (**Figure 6.2**).

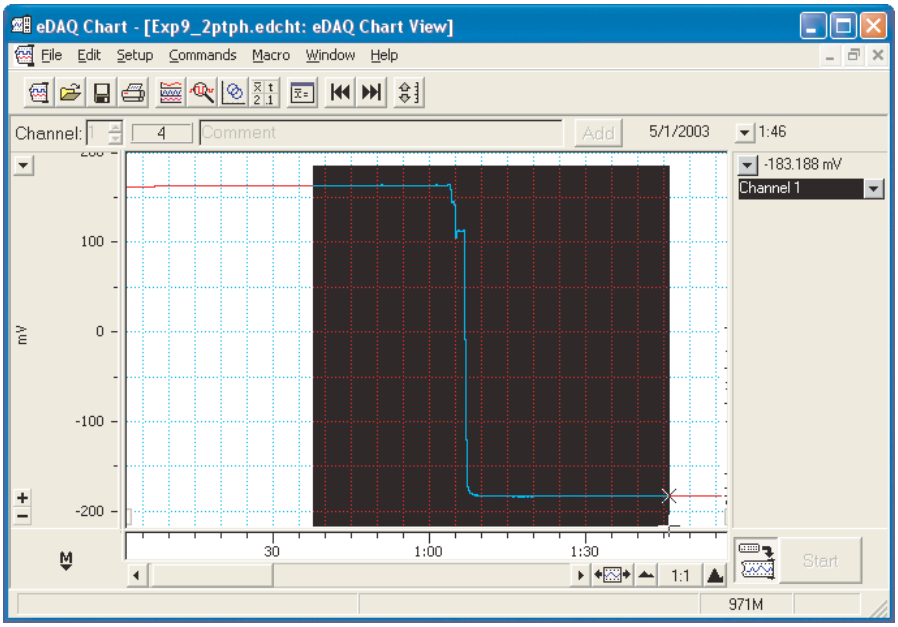


Figure 6.1 Selecting data from main Chart Window for two point pH calibration

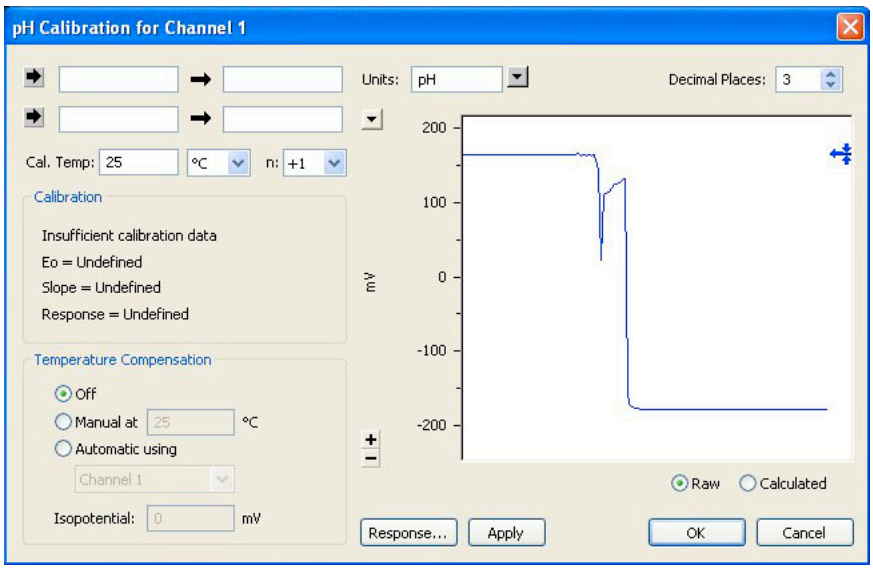


Figure 6.2 pH dialog Box

6.2.9 In the pH dialog box, choose pH as the unit, n = +1 and choose three decimal places, as the standards are known to three decimal places. Enter the laboratory temperature to the nearest 5 °C into the Temp box and make sure that the temperature compensation is off.

6.2.10 Select a region of the trace representative of the stabilised recording of the first buffer. Click the arrow button, to automatically enter the mean potential of the region into the adjacent box, then type in the pH value of the buffer into the linked box. The dialog box is updated assuming the electrode has 100 % Nernstian behaviour, and the E_0 value for the electrode is shown (**Figure 6.3**). This is the equivalent to a single point calibration. Click the **View Response...** button to view the View Response dialog box (**Figure 6.4**). Click OK to return to the pH dialog box.

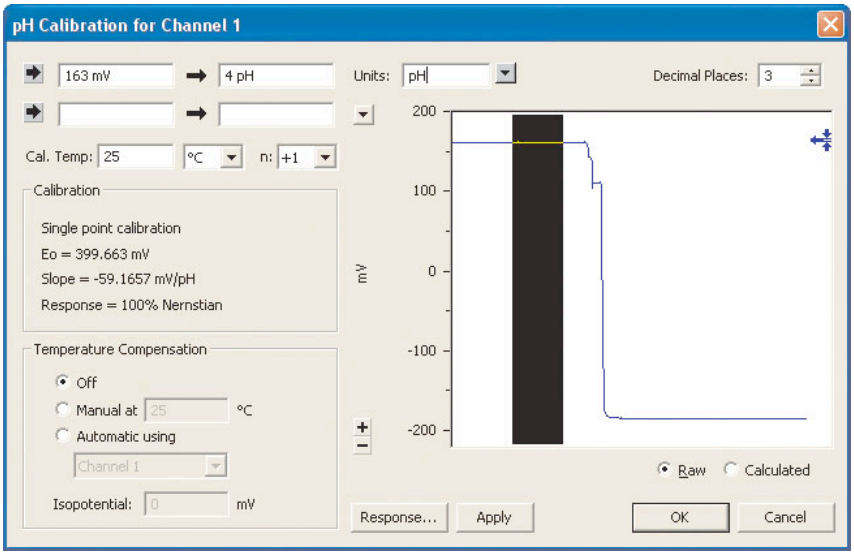


Figure 6.3 pH dialog box with single point calibration data

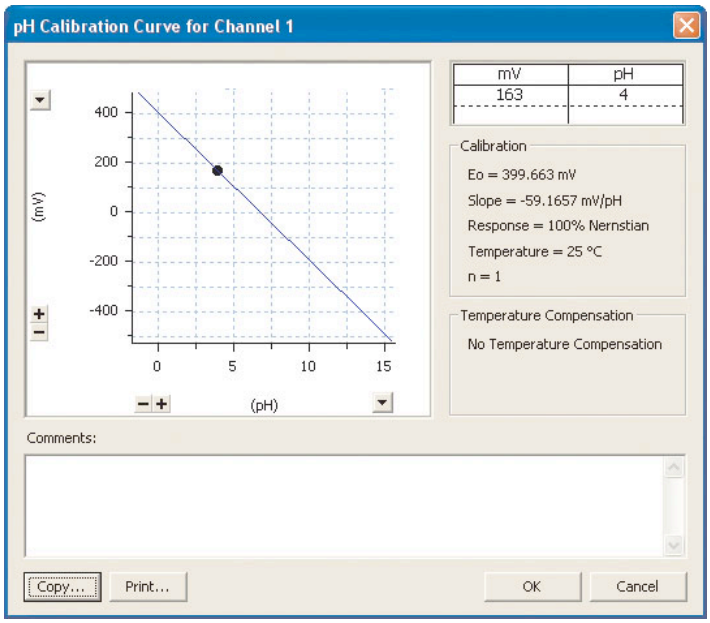


Figure 6.4 Calibration plot for single point calibration

6.2.11 For a two point calibration select a region of the trace that records the electrode response to the second buffer (**Figure 6.5**), enter the mean value into the lower text box, and type in the pH of the buffer in the linked box. The slope, E_0 and percentage Nernstian response of the electrode are displayed in the pH dialog box.

The View Response dialog box (**Figure 6.6**), now shows line fitted between the two points.

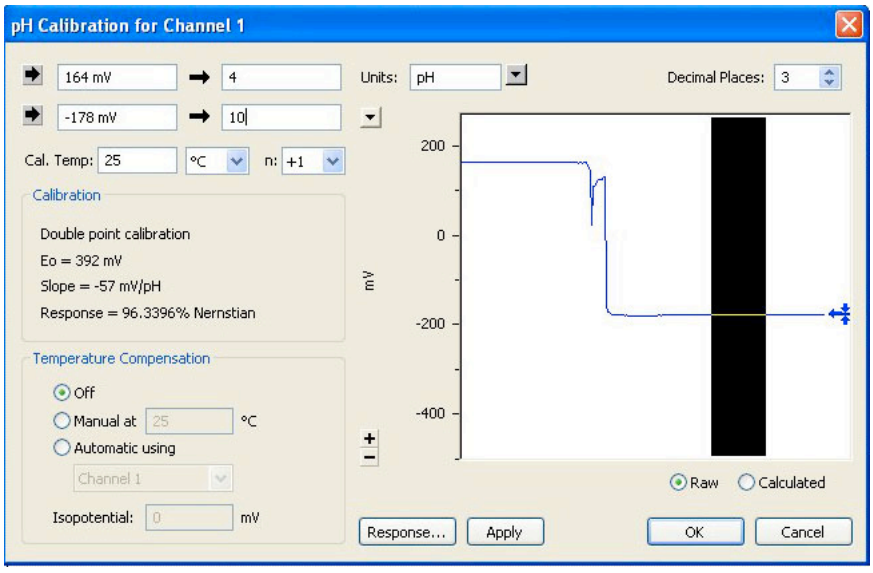


Figure 6.5 pH dialog box with two point calibration data

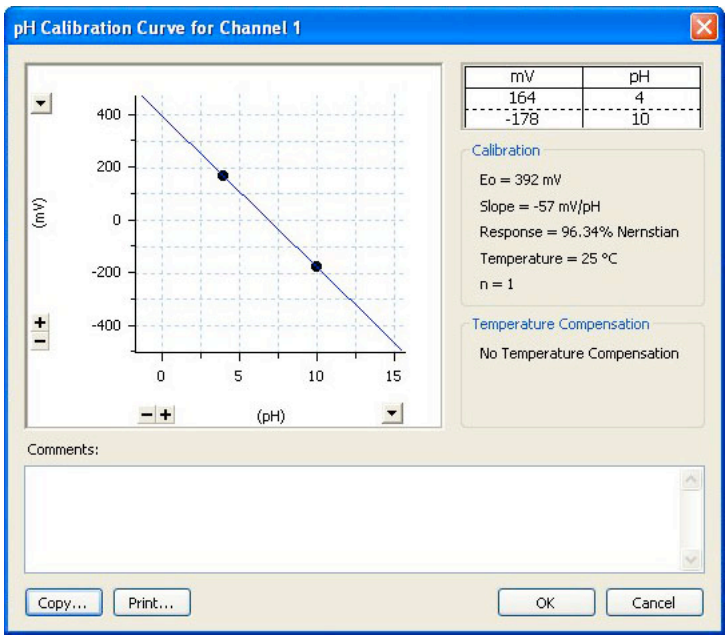


Figure 6.6 Calibration plot for two point calibration

6.2.12 Click the Apply button in the pH dialog box to show the calibrated pH values in the preview pane. Click OK to close the dialog box and apply the calibration to the whole channel (**Figure 6.7**). The pH of solutions can now be recorded directly on this channel.

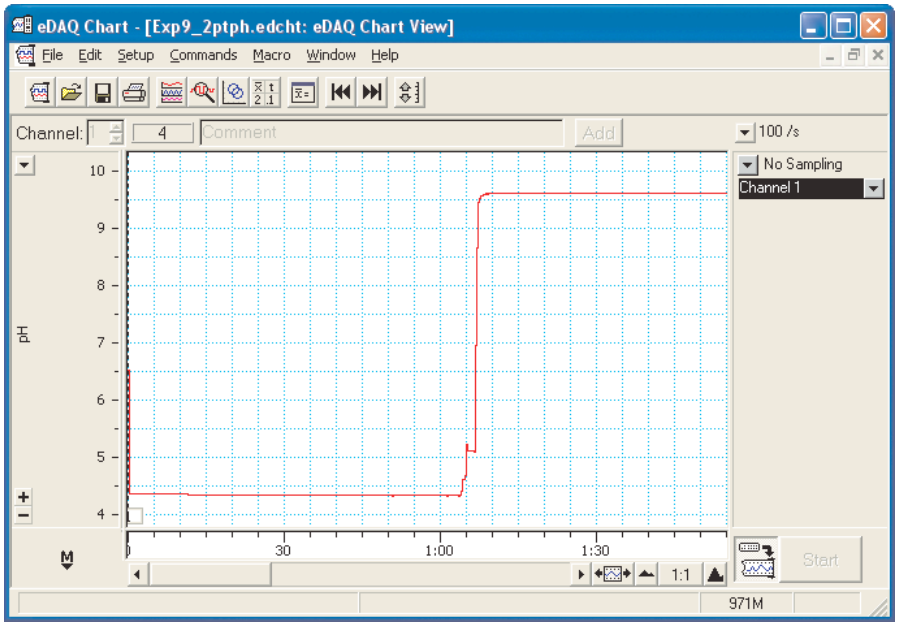


Figure 6.7 Chart channel displaying pH

6.3 Multipoint pH Electrode Calibration

Both single and double point calibration assume that the electrode has a linear response to ion activity. Experimentally there will always be some deviation from linearity in the response. If the deviation is systematic, a non-linear calibration technique can be used for accurate work.

6.3.1 Open up a new Chart file with the same data acquisition settings as those used in **Section 6.1**.

6.3.2 Record the pH electrode response to all the pH buffers listed in Table 1, Appendix A, and the 0.1 M and 1 M HCl and NaOH standards. Move from lowest to highest pH solution. After the last solution has been analysed, results similar to those shown in **Figure 6.8** should be obtained.

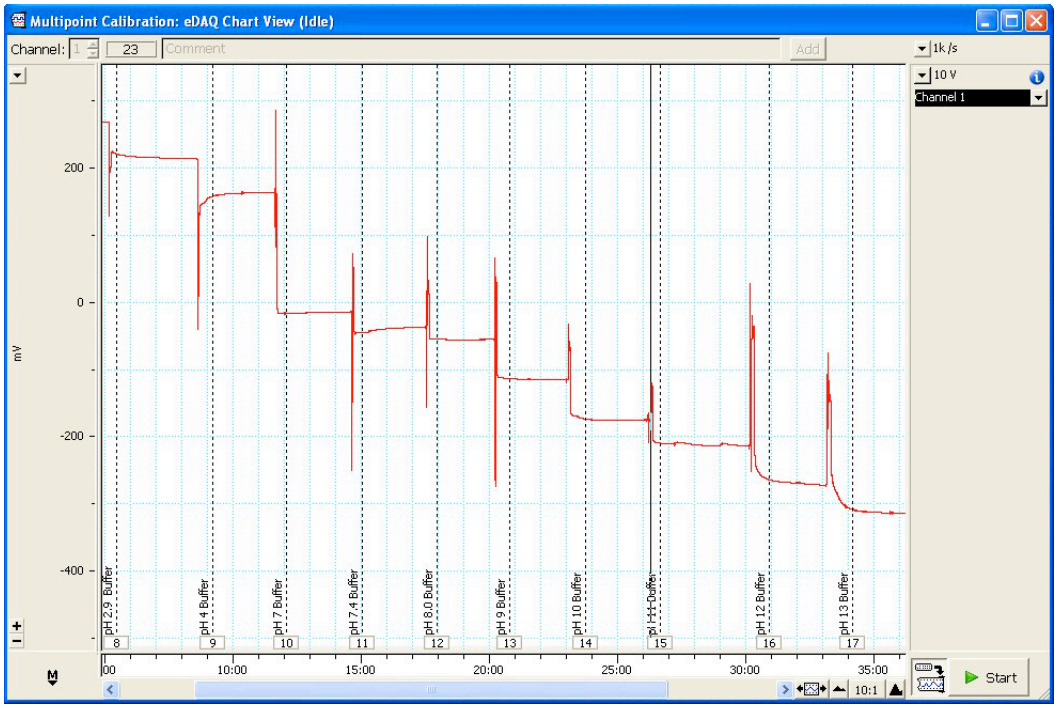


Figure 6.8 pH electrode response from a wide pH range

6.3.3 Make a selection of data covering all the signals recorded for all the pH buffers, then choose the **MPCalibration...** command from the Channel Function pop-up menu. The Multipoint Calibration dialog box will then appear (**Figure 6.9**).

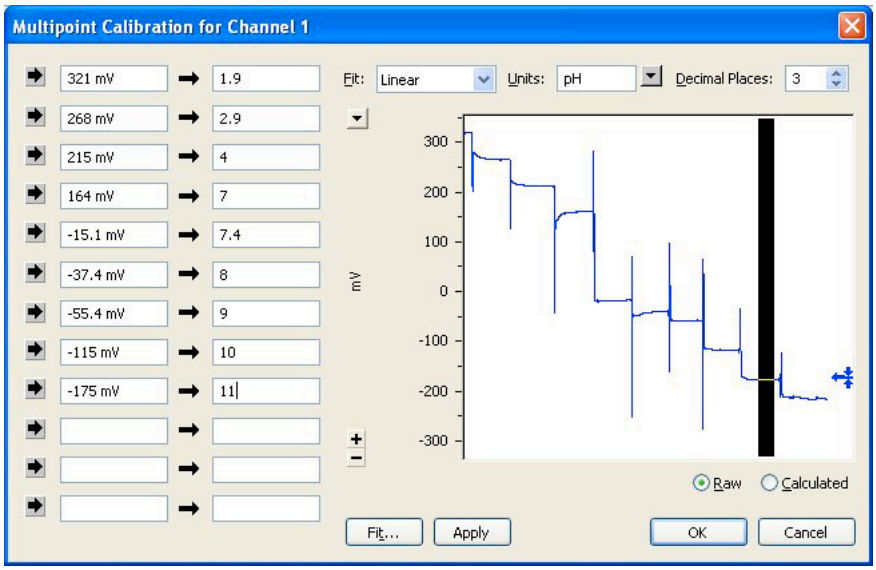


Figure 6.9 Multipoint Calibration dialog box

6.3.4 Select linear fit, pH as the measurement unit and set to three decimal places.

6.3.5 In the same manner as Steps **6.2.10** & **6.2.11**, select a representative proportion of the signal for each of the buffers in turn, and click on the arrow of the next available box to enter the average signal. Then manually enter the pH values in the linked boxes.

6.3.6 Click on Fit button to bring up the Fit dialog box (**Figure 6.10**).

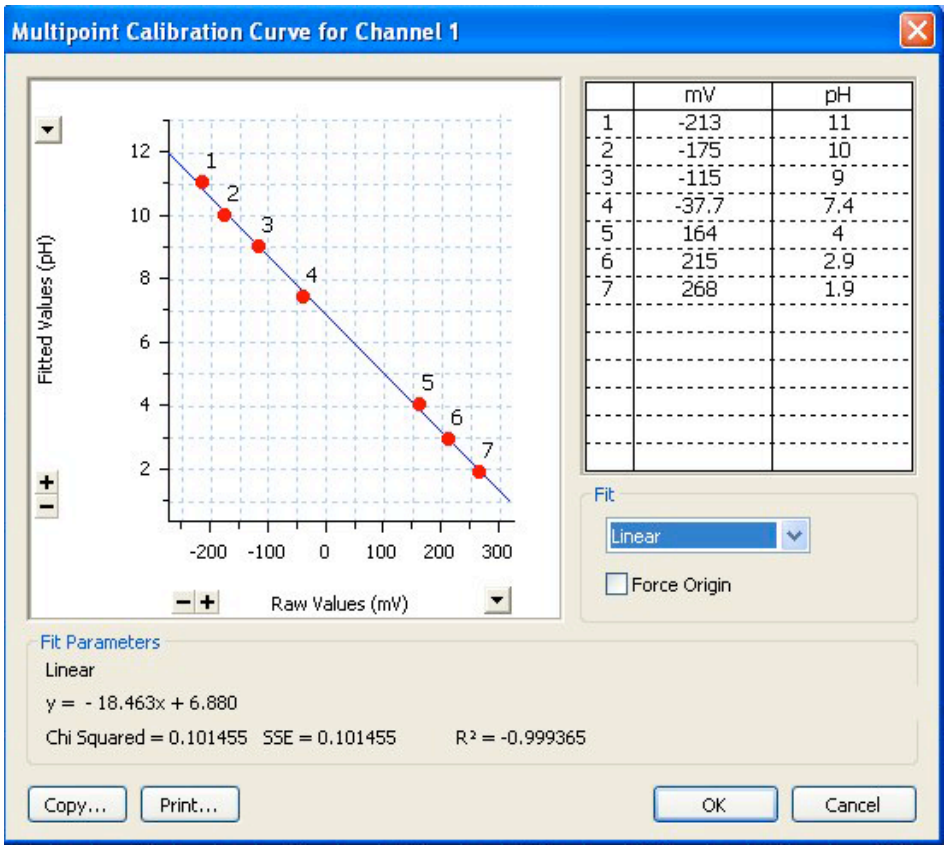


Figure 6.10 Multipoint Calibration fit dialog box

6.3.7 Initially perform a linear fit. Copy the graph to paste into a report or print. Experiment with different fitting functions.

6.3.8 Return to the linear fit and compare the slope of the curve to the theoretical Nernstian response. Note that the slope of the equation in the Multipoint Calibration Fit window is in pH. V^{-1}. Convert the slope to $V.pH^{-1}$ to calculate the percent Nernstian response.

$$\text{Hint: Theoretical Nernstian Slope} = \frac{2.3RT}{nF}$$

6.3.9 Click OK to return to the Multipoint Dialog box, then Click the Apply button to show the calibrated pH values in the preview pane. Click OK to close the dialog box and apply the calibration to the whole channel. The pH of solutions can now be recorded directly on this channel.

6.4 Calibrating the Drop Counter

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

6.4.1 In the **Setup** menu, turn off fixed duration recording.

6.4.2 Connect the Drop Counter to Input 1 of the e-corder, using the Pod port.

6.4.3 Fill your burette completely with tap water.

6.4.4 Place a beaker under the drop counter and open the stopcock on the burette to allow water to fill the tip. Slowly run about 5mL of water through your burette to remove any air bubbles and then close the stopcock.

6.4.5 Remove the beaker and refill your burette. You are now ready to calibrate the Drop Counter.

6.4.6 Weigh a clean, dry 125mL beaker and record the mass in **Table 7.4** of the results section. Next, place the 125mL beaker beneath the burette and drop counter. Leave the pH electrode in its beaker of distilled water, and out of the way of the burette tip, for now.

6.4.7 In the Channel Function drop down menu for Channel 1, select **Computed Input...** , which opens the Computed Input dialog box.

6.4.8 Select Counter from the Function menu of the Computed Input dialog box (**Figure 6.11**). Drag the Baseline tracking slider to Off, set the voltage range to 5 V and the count range to 2000 counts.

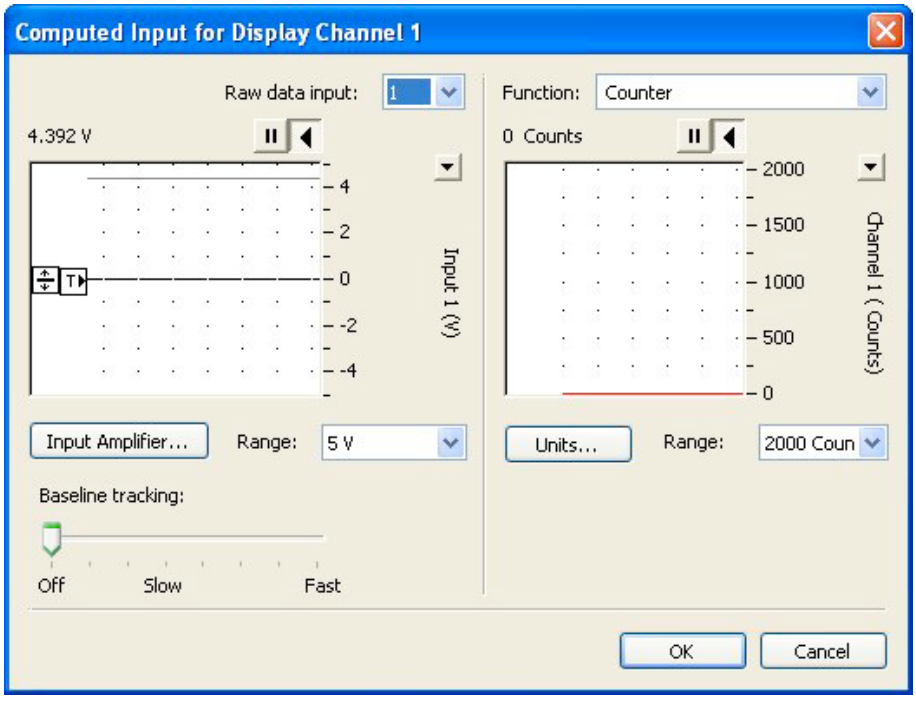


Figure 6.11 Computed Input dialog box with Counter selected

6.4.9 Carefully open the stop cock of the burette so a steady stream of drops (~ 1 – 2/s) fall past the sensor of the drop counter into the beaker. Adjust the threshold slider of the Computed Input dialog of Channel 1 to a position which intersects with the drop counter signal when a drop passes through the sensor (**Figure 6.12**). The Drop counter has an output voltage of approximately 4 V, which drops to 0 V as the drop passes through the sensor. Click on OK to return to the main Chart window.

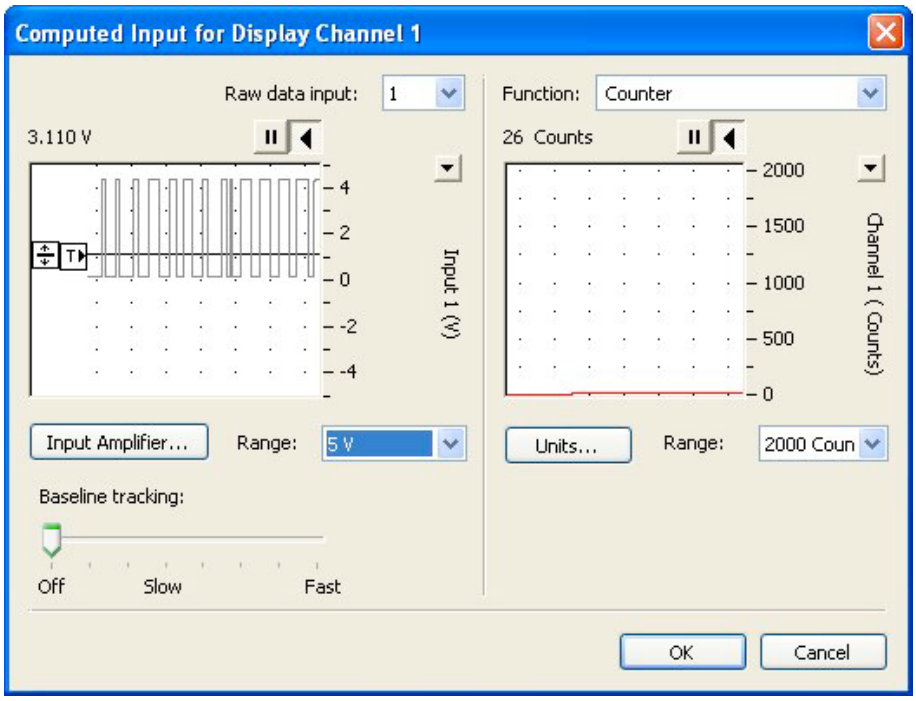


Figure 6.12 Adjusting the count threshold

6.4.10 Click on **Start** to begin recording the number of drops that pass through the counter. Pass approximately 20 mL through the counter.

6.4.11 Re-weigh the beaker that collected the drops and subtract the dry-weight measured in **6.4.6**, to determine the mass of water collected.

6.4.12 Convert the mass of the water collected into volume and divide by the number of drops counted in **6.4.9** to obtain the average volume of each drop.

6.4.13 Re-open the Computed Input dialog and click on the Units button to open Units Conversion for Channel 1 (**Figure 6.13**). Define mL as a unit and select to record to 3 decimal places. For Point 1 enter 0 counts and 0 mL and for point 2 enter 1 count and the volume calculated in **6.4.12**. Channel 1 is now calibrated to record directly in mL.

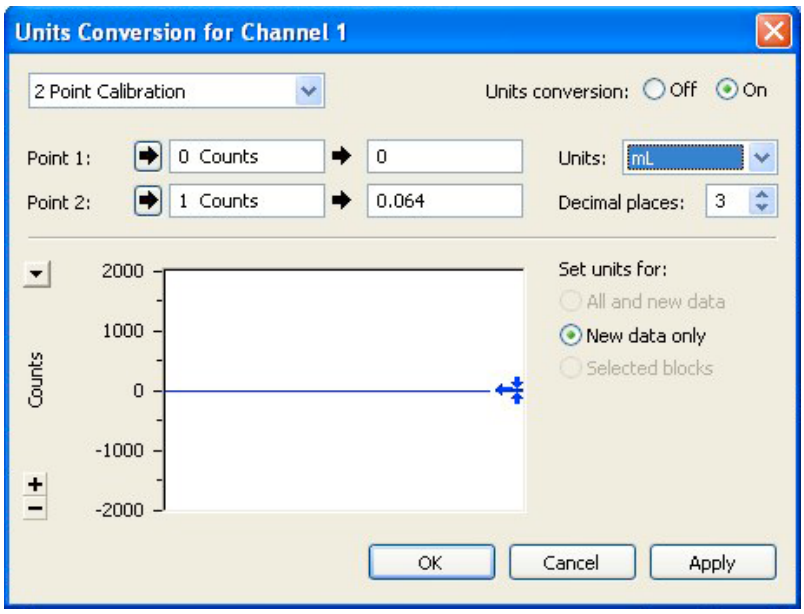


Figure 6.13 Units Conversion dialog box

6.5 A Simple Acid-Base Titration

In this exercise, you will titrate the unknown HCl solution with 0.1 M NaOH to determine the equivalence point and concentration.

6.5.1 Drain the excess water from the burette then rinse with approximately 10 mL of 0.1 M NaOH.

6.5.2 Close the stopcock and fill the burette with 0.1 M NaOH. Partially drain the burette into a beaker labelled "alkali waste" so that there are no bubbles trapped in the burette tip.

6.5.3 Obtain a clean, dry 250mL beaker. Place a Teflon stir bar into the beaker.

6.5.4 Pipette 50 mL of the unknown HCl solution into your beaker, and then place the beaker on the magnetic stirrer underneath the burette.

6.5.5 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.

6.5.6 Turn on the magnetic stirrer, and set it to a slow speed.

6.5.7 In Chart, click **Start**.

6.5.8 Enter a comment called "HCl titration" into your data trace.

6.5.9 Slowly open the stopcock on your burette until you get a drop rate of 1-2 drops per second.

6.5.10 Continue recording until the burette is completely empty.

6.5.11 When the burette is empty, click **Stop**.

6.5.12 Close the stopcock on your burette.

6.5.13 Save your data with an appropriate filename, such as "HCl Titration".

6.5.14 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.

6.5.15 Click the **X-Y Plot** button in the Chart toolbar.

6.5.16 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.

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

6.5.18 Record the volume of NaOH added to reach the equivalence point in **Table 7.5** of the results section.

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

$$\text{moles NaOH} = V_{NaOH} \times M_{NaOH} \qquad (6.1)$$

$$M_{HCl} = \frac{\text{moles NaOH}}{V_{HCl}} \qquad (6.2)$$

Where V = volume, in mL

M = molarity, in $mol.L^{-1}$

6.6 Determining the Concentration of Acetic Acid in Household Vinegar

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

6.6.1 Click the "New Chart Document" button in the Chart Toolbar Menu. Choose "Use the current settings" and make sure you have saved any previous data.

6.6.2 Pipette 5mL of household vinegar into a 100mL volumetric flask.

6.6.3 Fill the flask to the fill line with distilled water. This represents a 1:20 dilution of the vinegar.

6.6.4 Fill your burette with 0.1M NaOH standard. Make sure there are no air bubbles in the burette tip; use your waste beaker if necessary to drain air bubbles from the tip.

6.6.5 Place the Teflon stirrer bar into a clean 250mL beaker, and pour 50mL of diluted vinegar into it.

6.6.6 Place the beaker under the burette; rinse the pH electrode and place it into the diluted vinegar solution.

6.6.7 In Chart, click Start. Enter a comment called "Vinegar" into your data trace.

6.6.8 Slowly open the stopcock on your burette until you get a drop rate of 1-2 drops per second.

6.6.9 Continue recording until the burette is completely empty. When the burette is empty, click **Stop**.

6.6.10 Close the burette stopcock.

6.6.11 Save your data with an appropriate filename, such as "Vinegar Titration".

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

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

6.6.14 In the X-Y window, click the number 1 box on the x-axis and the number 2 box on the y-axis.

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

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

6.6.17 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.

$$M_{Hac} = \frac{\text{moles NaOH}}{V_{Hac}} \times 20 \qquad (6.3)$$

6.7 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

6.7.1 Click the "New Chart Document" button in the Chart Toolbar Menu. Choose "Use the current settings" and make sure you have saved any previous data.

6.7.2 Place 100mL of distilled water into a clean 250mL beaker and add a Teflon stir bar.

6.7.3 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.

6.7.4 Pipette 1.3 mL of 1.0M HCl into your beaker.

6.7.5 Fill your burette with 0.1M NaOH standard, and make sure there is no air in the burette tip.

6.7.6 Make sure the stopcock on the burette is closed, and place the beaker on the magnetic stirrer beneath the burette.

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

6.7.8 Turn on the magnetic stirrer at a slow speed.

6.7.9 Click **Start** to begin recording.

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

6.7.11 Continue recording until your burette is empty.

6.7.12 Close the burette stopcock and click **Stop** to end your data collection.

6.7.13 Remove the pH electrode from the beaker, rinse it, and return it to the beaker of distilled water.

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

6.7.15 Click the **X-Y plot** button in the Chart Toolbar.

6.7.16 In the X-Y window, select Channel 1 for the x-axis and Channel 2 for the y-axis.

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

6.7.18 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 pKa value. This is the pH at which the concentrations of conjugate base and undissociated acid are equal.

6.7.19 Click on OK to return to the main Chart window. Select **Channel Settings...** from the **Setup** menu and open a third channel.

6.7.20 In the **Channel functions** drop down menu of the new Channel, select **Derivative** with Channel 2 as the source channel, a window width of 15 points and 1st derivative selected. Adjust the axes of Channel 3 so the differential peaks are optimally displayed.

6.7.21 Place the cursor onto the first major peak on Channel 3 and record the volume of NaOH added and record as V1 in **Table 7.7**. Similarly record the volume at the second peak as V2.

6.7.22 Subtract V1 from V2 to get V3

6.7.23 Divide V3 by 2 to get V4

6.7.24 Add V4 to V1 to get V5. This will be the midpoint estimate.

6.7.25 Move the waveform cursor along the NaOH volume data until the number displayed is as close as possible to the midpoint estimate. Record the corresponding pH value, which is the pKa value of the buffer.

Examining the Carbonic Acid/ Bicarbonate Buffer System

6.7.26 Remove the burette from its holder.

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

6.7.28 Place the beaker on the magnetic stirrer, rinse the pH electrode tip and place the electrode into the beaker of distilled water.

6.7.29 Turn the magnetic stirrer on and set it to a slow speed.

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

6.7.31 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.

6.7.32 Click **Stop**, and enter the observed pH changes into **Table 7.8**.

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

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

6.7.35 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".

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

6.7.37 Click Stop to end your recording.

6.7.38 Save the data with an appropriate filename, such as "Bicarbonate data" and record your observations into **Table 7.8**.

6.8 The Potentiometric Titration Curve of L-Histidine

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

6.8.1 Click the **"New Chart Document"** button in the Chart Toolbar Menu. Choose **"Use the current settings"** and make sure you have saved any previous data.

6.8.2 Weigh out 0.1g of L-histidine HCl in a weigh boat. Using the electronic balance.

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

6.8.4 Add the histidine HCl to your beaker.

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

6.8.6 Use a pipette to transfer 3.0mL of 1M HCl to the beaker.

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

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

6.8.9 Turn on the magnetic stirrer to a slow speed. Do not proceed to the next step until you are sure the histidine is fully dissolved.

6.8.10 In Chart, click **Start**.

6.8.11 Enter a comment to your recording called "Histidine".

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

6.8.13 Continue recording until you reach a pH of at least 11.5. If you are using a 25mL.

6.8.14 When the pH of your solution is 11.5, close the stopcock on your burette.

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

6.8.16 Determine V1, V2 and V3 for the three peaks you should have obtained on Channel 3. Estimate pKa_1 as the pH at 1/2 V1 and record in **Table 7.9**.

6.8.17 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 **6.7**.

7. Results

7.1 Laboratory Temperature = _____

7.2 Two Point pH Electrode Calibration

E_0	
Slope ($mV.pH^{-1}$)	
Nernstian response	

7.3 Multipoint pH Electrode Calibration

Nernstian Response (full pH Range) = _____

7.4 Drop Counter Calibration

Mass of empty beaker (g)	
Mass of full beaker (g)	
Mass of water in beaker (g)	
Volume of water (mL)	
Number of drops	
Average drop volume (mL)	

7.5 Results of Simple Acid Base Titration

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

7.6 The concentration of acetic acid (Hac) in 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)	

7.7 The determination of the pKa value of bicarbonate

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

7.8 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				

7.9 pKa values for histidine

V1 (mL)	
V2 (mL)	
V2 (mL)	
1/2 V1 (mL)	
Midpoint V1 and V2 (mL)	
Midpoint V2 and V3 (mL)	
pKa_1	
pKa_2	
pKa_3	

8. Questions

8.1 Comment on the Nernstian response range of a the pH electrode tested.

pH electrodes typically have a Nernstian response between pH 1 and 14, which is a 13 orders of magnitude range. pH electrodes can analyse solutions with a very wide range of hydronium ion concentrations.

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

$pH = -\log [1.8 \times 10^{-3}] = 2.8$

8.3 A solution has a hydroxyl concentration of 3.4×10^{-8}M. What is the solution's hydronium ion concentration? What is the solution's pH?

3.4×10^{-8} M x $[H_3O^+] = 10^{-14}$

$[H_3O^+] = 2.9 \times 10^{-7}$

$pH = -\log [2.9 \times 10^{-7}] = 6.5$

8.4 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 6.7.

CO_2 reacts with water H_20 to form carbonic acid H_2CO_3, which dissociates to H^+ + HCO_3^-, increasing the hydronium ion concentration in solution, and hence pH.

8.5 What compound is formed when you exhale into water?

H_2CO_3 Carbonic acid.

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

When exhaling into water, the pH should have dropped rapidly and the pH value should have been slow to recover. When exhaling into the bicarbonate, the pH did not fall as rapidly and the initial pH value should have recovered after exhalation stoped.

In the bicarbonate solution, the following equilibrium conditions are established:

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ with a pKa of around 7.

When CO_2 is dissolved in solution, the HCO_3^- concentration increases and to establish equilibrium the reverse reaction is favoured, increasing pH.

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

Most physiological reactions occur at pH 7, which corresponds to the maximum buffering capacity of the bicarbonate system.

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

Histidine has three pKa values.

pKa_1 = 1.82 (ionisation of acidic carboxyl group)

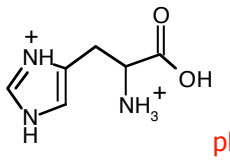
pKa_2 = 9.17 (ionisation of basic amine group)

pKa_3 = 6 (ionisation of acidic side group)

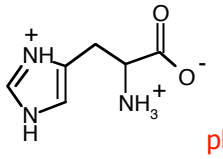
A histidine solution could be used as a buffer over a narrow pH band 1.82, 6 and 9.17.

8.9 Draw the structure of histidine as it appears at:

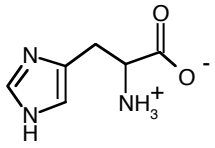
pH 0 pH 4 pH 7.5 pH 10.5



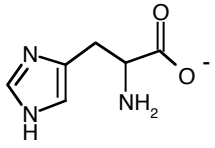
pH 0



pH 4



pH 7.5



pH 10.5

9. Appendix A

Table 1 NIST Standard pH Buffers at 25 °C		
pH		Preparation
3.776	Citrate (0.05 m)	Dissolve 11.41 g of potassium dihydrogen citrate in water and dilute to 1 L. Before weighing, dry the crystals at 80 °C for 1 h and cool in a desiccator.
4.008	Phthalate (0.05 m)	Dissolve 10.12 g of potassium hydrogen phthalate in water and dilute to 1 L.
6.865	Phosphate (1 + 1) Each salt is 0.025 m	Dissolve 3.388 g of potassium dihydrogen phosphate and 3.533 g of anhydrous disodium hydrogen phosphate in water and dilute to 1 L. Before weighing, dry each salt for 2 h at 110–130 °C and allow to cool in a desiccator
7.413	Phosphate (1 + 3.5) 0.008695 m KH_2PO_4 0.03043 m Na_2HPO_4	Dissolve 1.179 g of potassium dihydrogen phosphate and 4.302 g of anhydrous disodium hydrogen phosphate in water and dilute to 1 L. Before weighing, each salt should be dried in the same manner as the 6.865 buffer.
9.180	Borax (0.01 m)	Dissolve 3.80 g of sodium tetraborate decahydrate (borax) in carbon dioxide free water and dilute to 1 L
10.012	Carbonate (1 + 1) Each salt is 0.025 m	Dissolve 2.092 g of sodium hydrogen carbonate and 2.640 g of sodium carbonate in carbon dioxide free water and dilute to 1 L. Ignite the sodium carbonate at 270 °C for 1 h before use and cool in a desiccator
12.45	Calcium hydroxide (saturated)	Mix 2 g of CaO with 1 L of carbon dioxide free water to form a saturated $Ca(OH)_2$ solution. Filter and collect the supernatant.

NB: pH buffers above pH of 6 should be freshly prepared to prevent CO_2 contamination and algae growth. Store in sealed bottles to increase the shelf life of the buffer.

Table 2 Standard Values of pH Buffer Solutions at Temperature 0 – 95 °C

Temp °C	Citrate 0.05 m	Phthalate 0.05 m	Phosphate (1 + 1)	Phosphate (1 + 3.5)	Borax 0.01 m	Carbonate (1 + 1)	$Ca(OH)_2$ Sat'd
0	3.863	4.003	6.984	7.534	9.464	10.317	13.423
5	3.840	3.999	6.951	7.500	9.395	10.245	13.207
10	3.820	3.998	6.923	7.472	9.332	10.179	13.003
15	3.802	3.999	6.900	7.488	9.276	10.118	12.810
20	3.788	4.002	6.881	7.429	9.225	10.062	12.627
25	**3.776**	**4.008**	**6.865**	**7.413**	**9.180**	**10.012**	**12.454**
30	3.766	4.015	6.853	7.400	9.139	9.966	12.289
35	3.759	4.024	6.844	7.389	9.102	9.925	12.133
40	3.753	4.035	6.838	7.380	9.068	9.889	11.984
45	3.750	4.047	6.834	7.373	9.038	9.856	11.841
50	3.749	4.060	6.833	7.367	9.011	9.828	11.705
55	.....	4.075	6.834	.....	8.985	.....	11.574
60	.....	4.091	6.836	.....	8.962	.....	11.449
70	.....	4.126	6.845	.....	8.921	.....	.....
80	.....	4.164	6.859	.....	8.885	.....	.....
90	.....	4.205	6.877	.....	8.850	.....	.....
95	.....	4.227	6.886	.....	8.833	.....	.....

All buffers listed in Tables 1 & 2, except for the pH 12.45 buffer, were reported in Midgley *et al* (1991). The 12.45 buffer was reported in Weast (1997).

10. Bibliography

D.A. Skoog, F.J. Holler and T.A. Nieman, *Principle of Instrumental Analysis, 5th ed* (1998), Saunders College Publishing.

D. Midgley and K.Torrance, *Potentiometric Water Analysis, 2nd ed* (1991), John Wiley & Sons Ltd.

R.C. Weast, *CRC Handbook of Chemistry and Physics, 57th ed* (1997), CRC Press.