pH and Buffers Laboratory

Introduction

The control of pH is important in organisms and their cells because chemical reactions and processes are affected by the hydrogen ion concentration. For example, enzyme function is affected by the ambient pH. Some side chains of amino acids (R-groups) have ionizable groups like carboxyl or amine groups. Changes in pH can alter the number of positively and negatively charged groups. The net charge on the protein effects its three dimensional structure and thus the enzymatic activity.

When working with living systems in a laboratory it is necessary to pay close attention to the pH of solutions for these same reasons. Buffers are chemicals or combinations of chemicals that tend to prevent changes in the concentration of hydrogen ions. In this laboratory we will titrate some buffered solutions to gain an understanding of how buffers work and to observe the range of buffering capacity. In analyzing the data, we will make use of graphs and see the relationship of pH to pK_a using the Henderson-Hasselbalch equation.

Buffers are composed of mixtures of weak acids and their corresponding salts. Using the Lowry-Bronstead definition, an acid is a compound that can donate a hydrogen ion. A weak acid is one that does not completely ionize, or dissociate, in solution. The extent of dissociation is given by the equilibrium constant K_a for the reaction:

$$HA \leftrightarrow H^+ + A^-$$

The equilibrium constant for the ionization of this acid is then:

$$K_a = [H^+][A^-]/[HA]$$

This is the measure of the ease with which the acid donates its hydrogen ion. Higher K_a's indicate that the acid will dissociate more completely into ions.

The equilibrium constant equation may also be used to determine the hydrogen ion concentration:

$$[H^+] = (K_a)([HA]/[A^-].$$

And, since $pH = -\log[H^+]$ and
$pK_a = -\log K_a$, then,

$$pH = pK_a + \log([A^-]/[HA])$$

This last equation is the Henderson-Hasselbalch equation. It reveals the relationship of pH and pK_a. Remember that pH is a measure of the acidity of a solution while pKa is a measure of the affinity of a molecule for its proton. The former can be altered by various means, but the latter is an inherent property of a molecule. See the following table for pK_a's of some biologically important substances.

pK_a Values of selected acids at 25°C

Acetic acid	4.76		
Carbonic acid	6.36	10.24	
Citric acid	3.13	4.76	6.40
Glutamic acid	2.16	4.27	9.36
Glycine	2.35	9.77	
Lysine	2.18	8.95	10.53
Phosphoric acid	2.18	7.20	12.40
Succinic acid	3.46	5.10	

The Henderson-Hasselbalch equation permits the calculation of the pH from the ratio of the salt to the acid. Notice that the pH is equal to the pK_a when the salt and the acid are equal in concentration. If H^+ is added to such a solution, the acid concentration increases while the salt concentration decreases and the pH is lowered. Conversely, if OH^- is added, the salt concentration increases while the acid concentration decreases and the pH of the buffer mixture is raised.

The assignment of the appropriate pK_a to the correct step in the ionizaton of a polybasic acid can be thought of in the following terms. The first H^+ to be donated by an acid is the one which is most weakly bound and is the one which will be released

at the lowest pK_a. This first step, therefore, is the one with the lowest pK_a. All subsequent hydrogen ions that ionize from the acid will be released at higher pH's and these successive equilibria will occur with successively higher pKa's. For example, consider the case of phosphoric acid:

$$H_3PO_4 \leftrightarrow H^+ + H_2PO_4^{-1} \quad pK = 2.18$$
$$H_2PO_4^{-1} \leftrightarrow H^+ + HPO_4^{-2} \quad pK = 7.20$$
$$HPO_4^{-2} \leftrightarrow H^+ + PO_4^{-3} \quad pK = 12.40$$

When the concentration of H_3PO_4 equals the concentration of $H_2PO_4^{-1}$, then pH = pK_a = 2.18, which is a very acidic solution. When the concentration of HPO_4^{-2} equals the concentration of PO_4^{-3}, then pH = pK_a = 12.40, which is a very basic solution. The first H^+ ionizes in the acid solution; the last H^+ is not removed until the solution is very basic or low in H^+ concentration.

Hydrogen ion concentration is usually measured with a pH meter, a glass electrode and a reference electrode. The pH meter is a potentiometer, capable of accurately measuring small electrical potential differences. The glass electrode consists of a thin bubble of soft glass that contains a solution of KCl and acetic acid in which a platinum wire is immersed. An electrical potential is developed across the glass bubble, which is proportional to hydrogen ion concentration. The reference electrode is simply used as a standard against which the glass electrode can be compared. In practice, the pH meter and electrodes are calibrated against a buffer solution of known pH and potential differences are read directly in units of pH.

Overview of the Lab Exercise

First you will learn about the general operating techniques used with a pH meter and calibrate the meter at pH 10. Then a 20 ml sample of Na_3PO_4 will be titrated after setting up the burette, the stirrer and the electrode. The meter will be recalibrated twice with pH 7 and later pH 4 standard buffers as the pH of the phosphate solution drops. After the phosphate titration, either glycine or glutamate will be titrated. Because these solutions are near neutral pH, the pH meter will be calibrated at first with the pH 7 standard buffer. Also, to cover the entire pH scale, two samples must be titrated, one with HCl and the second with NaOH.

Operation of the pH Meter

1. The pH meter should already be plugged in. The FUNCTION switch should be on STANDBY and the electrode should be immersed in a soaking solution, either distilled water or buffer.
2. Check to see that the TEMPERATURE adjustment knob is set at room temperature.
3. Check to see if there is electrode-filling solution nearly filling the entire electrode.
4. Open the fill-hole by rotating a narrow ring near the top of the electrode.
5. Whenever you want to move the electrode from one solution to another to calibrate the meter or make a pH measurement, you must go through the following steps.
   A. The FUNCTION switch should be on STANDBY.
   B. The electrode must be thoroughly rinsed with distilled water from a squirt bottle. A waste container should be present to catch the water.
   C. The excess water should be dabbed dry from the bottom of the electrode with a lab wipe. The electrode should not be wiped dry.
   D. The electrode can then be placed in the new solution and the FUNCTION switch turned to pH.
   E. When you return the electrode to the soaking solution or to another solution these rinsing procedures must be repeated to prevent any contamination and erroneous pH measurements.
   F. In general, do not leave the electrode out of a solution.

Procedures for the Sodium Phosphate Titration

1. Calibrate the meter in the range you will be using it. Initially you will be measuring the pH of 0.1M Na_3PO_4. The pH of this solution is high, so you will need to calibrate the meter using a standard buffer of pH 10 or 11.
2. Place the electrode (using the procedures above) into a beaker of the standard buffer. Turn the FUNCTION switch to pH. Turn the STANDARDIZE knob until the correct pH is reached on the meter. For this lab you will be doing a "one-point" calibration so you will not need to use the SLOPE knob. You can now return the calibrated electrode to the soaking solution.
3. Set up a burette in a burette clamp on your lab bench. Fill the burette carefully with 0.1M HCl. Use a funnel and put on SAFETY GLASSES. Fill the burette a little above the top of the scale. This will permit you to get air bubbles out of the tip and practice with the stopcock as you release the excess HCl into the waste beaker. Bring the meniscus to the zero line at the top of the burette.
4. Put exactly 20 ml of Na_3PO_4 into a 50 ml beaker. Add a small stirring bar.
5. Assemble the pH meter, the magnetic stirrer and the burette together on your lab bench. Put the Na_3PO_4 beaker in the middle of the stirrer. Put the electrode into the beaker (after rinsing) and move all of the pieces around so that the burette will be able to dispense the HCl into the beaker also. This takes a little creativity, but saves time in the long run.
6. Once you have all the parts arranged and the stirring bar rotating, turn the FUNCTION knob to pH. Record the pH.
7. Add exactly 2 ml of HCl. After the meter has stopped changing, record the pH.
8. Repeat step 7 keeping record of the total number of milliliters added and the pH. At some point the 50 ml beaker will be very full. When this occurs, turn the meter to STANDBY and remove the electrode. Pour all of the solution from the 50 ml beaker into a 100 ml beaker, including the stirring bar. Reinsert the electrode and continue your measurements.
9. Continue until the pH is about 8 to 9. At this time you need to re-calibrate as you are leaving the accurate range of the standard buffer you initially used. So, remembering to use proper rinsing procedures, recalibrate the electrode using the standard pH 7 buffer.
10. Return the electrode to the Na_3PO_4 solution first taking a measurement without adding any additional HCl. If the measurement is different from the last measurement, you will need to average these values when you graph your data.
11. When the pH begins to leave the accuracy range of the pH 7 standard buffer, you will need to re-calibrate the electrode one more time, just as above, but with the standard pH 4 buffer.
12. You will know when you are finished adding HCl when the pH begins to change very little. This should occur after 60 to 70 ml of HCl have been added.
13. You may replace the electrode in the soaking solution and empty the beaker of the phosphate in the sink, being careful not to lose the stirring bar. Rinse both the 50 and 100 ml beakers and stirring bar with tap water, followed by a little distilled water and then dry them.

Procedures for the Glycine and Glutamate Titrations

Depending on the directions from your lab instructor, you may titrate either glycine or glutamate or both. The procedures are very similar to those for sodium phosphate. However, because both glycine and glutamate solutions have initial pH's that fall in the middle of the pH scale rather than near the top of the scale, there are some changes that need to be made.

1. You will want to calibrate your electrode initially with the standard pH 7 buffer.
2. Also, because you want to measure the pH change over the entire scale, you will need to run two separate samples for both glycine and

glutamate. Note that in the following directions glycine will be used for the example; the same procedures will be used for glutamate.

3. Put exactly 20 ml of 0.1M glycine into the 50 ml beaker.
4. Measure the pH of your sample. You will see that the pH changes dramatically at the beginning, so you must remember to recalibrate your meter to a pH of 4 shortly after adding a little of the HCl.
5. Also, you should add only very small aliquots of HCl initially. One pattern you could follow would be to add 0.1ml, 0.2ml, 0.2ml, 0.5ml and 1.0ml, followed by the normal 2ml additions. Because the pH changes rapidly, these small steps will provide better data.
6. When the pH is only changing by a few hundredths of a pH unit (after 30-40 ml of HCl) you may stop.
7. Now add 0.1M NaOH to your second burette.
8. Add a fresh 20 ml sample of 0.1M glycine to your rinsed and dried 50 ml beaker. Do not forget to recalibrate your meter to pH 7.
9. Measure the pH of the sample. Again, because the pH changes dramatically, follow the same initial pattern of addition of small aliquots of the titrant (NaOH in this case). Also remember to recalibrate with the standard pH 10 buffer when the pH goes above the accuracy range of the standard pH 7 buffer.
10. When the pH is no longer changing much, after about 30-40 ml of NaOH, you may stop and rinse and dry your beakers and stirring bar.

Clean-Up Procedures

1. Rinse beakers and stirring bar with tap water, then distilled and leave beakers upside down on a paper towel.
2. Place the pH electrode into the soaking buffer.
3. Make sure the pH meter, stirrer and bench top are clean and dry.
4. The instructor may ask you to drain your burettes into the waste containers, rinse them a little with distilled water and turn them upside down with the stopcock open to dry.

Data Analysis

1. You should graph your data with the pH on the Y-axis and the ml acid and/or base added on the X-axis.
2. For all graphs you should indicate where the pK_a's are.
3. For the sodium phosphate graph, indicate along the curve what constituents (ions) are present at different points.
4. For the glycine and glutamate, you will need to think carefully how to set up the X-axis to make the curve continuous from high pH to low pH. It should look like the sodium phosphate curve. Hint: start in the middle of the X-axis and go both left and right.
5. Draw the chemical structure of both the glycine and glutamate and indicate what hydrogens dissociate.

Sample pH Problems

1. The pK_a of an acid equals 3.8. Calculate the pH when the concentration of:
   a. the salt equals the concentration of the acid.
   b. the salt is twice that of the acid.
   c. the acid is twice that of the salt.
   d. the salt is ten times that of the acid.
   e. the acid is ten times that of the salt.
   f. Which of the preceding solutions is the best buffer? Why?

2. Calculate the pH when the K_a is 5×10^{-8} and the concentration of the acid is four times that of the salt.
3. Calculate the hydrogen ion concentration when the pK_a is 9.2 and the concentration of the salt is ¼ that of the acid.
4. Calculate the K_a when the pH is 8.5 and the salt is three times more concentrated than the acid.

This laboratory exercise is adapted from *Exercises in Cell Biology* by A. A. Parsons and H.C. Schapiro, McGraw-Hill, 1975.