Exploring Phosphate Buffers

Introduction

Reactions or procedures that have rates or equilibria that are pH dependent must be performed under controlled pH conditions in order to achieve reproducible and predictable results. Reactions performed under conditions that mimic biological conditions must have controlled pH in order to more closely resemble the *in vivo* conditions. Many compounds take different forms depending on the pH of their environment and will not behave as predicted without controlled pH. To achieve these controlled conditions, acid-base buffers are used.

A buffer is a solution that responds to the addition of strong acid or base by reacting to neutralize the addition. A combination of a weak acid (HA) and its conjugate base (A^-) are used to neutralize added strong base and strong acid, respectively. The pH of the system remains stable because a corresponding amount of the conjugate is made during the reaction, keeping the pH relatively constant. Buffers are most effective when made within ±1 pH unit of the pK_a of a weak acid. The Henderson-Hasselbalch equation (1), derived from the expression for K_a for a weak acid, can be used to predict the pH of a buffer if the pK_a of the weak acid and the concentrations of the weak acid and conjugate base are known.

$$pH = pK_a + log\frac{[A^-]}{[HA]} \tag{1}$$

Polyprotic acids can also be useful in making buffers that correspond to the various pK_a's of the acid. Polyprotic acids will have additional Henderson-Hasselbalch equations corresponding to each pK_a of the acid. For example, a diprotic acid, H_2A, would have two equations as shown below (2,3). In order to determine the pH of a buffer made from a system of two species from a polyprotic acid, the Henderson-Hasselbalch equation that involves the two specific species whose concentrations are known should be chosen. From there, it is treated the same way as a monoprotic acid.

$$pH = pK_{a1} + log\frac{[HA^-]}{[H_2A]} \tag{2}$$

$$pH = pK_{a2} + log\frac{[A^{2-}]}{[HA^-]} \tag{3}$$

In this experiment, you will explore the use of phosphate buffers. Phosphoric acid, H_3PO_4, is a triprotic acid with pK_as of 2.148, 7.198 and 12.375. Different combinations of the various forms of phosphoric acid can be used to achieve useful buffers anywhere from a pH of 2 to 13. Because of this range of possible pH's, phosphate buffers are very commonly used in chemistry and biology and are often used for making buffers near physiological pH.

Before coming to lab

While you will not know what pH buffer you will make until you come to lab, you should be able to set up the pH calculations for your buffer before arriving. You may either do this by hand in your notebook or on the computer. Make sure that you know how to use the appropriate pK_a, the appropriate Henderson-Hasselbalch equation and the total formality of phosphate in a buffer to determine the amount of the weak acid and its conjugate base that would be needed to make a buffer of a specific pH.

Chemicals and supplies needed
Hydrogen phosphate salt (may be a sodium or potassium salt, read the label carefully)
Dihydrogen phosphate salt (may be a sodium or potassium salt, read the label carefully)
1 M HCl
1 M NaOH
pH electrode
voltmeter or computer set-up for recording pH
pH 4.00 and pH 7.00 calibration buffers
stir bar
stir plate

A. Preparing a buffer from two different phosphates

In this section, you will determine the molar ratio of hydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$) needed to create a buffer of a specific pH. You will then attempt to create this buffer from solid salts of the two ions. Different student groups will be given different pH "goals" and you will be asked to compare the success of each group at achieving the pH "goal" as well as the behaviors of the different buffers.
   1. Obtain a pH "goal" from your instructor.
   2. Using the appropriate version of the Henderson-Hasselbalch equation, determine the concentration of each of the two phosphate ions needed to achieve this pH.
   3. Determine the mass of each phosphate salt needed to make 250 mL of a 0.100 F total phosphate solution of your buffer.
   4. Weigh out the salts and dissolve both in a single beaker in enough DDI water to make 200 mL of a solution (use a magnetic stir bar to help dissolve). Before measuring its pH, predict the actual pH of this solution (ignore ionic strength but use the accurate masses you weighed out to make the buffer).
   5. Measure the pH of the buffer (see instructor for how to calibrate the pH meter). How does it differ from your predicted pH? Why?
   6. Carefully adjust the pH of the buffer. Make sure not to change the total formality of phosphate in the solution. How can this be done? After adjusting the pH, dilute to ~250 mL with DDI water. Check the pH. Has it changed? <u>Do not discard this buffer.</u>

B. Preparing a buffer from one form of phosphate

Often, buffers are made starting with one form of the weak acid which is then titrated with strong acid or base until the desired pH is achieved. In this section, you will make a buffer of the same pH as the buffer created in the previous section (but a different formality) starting with a single form of phosphate.
   1. Determine the mass of <u>one</u> of the phosphate salts that would be needed to make 250 mL of a 0.0100 F (note different formality from the previous section) total phosphate solution.
   2. Weigh out the salt and dissolve it in ~100 mL DDI water. Before adjusting or measuring pH, predict the pH of this solution that was made with only one form of phosphate.
   3. Measure the pH of the solution. How does it differ from your predicted pH? Why?
   4. Carefully adjust the pH of the solution to your "goal" pH while making sure not to change the total formality of phosphate. After adjusting the pH, dilute to ~250 mL with DDI water. Check the pH. Has it changed?

C. Comparing buffer capacity

The two buffers you have created should both have the same pH but differ in buffer capacity due to differences in total concentration of phosphate. In this section, you will compare their ability to neutralize the addition of strong acid and base.

1. Using 1 F NaOH, make ~ 100 mL of a 0.1 F NaOH solution. Fill a buret with the 0.1 F NaOH.
2. Dispense 100 mL of your 0.100 F phosphate buffer into a 250 mL beaker.
3. Add a stir bar to the beaker and start it stirring on a stir plate. Slowly add 0.1 F NaOH from the buret to the beaker while monitoring the pH. Record the pH and volume after each addition. Start by adding approximately 0.5 mL at a time. If the pH isn't changing much with each addition, you may add 1 mL at a time. If the pH begins to jump quickly, slow down to about 0.2 mL at a time. Continue adding and recording information until about 40 mL of the NaOH has been added.
4. Repeat steps 1-3 with another 100 mL of the 0.100 F buffer but this time use 0.1 F HCl (make it from the 1 F HCl solution and dispense from a different buret – can be done at the same time as the NaOH reaction by your partner).
5. Repeat steps 1-4 with the 0.0100 F buffer.

Clean-up. All solutions from this lab may be disposed of in the sink.

Data Analysis

In your conclusion, make sure to discuss the differences in predicted and actual pH for the solutions you prepared. What factors does the Henderson-Hasselbalch equation fail to take into account in predicting pH? Determine the ionic strength of the buffer solution you prepared in Part A, step 4. Using ionic strength and activity correctly, re-calculate the predicted pH of the 200 mL solution you made before adjusting the pH. Was this pH correct? If not, what other factors may you not have taken into account?

Prepare a graph that compares the change in pH (from the initial buffer pH) and volume of added acid and base for each of your two buffers from Part C. What differences do you see in the two plots? What similarities? What can you say about the buffer capacity (change in moles of added acid or base per change in pH) of the two solutions? Why is buffer capacity an important property to know for a buffer?

Obtain data from Part C from the rest of the class for the buffers made at other pHs. Prepare similar plots for each as above. Compare the different graphs. What similarities do you see? What differences? Explain any differences that are observed. How can the nature of the triprotic acid be used to help explain these differences? What do these differences tell you about the appropriate pH range(s) for the use of phosphate buffers?