Teaching Buffering by Showing Its Effects

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In this laboratory, students identify "mystery" solutions as either distilled water, monobasic phosphate only, dibasic phosphate only, or combined phosphate buffer. The solutions are titrated with both acid and base, and the increase of hydrogen or hydroxide ions (not pH) is plotted against mL of acid of base added. These plots make the identification of the "mystery" solutions obvious. Solutions without buffering against acid (for example) show a linear increase in hydrogen concentration when titrated with acid. Solutions with buffering against acid show nearly no increase until the buffering is exhausted, and then the hydrogen ion concentration increases dramatically.

Key Words: buffering, phosphate, titration

Introduction

The classic phosphate buffer system consists of two components: $H_2PO_4^-$ (monobasic phosphate) and HPO_4^{2-} (dibasic phosphate). The first buffers against addition of bases and the second against addition of acids. When they are mixed together so that the concentration of $H_2PO_4^-$ and HPO_4^{2-} are equal, the solution can buffer against both acids and bases and has a pH near 7.0, perfect for many biological applications.

In this exercise, students are given a "mystery" solution that is either distilled water, 0.01 M $H_2PO_4^-$ alone, 0.01 M HPO_4^{2-} alone, or a mixture that contains 0.01 M $H_2PO_4^-$ and 0.01 M HPO_4^{2-}. These solutions, respectively, buffer against neither acid nor base, against base only, against acid only, or against both acid and base. The whole class solves the problem of solution identity. Student teams are assigned 50 mL of one of these solutions and titrate them with either 0.1 N HCl or 0.1 N NaOH. Then the teams enter their pH results into a spreadsheet (downloadable from a Clemson Web site—see Notes for the Instructor). The spreadsheet calculates the hydrogen ion concentration (if acid is being added) or the hydroxide ion concentration (if base is being added) and plots a graph of the molar concentration of these ions vs. the mL of acid or base added. The graphs plotted by the spreadsheet allow easy identification of the solutions, and also show the sharp difference between solutions that do and do not show buffering. The exercise takes about 30 minutes, and ideally would have one pH meter and associated equipment (such as a stir plate) per pair of students. More information on time and material requirements is contained in the "Notes for the Instructor."

Student Outline

This exercise asks you to distinguish between four "mystery solutions": distilled water, a solution containing $H_2PO_4^-$ only, a solution containing HPO_4^{2-} only, and a solution containing both $H_2PO_4^-$ and HPO_4^{2-}. The solutions are labeled A, B, C, and D, but not necessarily in that order.

Background

The phosphate buffer system consists of two components: $H_2PO_4^-$ (monobasic phosphate) and HPO_4^{2-} (dibasic phosphate). $H_2PO_4^-$ buffers against addition of bases by giving up a hydrogen ion and becoming HPO_4^{2-}; HPO_4^{2-} buffers against addition of acids by absorbing a hydrogen ion and becoming $H_2PO_4^-$. Fill in this table with either "acid," "base," "acid and base," or "neither acid nor base":

Table 1. Predicted buffering properties of the four "mystery" solutions.

Solution...	...will buffer against
Distilled water	
$H_2PO_4^-$	
HPO_4^{2-}	
$H_2PO_4^- + HPO_4^{2-}$	

You will titrate each solution above with both 0.1 N HCl and 0.1N NaOH, adding it in 0.5 mL increments, and recording the pH after each addition. Let's say you're adding acid. There are two possible situations for your "mystery" solution:
If there is no buffering against acid and you add 100 H^+ ions, you will expect to see about 100 more H^+ ions in the solution. Every time you add another 100 ions, the H^+ concentration will increase by the same amount. In other words, a graph of H^+ concentration (*not* pH) vs. acid added will look like this:
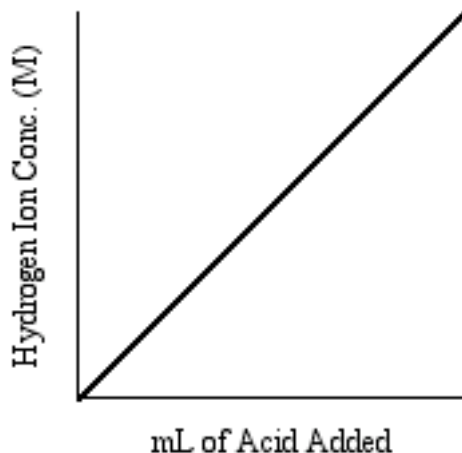

Figure 1. Expected change in hydrogen ion concentration if there is no buffering.

If there *is* buffering against acid and you add 100 H^+ ions, you will not see 100 more H^+ ions in the solution. You might only see one more because the other 99 have been absorbed by the buffer. Therefore, the H^+ concentration will increase much more slowly. However, when the buffering capacity is exhausted, suddenly the H^+ concentration will start increasing much faster, as fast as it did in the non-buffering case. Once the buffering is exhausted, no more H+ ions are being absorbed. In other words, a plot of H^+ concentration vs. acid added will look like Figure 2:
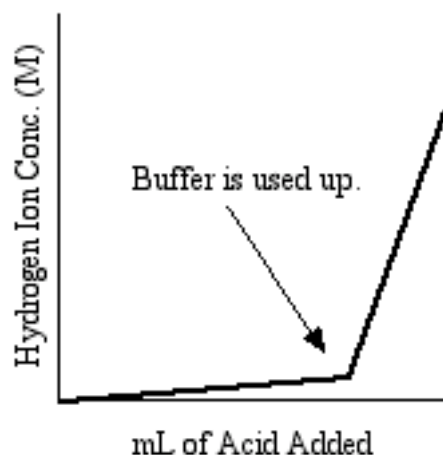
Figure 2. Expected pattern of change in hydrogen ion concentration if there is buffering against acids.

There is an identical set of expectations about the increase in hydroxide concentration as base is added, and the shape of the curves would be identical. The only difference would be that the x axis would refer to mL of base added and the y axis would refer to hydroxide ion concentration.

You will determine whether or not buffering exists in a solution by titrating with acid or base and determining whether the curves produced are similar to Figure 1 or to Figure 2. You will use this information to identify solutions A, B, C, and D as distilled water, $H_2PO_4^-$ alone, HPO_4^{2-} alone, or $H_2PO_4^- + HPO_4^{2-}$ (complete phosphate buffer). Suppose we see the results in Figure 3 for a solution. Can you see that the solution must be $H_2PO_4^-$ (buffers against base but not against acid)?
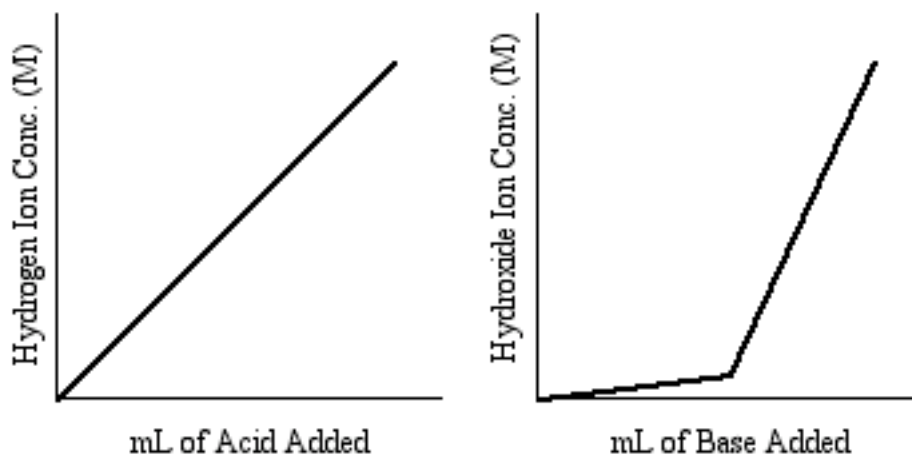

Figure 3. Expected results for $H_2PO_4^-$.

Procedure

1. You will work in pairs. You and your partner will be assigned mystery solution A, B, C, or D. Depending on what titrant (acid or base) is available at your pH meter, you will titrate this solution with either acid or base. Although you will work only with those solutions, you are responsible for putting together the whole story of solutions A, B, C and D in a wrap-up discussion.

2. Using the graduated cylinder for your solution, place 50 mL of your solution in a beaker, put in a stir bar, and place it on a stir plate. Start the stir bar spinning at a moderate rate of speed. If you have a VWR Model 2000 or a Beckman Model Φ240 handheld pH meter, do the following:

   a) Turn on the pH meter by pressing the power button.

   b) Remove the probe from the soak solution, hold it over the waste beaker and rinse it off using the distilled water squeeze bottle, and blot the tip dry with a Kimwipe tissue.

   c) Place the probe in the beaker, making sure that it is immersed up to the white line on the probe. However, don't let the stir bar hit it.

   d) Press the "Read" button. The pH will start to change. When the eye icon stops blinking, record the pH in Table 2 below. Once the eye stops blinking, the display is frozen, so every time you want to read another pH you must press the "Read" button again.

   e) Record the initial pH in Table 2 (next page).

3. Using a 1 mL pipet, add 0.5 mL or either 0.1 N NaOH or 0.1 N HCl, whichever one was at your pH meter. Record the pH after mixing in Table 2.

4. Continue to add 0.5 mL amounts of titrant, recording the pH after each addition, until you have added 10.0 mL.

Table 2. pH of solution _____ (A, B, C, or D) after addition of _____ (HCl or NaOH).

mL Titrant	pH
0.0	
0.5	
1.0	
1.5	
2.0	
2.5	
3.0	
3.5	
4.0	
4.5	
5.0	
5.5	
6.0	
6.5	
7.0	
7.5	
8.0	
8.5	
9.0	
9.5	
10.0	

5. After taking your last reading, turn off the pH meter's power. Rinse your electrode with the distilled water squeeze bottle, dry the electrode with a Kimwipe, and place it back in the electrode solution.

6. There will be a spreadsheet program on the display computer in the lab. Once you've finished your titration, add your pH data to sheet 1 of this spreadsheet. You should add your data under the "pH 1" column if you are first to add data for that solution, and under "pH 2" if you are the second team to add data. The spreadsheet will automatically calculate the hydrogen ion concentration (if you're adding acid) or the hydroxide ion concentration (if you're adding base).

7. The spreadsheet will plot graphs of both hydrogen and hydroxide concentration in solutions A, B, C, and D. The spreadsheet will also add a short segment of a line that shows how fast hydrogen or hydroxide ion would be increasing if there was no buffering. As a class, use these graphs to determine which solution is distilled water, which is $H_2PO_4^-$ alone, which is HPO_4^{2-} alone, and which is $H_2PO_4^- + HPO_4^{2-}$.

Notes for the Instructor

The four graphs in Figure 4 show student results as printed by the data entry spreadsheet for distilled water and combined buffer solution. The short line segment with the triangular points is a theoretical curve (plotted by the spreadsheet) of how fast the hydrogen or hydroxide concentration would increase if there were no buffering and the only factors affecting concentration were additions of hydrogen and hydroxide ions, dilution, and the volume increase produced by added titrant.

It is clear distilled water shows no buffering against either acid or base because the concentration of hydrogen or hydroxide goes up at the rate expected if there were no buffering. The combined buffer shows buffering against both acid and base because there is an extended region where addition of acid causes no visible increase in hydrogen ion concentration and addition of base causes no visible increase in hydroxide ion concentration. This in itself is a dramatic demonstration of how buffers can absorb hydrogen and hydroxide ions.

Time and Material Requirements

We make our solutions as follows:

Table 3. Solutions used in the exercise.

| Solution	Recipe
0.1 N HCl	8.3 mL concentrated HCl brought up to 1 L with distilled water.
0.1 N NaOH	4.00 g dissolved in < 1 L distilled water, brought up to 1 L.
0.02 M K_2HPO_4	3.48 g dissolved in < 1 L distilled water, brought up to 1 L.
0.02 M KH_2PO_4	2.72 g dissolved in < 1 L distilled water, brought up to 1 L.
0.01 M buffer	Mix 500 mL of the 0.02 M K_2HPO_4 solution and 500 mL of the 0.02 M KH_2PO_4 solution to make 1000 mL of 0.01 M phosphate buffer.
0.01 M K_2HPO_4	Dilute 0.02 M solution by 50%.
0.01 M KH_2PO_4	Dilute 0.02 M solution by 50%.
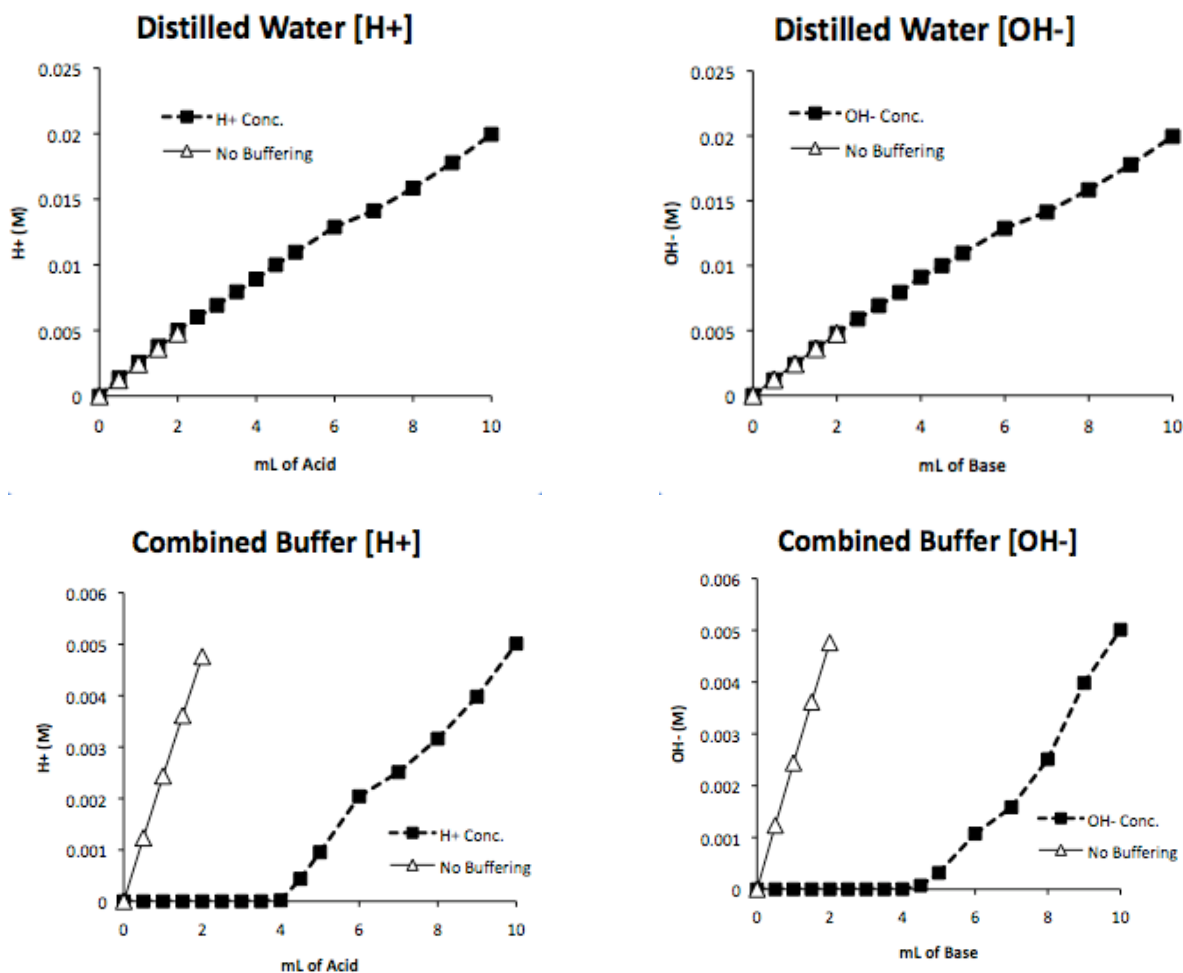
Figure 4. Results for the addition of both acid and base to distilled water (upper two graphs) and to combined buffer solution (lower two graphs). Note that the hydrogen or hydroxide concentration increases linearly if there is no buffering, but has a period of no increase followed by a sharp upward inflection if there is buffering.

Note that the 0.02 M solutions are used to make the combined buffer, and the 0.02 M solutions must be diluted to make the 0.01 M K_2HPO_4 and KH_2PO_4 solutions that the students will use as unknowns.

The students work in pairs, and each pair has a pH meter, stir plate, stir bar, rinse bottle, waste beaker, a 100 mL graduated cylinder, a 1 mL pipet, Kimwipes, a beaker that will hold about 100 mL of solution, etc. Each lab bench has four large stock bottles (about 1000 mL) containing distilled water, 0.01 M combined buffer, 0.01 M K_2HPO_4, or 0.01 M KH_2PO_4. At Clemson, these are marked as solutions B, C, A, and D, respectively. Each pH meter has either an HCl or NaOH stock bottle near it. Student teams are assigned to titrate one of the "mystery" solutions, but they use either acid or base, depending on which is at their pH meter. Therefore, each student team adds 10 mL of acid or base (but not both) to 50 mL their solution. They then type their pH results into a spreadsheet (supplied—see final section of these Instructor's notes). The spreadsheet calculates the hydrogen or hydroxide ion concentration and graphs it. This spreadsheet will accommodate two acid titrations and two base titrations per solution, and so could handle a laboratory with 16 pairs of students. The whole class then looks at the acid and base results for each solution and then decides what each solution is.

This exercise requires only about 30 minutes because the concept is simple and each team must do only one titration. The class identification of the solutions is very rapid—when we did this last at Clemson the class was shouting out which solution was which even before we had scrolled the spreadsheet down so they could see both graphs.

The Spreadsheet

A helpful spreadsheet can be downloaded from a Clemson Web site at <**http://biology.clemson.edu/bpc/bp/Lab/110/ buffering.html**>. Sheet 1 of the spreadsheet is used for data entry, and computes hydrogen ion and hydroxide ion concentrations and plots a concentration graph when pH values are inputted.

Sheet 2 of the spreadsheet is a model of buffering that allows the user to plot predicted titration curves and hydrogen and hydroxide ion concentration curves under different conditions. Using the leftmost two outlined cells at the upper left of the sheet, the user can input the initial concentrations of $H_2PO_4^-$ and HPO_4^{2-} in 50 mL of buffer. The rightmost two outlined cells were used for an exercise not described here in which buffer solutions were doctored by the initial addition of acid or base. The model is most useful for explaining the principles behind buffering and results expected. The best way to use it is to show the class a starting situation (e.g., 50 mL of buffer with 0.01 M $H_2PO_4^-$ and HPO_4^{2-}), make sure they understand what the curves are showing, and then propose various changes and ask the class how the curves would change. For example, if we cut the amount of $H_2PO_4^-$ in half, how will that affect the initial pH of the mixture? How would it affect the acid curve? The base curve?

The spreadsheet model is adequate for teaching purposes, and is fairly accurate when it is used close to it "home" conditions of 0.01 M $H_2PO_4^-$ and HPO_4^{2-} titrated with 10 mL of acid or base. That is, it is designed to simulate the experiments in this exercise, and may encounter glitches if it is used with inputs far from those conditions. For example, with 10 mL of titrant being added, if the initial concentration of HPO_4^{2-} is greater than 0.02 M, the HPO_4^{2-} will not be used up during the titration, the acid pH curve will appear to change little, and the [H^+] curve will appear to curve gently upward (not a pattern expected in the exercise). However, inspection of the y axis will reveal that the concentrations of hydrogen ions are so small that the H^+ curve really is in its "flat" phase. Only when the buffer is exhausted after 10 mL of titrant will there be large increases in hydrogen ions that will make this apparent.

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About the Authors

Robert J. Kosinski is a professor of Biology at Clemson University, where he is the sole lecturer in the Introductory Biology course for majors and also the coordinator of the laboratories for that course. He received his B.S. degree from Seton Hall University and his Ph.D. in Ecology from Rutgers University. His interests include laboratory development, investigative laboratories, and the educational use of computer simulations, all in introductory biology. He was chosen as the Alumni Master Teacher of Clemson University in 2007. He has attended every ABLE meeting since 1989, has presented at 15 of those meetings, and acted as the chair of the host committee for the 2000 ABLE meeting at Clemson University.

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