

BUFFER EXERCISE

Buffers are solutions of weak acids and their conjugate bases that resist changes in pH when strong acids or bases are added. They are most effective if substantial amounts of both the protonated and deprotonated forms of the weak acid are present. In this situation, if a strong acid is added, its protons can be "soaked up" by the conjugate base of the weak acid. If base is added, protons from the weak acid can neutralize it. The pH of a given mixture of a weak acid and its conjugate base is determined by the ratio of the concentrations of the two forms and a physical constant describing the affinity of the conjugate base for protons, the pK_a . The Henderson-Hasselbalch Equation is often used to calculate various quantities related to pH:

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) \quad \text{where HA = weak acid, A}^- = \text{conjugate base}$$

One frequently finds that the pH expected from a given ratio of weak acid to conjugate base is not attained in practice. If measurement errors are negligible, the cause of the discrepancy is that the "effective concentrations" of the two species do not equal their calculated concentrations due to nonideal behavior of the ions in solution. The proper name for "effective concentration" is thermodynamic activity. Phosphate ions, which are small and intensely charged, are notorious for having low thermodynamic activities, especially in the more highly charged forms. At a calculated concentration of 0.1 M, the "effective concentration" of the HPO_4^{2-} ion may be as low as 0.033 M, only 33% of the calculated value. Since it is the "effective concentration" that determines the ratio of conjugate base to weak acid, the observed pH for such solutions is quite different from that calculated using weighed amounts of ions.

Changes in solution temperature can cause significant changes in pH in some cases. The pH of buffers made from tris-hydroxymethylaminomethane (commonly called "Tris" by biochemists, but THAM by chemists) is particularly sensitive to temperature changes. The value of pK_a is dictated by the free energy change of the ionization process. Since $\Delta G = \Delta H - T\Delta S$, if ΔS for ionization is large, then ΔG and hence pK_a will be strongly temperature-dependent.

There are two main objectives in this experiment:

1. Learn how to prepare a series of phosphate buffers "from scratch", then how phosphate buffers of different pH values respond to challenges by strong acid and strong base. (You will see why calculation of ratios is not an efficient way to prepare buffers of a desired exact pH, but does help guess at "ballpark" amounts of partners to use.)
2. Prepare a pH 8 Tris buffer "from scratch", determine its pH/temperature dependence, and compare this to the temperature dependence of the pH 8 phosphate buffer previously prepared.

EXPERIMENTAL SECTION

Later in the experiment, you'll need one water bath at 0 °C and another near 40 °C. Locate or make these at the start of the period.

1. Prepare 500 mL stock solutions of 0.05 M mono- and dibasic sodium phosphate (2 separate solutions). Use information on bottle labels to determine how much to weigh out. Don't forget to include the mass of waters of crystallization. **Clean up spills in balances!!!** Prepare these solutions in labeled beakers after the instructor has checked your calculations.
2. Standardize your pH meter at pH 7 and 10. It may be necessary to go through more than one round of standardization before the meter reads pH 7.00 correctly – check using pH 7 buffer after completing each round.
3. Use the H-H Equation to calculate the volumes of stock solutions needed to prepare 120 mL of 0.05 M buffer at pH 8.0 (the pK_a for the pertinent ionization is 7.21). Mix these volumes together and measure the pH. The discrepancy is due to different thermodynamic activities of the singly and doubly charged ions. Note that if their activities were equal (whether high or low), no discrepancy would result.

4. Discard about 1/4 of the "pH 8" buffer and then add the proper stock solution to the remainder until its pH actually reads 8.0 ± 0.05 . Save this pH 8 buffer in a labeled beaker or flask. Use the same "pour until the pH is right" technique to prepare about 100 mL of buffer solutions of pH 7.5, 7.0, 6.5 and 6.0 (each ± 0.05).
Think before you pour: which stock solution will comprise the bulk of your buffer, and about how much of each will you need to get to a final volume of roughly 100 mL? Record the actual measured pH of each buffer.
5. Into separate small beakers pour two 40.0 mL portions of one of the buffers you made. To one portion add 0.50 mL of 0.5 M HCl; to the other add 0.50 mL of 0.5 M NaOH. Measure the resulting pH values. Repeat for the remaining four buffers. Save the unused part of the pH 8.0 buffer for later.
6. Prepare 100 mL of 0.01 M Tris Cl buffer at $\text{pH } 8.0 \pm 0.05$. **Since this compound interacts with Ag^+ ions, you must use a combination pH electrode that has a calomel reference, not an Ag/AgCl reference.** We do not have the acid partner of the buffer pair, so we use only the base and partially neutralize it with a strong acid. Generic technique: weigh the mass of Tris (TRIZMA) base needed for 100 mL of 0.01 M solution and dissolve it in about 80% of the desired final volume of water. Add HCl (whose concentration is at least 10 times higher than the final desired buffer concentration) dropwise with stirring until both the pH and the temperature are as desired. It is important to monitor the temperature of the solution because neutralization generates heat and the pH of Tris buffers depends on temperature. The warming effect is more pronounced in more concentrated solutions. Make your buffer read pH 8.0 at room temperature (measure the temperature!). Finally, add room temperature water to make the final volume 100 mL and recheck the pH (it should stay constant).
7. Divide the Tris buffer into two roughly equal portions. Equilibrate one portion at 0°C and one near 40°C . Also equilibrate a sample of your saved pH 8.0 phosphate buffer at each temperature. When at thermal equilibrium, measure the pH values of the buffers using equipment calibrated at these temperatures. **Don't forget, you must use electrodes with calomel references!** (*There is a problem at 0°C : the KCl in the outer electrode precipitates, so you can't cool the electrode. Calibrate using cold buffers and a room temp electrode. This will introduce a small but unavoidable error. Don't leave the electrode cold very long.*)

REPORT

Before you start writing, recall the objectives of this experiment. Organize your Experimental, Results, and Discussion sections to reflect these in a consistent order. Use subheadings to signal your organization.

Response to challenge by strong acid and base: Use a Figure (graph) to show the **change in pH** caused by challenge as a function of the initial ("unchallenged") pH you measured (not the target pH). This information should permit you to decide the pH at which the buffer most effectively resists changes in pH. Do the results support what you have been told about buffer behavior?

Temperature dependence of pH: On a single plot (another Figure), show pH of both buffers vs. temperature (you have data for 3 temperatures so you will have two lines with 3 points each). Can you quantitate the dependence of pH on temperature of phosphate buffer? of Tris buffer?

In an appendix, use your data to solve the following problem, clearly showing your method.

A 0.01 M Tris buffer has a pH of 7.90 at 25°C . What will its pH be at 5°C ? Assume that its pH – Temp dependence is the same as that of your buffer.

Caution: did your tris buffer have a pH of 7.9 at 25°C ? If not, what quantity from your results must you use to compute the answer, and what quantity must you NOT use?