

Biochemistry Laboratory I CHEM 4401

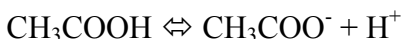
Texas A&M University-Corpus Christi

Buffers, pH & Practical Buffer Preparation

In addition to the Buffers & pH lab from your manual, we will learn a practical method for preparing buffers. A typical buffer would be described as:

0.1 M sodium acetate, pH 5.0

Let's examine the buffer description in detail. First notice that buffers are usually named after the conjugate base, in this case, acetate. So this buffer solution relies on the dissociation reaction of acetic acid.



where CH_3COOH is *acetic acid* and CH_3COO^- is *acetate*, the conjugate base. We expect the acid and conjugate base to be in approximate ratio of 1.0 in a well-designed buffer. Look back at the description of the sodium acetate buffer. What has a concentration of 0.1M? It's not the concentration of the acetate ion. It is the *total* concentration of acetic acid and acetate. And lastly, what does the sodium have to do with the buffer? Well, nothing, the sodium is just the counter ion added along with the acetate. It is not included in the dissociation equation. We could just as easily make a potassium acetate buffer, which would have the same buffering properties.

Preparing Buffers

We are going to use a method for preparing buffers which requires a minimum of calculation. Of course, it is always possible to use the Henderson-Hasselbach equation to calculate the exact amounts of acid and base concentrations needed for any pH. Our method requires only that we correctly prepare stock solutions of acids and their conjugate bases at known concentrations.

We have three choices when making a buffer

1. Start with the weak acid (acetic acid, etc.) and add base (e.g. sodium hydroxide) until the required pH is obtained.
2. Start with the conjugate base (sodium acetate, etc.) and add acid (e.g. hydrochloric acid) until the required pH is obtained.
3. Start with the conjugate base (sodium acetate) and weak acid (acetic acid) and mix the two solutions until the required pH is obtained.

Options 1 and 2 are tedious and time consuming. You do get a solution of the proper pH, which is a buffer, but with option 2 you have produced some unwanted sodium chloride

in the process. A quantity of sodium chloride will be produced during the neutralization of acetate to form acetic acid. This additional sodium chloride will change the *ionic strength* of the solution. Ionic strength describes the concentration of ions for a solution. Ionic strength affects protein folding, enzyme activity and biochemical interactions in general. The additional NaCl may not significantly affect the ionic strength, but why introduce it if unnecessary?

Alternative 3 is the easiest way to prepare most buffers. Simply mix solutions, of the same concentration, of the weak acid and its conjugate base until the desired pH is reached. This produces a buffer with *both* the desired pH and concentration. For example, a 0.1 M sodium acetate buffer, pH 5.0, could be prepared by adding a 0.1 M solution of sodium acetate to a 0.1 M solution of acetic acid until a pH of 5.0 is reached.

As a practical matter, always use alternative 3 whenever possible. For some buffers, unfortunately, either the acid or conjugate base are not readily available and you have to use alternative 1 or 2.

You will be assigned to prepare one buffer from a list supplied in class. Instructions for using the pH meters will also be provided. We will supply stock solutions of sodium hydroxide and hydrochloric acid.

Here is an example of how to prepare a 0.1 M buffer of a specified pH:

1. Prepare 50 ml of a 0.1 M solution of the conjugate base. Calculate the amount of base needed. Weigh out and dissolve in 30 ml of distilled water. Once it has completely dissolved bring the volume of the solution up to 50 ml with distilled water.
2. Prepare 50 ml of a 0.1 M solution of the weak acid. As for the conjugate base, calculate the amount of weak acid required. If your weak acid is a liquid, see if the concentration or solution % is listed. *Glacial acetic acid*, for instance, is 100% pure acetic acid. Because it is a 100% solution, we can treat it as if it were a dry solid for calculation purposes. Weigh out and dissolve your weak acid in 30 ml of distilled water. Once it has completely dissolved bring the volume of your solution up to 50 ml with distilled water.
3. Slowly add 0.1 M weak acid solution to 30 ml of the 0.1 M conjugate base solution (while stirring). Monitor the pH as you mix the components. As the final pH is approached, carefully add the weak acid solution with a disposable pipet. This will help to avoid overshooting the targeted pH.

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Buffers & pH Lab Prep sheet

Material & Reagents (per student team)

pH Standards (4, 7, 10)	50 ml
Sodium Acetate (trihydrate)	Calculate
Glacial Acetic Acid	Calculate
Sodium Phosphate dibasic (Na ₂ HPO ₄)	Calculate
Sodium Phosphate monobasic (NaH ₂ PO ₄)	Calculate
Hydrochloric acid (0.1 N)	1 small bottle (20-50 ml) per bench
NaOH (0.2 N)	40 ml (8g/L)
Glycine (0.1 M, titrate to pH 2 w/HCl)	25 ml (7.5 g/L)
Albumin (1% solution)	20 ml (10g/L)
disposable pipets (glass) & bulbs	2-5
50 mL burets w/stopcocks (CS 219)	1
Buret Stands (CS 228)	1
pH meters (CS 219)	1

Modifications to *Experimental Procedures* in lab manual:

1. Same as in manual
2. Student teams will prepare 50 ml of either acetate or phosphate buffer as assigned.
3. Same as in manual
4. Place glycine solution on a stir plate. Add small stir bar and mix while titrating.

Assignment (17 pts)

1. Example calculation for preparation of buffer (2 pt)
2. Table 1(computer generated): record of pH values for tap water, buffer, albumin and glycine titration (2 pt)
3. Figure 1: Titration curve (computer generated):
 - (i) Graph title (1 pt)
 - (ii) axis labels (2 pt)
 - (iii) titration curve from plotted pH values. Curve can be hand drawn but must connect plotted points (1 pt)
 - (iv) pK₁ and pK₂ values (Identify and estimate from graph) (2 pt)
 - (v) drawing of correct glycine ionization reactions at appropriate pK₁ and pK₂ points on graph. Circle functional group involved in loss of protons (2 pt)
4. What is the [H⁺] of a solution that shows a pH = 8.92? (show work) (1 pt).

5. What is the pK_a of a buffer solution where the acid $(HA) = 0.125\text{ M}$, the conjugate base $[A] = 0.050\text{ M}$ and the $pH = 5.95$ (show work)? Will the buffering capacity of this solution be maximized at this pH ? Why or why not (2 pt).

7. Lab performance (2 pt)