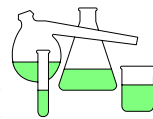# 3 The Preparation of Buffers at Desired pH

Objectives: To become familiar with operating a pH meter, and to learn how to use the Henderson-Hasselbalch equation to make buffer solutions at a desired pH value.

Introduction: A buffer system is a mixture of a _weak_ acid or a _weak_ base and its salt (conjugate base or conjugate acid, respectively) that permits solutions to resist large changes in pH upon the addition of small amounts of hydrogen ions (H^+) or hydroxide ions (OH^-). What does this mean? It means a buffer helps maintain a _near constant pH_ upon the addition of small amounts of H^+ or OH^- to a solution.
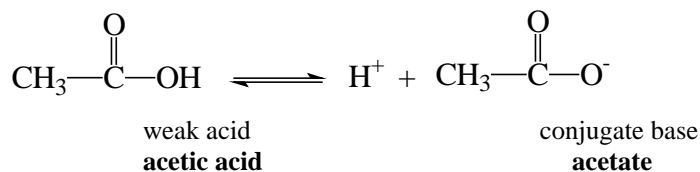
In the definition of a buffer system, it should be noted that reference is made to weak acids and bases. What is the distinction between a _weak_ and a _strong_ acid or base? **Strong** acids (e.g., HCl) and bases (e.g., NaOH) almost completely ionize in water:

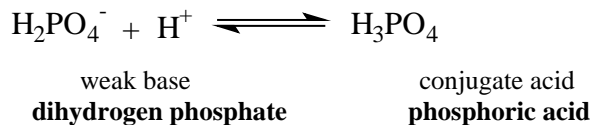$$HCl \longrightarrow H^+ + Cl^- ; \quad NaOH \longrightarrow Na^+ + OH^-$$

Many acids and bases, however, do not undergo complete ionization in water. These compounds, then, are called **weak** acids and bases. Many organic acids are weak acids. Weak acids do not completely dissociate in water. The dissociation of an organic acid is described by the following reaction:

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

weak acid   **conjugate base**
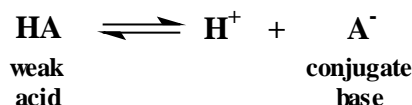
Note that the unprotonated product of the acid dissociation reaction is referred to as a conjugate base. Recall the Bronsted-Lowry definition of acids and bases. An acid is defined as a proton (H^+) donor, whereas the base is the H^+ acceptor. In a conjugate acid/base pair such as that indicated above, the weak acid always has _one more_ H^+ than its conjugate base. Likewise, a weak base will have _one less_ H^+ than its conjugate acid.

$$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-OH \rightleftharpoons H^+ + CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-O^-$$

weak acid   conjugate base
acetic acid   **acetate**

$$\text{H}_2\text{PO}_4^- \;+\; \text{H}^+ \;\rightleftharpoons\; \text{H}_3\text{PO}_4$$

<div align="center">
weak base   conjugate acid

dihydrogen phosphate   **phosphoric acid**
</div>

One can analyze the strength of a weak acid. This means that the amount of hydrogen ion released can be determined. To do this, one can use the following expression:

$$K_a = \frac{[\text{H}^+]\,[\text{A}^-]}{[\text{HA}]}$$

where K_a is defined as the acid dissociation constant.

The larger the value of K_a, the stronger the acid is. Because K_a values vary over a wide range, they are usually expressed using a logarithmic scale:

$$pKa = -\log Ka$$

The hydrogen ion is one of the most important ions in biological systems. The concentration of this ion affects most cellular processes. For example, the structure and function of most biological macromolecules and the rates of most biochemical reactions are strongly affected by $[\text{H}^+]$. The pH scale has been devised as a convenient method of expressing hydrogen ion concentration. pH has been defined as the negative logarithm of the hydrogen ion concentration:

$$pH = -\log[\text{H}^+]$$

The **Henderson-Hasselbalch** equation provides a convenient way to think about buffers and pH:

$$pH = pKa + \log\frac{[\text{A}^-]}{[\text{HA}]}$$

If one were examining the dissociation of acetic acid, the Henderson-Hasselbalch relationship, then, would be:

$$pH = pKa + \log\frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

The Henderson-Hasselbalch equation can be used to determine if an aqueous solution of a conjugate acid/base pair is functioning as a buffer. If the concentration of the weak acid is equal to that of its conjugate base, the ratio of these two components is one. When this is the case, the Henderson-Hasselbalch equation reduces to

<div align="center">

pH = pKa

</div>

because the log(1) is equal to zero.

When the pH of the solution is equal to the pKa of the ionizing group, the solution is functioning at maximum buffering capacity (*best buffer*). An aqueous solution of a conjugate acid/base pair functions as a *good buffer* when the ratio of the conjugate base to weak acid ranges from *1:9 to 9:1*. Substituting these ratios into the Henderson-Hasselbalch equation, one finds that this aqueous solution functions as a good buffer when the pH of the solution is within approximately one pH unit of the ionizing group's pKa.

<div align="center">

pH = pKa $\pm$ 1

</div>

because the log (1/9) is -0.999 and the log of (9/1) is +0.999.

Using the Henderson-Hasselbalch Equation

Example A: *This is a two-component buffer system meaning that the weak acid and its conjugate base are added separately.* How would you prepare 10mL of a 0.01M phosphate buffer, pH 7.40, from stock solutions of 0.10M KH_2PO_4 and 0.25M K_2HPO_4? pKa of KH_2PO_4 = 7.20. This problem is similar to Problem 1 in **Experimental Procedures**. The following approach may be helpful in solving this type of buffer problem in which both the conjugate acid and base are added separately. Please note that the numbers are not rounded off until the very end and are rounded based on the limits of the pipets, cylinders, etc. that are required to accurately measure the calculated volumes.

1. Use the Henderson Hasselbalch equation to find the ratio of A^- to HA.

   pH = pKa + log $[A^-]$ / [HA]
   7.40 = 7.20 + log $[A^-]$ / [HA]
   0.20 = log $[A^-]$ / [HA]
   1.584893192 = $[A^-]$ / [HA]*

   *Since $[A^-]$ / [HA] = 1.584893192, we can say that $[A^-]$ / [HA] = 1.584893192/ 1. In this case $[A^-]$ = 1.584893192; [HA] = 1.

2. Calculate the decimal fraction (part/whole) of each buffer component.

   A^- = 1.584893192 / (1.000 + 1.584893192) = 1.584893192 / 2.584893192= 0.61313682
   HA = 1.000 / 2.584893192= 0.38686318

3. Find the **molarity (M)** of each component in the buffer by simply multiplying the molarity of the buffer by the decimal fraction of each component.

   M_{A^-} = 0.01M x 0.61313682 = 0.006131368M
   M_{HA} = 0.01M x 0.38686318 = 0.003868632M

4. Calculate the moles of each component in the buffer. **Moles = Molarity x Liters** of buffer

   $moles_{A^-}$ = 0.006131368M x 0.01L = 6.131 x 10^{-5} moles

moles$_{HA}$ = 0.003868632M x 0.01L = 3.869 x 10^{-5} moles

5. Calculate the volume of each stock solution required to make the buffer

    Liters of stock = moles of the buffer component / Molarity of the stock
    L$_{A-}$ = 6.131 x 10^{-5} moles / 0.25 M = 2.452 x 10^{-4} L = 245μL
    L$_{HA}$ = 3.869 x 10^{-5} moles / 0.10 M = 3.869 x 10^{-4} L = 387μL

6. To prepare this buffer, one would use appropriately-sized pipets to measure and transfer each component to a 10mL volumetric flask and bring the solution to volume with dH$_2$O.

Example B: *This is a single-component buffer system. This means that one component of the conjugate acid/base pair will be generated in situ (in solution). In the following example, the conjugate base will be generated by reaction between the weak acid (acetic acid) and strong base (sodium hydroxide).* How would you prepare 10mL of a 0.02M acetate buffer, pH 4.30, from stock solutions of 0.05M acetic acid (HAc) and 0.05M NaOH? pKa $_{acetic\ acid}$ = 4.76. This problem is similar to Problem 2 in **Experimental Procedures**.

1. Use the Henderson Hasselbalch equation to find the ratio of A$^-$ to HA.

    pH = pKa + log [A$^-$] / [HA]
    4.30 = 4.76 + log [A$^-$] / [HA]
    -0.46 = log [A$^-$] / [HA]
    0.34673685 = [A$^-$] / [HA]

2. Calculate the decimal fraction (part/whole) of each buffer component.

    A$^-$ = 0.34673685 / (1.00 + 0.34673685) = 0.34673685 / 1.34673685 = 0.257464441
    HA = 1.00 / 1.34673685 = 0.742535559

3. Find the **molarity (M)** of each component in the buffer by simply multiplying the molarity of the buffer by the decimal fraction of each component.

    M$_{A-}$ = 0.02M x 0. 257464441= 0.005149289 = 5.15 x 10^{-3} M
    M$_{HA}$ = 0.02M x 0. 742535559= 0.014850711 = 1.49 x 10^{-2} M

4. Calculate the moles of each component in the buffer. Moles = Molarity x Liters of buffer

    moles$_{A-}$ = 0.005149289M x 0.01L = 5.14929 x 10^{-5} moles
    moles$_{HA}$ = 0.014850711M x 0.01L = 1.485071.47 x 10^{-4} moles

5. Note: Since this buffer is prepared by the reaction of a weak acid (HAc) with a strong base (NaOH), you must determine the **total moles** of the weak acid component needed because the conjugate base is made *in situ*.

    Total moles = 5.15 x 10^{-5}moles NaOH + 1.49 x 10^{-4} moles *HAc* = **2.00 x 10^{-4} moles** HAc. *This sum indicates that, although in the buffer one only needs 1.49 x 10^{-4} moles HA, an additional 5.15 x 10^{-5}moles is needed to generate the conjugate base in situ.*

6. Calculate the volume of each stock solution required to make the buffer

Liters of stock = Moles of the buffer component / Molarity of the stock

L_{A^-} = 5.14929 x 10^{-5} moles / 0.05 M = 1.03 x 10^{-3}L NaOH = 1.0mL

L_{HA} = **2.00 x 10^{-4} moles** / 0.05 M = 4.00 x 10^{-3} L CH_3COOH = 4.0mL

7. To prepare this buffer, one would use appropriately sized pipets or cylinders to measure and transfer each component to a 10 mL volumetric flask and bring the solution to volume with dH_2O.

Example C: What is the pH of a solution resulting from a mixture of 200 mL of 0.1 M NaOH and 100 mL of 0.3 M HAc? This problem is similar to Problem 3 in **Experimental Procedures**. It is a *limiting reagent* type problem.

1. Calculate the initial moles of each reactant. Moles = Molarity x Liters

$Moles_{NaOH}$ = 0.1M x 0.2L = 0.02moles

$Moles_{HAc}$ = 0.3M x 0.1L = 0.03moles

2. Write the balanced equation for this reaction. *Note: This is a neutralization reaction.*

$$NaOH + HAc \longrightarrow Na^+Ac^- + H^+OH^-$$
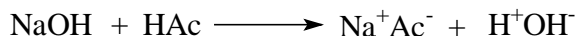
3. Determine the moles of each reactant remaining after the reaction. *Recall: This is a limiting reagent type problem.* Since NaOH is present in the least amount and, according to the balanced equation the reactants react in a 1:1 ratio, the NaOH is completely consumed (i.e., zero moles of NaOH remain). Because the reactants react in a 1:1 ratio,

$Moles_{HAc\ remaining}$ = $Moles_{HAc\ initial}$ – $Moles_{HAc\ reacted}$

= 0.03moles – 0.02moles = 0.01moles

4. Determine the molarity of the buffer components (i.e., the conjugate acid-base pair).

M_{HAc} = $moles_{HAc\ remaining}$ / Liters solution = 0.01moles/0.3L = 0.033M

The molarity of the conjugate base Ac^- resulting from the dissolution of the product NaAc is:

M_{Ac^-} = moles formed / Liters solution = 0.02moles / 0.3L = 0.067M

5. Use the H-H equation to calculate the pH.

pH = pKa + log [A^-] / [HA]

= 4.76 + log (0.067/0.033)

= 4.76 + log 2.02

= 4.76 + 0.31

= 5.07

Try this one: Determine the pH of a solution resulting from a mixture of 5mL of 0.1M KOH and 30mL of 0.1M HAc.

Experimental Procedures:

1.   Prepare 10 mL of a 0.01 M phosphate buffer, pH 7.70, from stock solutions of 0.1 M K_2HPO_4 and 0.2 M KH_2PO_4. (pKa for the weak acid = 7.20).

   A.   Use the Henderson-Hasselbalch equation to calculate the volume of each stock solution needed.

pH = pKa + log [conjugate base] / [weak acid]

   B.   Check your calculations with other students. See the instructor if there is uncertainty.

   C.   Make the solution and check the pH of a portion of your buffer solution using the pH meter. (Instructions are on **page 33**)

2.   Prepare 10 mL of 0.01 M acetate buffer, pH 3.80, from stock solutions of 0.1 M acetic acid and 0.02 M sodium hydroxide. $pKa_{acetic\ acid} = 4.76$.

   A. – C. See above.

   D.   Calculate the exact volume of the 0.01 M acetate buffer required to make 10 mL of a 0.0005 M acetate buffer.

   1.   Prepare this new buffer using the following equation to aid you in your calculations.

$$V_1 = \frac{(V_2 \ x \ M_2)}{M_1}$$

where: V_1 = the volume of the concentrated solution (liters, L)

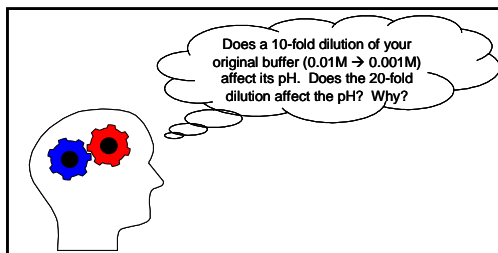V_2 = the volume of the diluted solution (liters, L)

M_1 = the molar concentration of the concentrated solution (moles/L)

M_2 = the molar concentration of the diluted solution (moles/L)

   2.   Check the pH of this new buffer.

   E.   Calculate the exact volume of the 0.01 M acetate buffer required to make 10 mL of a 0.001 M acetate buffer.

   1.   Prepare this new buffer.

   2.   Check its pH.

Does a 10-fold dilution of your original buffer (0.01M → 0.001M) affect its pH. Does the 20-fold dilution affect the pH? Why?

3. What is the pH of a solution resulting from the addition of 2.5 mL of 0.02M NaOH to 5.0 mL 0.25M acetic acid? Use the Henderson-Hasselbalch equation to calculate the pH of the resulting solution, and then follow directions B and C above. $pKa_{acetic\ acid} = 4.76$.

Record the data in your notebook (you should have 5 pH values):

The Preparation of Buffer Solutions of Desired pH STUDY GUIDE

1. The hydrogen ion concentration of tomato juice (pH 4) is how many times higher than the hydrogen ion concentration of 0.01 M NaOH (pH 12)?

2. Phosphoric acid H_3PO_4 has three dissociable protons (it is triprotic) thus three pKa values; 2.12, 7.21, and 12.32). What volume of 200 mM phosphoric acid and what volume of 500 mM NaOH are required to make 100 mL of 20 mM phosphate buffer pH 12?

3. What must be done to the electrode of the pH meter before placing it into a solution to measure its pH?

4. In the phosphate buffer system containing K_2HPO_4 and KH_2PO_4, what is the weak acid? What is its conjugate base?

5. If the weak acid in a buffer system has pKa 10.5, in what pH range is the system a most effective buffer?

6. On the laboratory shelf are 250mM solutions of both acetic acid and sodium hydroxide. How would you make a 100 mL solution of 25mM acetate buffer of pH 5.50 using these stock solutions?

7. How would you prepare a liter of 25% NaCl (w:w) aqueous solution?

8. How would you prepare a 1.5% NaBr (w:v) aqueous solution?

9. How would you prepare a 65% acetone (v:v) aqueous solution?

10. How would you prepare 500 mL of 0.12μM aqueous solution of glucose ($C_6H_{12}O_6$)?

11.   What is the percent conjugate acid in an acetate buffer of pH 3.80? (pKa = 4.76)

12.   How many μmoles are equivalent to 100 pmoles?

13.

14. What is the ratio of conjugate base to weak acid in a buffer when the pH drops to two units below the pKa?