Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 4: More on Solutions and Buffers

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Clicker Question #1: For Polling Purposes Only!

Given the current high level of COVID infections in Utah, do you prefer attending lecture classes in-person or remotely?

A) In-person

B) Remote

Given the current high level of COVID infections in Utah, do you prefer
attending laboratory classes in-person or remotely?

A) In-person

B) Remote

Temporary Change to Clicker Policy

To Minimize Pressure to Attend Lectures:

- Clicker points will not be counted until the Omicron coronavirus surge has passed.
  Early to mid February?

- We will still continue to use the clickers as a learning tool.
  Don't just guess!

- Lowest four scores (from when the points count) will still be dropped.

Calculating Dilutions

- Solutions used in biochemical experiments are often rather complicated, with multiple solutes at different concentrations.

- A solution for an experiment in this course might contain:

  0.1 M Tris-Cl buffer

  20 mM $CaCl_2$

  0.05 μg/mL Enzyme

  125 μM Substrate

- A common practice:

  - Make stock solutions at concentrations higher than the compounds will be used.

  - Dilute and mix stock solutions to make specific solutions for different experiments.

Calculating Dilutions

- Suppose that have a 2 mM stock of substrate solution, and we want to use it to make 0.8 mL of a solution that has a substrate concentration of 125 μM.

  How much of the stock solution do we use?

- A back-to-basics calculation:

  1. Calculate the number of moles in the final solution:

     moles = volume (L) $\times$ concentration (moles/L)

     volume = 0.8 mL $\times$ 10^{-3} L/mL = 8×10^{-4} L

     concentration = 125 μmoles/L $\times$ 10^{-6} moles/μmoles = 1.25×10^{-4} moles/L

     moles = 8×10^{-4} L $\times$ 1.25×10^{-4} moles/L = 10^{-7} moles = 0.1 μmoles

  2. Calculate the volume of stock solution that contains 1×10^{-7} moles
     volume (L) = moles $\div$ concentration (moles/L)

     $$= 10^{-7} \text{ moles} \div 2 \times 10^{-3} \text{ moles/L} = 5 \times 10^{-5} \text{ L}$$

     $$= 5 \times 10^{-5} \text{ L} \times 10^{6} \, \mu\text{L/L} = 50 \, \mu\text{L}$$

- An equation:

$$C_1 V_1 = C_2 V_2$$

C_1 = concentration of stock solution

V_1 = volume of the stock solution to be used

C_2 = concentration of the dilute solution

V_2 = volume of the dilute solution.

Why this equation works: The number of moles is the same in the volume of stock solution used and in the final dilute solution.

- The equation rearranged:

$$V_1 = \frac{C_2 V_2}{C_1}$$

Another Way to Calculate Dilutions

- The same example: What volume of a 2 mM stock solution of substrate do we use to make 0.8 mL of a solution that has a substrate concentration of 125 μM?

$$V_1 = \frac{C_2 V_2}{C_1}$$

$$C_1 = 2\,\text{mM} = 2,000\,\mu\text{M}$$
$$C_2 = 125\,\mu\text{M}$$
$$V_2 = 0.8\,\text{mL} = 800\,\mu\text{L}$$

$$V_1 = \frac{C_2 V_2}{C_1} = \frac{125\,\mu\text{M} \times 800\,\mu\text{L}}{2,000\,\mu\text{M}}$$
$$= 50\,\mu\text{L}$$

- This method just skips the step of calculating the number of moles.

- Keep track of the units and make sure that the result makes sense!

- For our example, 50 μL of the stock solution is diluted into a final volume of 800 μL:

$$\frac{V_2}{V_1} = \frac{800\,\mu\text{L}}{50\,\mu\text{L}} = 16$$

$$\frac{C_1}{C_2} = \frac{2,000\,\mu\text{M}}{125\,\mu\text{M}} = 16$$

- Commonly call this a "16-fold" dilution, or a "dilution factor of 16".

- Does a "1 to 16 dilution" (or 1:16) mean the same thing?
  - Does this mean 1 volume of stock solution diluted to a total of 16 volumes?
  - Does it mean 1 volume of stock solution **plus** 16 volumes of other components?
  - There's no general agreement!
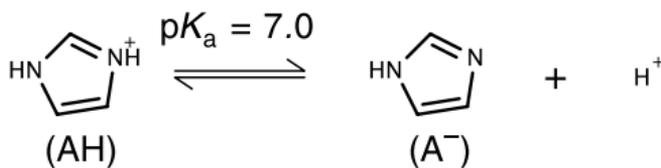 - "1 + 15" seems clearer, as the equivalent of "16-fold".

Warning!
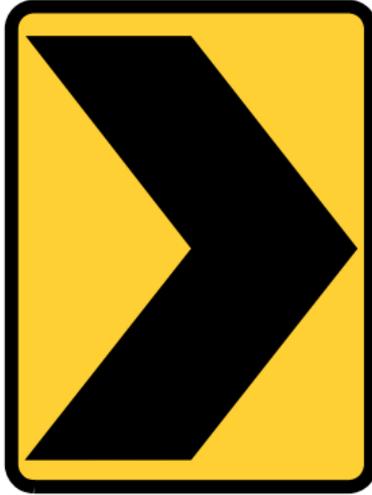
Direction Change

Back to Buffers

For the equilibrium:

$pK_a = 7.0$

(AH) (A$^-$) + H$^+$

What is the fraction of imidazole in the protonated state at pH 8?

A) $\sim 1\%$

B) $\sim 10\%$

C) $\sim 50\%$

D) $\sim 90\%$

E) $\sim 99\%$

$$pH - pK_a = -\log \frac{[A^-]}{[AH]}$$

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

- The fraction protonated:

$$f_p = \frac{[AH]}{[A^-] + [AH]}$$

A number between 0 and 1.

- Compare to the ratio of protonated and deprotonated molecules:

$$r = \frac{[AH]}{[A^-]}$$

A number between 0 and ∞. Directly related to the Henderson-Hasselbalch equation.

■ From the previous slide:

$$f_p = \frac{[AH]}{[A^-] + [AH]}$$

$$r = \frac{[AH]}{[A^-]}$$

■ With some rearranging, substituting and rearranging:

$$[AH] = r[A^-]$$

$$f_p = \frac{r[A^-]}{[A^-] + r[A^-]}$$

$$f_p = \frac{r}{1+r}$$

Why Calculate the "Fraction of . . ."?

- Generally, we know the total concentration of a compound that undergoes ionization, or other reaction.

- What is often most relevant is the concentration of one form of the compound, say the protonated form.

- If we know the fraction of molecules in the form of interest, and the total concentration, it is easy to calculate the concentration we are most interested in.

    $[AH] = f_p \times$ Total concentration

- Expressing things this way also helps in thinking clearly about what is going on.

Choosing a Buffer Compound

- Concentrations AH and A^- should be roughly equal.
  - $[AH] = [A^-]$ when $pH = pK_a$.
  - Decide on pH for experiment, then choose buffer with pK_a close to pH.

- A common rule of thumb:
  pK_a of buffer should be within 1 pH unit of solution pH.
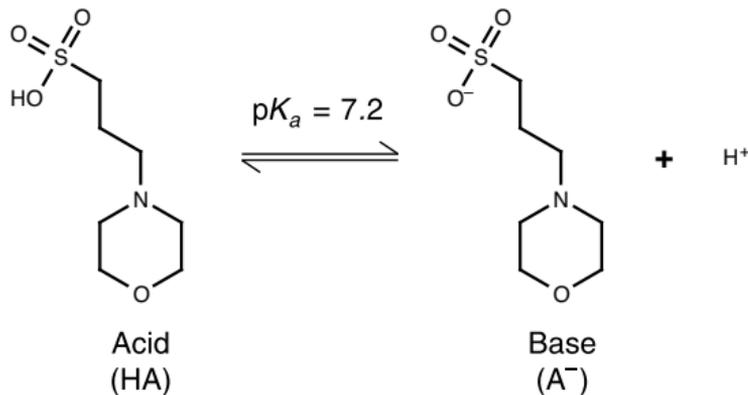
$$0.1 \lesssim \frac{[A^-]}{[AH]} \lesssim 10$$

- A better rule of thumb:
  pK_a of buffer should be within 0.5 pH unit of solution pH.

$$0.3 \lesssim \frac{[A^-]}{[AH]} \lesssim 3$$

A Buffer Calculation Example

MOPS: 3-morpholinopropane-1-sulfonic acid

Acid
(HA)

Base
(A⁻)

$pK_a = 7.2$

$+$ H^+

- Suppose that I want to make 500 mL of a 0.15 M MOPS solution, with a pH of 7.

- I dissolve 0.075 moles of MOPS (acid form) in ≈ 400 mL of water.
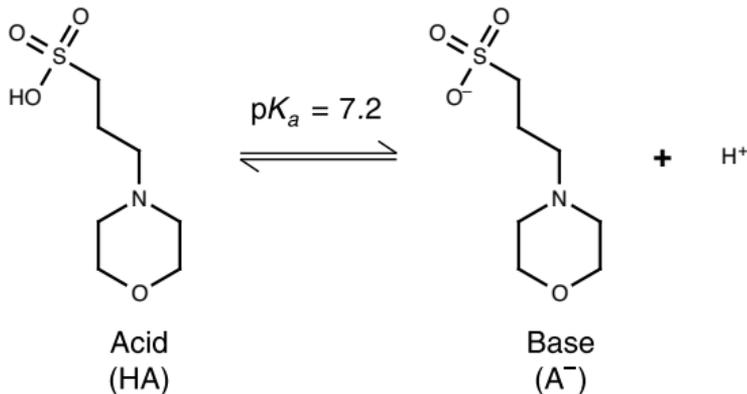
- What will the pH be?

Clicker Question #3:

After dissolving the MOPS (acid), what will the pH be?

A) Less than 7

B) 7

C) 7.2

D) Greater than 7.2

$pK_a = 7.2$

Acid
(HA)

Base
(A⁻)

+ H⁺

A Buffer Calculation Example: How do we adjust the pH to 7?

- The ionization equilibrium: $AH \rightleftharpoons A^- + H^+$
- Calculate the ratio of $[A^-]$ and $[AH]$ at pH 7.

$$pH - pK_a = \log \frac{[A^-]}{[AH]}$$

$$7 - 7.2 = -0.2 = \log \frac{[A^-]}{[AH]}$$

$$\frac{[A^-]}{[AH]} = 10^{-0.2} = 0.631$$

- How do we make the concentrations of A^- and AH satisfy this condition?
- Add a strong base (*e.g.,* NaOH) to convert some of the AH to A^-.

$$AH + OH^- \rightleftharpoons A^- + H_2O$$

How Much NaOH Should We Add?

- Use moles instead of concentrations.

$$\frac{[A^-]}{[AH]} = \frac{\text{moles } A^-/L}{\text{moles } AH/L} = \frac{\text{moles } A^-}{\text{moles } AH}$$

Both species are in the same volume, so the volume cancels out.

- Assume that very little of the MOPS is initially ionized. (MOPS is a *weak* acid.)

Starting moles of AH = 0.075

Starting moles of $A^- \approx 0$

- Assume that each mole of NaOH added drives the ionization of one mole of MOPS. (OH^- is a *strong* base.)

$$AH + OH^- \rightleftharpoons A^- + H_2O$$

How Much NaOH Should We Add?

- After adding x moles of NaOH:

    moles AH = 0.075 - x

    moles A$^-$ = x

- at pH 7:

    $$\frac{\text{moles A}^-}{\text{moles AH}} = \frac{x}{0.075 - x} = 0.631$$

- Solve for x:

    $x = 0.631(0.075 - x)$

    $x = 0.0473 - 0.631x$

    $1.631x = 0.0473$

    $x = 0.029 \text{ moles NaOH}$

- Does this make sense?

A Reality Check: The "ICE" Table

	Moles AH	Moles A$^-$
Initial	0.075	~ 0
Change	$-x = -0.029$	$x = 0.029$
Equilibrium	0.046	0.029

- Added NaOH converts a bit less than half of AH to A$^-$.

- Final pH (7) is a bit less than pK_a (7.2)
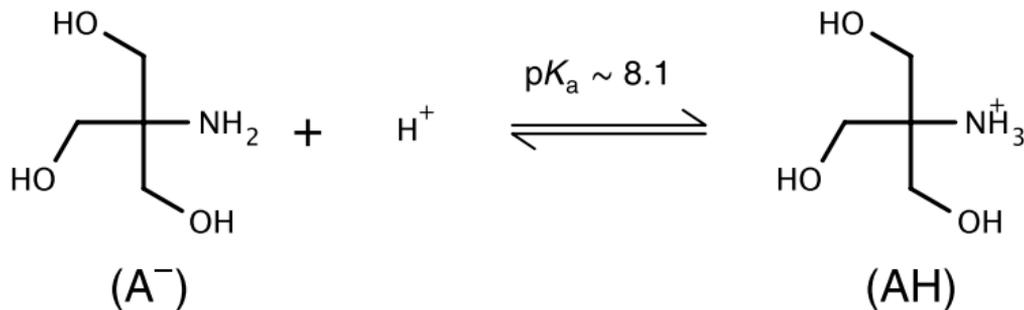
- Sounds about right!

A Common Mistake

- Start with Henderson-Hasselbalch:

$$\mathrm{pH} - \mathrm{p}K_\mathrm{a} = \log \frac{[\mathrm{A}^-]}{[\mathrm{AH}]}$$

- moles A^- = moles NaOH (the base, x moles)
- moles AH = moles MOPS (the acid, 0.075 moles)
- $[\mathrm{A}^-]$ and $[\mathrm{AH}]$ are the **final equilibrium concentrations** of the base and acid forms of the MOPS.
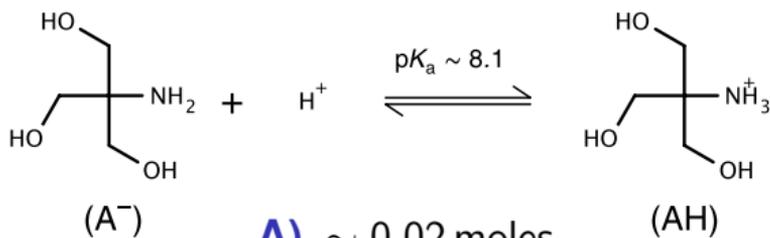
■ Tris: tris(hydroxymethyl)aminomethane

$$pK_a \sim 8.1$$

(A^-) (AH)

■ Suppose that we dissolve 0.1 moles of tris base in 0.4 L of water, and we want to adjust the pH to 7.5.

■ How much HCl should we add?

How much HCl should we add to a solution containing 0.1 moles of tris base to adjust the pH to 7.5?

$pK_a \sim 8.1$

(A^-)   (AH)

A) ~ 0.02 moles

B) ~ 0.05 moles

C) ~ 0.08 moles

D) ~ 0.1 moles

E) ~ 0.15 moles