Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 3:
pH and Buffers

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Lab Safety

- Always be aware of your environment and what you are working with!
- In the lab:
  - No food or drink in the lab.
  - Safety glasses are required for all laboratory sessions.

    Prescription glasses are not adequate.
  - Full-length trousers or equivalent are required.
  - Shoes must fully cover your feet.
  - Lab coats must be worn in the lab. They are provided at no cost.
  - Latex or nitrile gloves must be worn when working with hazardous chemicals.
  - Personal electronic devices will not be allowed in the laboratory (except when used for an experiment).

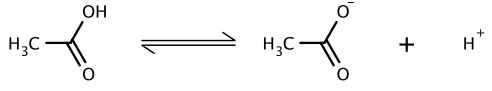
H^+ Concentration Determines Equilibria Between Protonated and De-protonated Species

- General representation of an acid-base equilibrium:

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

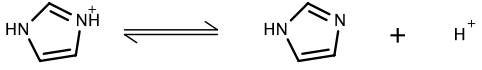
- Brønsted definition of acids and bases:
  Acids release H^+ ions to solution. (AH)
  Bases accept H^+ ions from solution. (A^-)

- Some examples:
  - Acetic acid/acetate



(AH) (A^-)

  - Imidazole



(AH) (A^-)

- Chemical properties of protonated and de-protonated functional groups are radically different!

The Equilibrium Between Protonated and De-protonated Species Also Depends on Affinity for H^+ Ions

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

- The acid dissociation constant:

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

A **large** value of K_a means that HA likes to give up its H^+.

- Commonly expressed in logarithmic form:

$$pK_a = -\log K_a$$

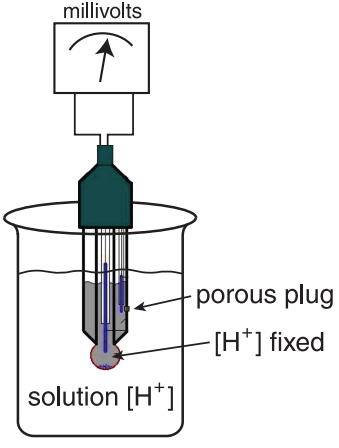
by analogy to pH:

$$pH = -\log [H^+]$$

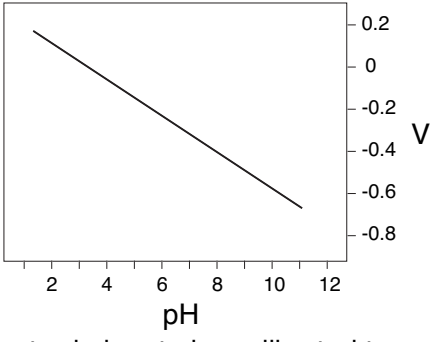
But, don't confuse $pK_a$and pH!

A **small** value of pK_a means that HA likes to give up its H^+.

pH Meter with Combination Electrode



- Difference in $[H^+]$ creates voltage difference across glass membrane.



- Electrode has to be calibrated to establish slope and intercept.

Figure adapted from Wikipedia: http://en.wikipedia.org/wiki/Glass_electrode

pH Buffers

- The basic idea: A weak acid and it's conjugate base in equilibrium:

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

  - If $[H^+]$ increases, A^- combines with H^+, and pH is (mostly) restored.
  - If $[H^+]$ decreases, AH dissociates, and pH is (mostly) restored.
- In order for a buffer to be effective:
  - Concentrations of AH and A^- must be greater than potential change in H^+ concentration.
  - Concentrations of AH and A^- must be roughly equal.
- Relative concentrations of AH and A^- are determined by $[H^+]$ and K_a (pH and pK_a).

The Henderson-Hasselbalch Equation

- The acid dissociation equilibrium: $K_a = \frac{[H^+][A^-]}{[HA]}$
- Take logarithms of both sides: $\log K_a = \log \frac{[H^+][A^-]}{[HA]}$
- Separate out $\log [H^+]$ on the right-hand side: $\log K_a = \log [H^+] + \log \frac{[A^-]}{[HA]}$
- Substitute $\log K_a = -pK_a$ and $\log [H^+] = -pH$, and rearrange things a bit:

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

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

- It's just an equilibrium expression!

The Henderson-Hasselbalch Equation

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

- If $\mathrm{pH} = \mathrm{p}K_\mathrm{a}$:

$$\log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} = 0, \qquad \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} = 1, \qquad [\mathrm{A}^-] = [\mathrm{HA}]$$

- If $\mathrm{pH} > \mathrm{p}K_\mathrm{a}$:

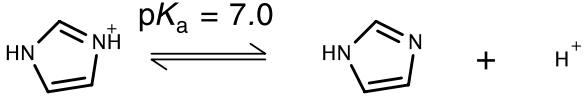
$$\log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} > 0, \qquad \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} > 1, \qquad [\mathrm{A}^-] > [\mathrm{HA}]$$

- If $\mathrm{pH} < \mathrm{p}K_\mathrm{a}$:

$$\log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} < 0, \qquad \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} < 1, \qquad [\mathrm{A}^-] < [\mathrm{HA}]$$

Clicker Question #1

For the equilibrium:



(AH) (A⁻)

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

A) ~ 1%

B) ~ 10%

C) ~ 50%

D) ~ 90%

E) ~ 99%

Calculating the “Fraction of . . .”

$$\mathrm{AH} \rightleftharpoons \mathrm{A^-} + \mathrm{H^+}$$

- The fraction protonated:

$$f_\mathrm{p} = \frac{[\mathrm{AH}]}{[\mathrm{A^-}] + [\mathrm{AH}]}$$

  A number between 0 and 1.

- Compare to the ratio of protonated and deprotonated molecules:

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

  A number between 0 and ∞.
  Calculated from the Henderson-Hasselbalch equation, given $\mathrm{pH} - \mathrm{p}K_\mathrm{a}$

Calculating the “Fraction of . . .”

- 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 rapid equilibrium.
- 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.

$$[\mathrm{AH}] = f_{\mathrm{p}} \times \text{Total concentration}$$

- 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.

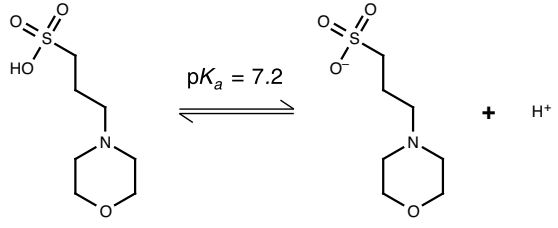
$$0.1 \lesssim \frac{[A^-]}{[AH]} \lesssim 10$$

- 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



- 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 $\approx$ 400 mL of water.
- What will the pH be?

Clicker Question #2:

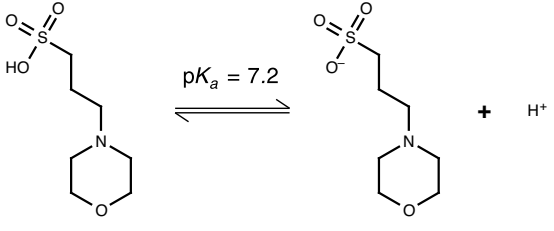
After dissolving the MOPS (acid), what will the pH be?

A) Less than 7

B) 7

C) 7.2

D) Greater than 7.2



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

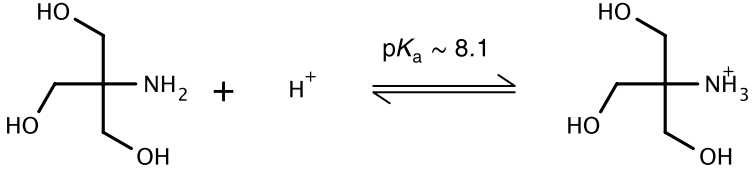
- Start with Henderson-Hasselbalch:

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

- 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.

The Buffer We Will Use for Most of Our Experiments

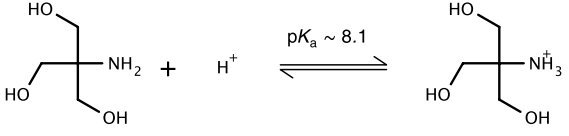
- Tris: tris(hydroxymethyl)aminomethane



- Works well at pH 8, where we will do most of our experiments.
- Largely unreactive with biological molecules.
- Relatively inexpensive.

Clicker Question #3

What will the pH be if we make a solution of 0.2 M tris base?



A) pH 4

B) pH 6

C) pH 7

D) pH 8

E) pH 10

Protocol for Preparing Tris Buffer

- Measure out tris base to make 50 mL of a 0.2 M solution.
- Dissolve tris in about 40 mL of water.
- Adjust pH to 8.0, at 25°C by adding HCl and monitoring with a pH meter.
- Adjust final volume to 50 mL, using a graduated cylinder.
- Filter solution and store in a carefully labeled vessel.