

Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
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Lecture 3: pH and Buffers

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©David P. Goldenberg
University of Utah
goldenberg@biology.utah.edu

A Special Measure of Concentration for Hydrogen Ions

- Hydrogen ion concentration expressed as pH

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

with $[\text{H}^+]$ expressed in molar units

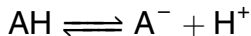
- To convert from pH to molar concentration:

$$[\text{H}^+] = 10^{-\text{pH}}\text{M}$$

- Why does H^+ concentration get special treatment?

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

- General representation of an acid-base equilibrium:

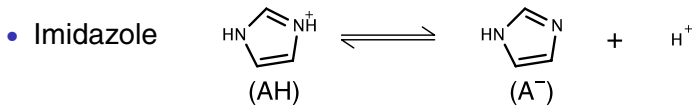
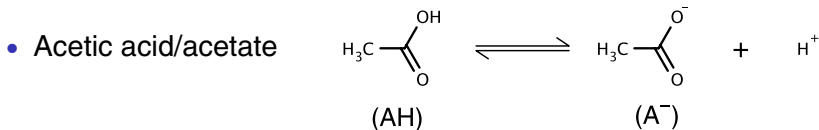


- Brønsted definition of acids and bases:

Acids release H⁺ ions to solution. (AH)

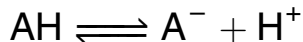
Bases pick up H⁺ ions from solution. (A⁻)

- Some examples:



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

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



- The acid dissociation constant:

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

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

- Commonly expressed in logarithmic form:

$$\text{p}K_a = -\log K_a$$

by analogy to pH:

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

But, don't confuse $\text{p}K_a$ and pH!

A **small** value of $\text{p}K_a$ means that HA likes to give up its H⁺.

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 $pK_a = -\log K_a$ and $pH = -\log [H^+]$, 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

$$\text{pH} - \text{p}K_a = \log \frac{[\text{A}^-]}{[\text{HA}]}$$

- If $\text{pH} > \text{p}K_a$:

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

- If $\text{pH} = \text{p}K_a$:

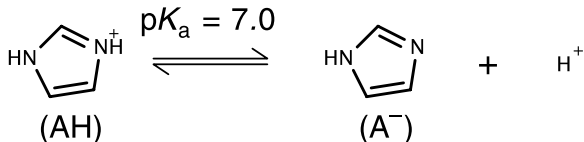
$$\log \frac{[\text{A}^-]}{[\text{HA}]} = 0, \quad \frac{[\text{A}^-]}{[\text{HA}]} = 1, \quad [\text{A}^-] = [\text{HA}]$$

- If $\text{pH} < \text{p}K_a$:

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

Clicker Question #1

For the equilibrium:

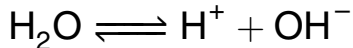
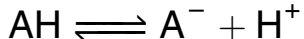


What is the fraction of imidazole in the protonated state at pH 8?

- 1 ~ 1%
- 2 ~ 10%
- 3 ~ 50%
- 4 ~ 90%
- 5 ~ 99%

Why pH Requires Special Attention

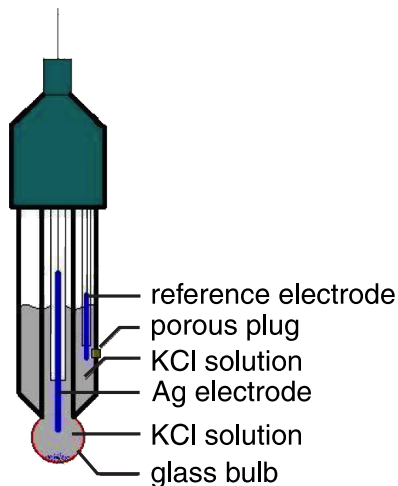
- Why not just add H^+ ions to the desired concentration?
 - The H^+ concentration is usually very low. pH 7 $\rightarrow 10^{-7}$ M
 - Adding H^+ ions will shift equilibria:



Generally, the H^+ concentration will increase less than expected from the addition of H^+ .

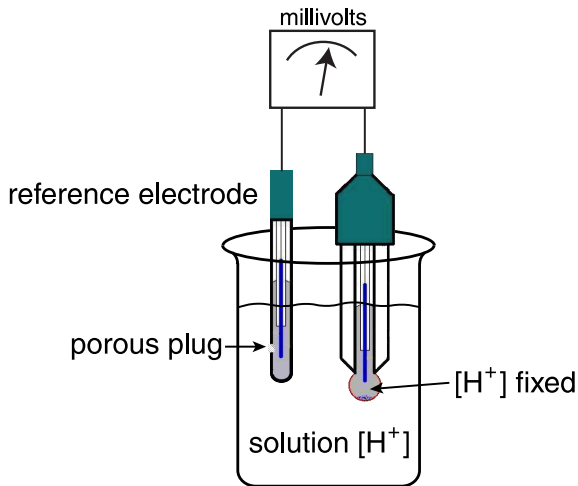
- Two special means of dealing with pH:
 - pH meter, directly measures pH of solution
 - pH buffers, compounds added to solutions to establish and maintain pH

A Combination pH Electrode

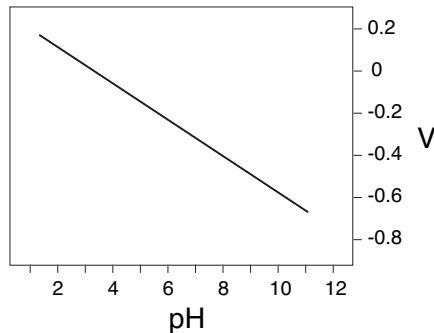


- Contains 2 electrodes:
 - pH-sensitive electrode
 - Reference electrode
- Reference electrode is electrically connected to test solution (porous plug)
- H^+ ions cannot cross glass membrane of bulb.
- $[H^+]$ inside bulb is fixed.

Separate pH and Reference Electrodes



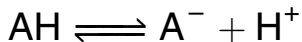
- Difference in $[H^+]$ creates voltage difference across glass membrane.



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

pH Buffers

- The basic idea: A weak acid and its conjugate base in equilibrium:



- If $[\text{H}^+]$ increases, A^- combines with H^+ , and pH is (mostly) restored.
- If $[\text{H}^+]$ 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 AH and A^- must be roughly equal.
- Relative concentrations of AH and A^- are determined by $[\text{H}^+]$ and K_a (pH and $\text{p}K_a$).

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.

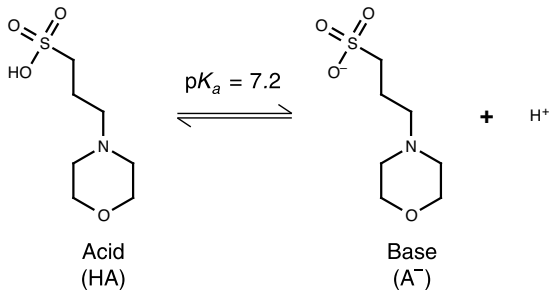
$$0.1 \lesssim \frac{[A^-]}{[AH]} \lesssim 10$$

- 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:

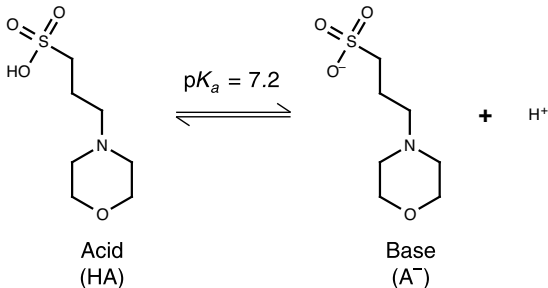
After dissolving the MOPS (acid), what will the pH be?

1 Less than 7

2 7

3 7.2

4 Greater than 7.2



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

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

$$\text{pH} - \text{p}K_a = \log \frac{[\text{A}^-]}{[\text{AH}]}$$

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

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

- 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^- .



How Much NaOH Should We Add?

- Use moles instead of concentrations.

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

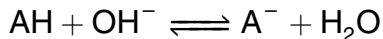
Both species are in the same volume, so the volume cancels out.

- 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.)



How Much NaOH Should We Add?

- After adding x moles of NaOH:

$$\text{moles AH} = 0.075 - x$$

$$\text{moles A}^- = x$$

- 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 ⁻
<u>I</u> nitial	0.075	~ 0
<u>C</u> hange	-x = -0.029	x = 0.029
<u>E</u> quilibrium	0.036	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

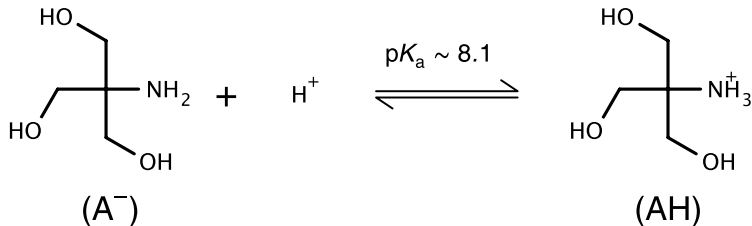
- Start with Henderson-Hasselbalch:

$$\text{pH} - \text{p}K_a = \log \frac{[\text{A}^-]}{[\text{AH}]}$$

- moles A^- = moles NaOH (the base, x moles)
- moles AH = moles MOPS (the acid, 0.075 moles)
- $[\text{A}^-]$ and $[\text{AH}]$ are the **final equilibrium concentrations** of the base and acid forms of the MOPS.

Another Example

- Tris: tris(hydroxymethyl)aminomethane



- 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?

Other Considerations with Tris Buffers

- pK_a of tris is sensitive to temperature.
 - Generally true of amines. Carboxylate and sulfonates (like MOPS) are much better in this regard.
 - A 1°C increase in temperature causes decrease in pK_a (and pH) of 0.03 pH units.
 - A tris buffer adjusted to pH 8 at 25°C will have a pH of 7.4 at 4°C , a 4-fold increase in $[\text{H}^+]$.
 - Adjust pH of buffer at the temperature it is to be used!
- Tris does funny things to some pH electrodes! Often makes response to other solutions sluggish.
- Good things about tris:
 - Does not interact with most biochemical molecules or reactions.
 - Inexpensive!