Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 3:
Concentration, pH and pK_a

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Announcements

- Instructor's office hours:
  - Regularly scheduled times (via Zoom):
    Tuesdays, 11:00 AM to noon
    Wednesdays, 2:00 to 3:00 PM
    Zoom links on Canvas
  - Other times by appointment.
    Send me an E-mail: goldenberg@biology.utah.edu
- Lab sessions: Begin at 1:00 PM in room 138 CSC.

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).

Units of Concentration

- Most convenient: amount of solute per volume of solution:
  - g/L (= mg/mL): 1 g solute in 1 L final volume of solution
  - molar (M) = mole/L: 1 mole of solute in 1 L final volume of solution
- Some less commonly used units of concentration:
  - molal: 1 mole of solute dissolved in 1 kg solvent
  - 1%(m/v): 1 g solute in 100 mL final volume of solution
  - 1%(v/v): 1 mL pure liquid in 100 mL final volume of solution

A Source of Confusion: Units for "Molecular Weight"

- Molecular weight or molecular mass:
  - The mass of a single molecule
  - Units: atomic mass unit (u or amu) *or* dalton (Da) or kilodalton (kDa)

    1 amu = 1 Da = mass of one atom of $^{12}C \div 12$
  - Units are often not included, because it is a relative mass, M_r.
  - amu is commonly used in mass spectrometry
  - Da and kDa are very commonly used in biochemistry and molecular biology, especially for proteins and other macromolecules.
- Molar mass:
  - Mass of one mole of a compound
  - Units: g/mol (which doesn't completely make sense)
- Molecular mass of 100 Da $\longrightarrow$ molar mass of 100 g/mol

To Calculate the Amount of Solute in a Solution

- The number of grams in 53 mL of a 5 g/L solution:

$$53\,\mathrm{mL} \times 0.001\,\mathrm{L/mL} = 0.053\,\mathrm{L}$$
$$0.053\,\mathrm{L} \times 5\,\mathrm{g/L} = 0.26\,\mathrm{g}$$

- The number of moles in 1.3 L of a 15 mM solution ($1\,\mathrm{mM} = 0.001\,\mathrm{M}$):

$$15\,\mathrm{mM} \times 0.001\,\mathrm{M/mM} = 0.015\,\mathrm{M} = 0.015\,\mathrm{mol/L}$$
$$1.3\,\mathrm{L} \times 0.015\,\mathrm{mol/L} = 0.0195\,\mathrm{mol}$$

- The number of molecules in 1.3 L of a 15 mM solution:

$$1\,\mathrm{mol} = 6.02 \times 10^{23}\,\mathrm{molecules}$$
$$0.0195\,\mathrm{mol} \times 6.02 \times 10^{23}\,\mathrm{molecules/mol} = 1.17 \times 10^{22}\,\mathrm{molecules}$$

Clicker Question #1

How many moles of water molecules ($M_r = 18$) are in 1 L ($\approx 1000\,\mathrm{g}$)?

A) ~ 10

B) ~ 30

C) ~ 50

D) ~ 70

How Many Moles of Water in 1 L?

- Liters to grams:

$$1\,\text{L} \times 1,000\,\text{ml}/\text{L} = 1,000\,\text{mL}$$

$$1,000\,\text{mL} \times 1\,\text{g}/\text{mL} = 1,000\,\text{g}$$

- Grams to moles:

$$1,000\,\text{g} \div 18\,\text{g}/\text{mol} = 1,000\,\text{g} \times 1\,\text{mol}/(18\,\text{g})$$

$$= 56\,\text{mol}$$

Other Units of Concentration Commonly Used in Biochemistry

- Based on molar units:
  - 1 mM = 1×10^{-3} M
  - 1 μM = 1×10^{-6} M = 1×10^{-3} mM
  - 1 nM = 1×10^{-9} M = 1×10^{-3} μM
  - 1 pM = 1×10^{-12} M = 1×10^{-3} nM
- Based on mass units:
  - 1 mg/mL = 1 g/L
  - 1 μg/mL = 1×10^{-3} mg/mL = 1×10^{-3} g/L
  - 1 μg/μL = 1 mg/mL = 1 g/L

A Special Measure of Concentration for Hydrogen Ions

- Hydrogen ion concentration expressed as pH

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

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

- To convert from pH to molar concentration:

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

- Why does H^+ concentration get special treatment?

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

- General representation of an acid-base equilibrium:

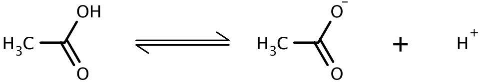
$$AH \rightleftharpoons A^- + H^+$$

- Brønsted definition of acids and bases:

  Acids release H^+ ions to solution. (AH)

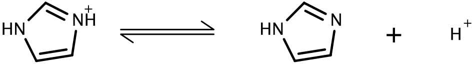
  Bases accept H^+ ions from solution. (A^-)

- 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^+.

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:

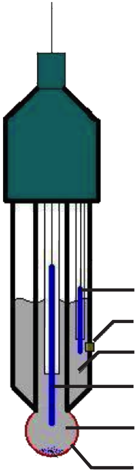
$$H^+ + A^- \rightleftharpoons AH$$

$$H^+ + OH^- \rightleftharpoons H_2O$$

  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

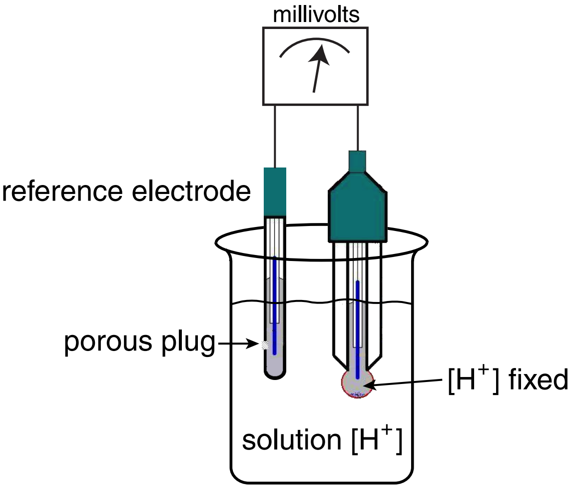
A Combination pH Electrode



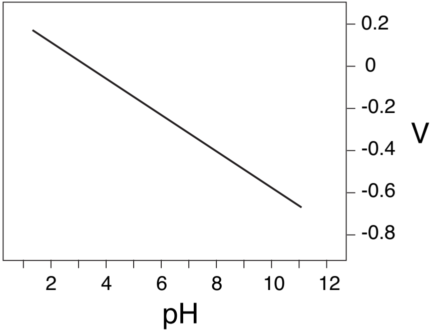
- Contains 2 electrodes:
  - pH-sensitive electrode
  - Reference electrode
- Reference electrode is electrically connected to test solution (porous plug)
- H^+ ions cannot cross glass membrane of bulb.
- $[H^+]$ inside bulb is fixed.

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

Separate pH and Reference Electrodes



- 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: $K_\mathrm{a} = \dfrac{[\mathrm{H}^+][\mathrm{A}^-]}{[\mathrm{HA}]}$
- Take logarithms of both sides: $\log K_\mathrm{a} = \log \dfrac{[\mathrm{H}^+][\mathrm{A}^-]}{[\mathrm{HA}]}$
- Separate out $\log\,[\mathrm{H}^+]$ on the right-hand side: $\log K_\mathrm{a} = \log\,[\mathrm{H}^+] + \log \dfrac{[\mathrm{A}^-]}{[\mathrm{HA}]}$
- Substitute $\log K_\mathrm{a} = -\mathrm{p}K_\mathrm{a}$ and $\log\,[\mathrm{H}^+] = -\mathrm{pH}$, and rearrange things a bit:

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

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

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

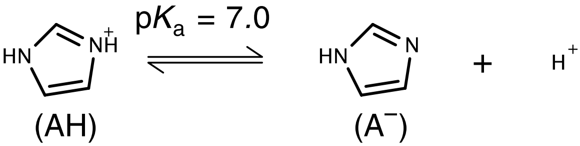
$$\log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} > 0, \qquad \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} > 1, \qquad [\mathrm{A}^-] > [\mathrm{HA}]$$

- 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 #2

For the equilibrium:



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%

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.

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