Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2017

Lecture 2:
Units, Concentrations, Acid-base Equilibria

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

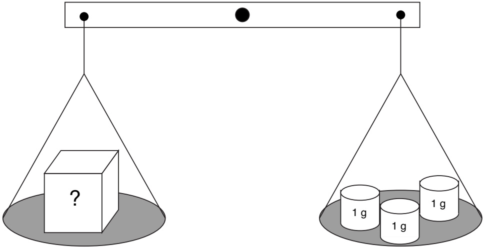
Clicker Question #1:

What time does class start?

1. 9:10 AM
2. $\boxed{\text{9:40 AM}}$
3. 9:45 AM
4. 9:50 AM
5. What class?

A Bit on Units and Conversion Factors

- All measurements are comparisons.



- Units of measurement are defined by the reference for comparison.
- Suppose that we want to use other units, such as mg or ounces? (what kind of ounces?)

Conversion Factors

- As instructions:
  - To convert from g to mg, multiply by 1,000
  - To convert from g to ounces (U.S.), multiply by 0.035274
- As equations:
  - $1\,\mathrm{g} = 1,000\,\mathrm{mg}$
  - $1\,\mathrm{g} = 0.035274\,\mathrm{ounce}$
- As ratios equal to 1:

$$\frac{1\,\mathrm{g}}{1,000\,\mathrm{mg}} = 1 \qquad \frac{1,000\,\mathrm{mg}}{1\,\mathrm{g}} = 1$$

$$\frac{1\,\mathrm{g}}{0.035274\,\mathrm{ounce}} = 1 \qquad \frac{0.035274\,\mathrm{ounce}}{1\,\mathrm{g}} = 1$$

We can always multiply or divide by 1 without changing anything!

Conversion by Multiplication (or Division?)

- Convert 3 g to ounces

  - Multiply

$$3\,\mathrm{g} \times \frac{0.035274\,\mathrm{ounce}}{1\,\mathrm{g}} = 0.1\,\mathrm{ounce}$$

  - or divide?

$$3\,\mathrm{g} \div \frac{0.035274\,\mathrm{ounce}}{1\,\mathrm{g}} = 85\,\mathrm{g}^2/\mathrm{ounce}$$

- Units can be treated as algebraic entities (and should cancel sensibly in the result!)
- "Dimensional analysis" or "unit factor analysis"

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

    1 mole = amount of a substance containing the number of atoms or molecules equal to the number of atoms in 12 g of ^{12}C.

    Number of atoms or molecules in 1 mole of a substance is called Avogadro's number, $N_A \approx 6.02 \times 10^{23}$
- Some less convenient (for purposes of calculation) 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:

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

Other Units of Concentration Commonly Used in Biochemistry

- Based on molar units:
  - $1\ \text{mM} = 1 \times 10^{-3}\ \text{M}$
  - $1\ \mu\text{M} = 1 \times 10^{-6}\ \text{M} = 1 \times 10^{-3}\ \text{mM}$
  - $1\ \text{nM} = 1 \times 10^{-9}\ \text{M} = 1 \times 10^{-3}\ \mu\text{M}$
  - $1\ \text{pM} = 1 \times 10^{-12}\ \text{M} = 1 \times 10^{-3}\ \text{nM}$

- Based on mass units:
  - $1\ \text{mg/mL} = 1\ \text{g/L}$
  - $1\ \mu\text{g/mL} = 1 \times 10^{-3}\ \text{mg/mL} = 1 \times 10^{-3}\ \text{g/L}$
  - $1\ \mu\text{g}/\mu\text{L} = 1\ \text{mg/mL} = 1\ \text{g/L}$

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) × concentration (moles/L)

     $\text{volume} = 0.8\,\text{mL} \times 10^{-3}\,\text{L/mL} = 8\times10^{-4}\,\text{L}$

     $\text{concentration} = 125\,\mu\text{moles/L} \times 10^{-6}\,\text{moles}/\mu\text{moles} = 1.25\times10^{-4}\,\text{moles/L}$

     $\text{moles} = 8\times10^{-4}\,\text{L} \times 1.25\times10^{-4}\,\text{moles/L} = 10^{-7}\,\text{moles} = 0.1\,\mu\text{moles}$
  2. Calculate the volume of stock solution that contains 1.25×10^{-4} moles

     volume (L) = moles ÷ concentration (moles/L)

     $= 10^{-7}\,\text{moles} \div 2\times10^{-3}\,\text{moles/L} = 5\times10^{-5}\,\text{L}$

     $= 5\times10^{-5}\,\text{L} \times 10^{6}\,\mu\text{L/L} = 50\,\mu\text{L}$

Another Way to Calculate Dilutions

- 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?

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

$$C_1 = 2\,\mathrm{mM} = 2,000\,\mu\mathrm{M}$$

$$C_2 = 125\,\mu\mathrm{M}$$

$$V_2 = 0.8\,\mathrm{mL} = 800\,\mu\mathrm{L}$$

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

$$= 50\,\mu\mathrm{L}$$

- 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!

Describing a Dilution

- 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 “1:16 dilution” 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”.

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?

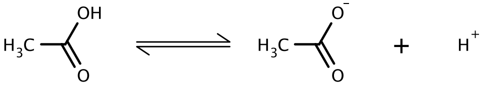
H^+ Concentration Determines Equilibria Between Protonated and De-protonated Species

- General representation of an acid-base equilibrium:

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

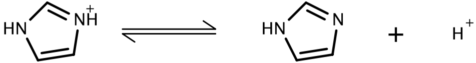
- Brønsted definition of acids and bases:
  Acids release H^+ ions to solution. (AH)
  Bases pick up H^+ ions from solution. (A^-)

- Some examples:
  - Acetic acid/acetate



(AH) (A^-)

  - Imidazole



(AH) (A^-)

- 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

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

- 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^+.