Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 5:
A Bit More on Buffers
and
Introduction to UV-visible Spectrophotometry

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Lab Reports

- Read the instructions on lab reports in the lab manual!
  (also available on LabArchives)
- Report for Experiment 1: Due the week of 31 January, at 1:00 PM on the day of your lab section.
- From LabArchives to Canvas:
  1. Save the folder for the experiment as a pdf file.
  2. submit the pdf file to Canvas.

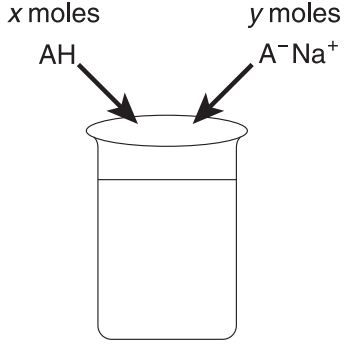
A General Approach to Making Buffers

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

1. Choose buffer compound to match pK_a to working pH.
2. Start with a solution of weak acid (or base).
3. Adjust pH to desired value by adding strong base (or acid).
   Two ways:
   a. Follow pH with meter.
      - Advantage: No math required!
      - Disadvantage: Subject to pH-meter errors.
      - Variability in final salt concentration, because pH is relatively insensitive to amount of strong base (or acid) added. It's a buffer solution!
   b. Calculate the amount of strong base (or acid) to add.
      - Advantage: Independent of pH meter.
      - Advantage: More consistent with respect to final concentrations.
      - Disadvantage: Depends on accurately determined concentration of strong base (or acid).
      - pK_a can depend on solution conditions.

Another Way to Make a Buffer Solution

- Directly mix weak acid and salt of weak base.
  (or weak base and salt of weak acid)



- Because they are weak, the acid and base do not release or take up a significant number of H^+ ions when dissolved. So, their concentrations don't change significantly.
- Ratio of $[A^-]$ and $[AH]$

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

- pH:

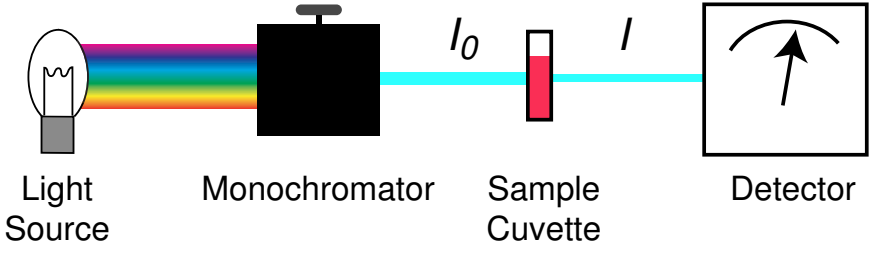
$$\text{pH} = \log \frac{y}{x} + \text{p}K_\text{a}$$

Warning!

Direction Change

UV-Visible Spectrophotometry

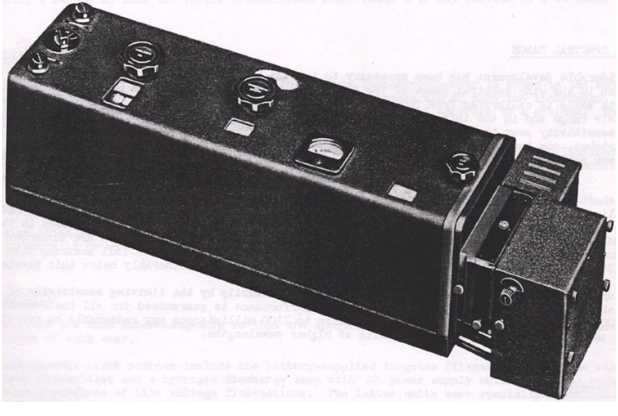
A UV-Visible Spectrophotometer



$$\% \, Transmittance = \frac{I}{I_0} \times 100$$

$$A = \log \frac{I_0}{I}$$

The *Classic* Spectrophotometer

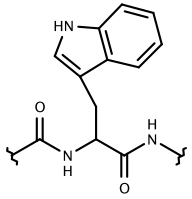


The Beckman DU: Produced from 1941 to 1975

Cary, H. H. & Beckman, A. O. J. (1941) *J. Opt. Soc. Am.* **31**, 682-689.

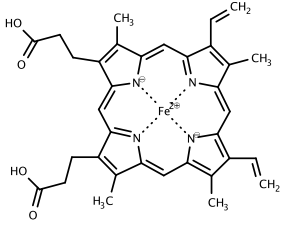
UV or Visible Absorbance Usually Arises from:

- Systems of conjugated double bonds
  - Peptide bonds in proteins.
  - Aromatic amino acid residues in proteins
  - Bases in nucleic acids



Tryptophan

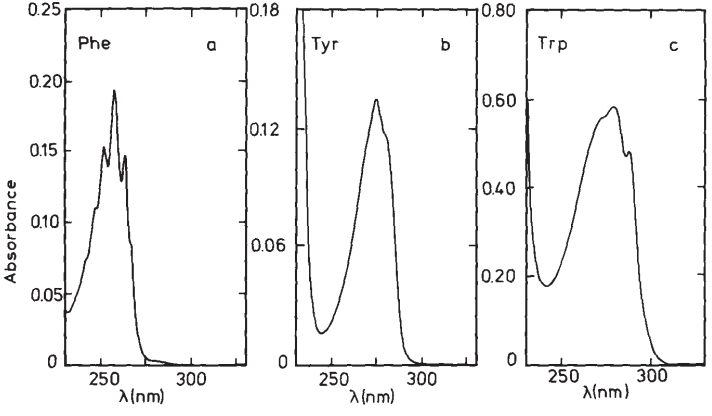
- Coordinated metal ions
  - Heme
  - Chlorophyl



Heme

Larger conjugated systems $\rightarrow$ longer wavelength of light absorbed

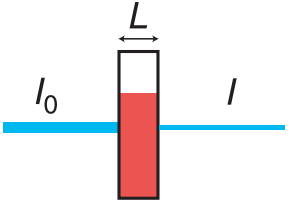
UV Absorbance of Aromatic Amino Acids



- λ_{max} Wavelength of maximum absorbance

Spectra from: Schmid, F. X. (1997). In *Protein Structure: A Practical Approach* (Creighton, T. E., ed.), pp. 261-297. IRL Press, Oxford. (A good introduction to optical spectroscopy of proteins)

The Beer-Lambert Law



$$A = \log \frac{I_0}{I} = C \cdot L \cdot \epsilon$$

$C =$ concentration (M)

$L =$ cuvette path length (cm)

$\epsilon =$ extinction coefficient ($M^{-1}cm^{-1}$)

$$C = \frac{A}{\epsilon \cdot L}$$

- Allows us to measure concentration by measuring absorbance.
- How do we know what the extinction coefficient is?

Units for the Extinction Coefficient

$$A = C \cdot L \cdot \epsilon$$

- A is dimensionless
- Most cuvettes have a path length of 1 cm, so it is convenient to use cm as the dimension of length.
- If concentration is expressed in molar units, then ϵ should have units of $\mathrm{M}^{-1}\mathrm{cm}^{-1}$, so that:
  $\mathrm{M} \times \mathrm{cm} \times \mathrm{M}^{-1}\mathrm{cm}^{-1}$ is dimensionless
- If concentration is expressed in units of mg/mL, then ϵ should have units of $\mathrm{cm}^{-1}(\mathrm{mg/mL})^{-1} = \mathrm{cm}^{-1}(\mathrm{mL/mg})$.
- If concentration is expressed as % (m/v) solute, then ϵ should have units of $\mathrm{cm}^{-1}\%^{-1} = \mathrm{cm}^{-1}(\mathrm{g}/100\mathrm{mL})^{-1} = \mathrm{cm}^{-1}(100\mathrm{mL/g})$.

Clicker Question #1

- Someone gives you a solution of a mystery compound and tells you that the extinction coefficient at 535 nm is $3\,\mathrm{cm}^{-1}(\mathrm{g/L})^{-1}$
- Using a 1 cm cuvette, the absorbance is 1.2.
- The concentration of the sample is:

A) 0.04 mg/mL

B) 0.4 mg/mL

C) 4 mg/mL

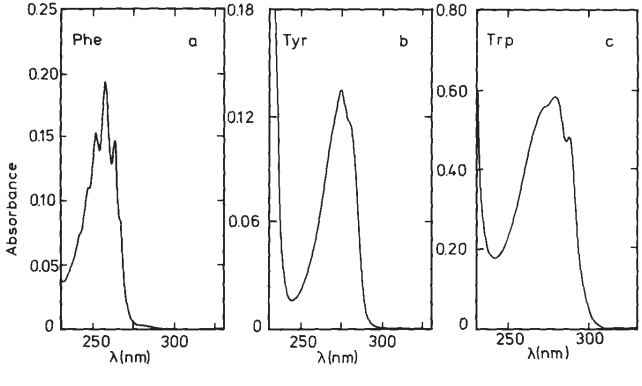
D) 0.04 g/mL

E) 0.4 g/mL

$$A = C \cdot L \cdot \epsilon$$
$$1.2 = C \times 1\,\mathrm{cm} \times 3\,\mathrm{cm}^{-1}(\mathrm{g/L})^{-1}$$
$$1.2 = C \times 3\,(\mathrm{g/L})^{-1}$$
$$C = 1.2 \div 3\,(\mathrm{g/L})^{-1}$$
$$C = 0.4\,\mathrm{g/L} = 0.4\,\mathrm{mg/mL}$$

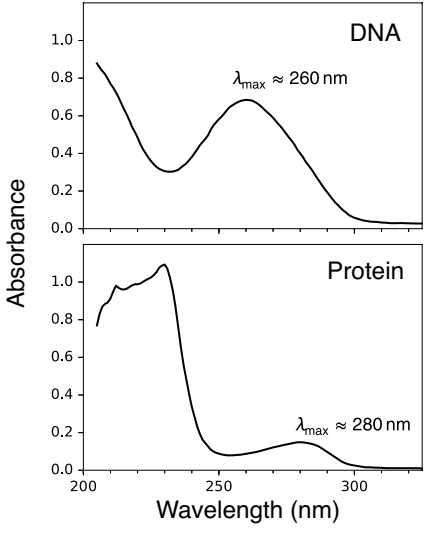
What if Solution Contains Multiple Compounds that Absorb Light?

- Peaks in UV-visible absorption spectra are quite broad:



- Peaks from different compounds often overlap.
- Absorption at a given wavelength may contain contributions from multiple compounds.

UV Absorption Spectra of Proteins and DNA



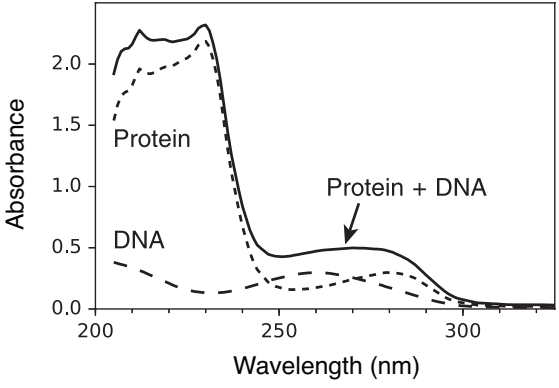
- DNA spectra do not depend much on sequence.
- Protein spectra do depend on amino acid composition, and a bit on three-dimensional structure.
- DNA and protein spectra, between 250 and 300 nm, overlap extensively.
- Concentrations:

  $[\text{DNA}] \approx 0.03\,\text{mg/ml}$

  $[\text{Protein}] \approx 0.16\,\text{mg/ml}$

Spectra adapted from Spectrophotometry Handbook (2012) GE LifeScience

Spectra of DNA, Protein and a Mixture



- Absorbances of different components add.
- Assumes components don't interact and alter spectra.
- Can we interpret absorbance of mixtures?

Estimating Concentrations of Protein and DNA in a Mixture

- Between 250 and 300 nm

  For Protein: $\lambda_{max} \approx 280\,\text{nm}$

  For DNA: $\lambda_{max} \approx 260\,\text{nm}$

- At 260 nm (assuming 1-cm cuvette):

$$A_{260} = [\text{Protein}] \cdot \epsilon_{260}^{\text{Protein}} + [\text{NA}] \cdot \epsilon_{260}^{\text{NA}}$$

- At 280 nm:

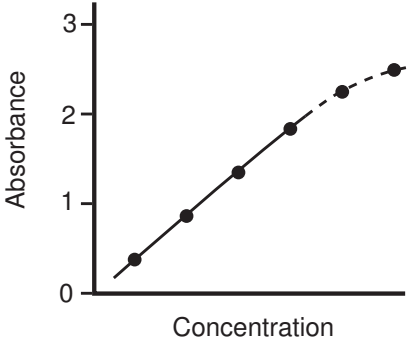
$$A_{280} = [\text{Protein}] \cdot \epsilon_{280}^{\text{Protein}} + [\text{NA}] \cdot \epsilon_{280}^{\text{NA}}$$

- If all four extinction coefficients are known, and we measure A_{260} and A_{280}, we have two equations in two unknowns.

  Solve for [Protein] and [NA].

- What could go wrong?

Absorbance versus Concentration



The most reliable measurements are obtained when $0.1 \lesssim A \lesssim 1.5$

Some Practical Points

- The cuvettes must be transparent to light of the wavelength of interest.
  - Glass or plastic work well for visible light ($\lambda > \approx 350$ nm.)
  - Fused silica (quartz) ~~is~~ **was** necessary for UV light ($200 \text{ nm} < \lambda < \approx 350$ nm). Quartz cuvettes are very expensive!
  - Very recent: There are UV-transparent plastic cuvettes! (down to about 220 nm)
- Absorbances are measured relative to that for a “blank” solution that contains everything *except* the compound of interest.