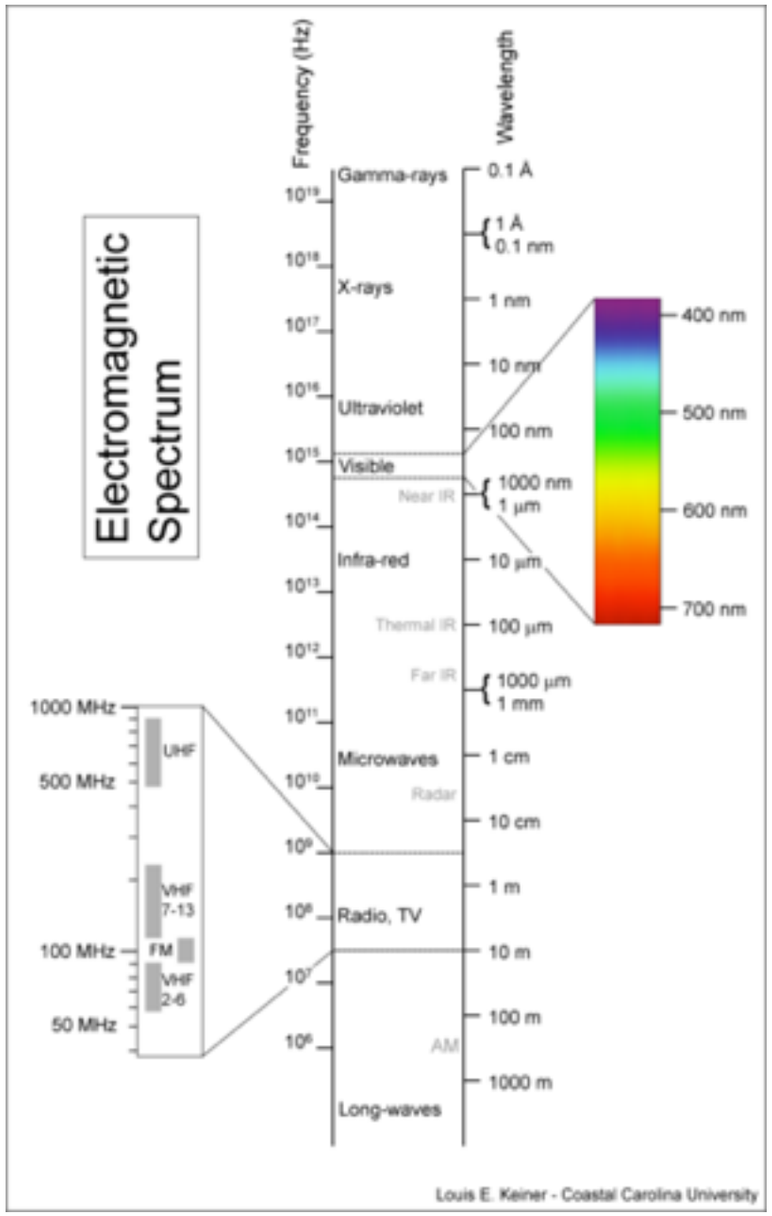
Spectroscopy
Chapter 13

Electromagnetic Spectrum

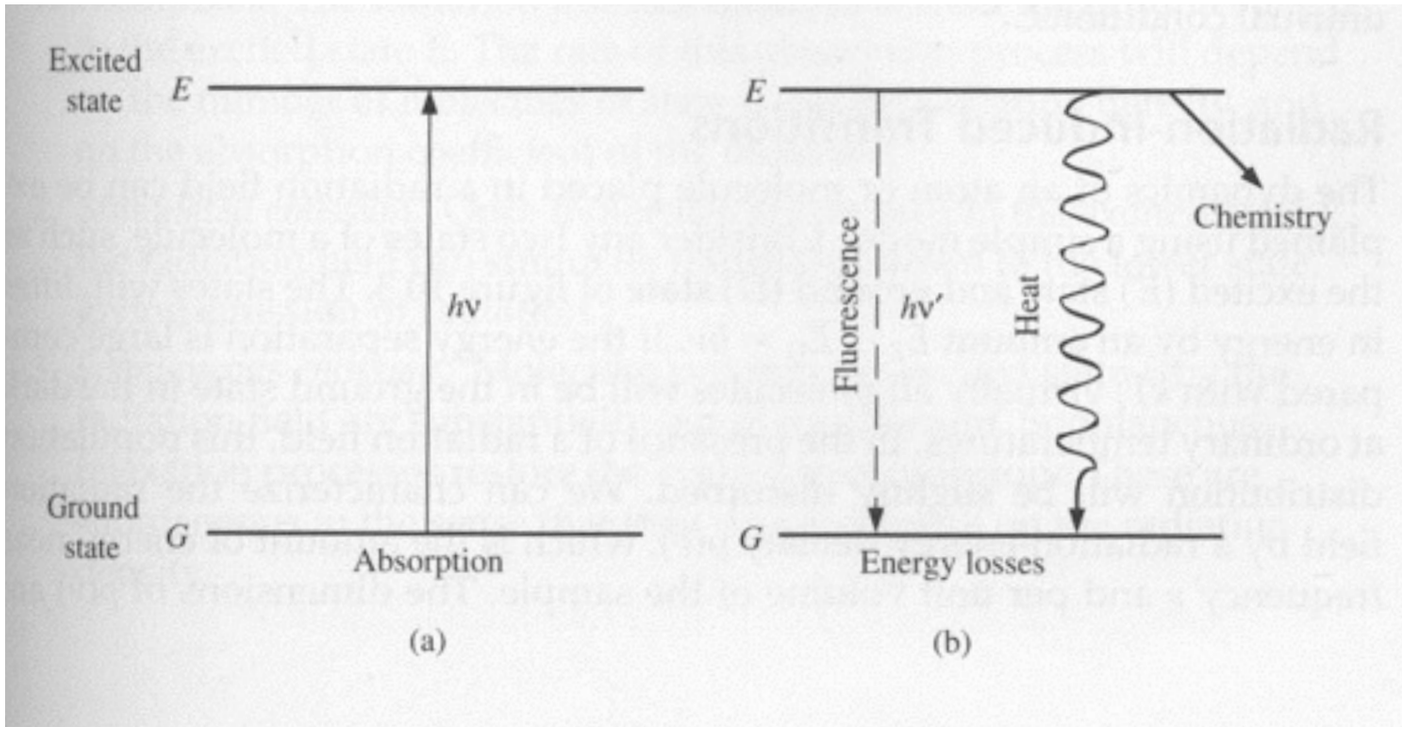


- Electromagnetic spectrum in terms of wavelength, frequency and Energy
- $c=\lambda v$
  - c= speed of light in a vacuum $3x10^8$ m/s
  - v= frequency in Hertz (Hz s^{-1})
  - λ= wavelength
- Energy=hv
  - h = Planck constant $6.6261x10^{-34}$Js

Definitions

- Spectroscopy is the study of the interaction of electromagnetic radiation with matter.
- Absorption- radiation strikes a molecule and causes a shift from ground state to higher energy.
- Emission - going from higher to lower energy

Graphical Representation of Absorption and Emission

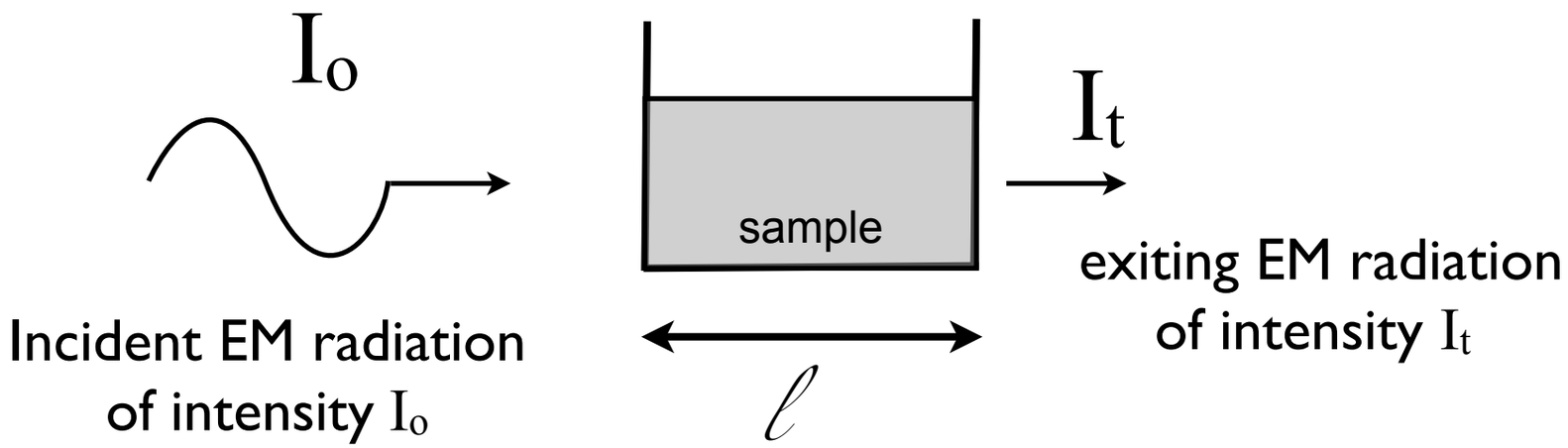


- Absorption depends on the Energy of radiation

Types of Spectroscopy

- Ultraviolet and Visible spectroscopy monitors changes in electronic state (measure concentration)
- Circular dichroism - absorption of polarized light (secondary structure prediction)
- Fluorescence and phosphorescence are types of emissions
- NMR - nuclear magnetic resonance - the absorption of radiofrequency by nuclei in a magnetic field

Beer-Lambert Law

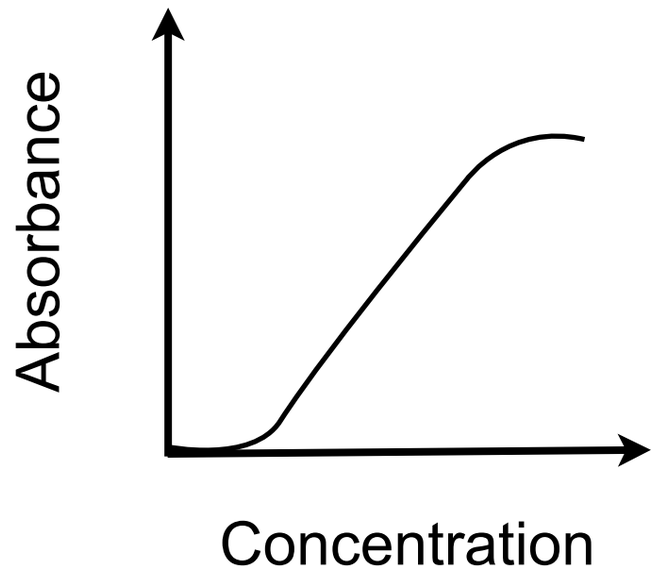


$$A=\log(I_o/I_t)=\varepsilon c\ell$$

- Absorption is A
- ε is the molar absorptivity or molar extinction coefficient. Units M^{-1} cm^{-1}. This is a characteristic of the sample.
- ℓ is the path length. Usually given in cm (1 cm is standard)
- sample contains absorbing substance of concentration c
- solution does not absorb
- walls of the cell do not absorb at the given wavelength

Breakdown of Beer-Lambert

- Beer-Lambert should be linear but it does not hold at high and low concentrations
- Need to stay within the linear range

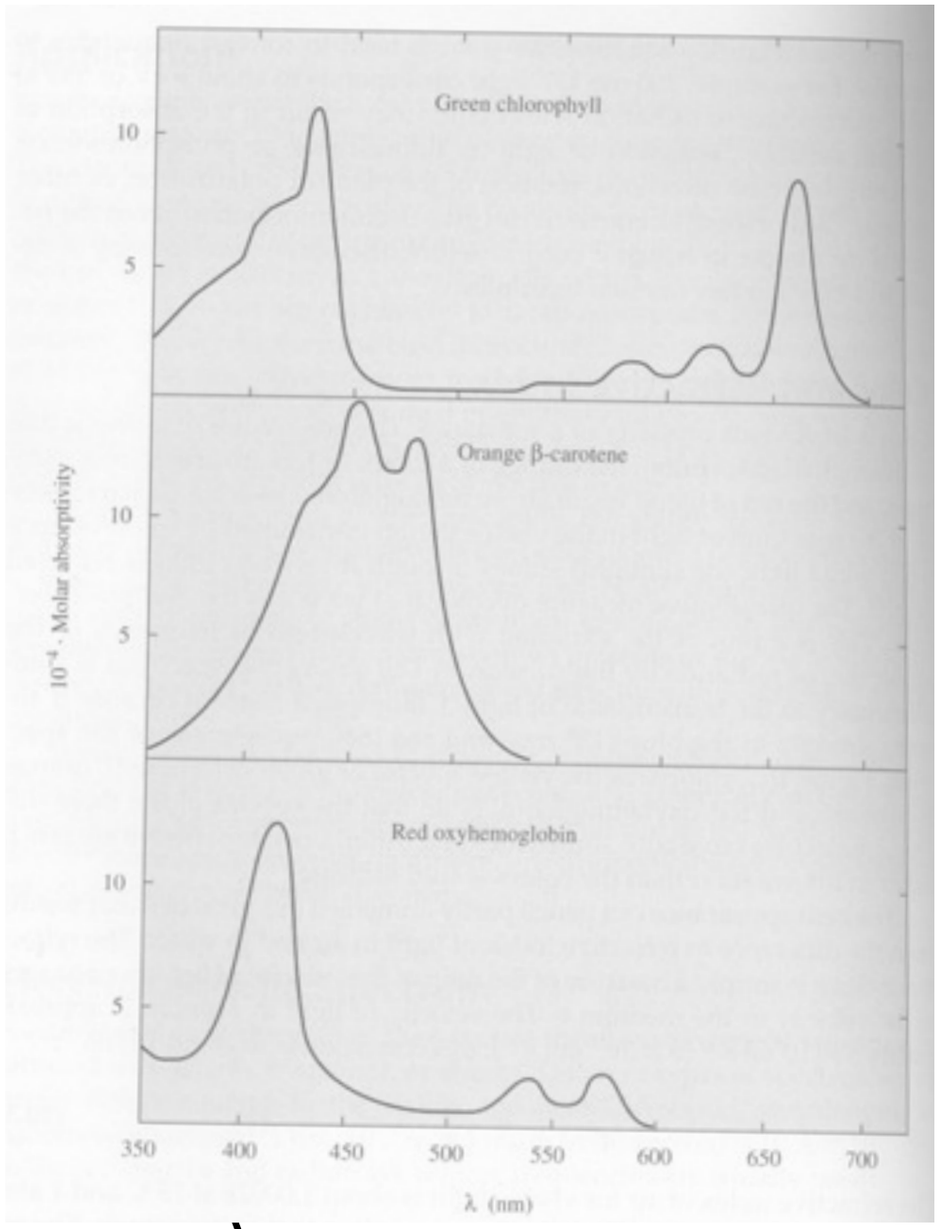


- Also light scattering instead of absorption

Absorption of Electromagnetic Radiation

Absorption

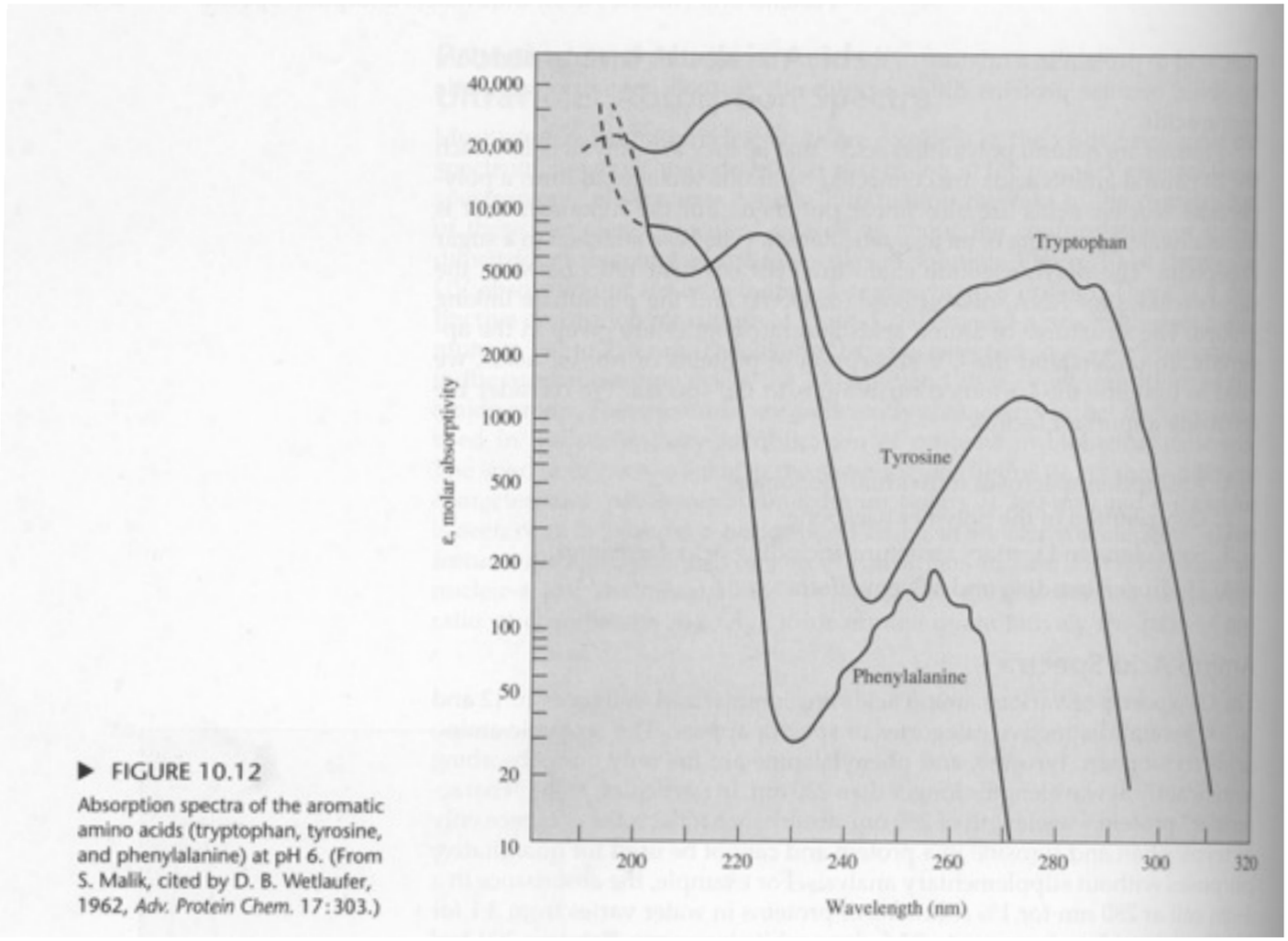
Transmission



λ

E

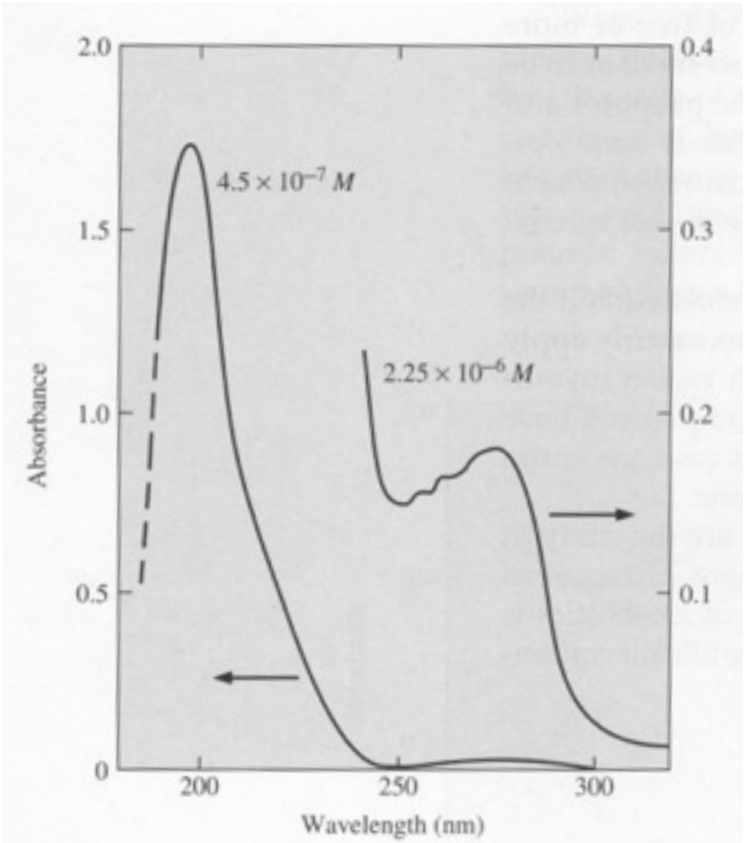
Absorption Spectra of Amino Acids



▶ FIGURE 10.12
Absorption spectra of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) at pH 6. (From S. Malik, cited by D. B. Wetlaufer, 1962, *Adv. Protein Chem.* 17:303.)

- Amino acids tryptophan, tyrosine, and phenylalanine are the only ones that absorb at wavelengths greater than 230nm
- $\pi \rightarrow \pi^*$ transitions of the aromatic ring

Ultraviolet Absorption Spectra:Proteins

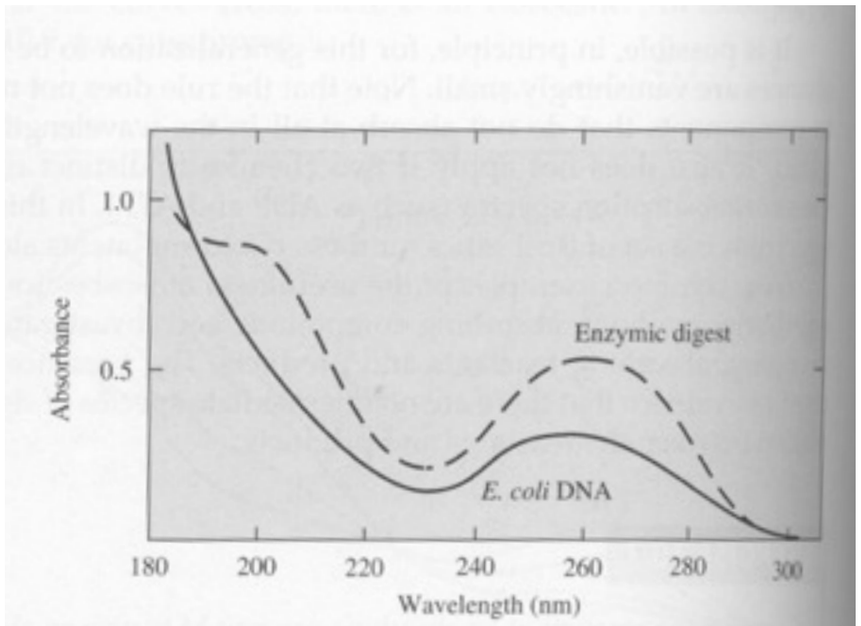


▲ FIGURE 10.10

UV-absorption spectrum of bovine serum albumin. Solution in 10^{-3} *M* phosphate buffer pH 7.0, 1.0-cm path. The wavelength region above 240 nm was measured at a higher concentration and on an expanded absorbance scale (right) so that the weaker absorption bands in that region would be visible. (From E. Yang, unpublished results.)

- Protein two maxima: 200nm and 280nm
- 200 is due to π→π* of the peptide bond
- 200 gives a stronger signal
- buffer effects at 200nm

Ultraviolet Absorption Spectra: Nucleic Acids

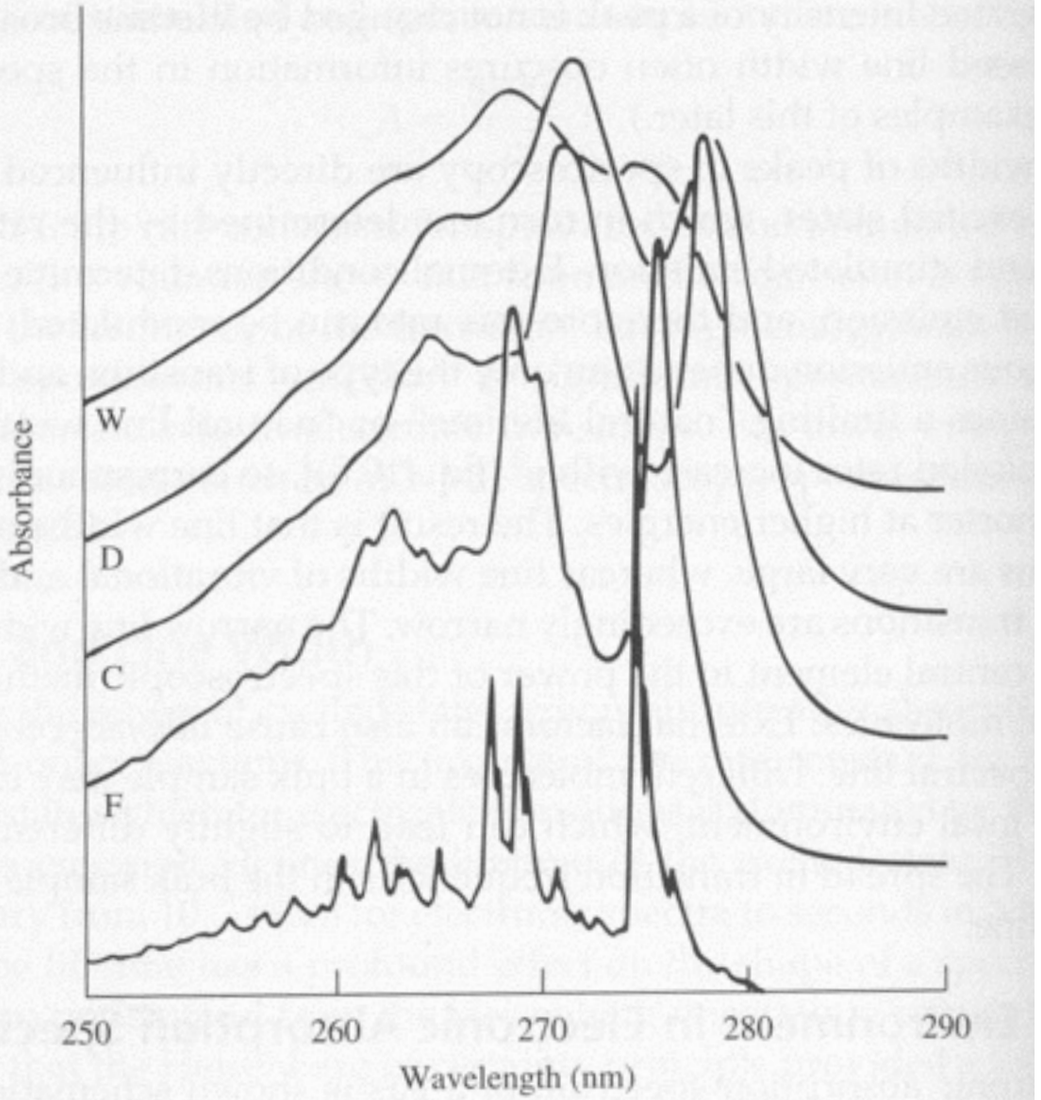


▲ FIGURE 10.11

UV-absorption spectrum of DNA from *E. coli* in the native form at 25°C (solid curve) and as an enzymic digest of nucleotides (dashed curve). (From D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, 1963, *Biopolymers* 1:193. Reprinted with permission of John Wiley and Sons, Inc.)

- $\pi \rightarrow \pi^*$ of the of the base
- this time the maximum is at 260nm not 280nm as with protein
- Digestion of DNA into nucleotides affects the signal.

Role of Environment



▶ FIGURE 10.7

Absorption spectra of anisole vapor and of anisole dissolved in perfluorooctane, F; in cyclohexane, C; in dioxane, D; and in water, W. All at 30°C. The spectra of anisole solutions are displaced vertically to decrease overlap. [From G. L. Tilley, Ph.D. thesis, Purdue University, 1967; cited by M. Laskowski, Jr., 1970, in *Spectroscopic Approaches to Biomolecular Conformation,* ed. D. W. Urry (Chicago: American Medical Association).]

- Pure vapor gives the best signals because the sample is dilute and not interacting with solvent
- Profiles changes with the environment the sample is in.
- This effect is important for proteins and nucleic acids that are folded.

Quantification of Proteins

- Measuring protein concentration using absorbance at 280nm
  - Advantage - Nondestructive
  - Disadvantage - depends on the number of Tryptophan, Tyrosine and cysteine
- Estimate ε_{280} (M^{-1} cm^{-1})=(#W)(5,500) + (#Y)(1,490) + (#C)(125)
  - Dependent on knowing the protein sequence
- [protein] (mg/ml) = (1.55 x A_{280}) – (0.76 x A_{260})
  - Estimation if protein sequence is unknown.
- Measure at the peptide bond (200-220nm)
  - Advantage - more sensitive, not dependent on amino acid composition
  - Disadvantage - buffer effects, some amino acids absorb in this range, influences of secondary structure

Absorption Properties of Nucleotides

	λ_{max}(nm)	ε_{max} ($M^{-1}cm^{-1}$) 10^{-3}
• Ribonucleotides		
• AMP	259	15.4
• CMP	271	9.1
• GMP	252	13.7
• UMP	262	10.0
• Deoxyribonucleotides		
• deoxyAMP	258	15.3
• deoxyCMP	271	9.3
• deoxyGMP	252	13.7
• TMP	267	10.2

Hyperchromicity

- base, nucleoside, nucleotides, and polynucleotides absorb in the same region
- polynucleotides and nucleic acids absorb less than the same amount of free nucleotides
- stacked bases absorb less than unstacked bases
- due to stacking interactions between adjacent bases in the helical polymer
- ENVIRONMENT MATTERS!!!

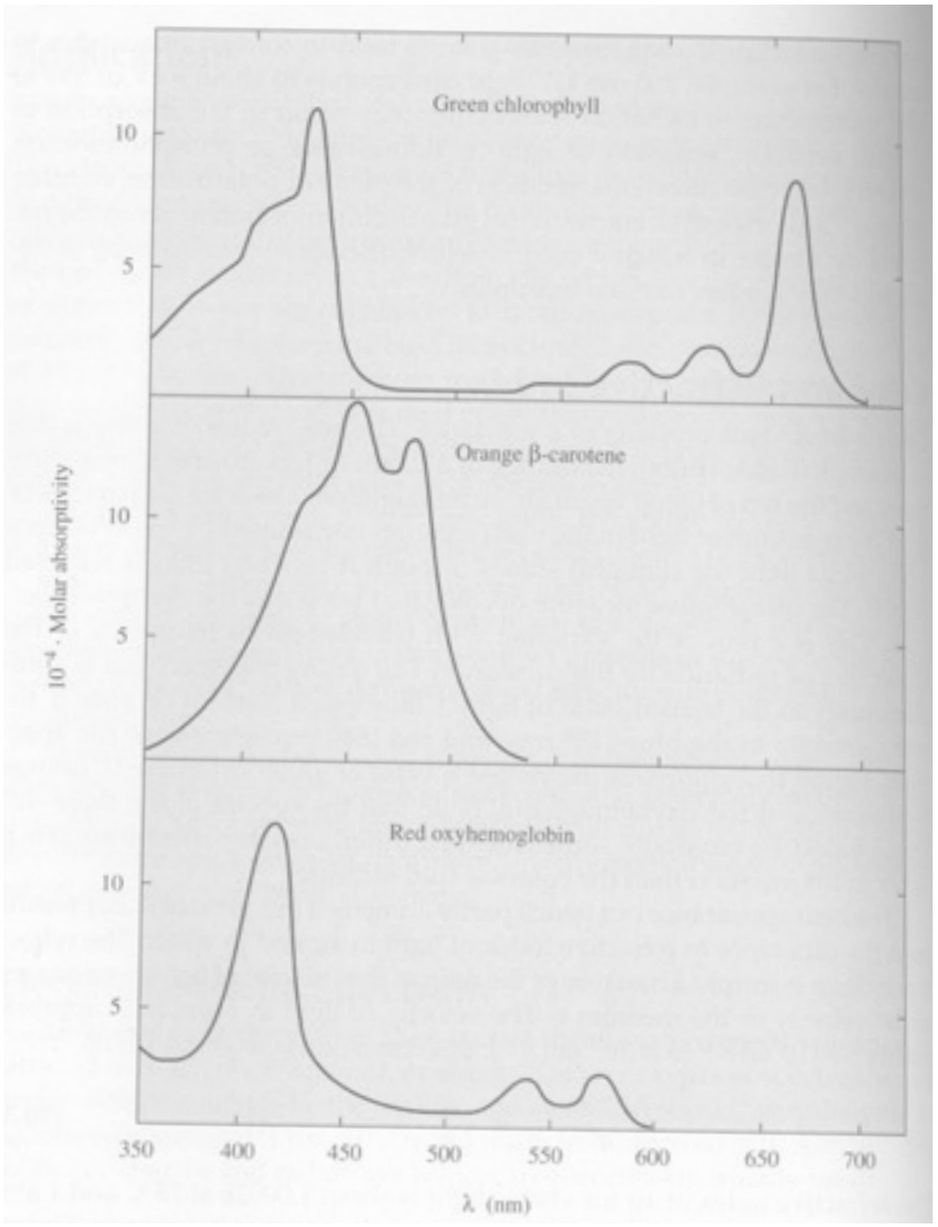
Quantification of Nucleic Acids

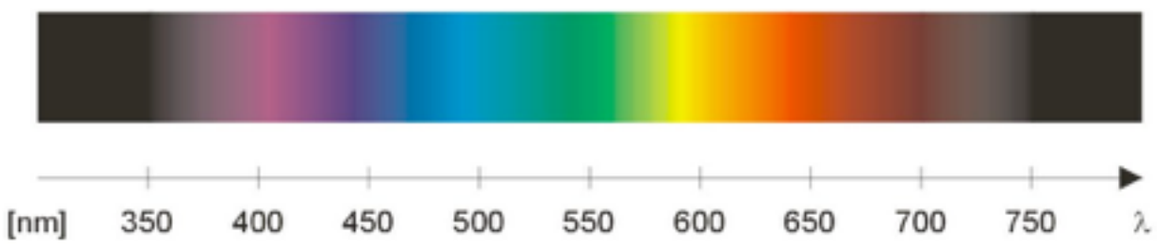
- Absorbance at 260nm (A_{260})
  - $1A_{260}$ dsDNA=50µg/mL
  - $1A_{260}$ ssDNA=37µg/mL
  - $1A_{260}$ ssRNA=40µg/mL

Absorption in the Visible Spectrum

- Chemical or enzymatic reactions that result in the formation of color
- Use visible spectrum to quantitate
- white light - certain colors are absorbed and other pass through giving off the color

Absorption in the visible spectrum





Ellman Reagent

- Ellman’s reagent is used for determining the quantity of free thiols
- Ellman’s reagent is clear
- reacts with free cysteines
- the product is a thiolate ion which is colored (yellow)
- measure at 412nm
- to determine free sulfhydryl content
  - standard curve
  - ε_{412} = 14,150 $M^{-1}cm^{-1}$