Visible and IR Absorption Spectroscopy

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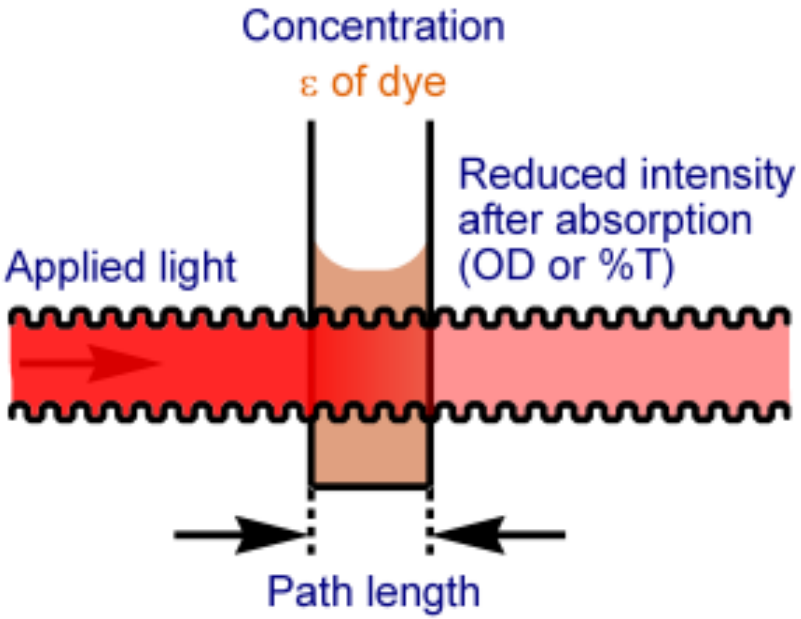
The Basics



wavelength= (λ)

original intensity= I_o

sample slab thickness= dI

Final intensity= I_f

ε'= molar extinction coefficient

$$-dI = C\varepsilon'(\lambda)I\,dI$$

How we get Absorbance

- $-dI = C\varepsilon'(\lambda)I\,dl$
- $\ln(I_o/I_f) = C\varepsilon'(\lambda)l$
- $\log(I_o/I_f) = C\varepsilon'(\lambda)l = A(\lambda)$

A(λ) is known as absorbance or Optical Density (OD)

When $A(\lambda) = 3\ldots1000 = (I_o/I_f)$

When $A(\lambda) = 4\ldots10000 = (I_o/I_f)$

Absorbance must be much lower than these to get any usable data

From Energy to Wavelength

UV visible spectrum responds to energies between 100-1000kJ $mole^{-1}$

frequency range= 2.5e14- 2.5e15

Wavelength range= 1.2e-6m- 1.2e-7m= 120nm-1200nm

E=hf

E= energy

h= planck's constant

f= frequency

c= fλ

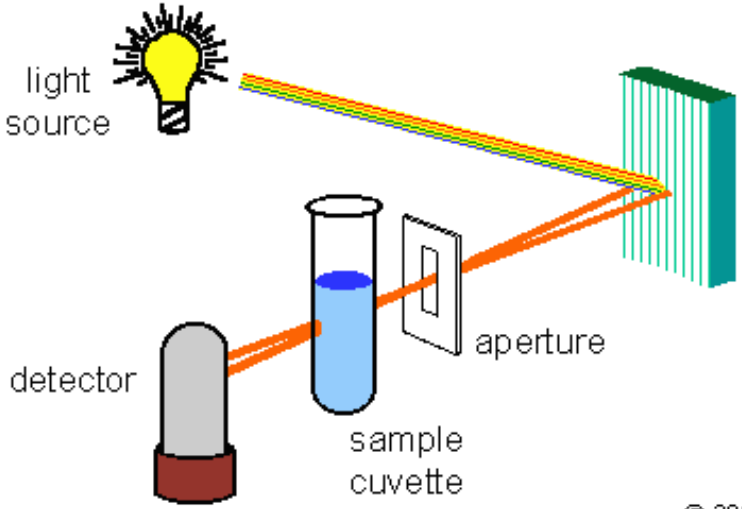
c= speed of light

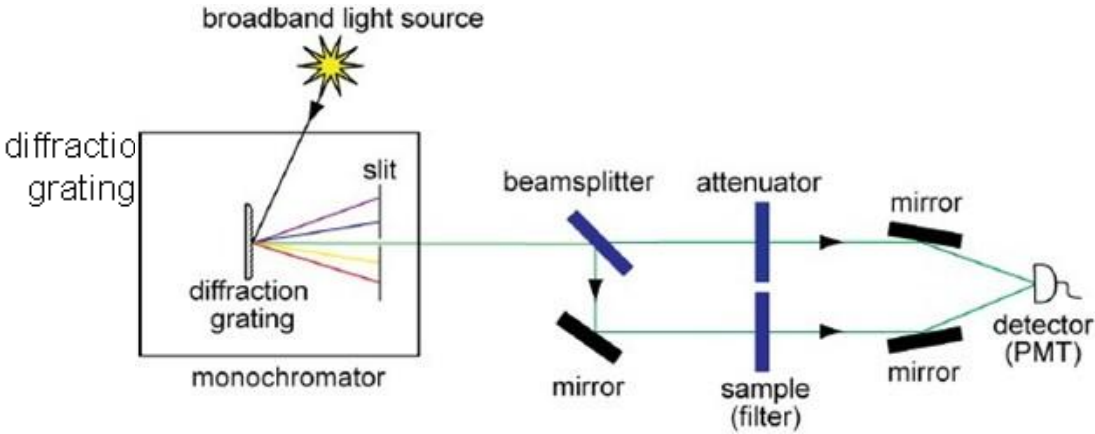
Macromolecules Studied in Water

Water absorbs 170nm wavelength, so measurements must be made above this wavelength

UV is largest change in energy, which is why we use it to measure absorbance. Vibrational and rotational are two small of a change to measure

Single Split Beam-Double Split Beam





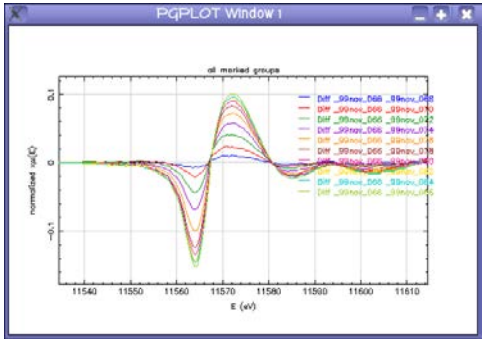
Kinetics and Difference Spectra

Kinetics

A+B→ C

Measuring the absorbance of C vs time gives information about A and B

Difference Spectra



Two absorbance graphs are subtracted from each other

UV Absorption of Proteins

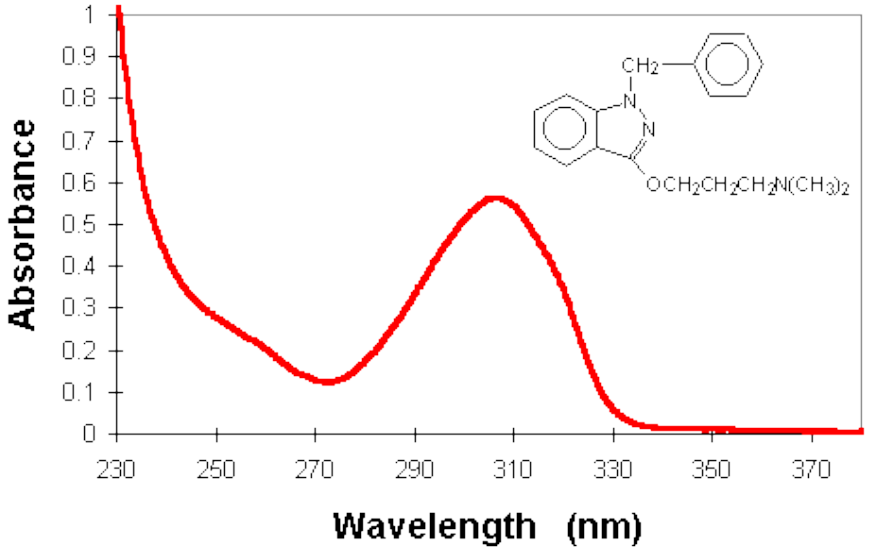
Main absorption areas

peptide group- 170-220nm

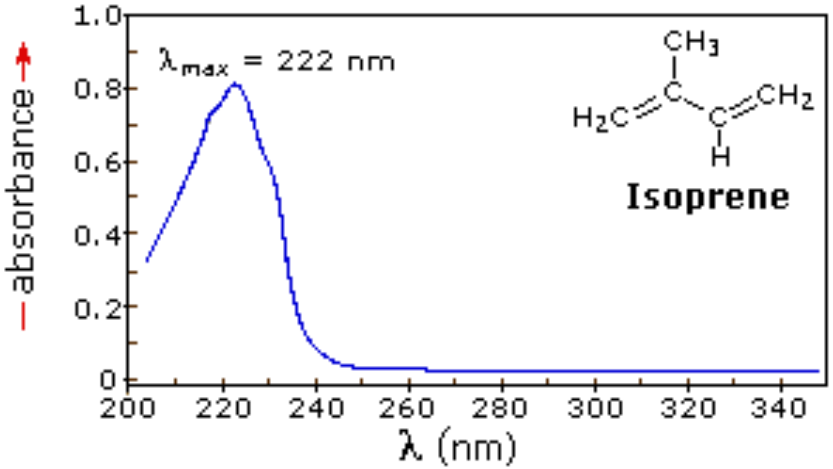
Aromatics- 280nm

Prosthetic groups, cofactors, enzymes, etc- vary

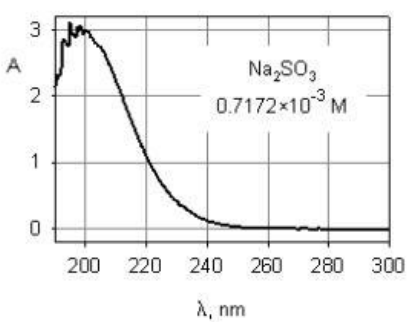
Can use Absorbance to calculate concentration (first equation)

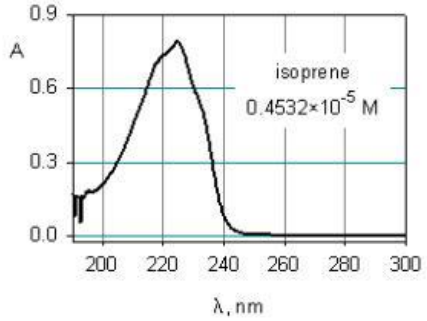


Light Scattering



Has a “tail” because of light scattering





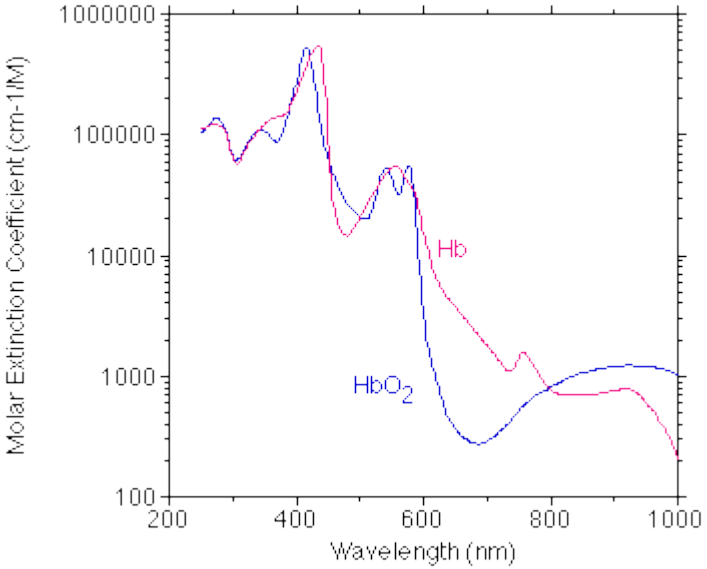
Can either set zero higher up (done here), or graph can simply be fixed to have zero absorbance at end

Flash Photolysis

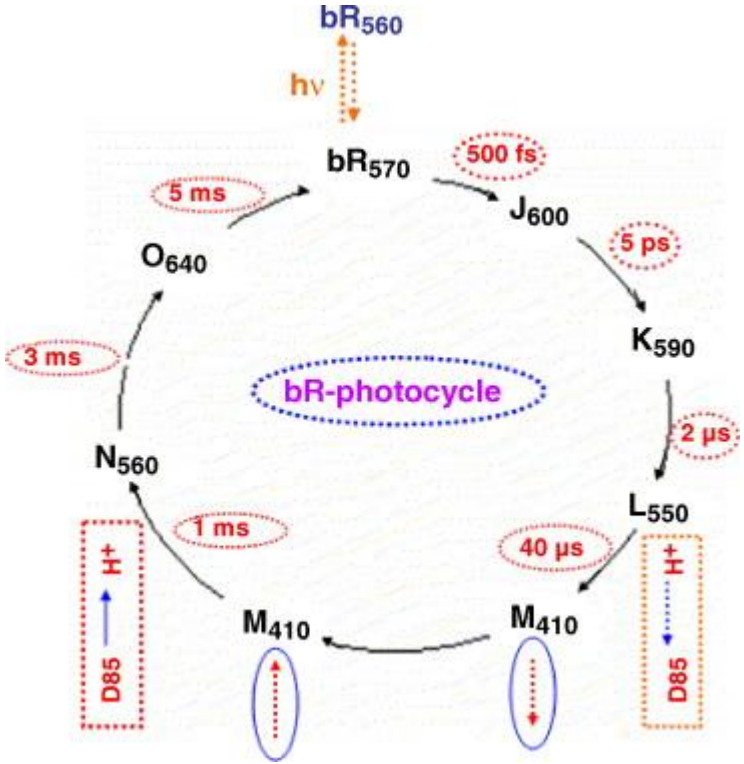
Bond is broken by laser flash

re-binding kinetics are followed through changes in absorption spectrum

Graph shows absorption is higher when bond was broken



Bacteriorhodopsin



Green light (569nm) initiates cycle

Each letter represents a change in absorbance, and a shape change

M has two shapes with the same absorbance

Bacteriorhodopsin is a light activated Proton Pump

When it is in the dark, retinal configuration is all trans except for one cis configuration (dark adapted state)

When light hits, the cis carbon turns trans, and the photocycle begins (light adapted state)

The “Schiff” base changes configuration as the absorbance increases

L to M shape change causes a loss of a hydrogen

M to N shape change causes a gain of a hydrogen

K shape causes carbon to go back to cis

O shape causes it to go back to trans

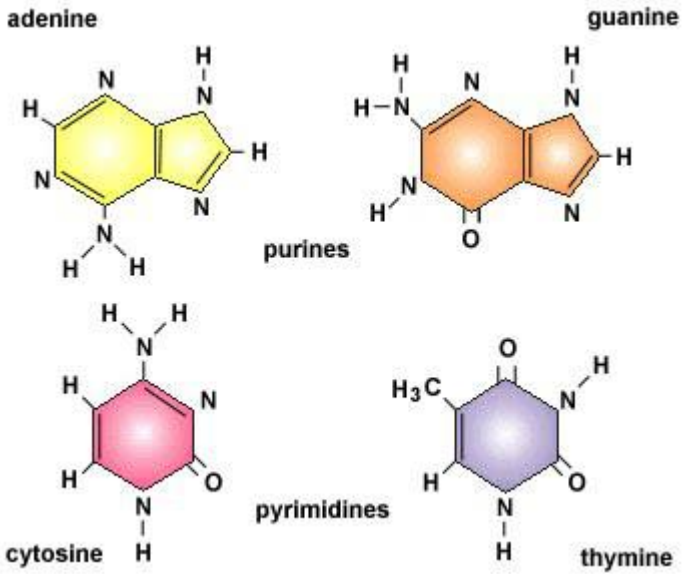
This is directly related to how we detect light

Nucleic Acids

Different absorption peaks than in proteins

Base causes the greatest absorbance- 260-290nm

DNA and protein together in a sample will give at least two distinct absorption peaks

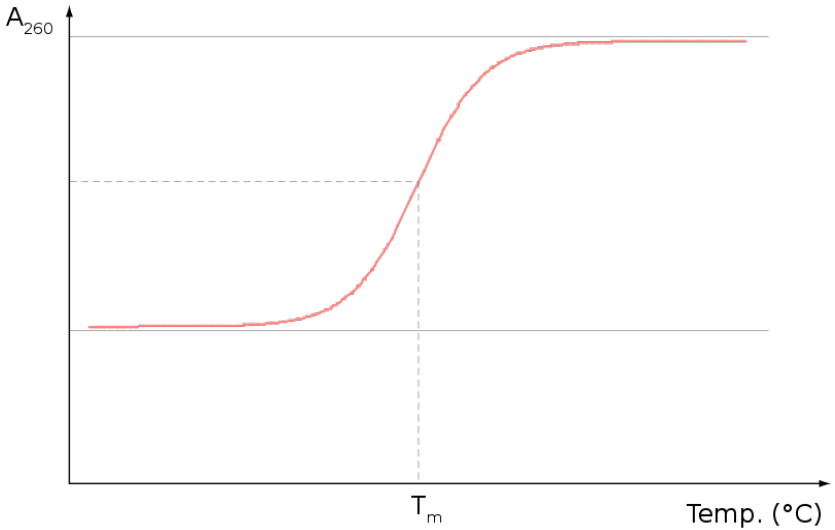


Melted DNA

Extinction coefficient of nucleic acid is smaller than that of free nucleotide

This means that absorbance increases when DNA is melted

Hyperchromic effect- the increase in absorbance upon DNA denaturing



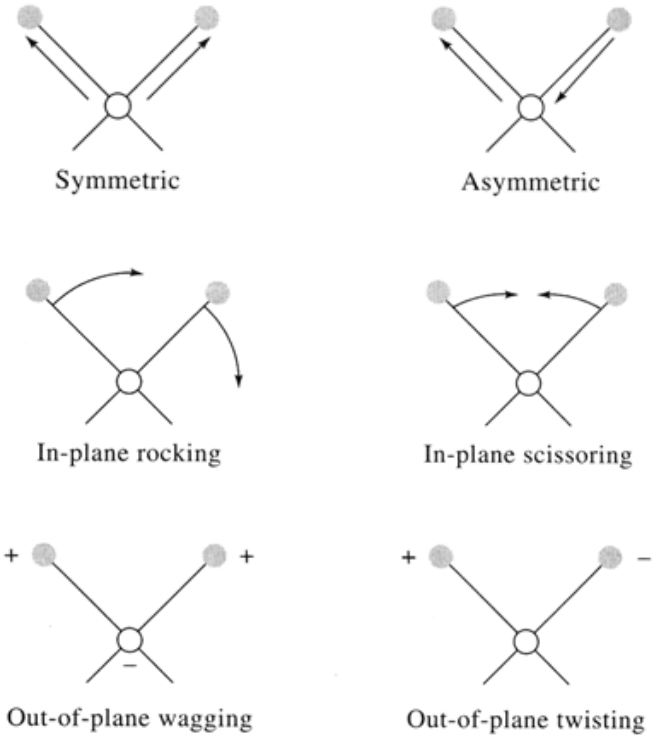
Infrared Absorption Spectroscopy

- IR photons have low energy.
- The only transitions that have comparable energy differences are molecular vibrations and rotations.
- Triggering molecular vibrations through irradiation with infrared light. Provides mostly information about the presence or absence of certain functional groups.

Region	Wavelength (λ) Range, μm	Wavenumber ($\bar{\nu}$) Range, cm^{-1}	Frequency (ν) Range, Hz
Near	0.78 to 2.5	12,800 to 4000	3.8×10^{14} to 1.2×10^{14}
Middle	2.5 to 50	4000 to 200	1.2×10^{14} to 6.0×10^{12}
Far	50 to 1000	200 to 10	6.0×10^{12} to 3.0×10^{11}
Most used	2.5 to 15	4000 to 670	1.2×10^{14} to 2.0×10^{13}

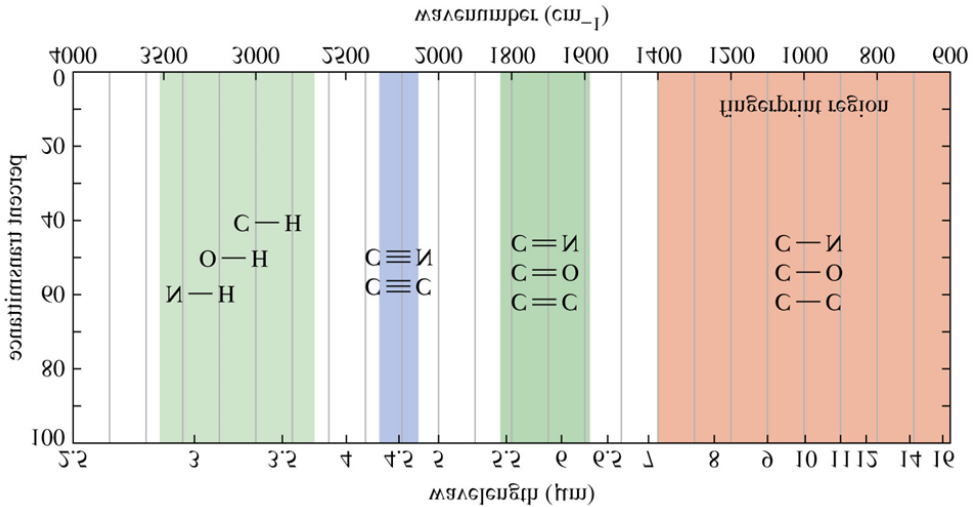
Vibrational Mode

- Stretching - the rhythmic movement along a bond axis with a subsequent increase and decrease in bond length.
- Bending - a change in bond angle or movement of a group of atoms with respect to the rest of the molecule.

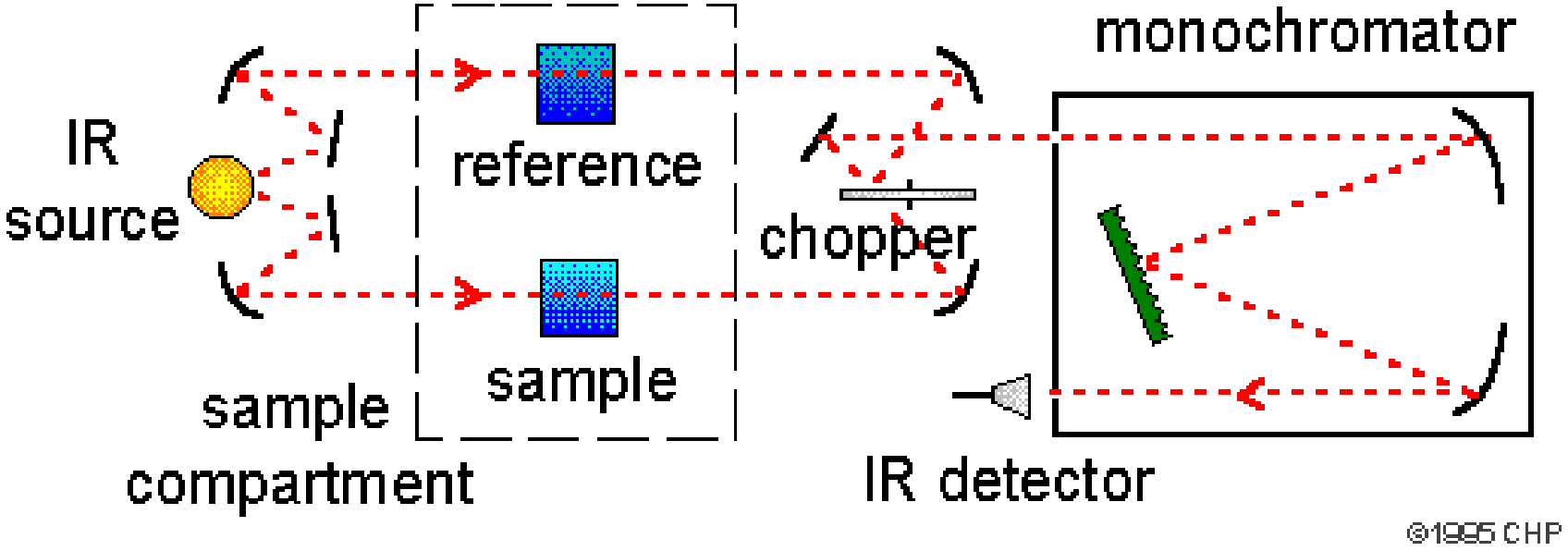


Application

- Used to identify organic compounds
- Provides information about the types of bonds present
- Used to measure the vibrational frequencies of bonds in the molecule
  - Each bond has a characteristic frequency



Infrared Spectroscope Schematic



Infrared Spectrum

- **A plot of % transmittance vs vibrational frequency in wavenumbers.**

- **The higher the wavenumber, the shorter the wavelength.**

$$wavenumber = \frac{1}{\lambda}$$

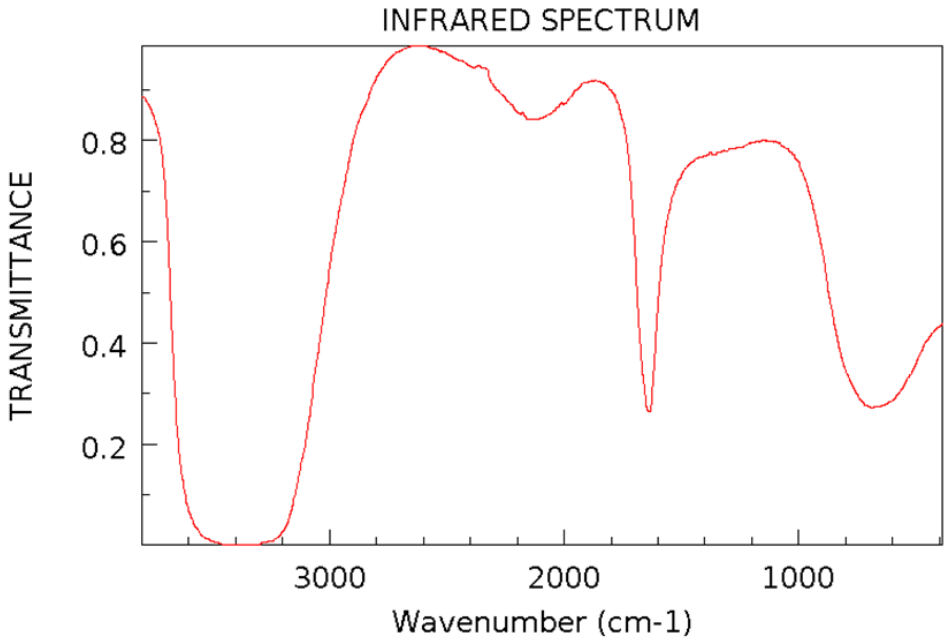
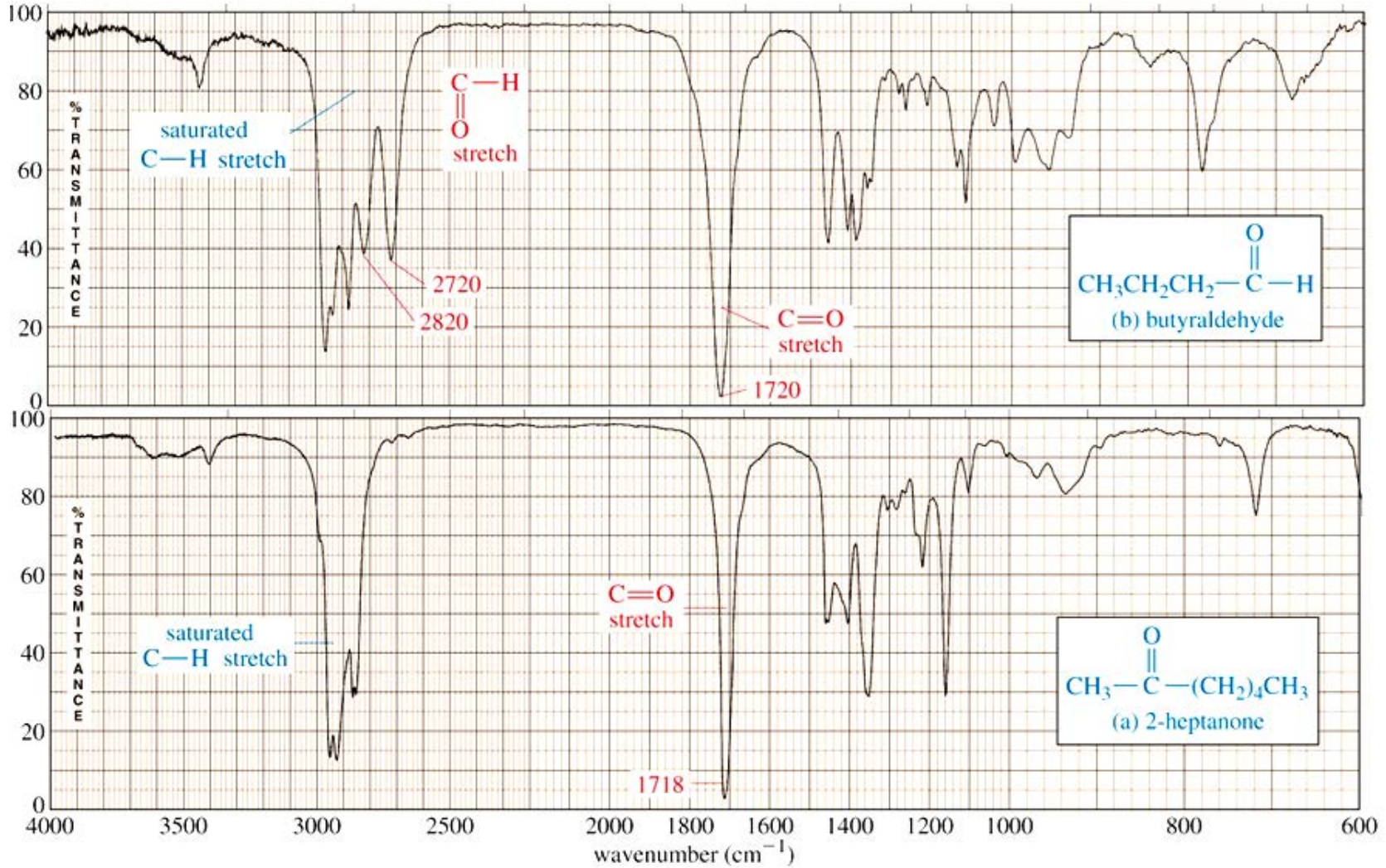
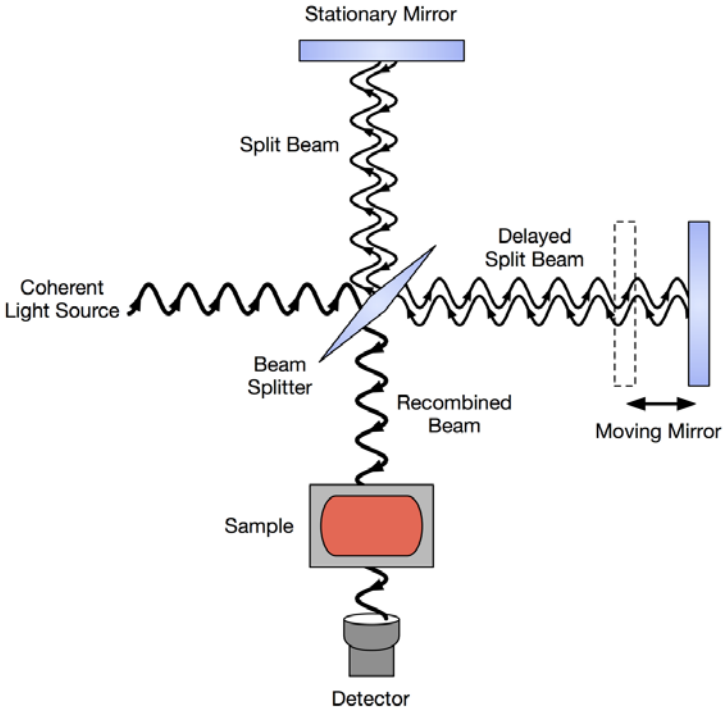


Table 13.4 Important IR Stretching Frequencies

Type of bond	Wavenumber (cm^{-1})	Intensity
C≡N	2260–2220	medium
C≡C	2260–2100	medium to weak
C=C	1680–1600	medium
C=N	1650–1550	medium
	~1600 and ~1500–1430	strong to weak
C=O	1780–1650	strong
C—O	1250–1050	strong
C—N	1230–1020	medium
O—H (alcohol)	3650–3200	strong, broad
O—H (carboxylic acid)	3300–2500	strong, very broad
N—H	3500–3300	medium, broad
C—H	3300–2700	medium

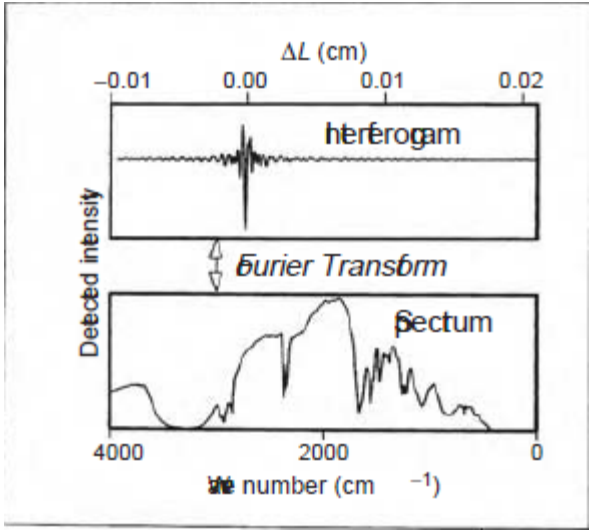

100
80
60
40
20
0
% TRANSMITTANCE
saturated C—H stretch
C—H
O
stretch
2720
2820
C=O stretch
1720
O
CH3CH2CH2—C—H
(b) butyraldehyde
saturated C—H stretch
C=O stretch
1718
O
CH3—C—(CH2)4CH3
(a) 2-heptanone
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
wavenumber (cm−1)

FTIR Spectrophotometers Schematic



FTIR Spectrophotometers

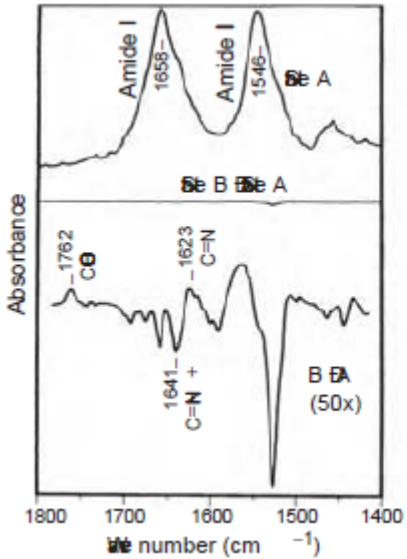
- **Uses an interferometer and polychromatic light (all frequencies used at one time, instead of one at a time) to generate an interferogram.**



IR Difference Spectrum

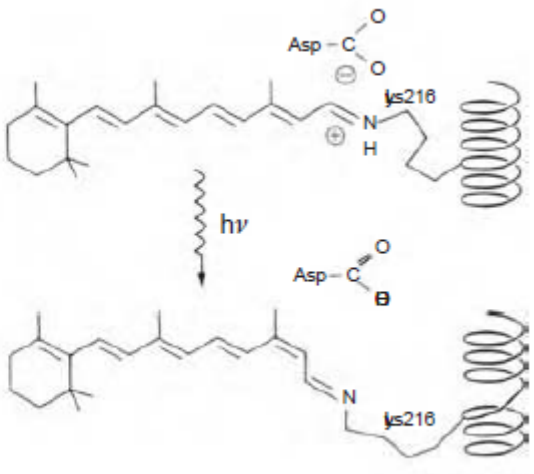
- IR spectra are very sensitive to structural alteration
  - A change in hydrogen bonding distance of 0.002Å shifts the frequency
- However, it is very difficult, in practice, to detect small, localised structural changes in a biological macromolecule, by IR spectroscopy.
  - All groups in the molecule essentially have IR-active vibrations
  - Multitude of overlapping spectral bands

IR Difference Spectrum

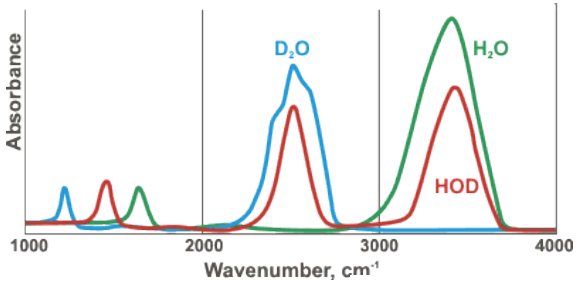


A (bR $_{570}$):

B (M $_{412}$):



IR Spectroscopy and Water



- Water peak at 3000-3700 cm-1