# NEPHAR 407

# PHARMACEUTICAL CHEMISTRY III LAB

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# SPECTROSCOPY

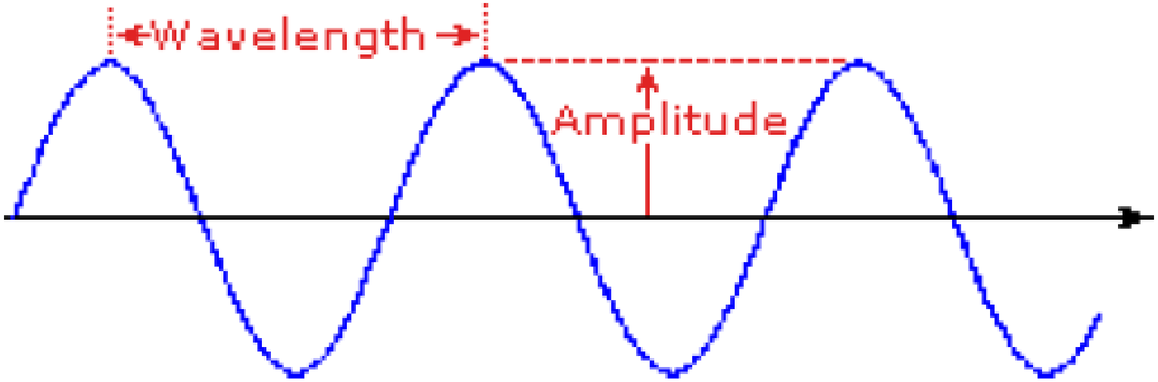
- Spectroscopy is the use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes.

**Absorption:** A transition from a lower level to a higher level with transfer of energy from the radiation field to an absorber, atom, molecule, or solid.

**Emission:** A transition from a higher level to a lower level with transfer of energy from the emitter to the radiation field. If no radiation is emitted, the transition from higher to lower energy levels is called nonradioactive decay.

**Scattering:** Redirection of light due to its interaction with matter. Scattering might or might not occur with a transfer of energy, i.e., the scattered radiation might or might not have a slightly different wavelength compared to the light incident on the sample.

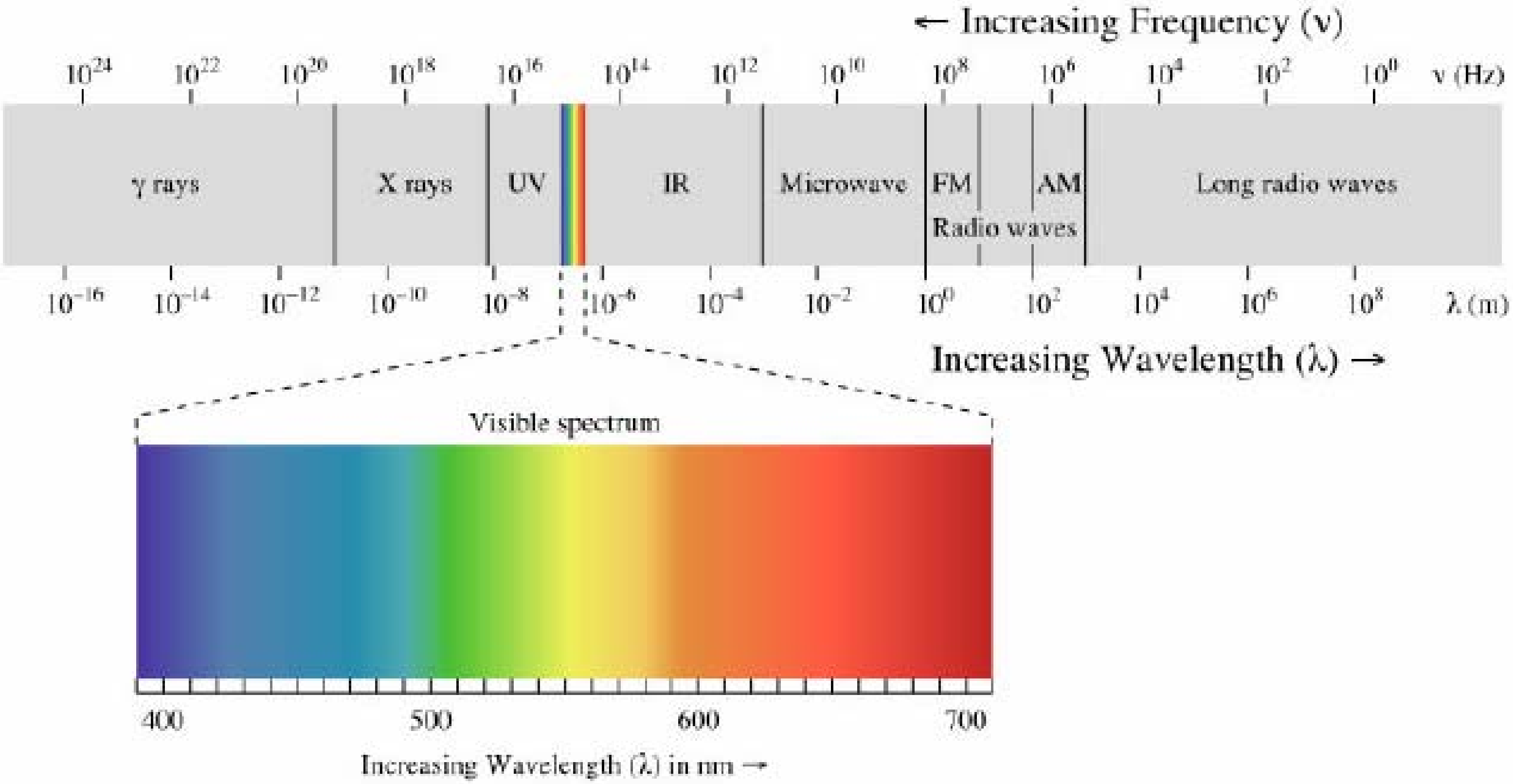
# Electromagnetic radiation is commonly treated as a wave phenomenon, characterized by a wavelength (λ) or frequency (ν).



***Frequency***, ν (nu), is the number of wavecycles that pass through a point in one second. It is measured in Hz, where 1 Hz = 1 cycle/sec.

***Amplitude*** is the magnitude of change in the oscillating variable with each oscillation within an oscillating system.

# ELECTROMAGNETIC SPECTRUM



The relationship between the light velocity, wavelength, and frequency is given below.

$E = h\nu$

$\nu = c / \lambda$

$E = hc / \lambda$

$h = 6.62618 \times 10^{-34}$ J·s

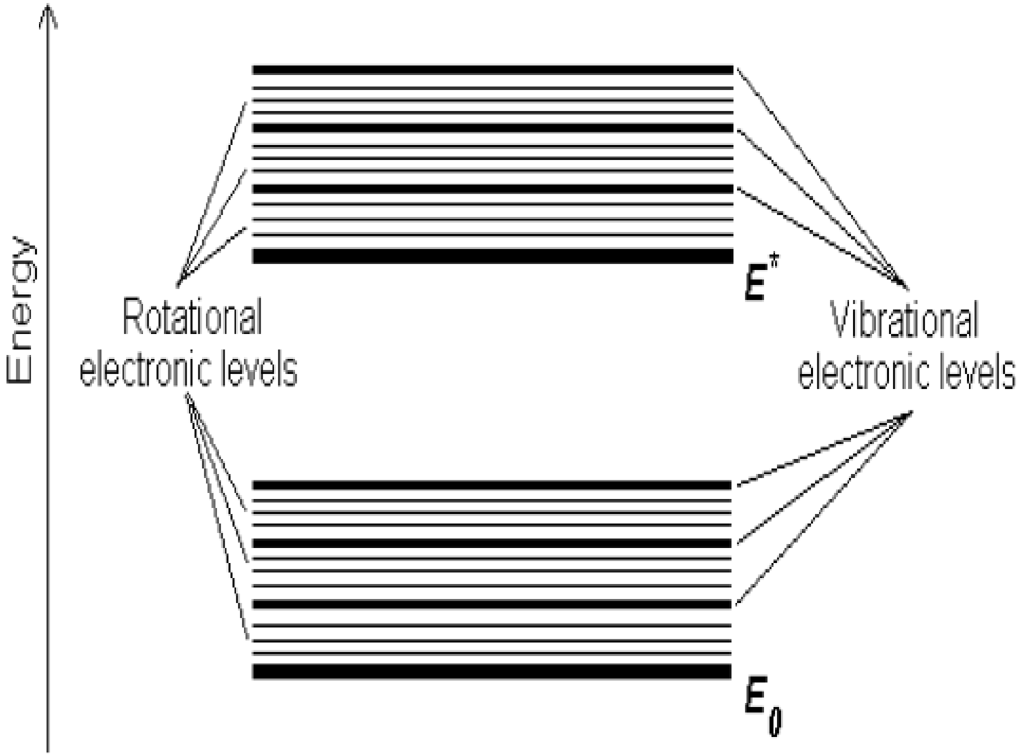
# Molecular Spectroscopy

The study of which frequencies of electromagnetic radiation are absorbed or emitted by a particular substance and the correlation of these frequencies with details of molecular structure.

| Energy Region (wavelength) | Frequency (Hz) | Type of Spectroscopy | Transitions |
|---|---|---|---|
| Radio frequency (0.1-1m) | $3x10^7$ - $9x10^8$ | nuclear magnetic resonance (NMR) | nuclear spin states |
| Infrared (1000-10000 nm) | $1x10^{13}$-$1x10^{14}$ | infrared (IR) | vibrational energy levels |
| Ultraviolet-visible (100-800 nm) | $2.5x10^{14}$ - $1.5x10^{15}$ | ultraviolet-visible (UV-Vis) | electronic energy levels |

A molecular energy state is the sum of an electronic, vibrational, rotational, nuclear and translational component.

$$E = E_{electronic} + E_{vibrational} + E_{rotational} + E_{translational} + E_{nuclear}$$



# UV-VIS SPECTROSCOPY

✓ Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface.

✓ Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels. Ultraviolet (UV) spectrometry is the spectrometry of electronic transitions.

***What kind of organic structure gives rise to UV absorption?***

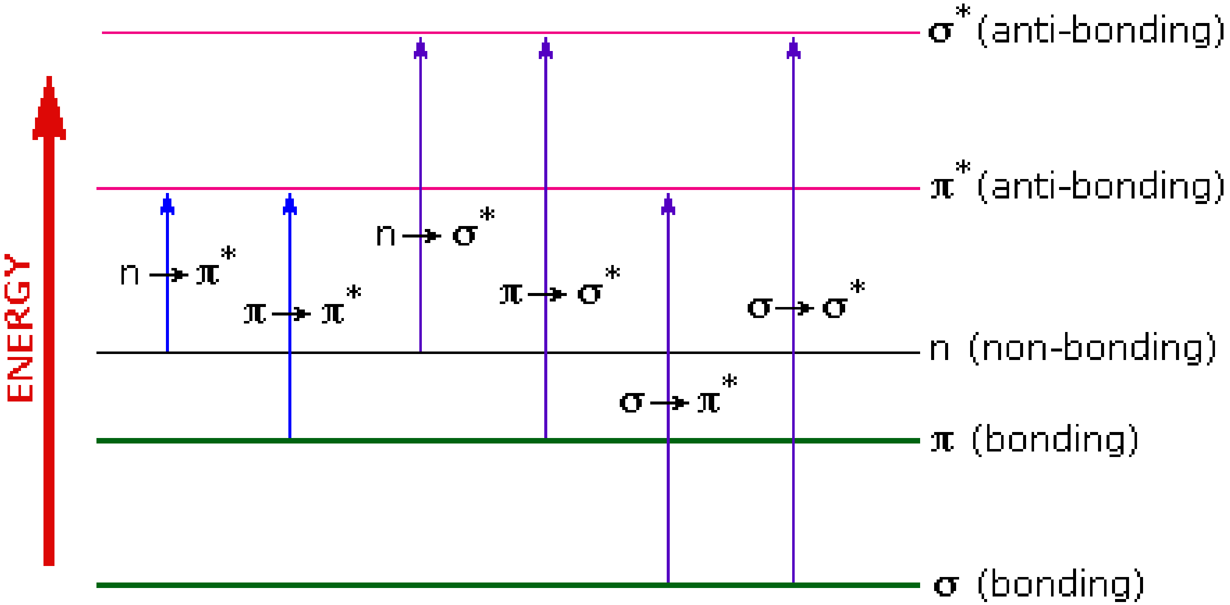
- It should be no surprise that the molecule should contain a structural unit with nonbonded (n) electrons or π-electrons. Such a unit giving rise to a UV absorption band is called a ***chromophore***. ***Chromophore*** is used to describe any structural feature which leads to absorption in the UV-Vis region and includes groups in which $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, $n \rightarrow \sigma^*$ transitions are possible.

- The term ***auxochrome*** is used to designate groups possessing non-bonding electron pairs which are conjugated with a π–bond system; an example is a hydroxyl or amino group attached to an aromatic ring system.

UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Absorption spectroscopy carried out in UV-V region is sometimes called "electronic spectroscopy".

# Various possible electronic transitions in an organic molecule



# $\sigma \rightarrow \sigma^*$ Transitions

($\lambda < 200$ nm)

The energy required is large.

Methane (which has only C-H bonds, and can only undergo $\sigma \rightarrow \sigma^*$ transitions) shows an absorbance maximum at 125 nm.

Absorption maxima due to $\sigma \rightarrow \sigma^*$ transitions are not seen in typical UV-V spectra (200 - 700 nm)

# $n \rightarrow \sigma^*$ Transitions

Saturated compounds containing atoms with lone pairs (non-bonding electrons).

They can be initiated by light whose wavelength is in the range 150 - 250 nm.

The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in the UV region is small.

# $n \rightarrow \pi^*$
# $\pi \rightarrow \pi^*$
# Transitions

Most absorption spectroscopy of organic compounds is based on these transitions.

This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm). These transitions need an unsaturated group in the molecule to provide the $\pi$ electrons.

Molar absorbtivities from $n \rightarrow \pi^*$

are relatively low, 10 to100 L $mol^{-1}$ $cm^{-1}$ .

$\pi \rightarrow \pi^*$ normally give molar absorbtivities

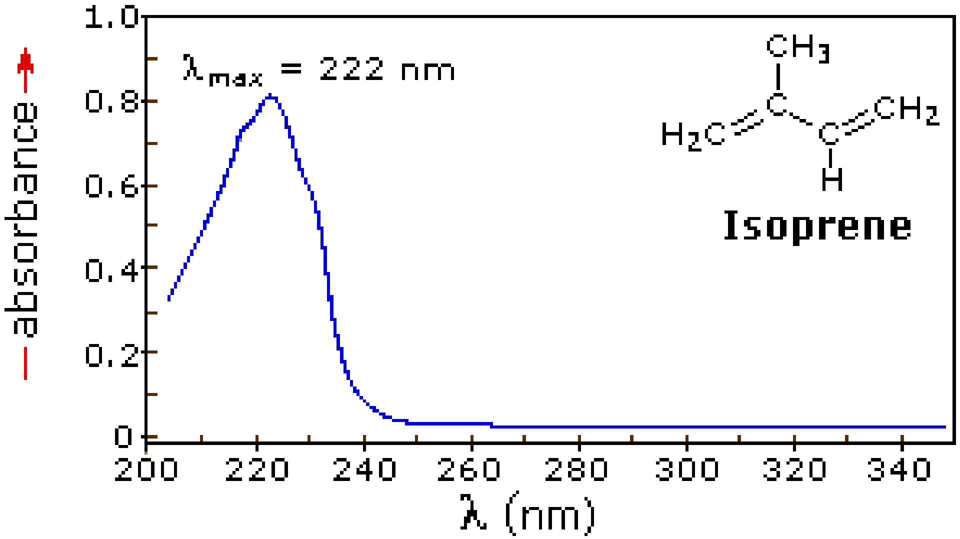
between 1000 and 10,000 L $mol^{-1}$ $cm^{-1}$ .

Molar absoptivities ,ε, may be very large for strongly absorbing chromophores (>10,000) and very small if absorption is weak (10 to 100).

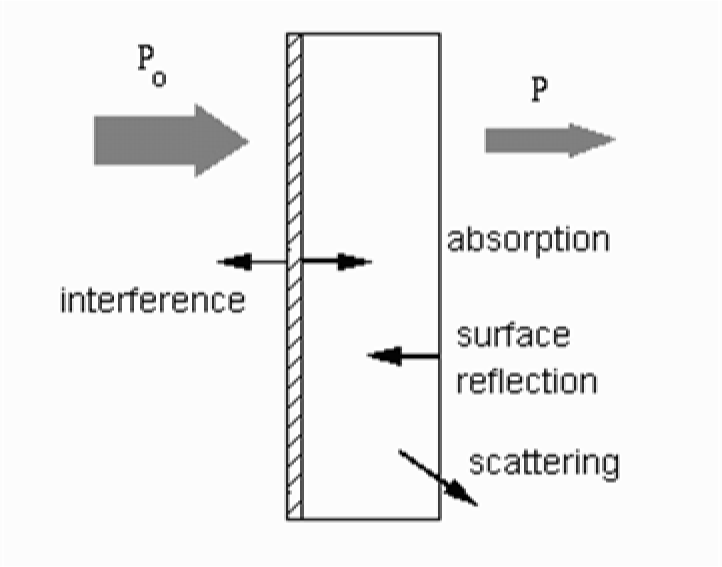
The magnitude of ε reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore.

✓ When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength.

## Absorption spectrum of isoprene



# Absorption of light through an optical filter

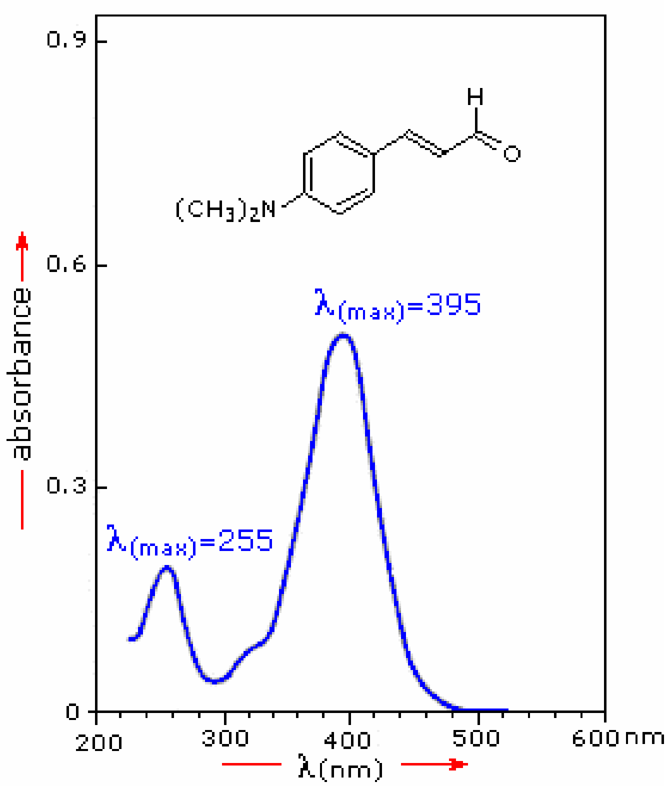


- **Dilute solutions must be used**
- **Most commonly used solvents are water, ethanol, hexane and cyclohexane**

***Beer-Lambert law*** $A = \varepsilon \times b \times c$

A = measured absorbance
ε = wavelength-dependent molar absorptivity coefficient with units of $M^{-1}$ $cm^{-1}$
b= length of light path through the sample in cm
c= analyte concentration in molarity units

# UV-Vis spectrum of an unsaturated aldehyde in ethanol



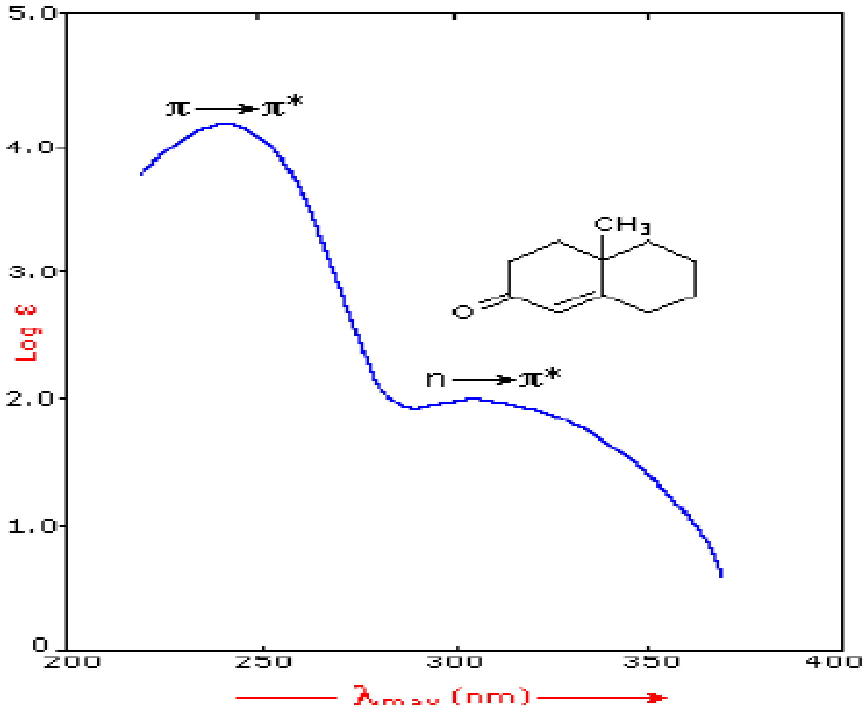
$A = \varepsilon \times b \times c$

$C = 1.42.10^{-5}$ M solution

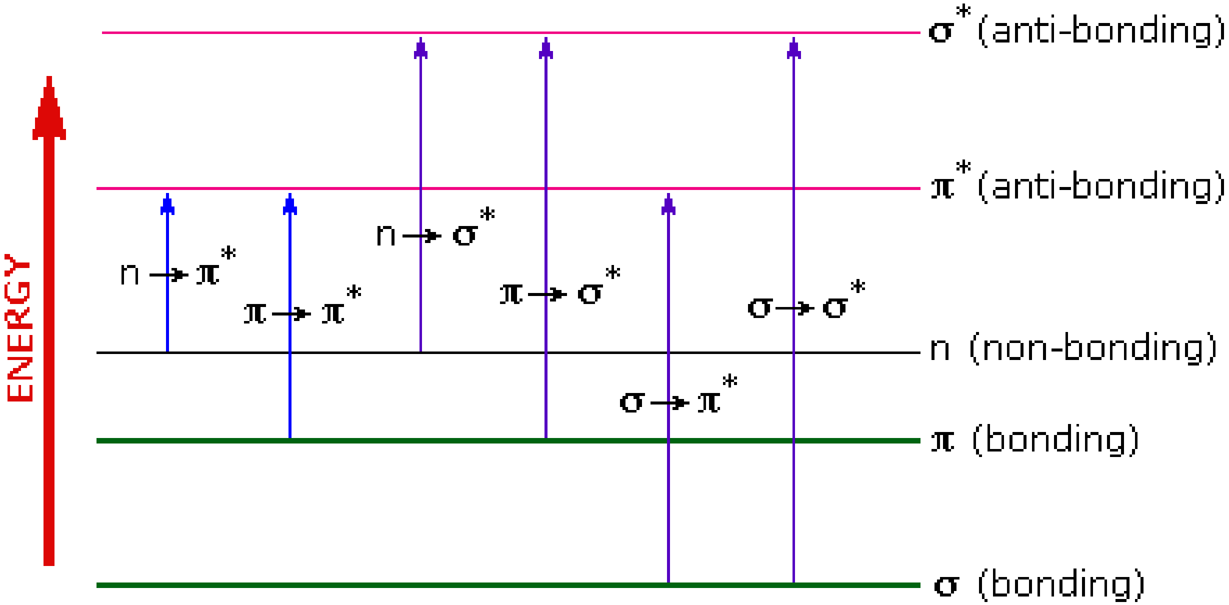
ε = 36,600 for the 395 nm peak and 14,000 for the 255 nm peak

The π —> π* absorption located at 242 nm is very strong, with an ε = 18,000.

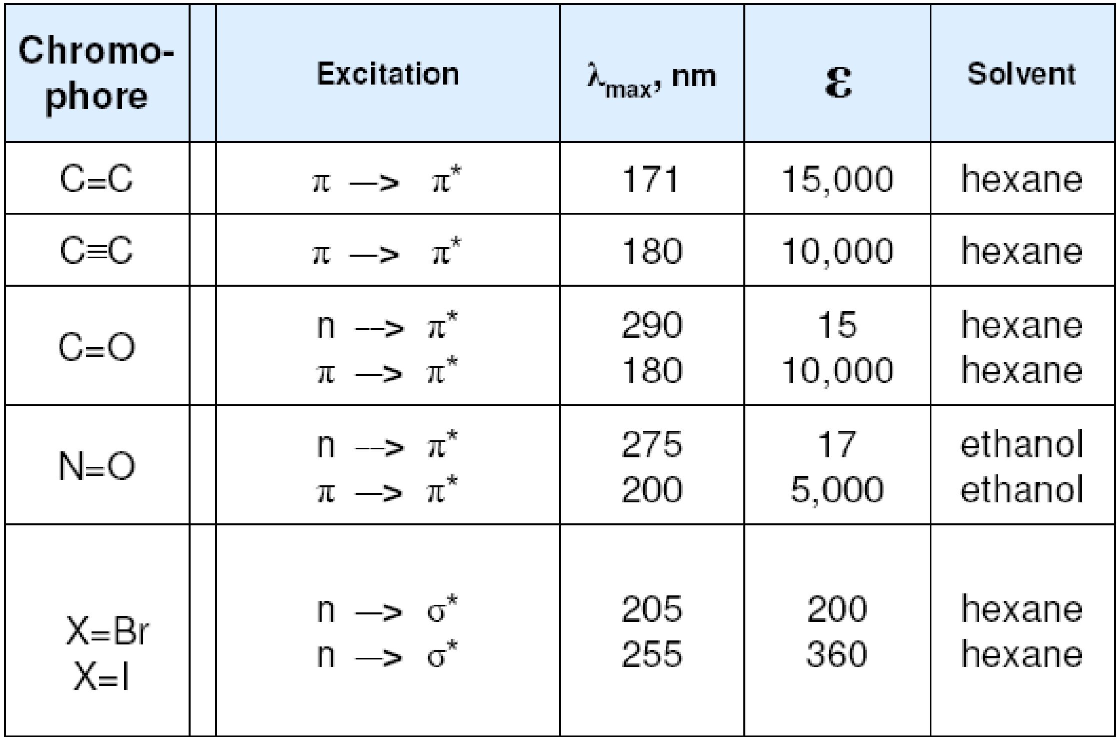
The weak n —> π* absorption near 300 nm has an ε = 100.



# Various possible electronic transitions in an organic molecule



# Some Simple Chromophores and Their Light Absorption Characteristics



| Chromophore | | Excitation | $\lambda_{max}$, nm | $\varepsilon$ | Solvent |
|---|---|---|---|---|---|
| C=C | | $\pi \rightarrow \pi^*$ | 171 | 15,000 | hexane |
| C≡C | | $\pi \rightarrow \pi^*$ | 180 | 10,000 | hexane |
| C=O | | $n \rightarrow \pi^*$<br>$\pi \rightarrow \pi^*$ | 290<br>180 | 15<br>10,000 | hexane<br>hexane |
| N=O | | $n \rightarrow \pi^*$<br>$\pi \rightarrow \pi^*$ | 275<br>200 | 17<br>5,000 | ethanol<br>ethanol |
| X=Br<br>X=I | | $n \rightarrow \sigma^*$<br>$n \rightarrow \sigma^*$ | 205<br>255 | 200<br>360 | hexane<br>hexane |

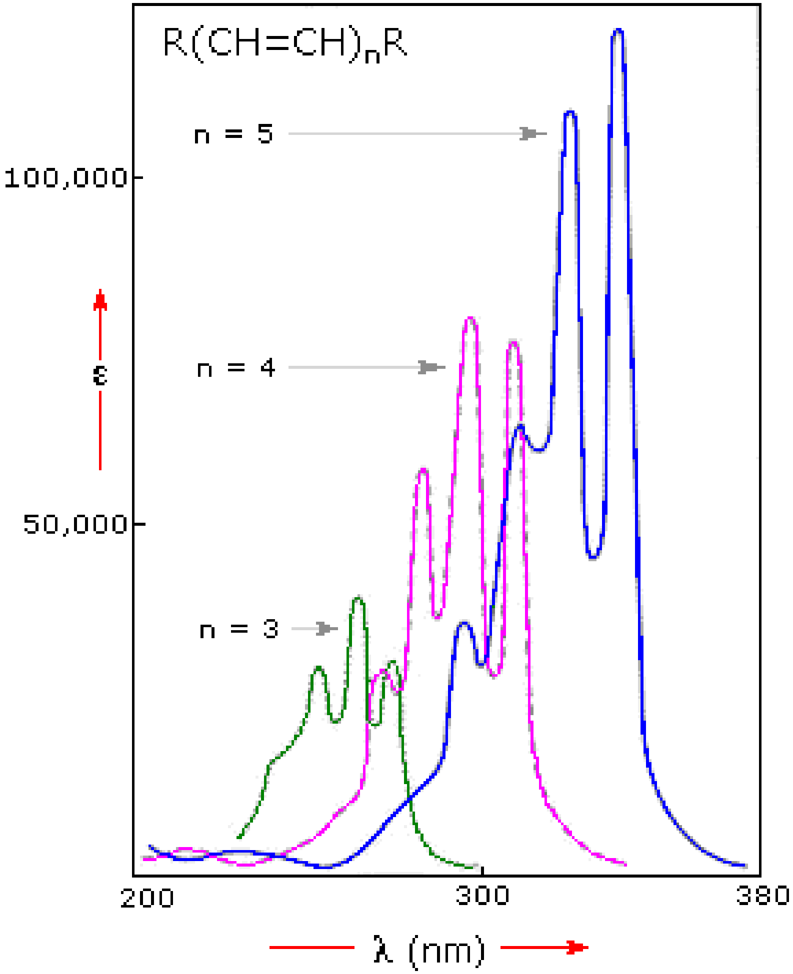
✓Only molecular moieties likely to absorb light in the 200 to 800 nm region are pi-electron functions and hetero atoms having non-bonding valence-shell electron pairs. Such light absorbing groups are referred to as **chromophores**.

# Terminology for Absorption Shifts

| Nature of Shift | Descriptive Term |
|---|---|
| To Longer Wavelength | Bathochromic |
| To Shorter Wavelength | Hypsochromic |
| To Greater Absorbance | Hyperchromic |
| To Lower Absorbance | Hypochromic |

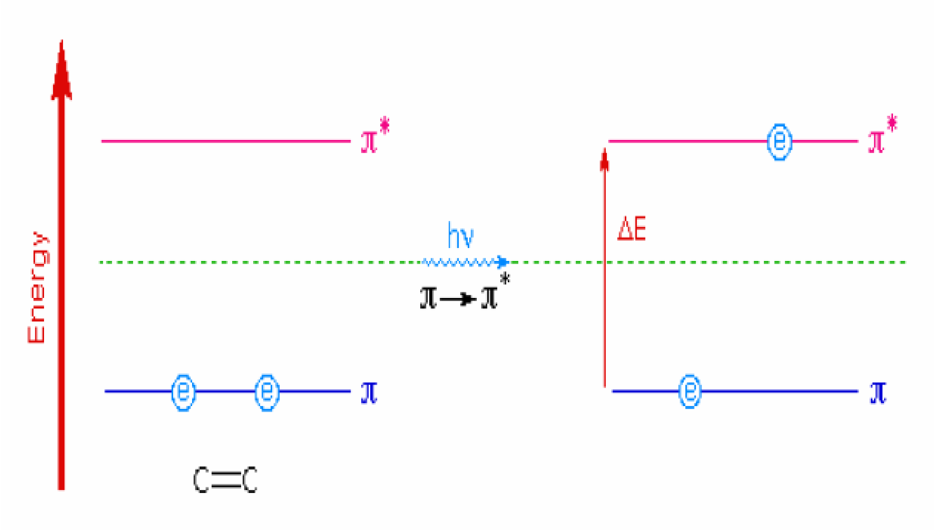
# The Importance of Conjugation

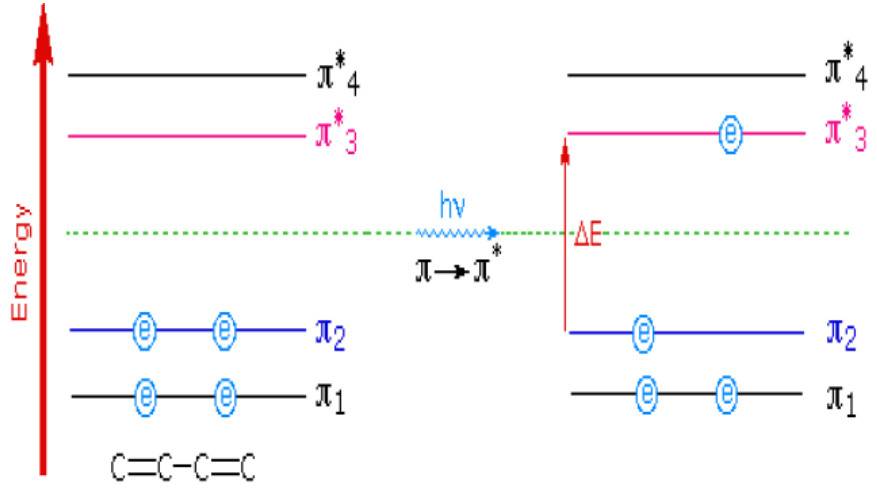
- ✓ conjugation generally moves the absorption maxima to longer wavelengths



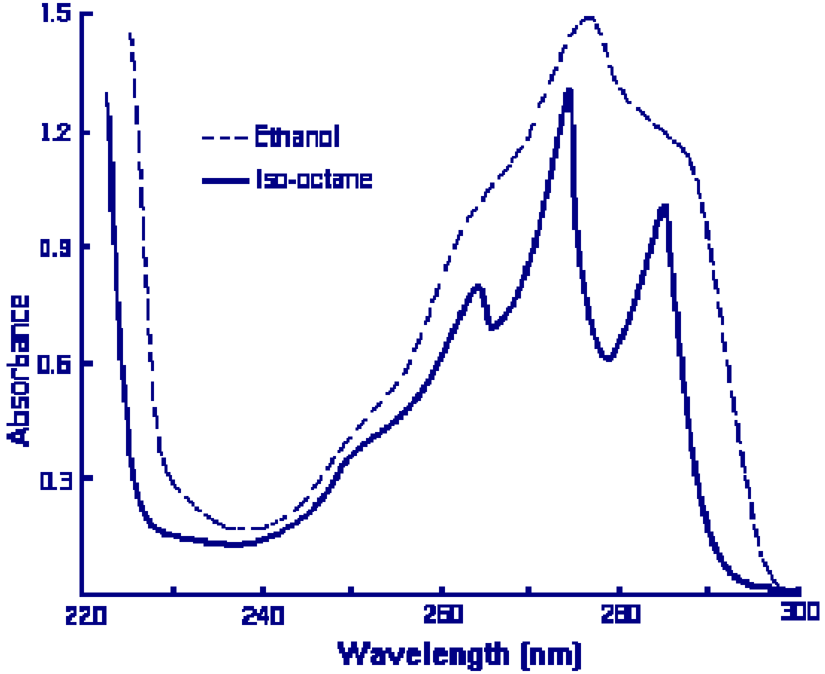
- ✓ Polyene spectra displayed in the center diagram, it is clear that each additional double bond in the conjugated pi-electron system shifts the absorption maximum about 30 nm in the same direction

✓ Increased conjugation brings the HOMO and LUMO orbitals closer together. The energy (ΔE) required to effect the electron promotion is therefore less, and the wavelength that provides this energy is increased

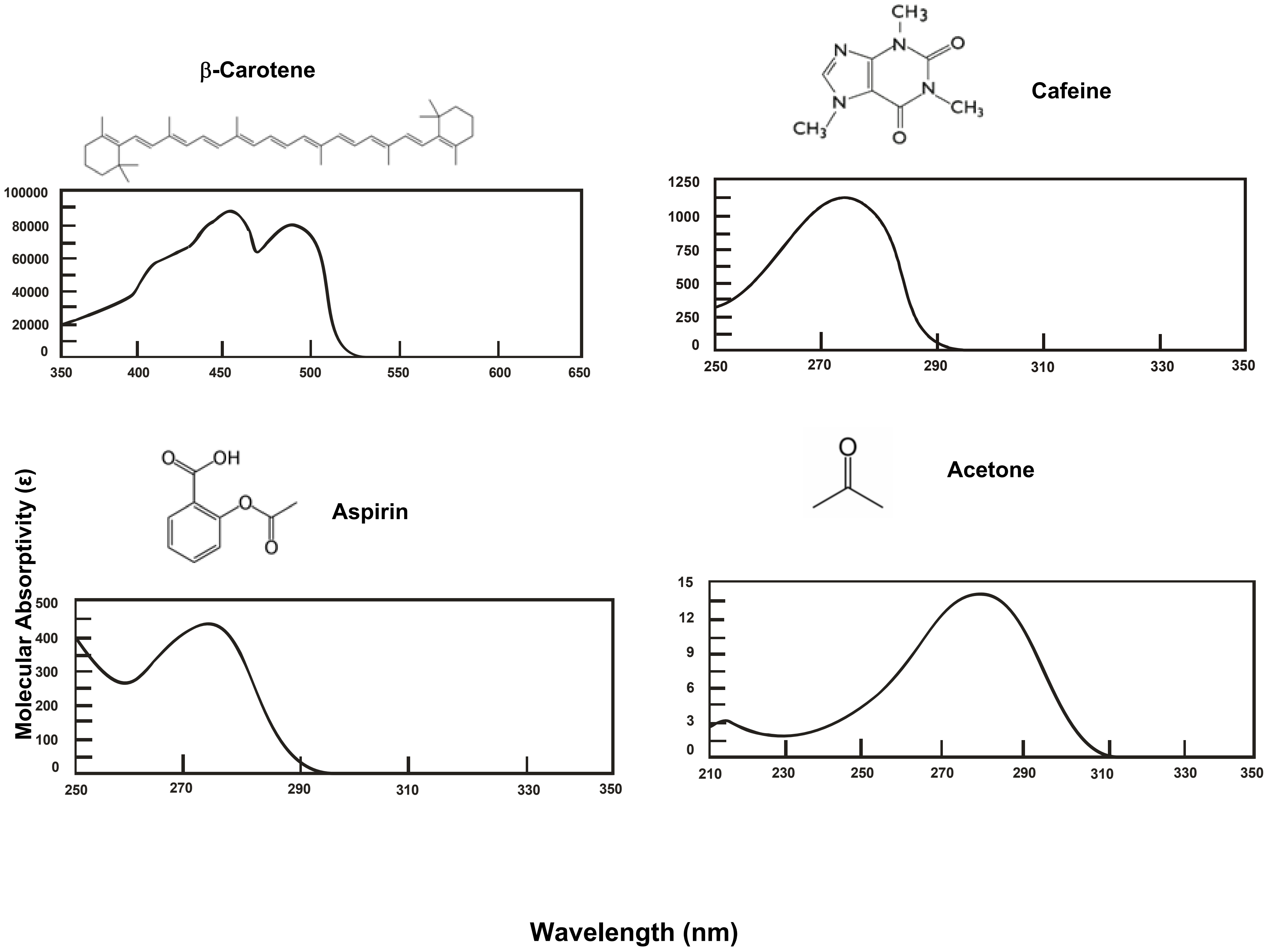




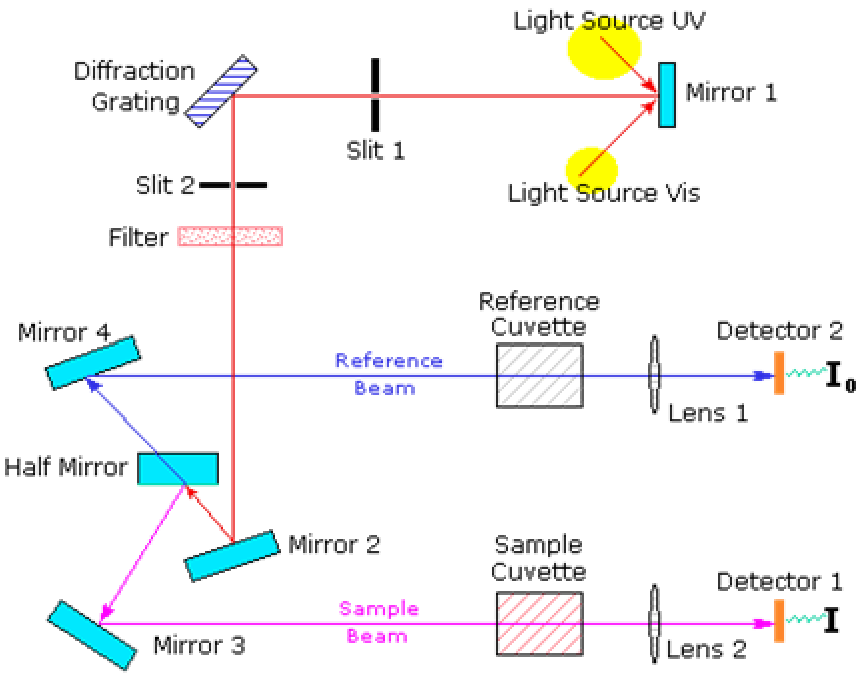
# Effect of Solvent on Absorption Spectrum



- ✓ Generally speaking, non-polar solvents and non-polar molecules show least effect.
- ✓ Thus care must be taken to avoid an interaction between the solute and the solvent.

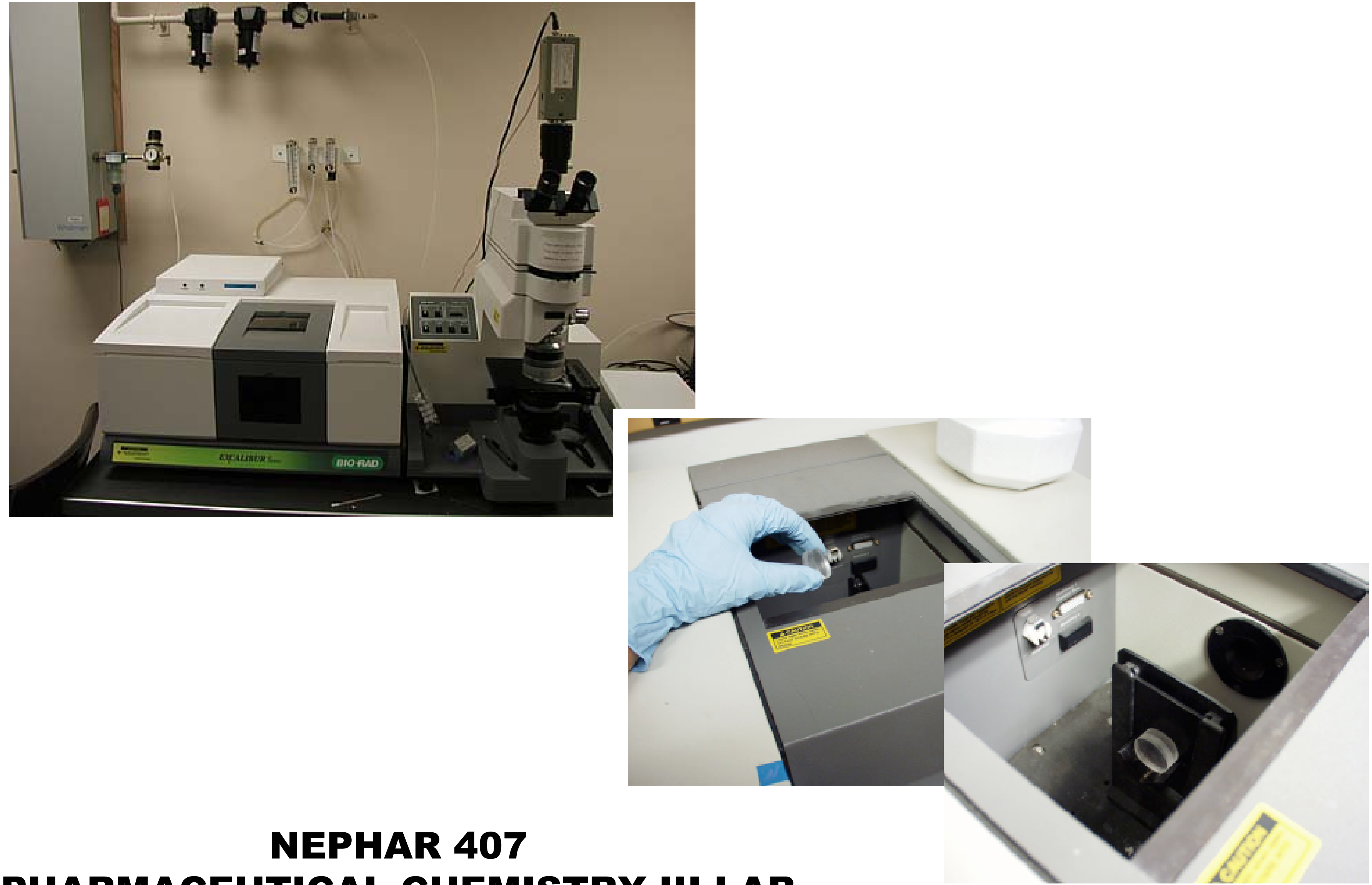

β-Carotene
100000
80000
60000
40000
20000
0
350
400
450
500
550
600
650
Cafeine
CH3
N
N
O
N
CH3
N
CH3
O
1250
1000
750
500
250
0
250
270
290
310
330
350
Aspirin
O
OH
O
O
500
400
300
200
100
0
250
270
290
310
330
350
Acetone
O
15
12
9
6
3
0
210
230
250
270
290
310
330
350
Molecular Absorptivity (ε)
Wavelength (nm)

# Components of a typical UV-Vis Spectrometer



✓ The light source is usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible and NIR measurements

# INFRARED (IR) SPECTROSCOPY



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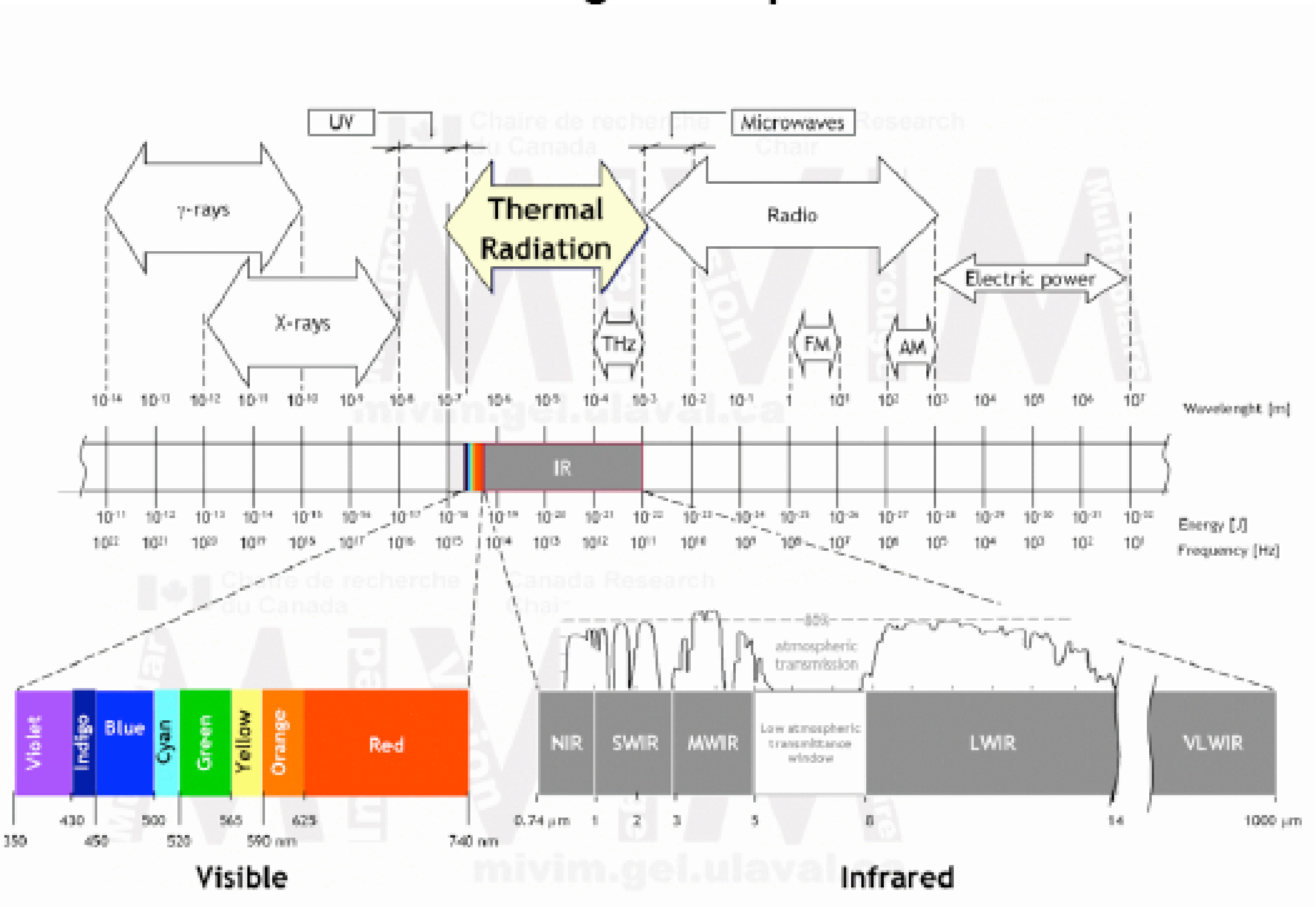
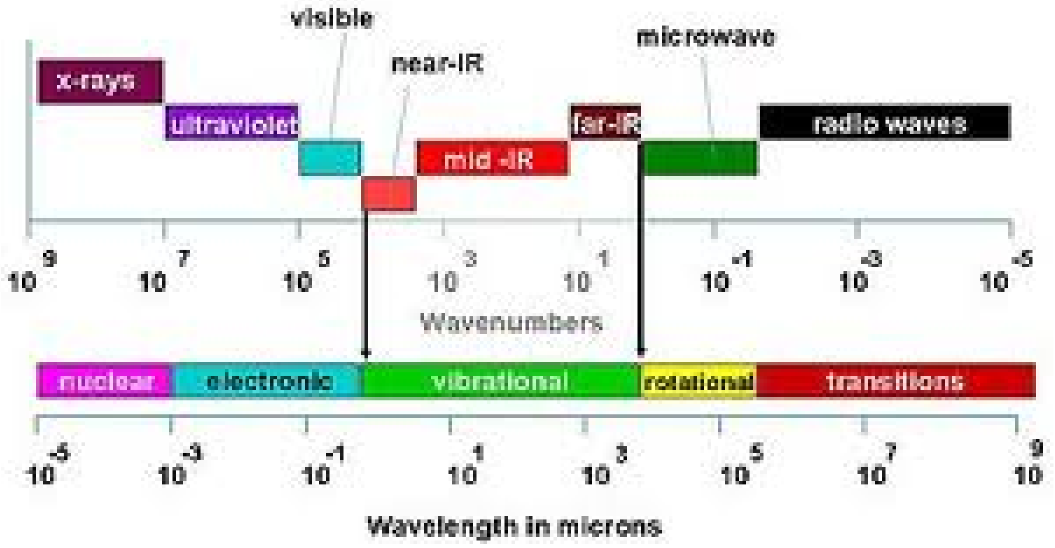
# Electromagnetic Spectrum



✓ Of greatest practical use is the limited portion between 4000 and 400 $cm^{-1}$ which is **mid IR**. There has been some interest in the **near-IR** (12820-4000 $cm^{-1}$) and the **far IR** region, 400-33 $cm^{-1}$ .



# Infrared (IR) Spectroscopy

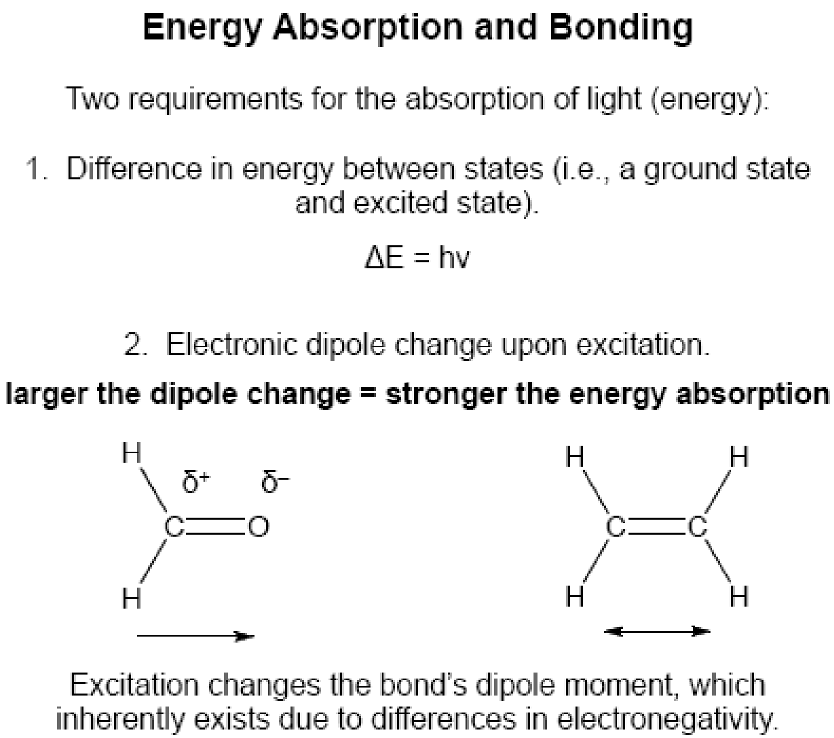
Infrared spectroscopy measures the **vibrational excitation** of atoms around the bonds that connect them. Specifically, middle infrared light is most useful

IR absorption bands are described by either the wavelength of light absorbed in micrometers (2.5 - 25.0 μM) or more commonly as its reciprocal value, called the **wave number**; which ranges from 4000 to 400 $cm^{-1}$.

**Wave Number ($v$) = $1/\lambda$**

**The position of absorption lines is related to the types of functional groups present in a molecule.**

Since many organic reactions are simply functional group transformations, IR spectroscopy is useful for probing molecular structure.



## Energy Absorption and Bonding

Two requirements for the absorption of light (energy):

1. Difference in energy between states (i.e., a ground state and excited state).

$$\Delta E = h\nu$$

2. Electronic dipole change upon excitation.

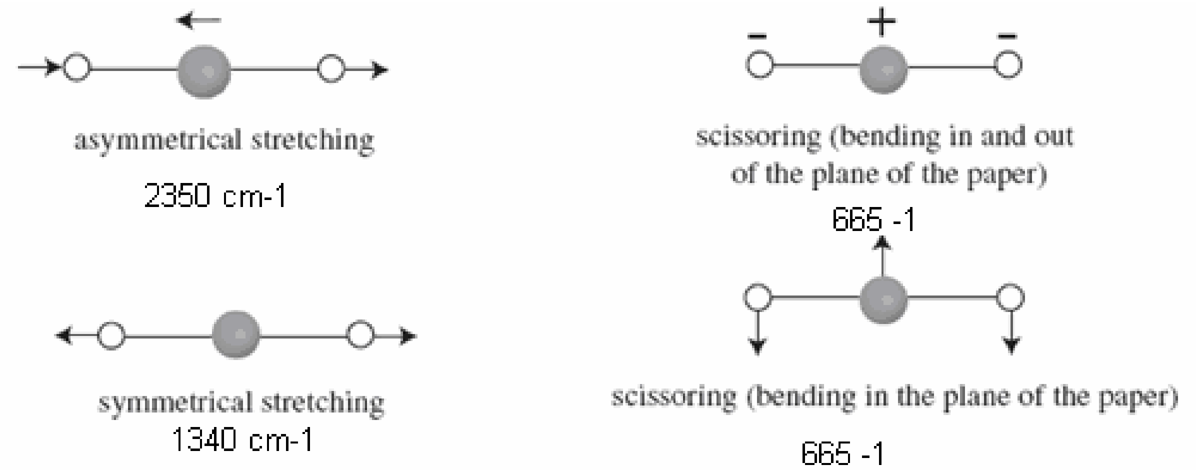
**larger the dipole change = stronger the energy absorption**

Excitation changes the bond's dipole moment, which inherently exists due to differences in electronegativity.

✓ **In order to be IR active, a vibration must cause a change in the dipole moment of the molecule**

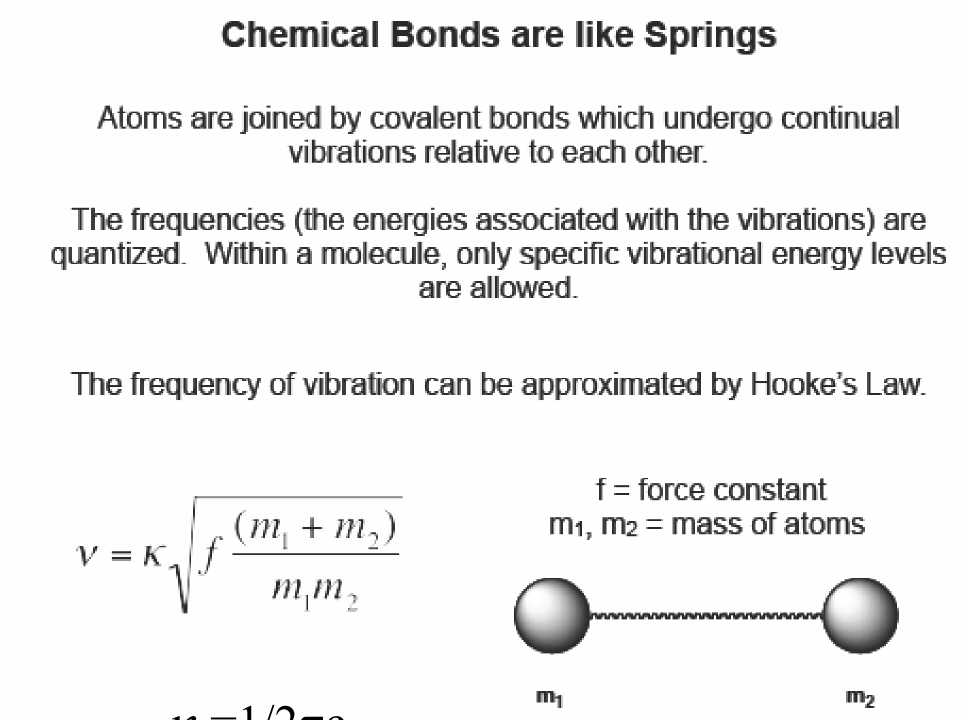
| | |
|---|---|
| C=O ICl | $H_2$ $N_2$ $Cl_2$ |
| absorb in IR | do not absorb in IR |

# Stretching and bending vibrational modes for $CO_2$



✓Only two IR bands (2350 and 666 $cm^{-1}$) are seen for carbon dioxide, instead of four corresponding to the four fundamental vibrations.

✓In the case of $CO_2$, two bands are degenerate, and one vibration does not cause a change in dipole moment.



## Chemical Bonds are like Springs

Atoms are joined by covalent bonds which undergo continual vibrations relative to each other.

The frequencies (the energies associated with the vibrations) are quantized. Within a molecule, only specific vibrational energy levels are allowed.

The frequency of vibration can be approximated by Hooke's Law.

$$\nu = \kappa\sqrt{f\,\frac{(m_1 + m_2)}{m_1 m_2}}$$

f = force constant
$m_1$, $m_2$ = mass of atoms

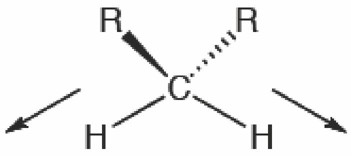
$\kappa = 1/2\pi c$

- higher mass of atoms = lower frequency
  Example: C–H (3000 $cm^{-1}$) > C–Cl (700 $cm^{-1}$) > C–Br (550 $cm^{-1}$)

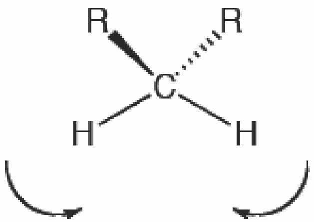
- higher bond strength = higher frequency
  Example: C≡C (2200 $cm^{-1}$) > C=C (1650 $cm^{-1}$)

# Types of Bond Vibrations

Stretching



Bending

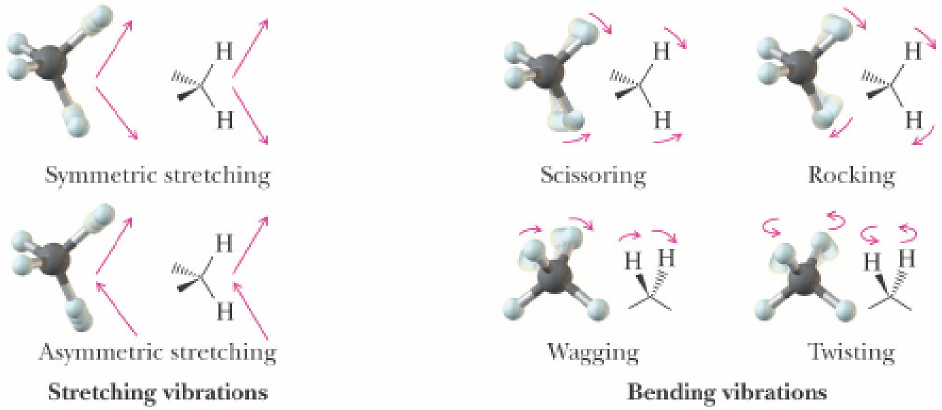


Many different vibrations for a molecule with *n* atoms.

$3n$-6 vibrational modes (nonlinear molecules)
$3n$-5 vibrational modes (linear molecules)

# Molecular Vibrations

Fundamental stretching and bending vibrations for a methylene group.



# Correlation Tables

Infrared stretching frequencies of selected functional groups:

| Bond | Stretching Frequency ($cm^{-1}$) | Intensity |
|---|---|---|
| O-H | 3200-3650 | weak to strong (broad) |
| N-H | 3100-3550 | medium |
| C-H | 2700-3300 | weak to medium |
| C=C | 1600-1680 | weak to medium |
| C=O | 1630-1820 | strong |
| C-O | 1000-1250 | strong |

# Wavenumber and Intensity

## Wavenumber

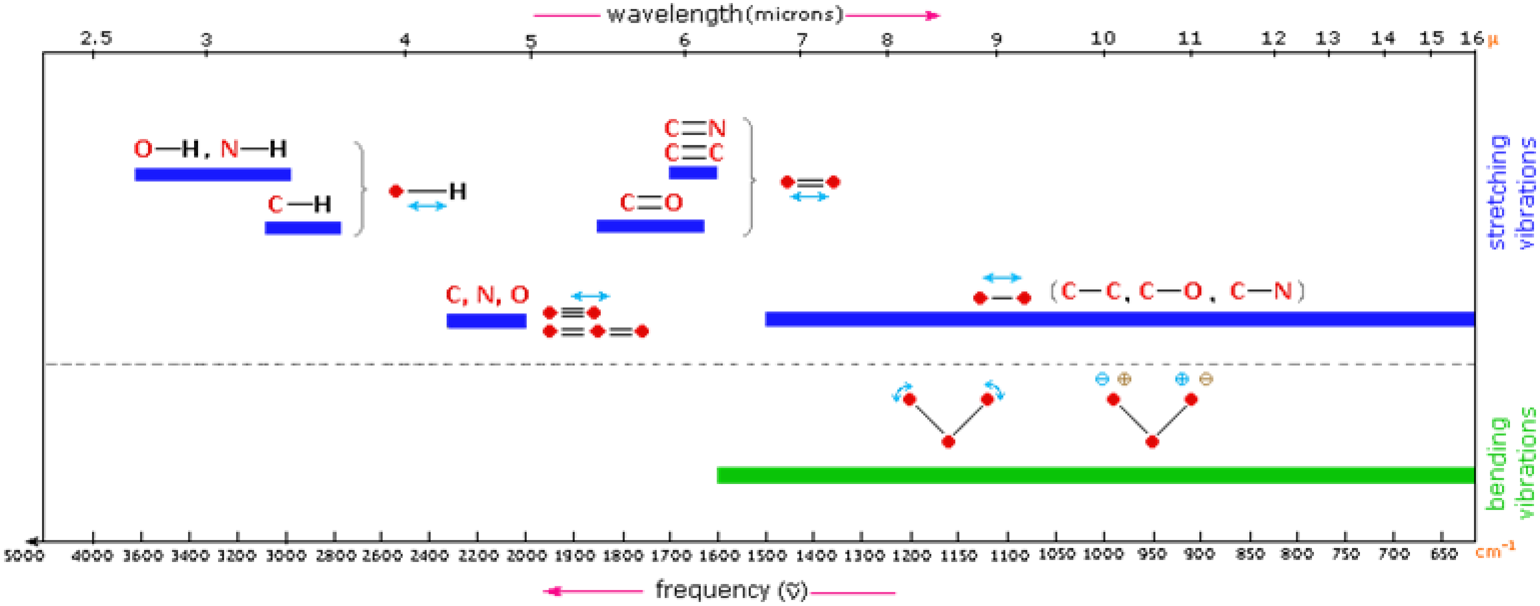
The shorter the bond; higher the frequency (energy)

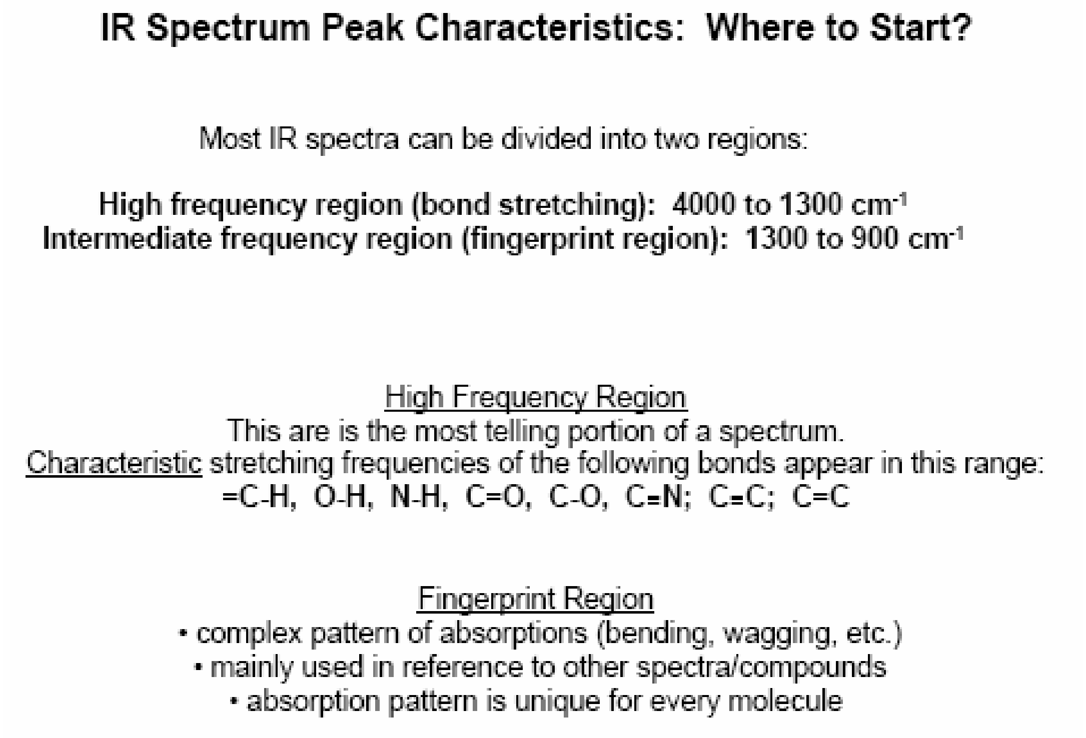
| Bond Type | Bond Length | $\nu$ (cm$^{-1}$) |
|---|---|---|
| C-C single bond | 1.54 A | 800 - 1000 |
| C-C double bond | 1.34 A | 1640 - 1680 |
| C-C triple bond | 1.20 A | 2100 - 2150 |

## Intensity

**The larger a bond's dipole moment, the more intense the energy absorption**. Certain covalent bonds do not meet this criteria (like double and triple bonds of symmetric alkenes and alkynes) and are said to be IR inactive.

# Group Frequencies





## IR Spectrum Peak Characteristics: Where to Start?

Most IR spectra can be divided into two regions:

**High frequency region (bond stretching): 4000 to 1300 cm$^{-1}$**
**Intermediate frequency region (fingerprint region): 1300 to 900 cm$^{-1}$**

### High Frequency Region

This are is the most telling portion of a spectrum.
Characteristic stretching frequencies of the following bonds appear in this range:
=C-H, O-H, N-H, C=O, C-O, C≡N; C≡C; C=C

### Fingerprint Region

- complex pattern of absorptions (bending, wagging, etc.)
- mainly used in reference to other spectra/compounds
- absorption pattern is unique for every molecule

✓ the most useful information obtained from an IR spectrum is what functional groups are present within the molecule

# Characteristic IR Stretches/Bends

Alkanes

C-H (s, stretch): 2850-3000 $cm^{-1}$

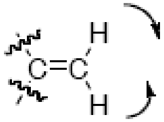
C-H (b, bend): 1450-1470; 1370-1380 $cm^{-1}$

(usually too many C-H's to be of any use)

Alkenes

C-H (s): 3020-3140 $cm^{-1}$ (very indicative; > 3000 $cm^{-1}$)

C=C (s): 1640-1680 $cm^{-1}$ (often weak due to small dipole)



(b): 900-1000 $cm^{-1}$

Amines

N-H (s): 3250-3500 $cm^{-1}$ (broad; similar to alcohols)

# Characteristic IR Stretches/Bends

## Alkynes

C-H (s, stretch): 3300 $cm^{-1}$
C-C triple bond (s): 2100-2150 $cm^{-1}$ (very diagnostic)

## Nitriles

C-N triple bond (s): 2250-2300 $cm^{-1}$ (strong due to dipole change)

## Alcohols

C-O (s): 1050-1200 $cm^{-1}$
O-H (s): 3250-3500 $cm^{-1}$ (often very broad due to hydrogen bonding)

## Characteristic IR Stretches/Bends for Carbonyls

Ketones

C=O (s): 1705-1745 $cm^{-1}$ (special cases exceed this range)

Aldehydes

C=O (s): 1715-1725 $cm^{-1}$

C-H (s): 2700-2800 $cm^{-1}$ (diagnostic, but weak C-H stretch)

Carboxylic Acids

C=O (s): 1720-1760 $cm^{-1}$

O-H (s): 2400-3100 $cm^{-1}$ (very broad due to hydrogen bonding)

Acyl Halides

C=O (s): 1800 $cm^{-1}$

Esters

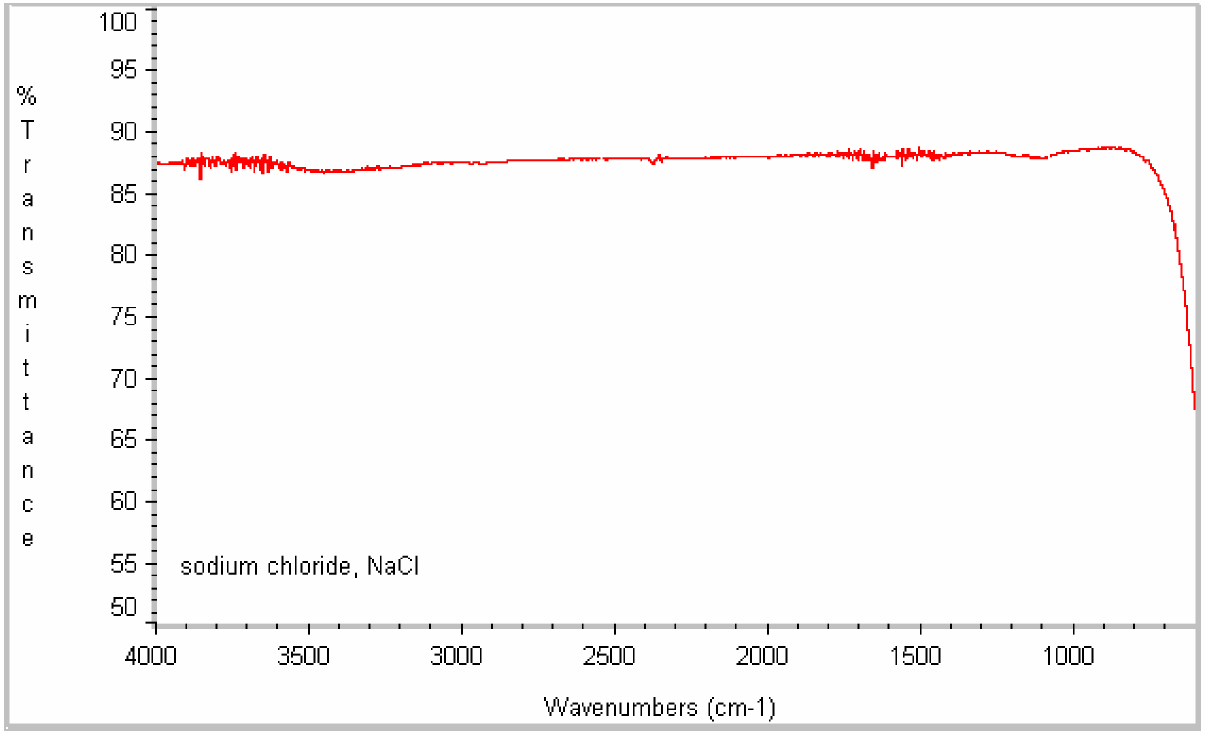
C=O (s): 1735-1750 $cm^{-1}$

Amides

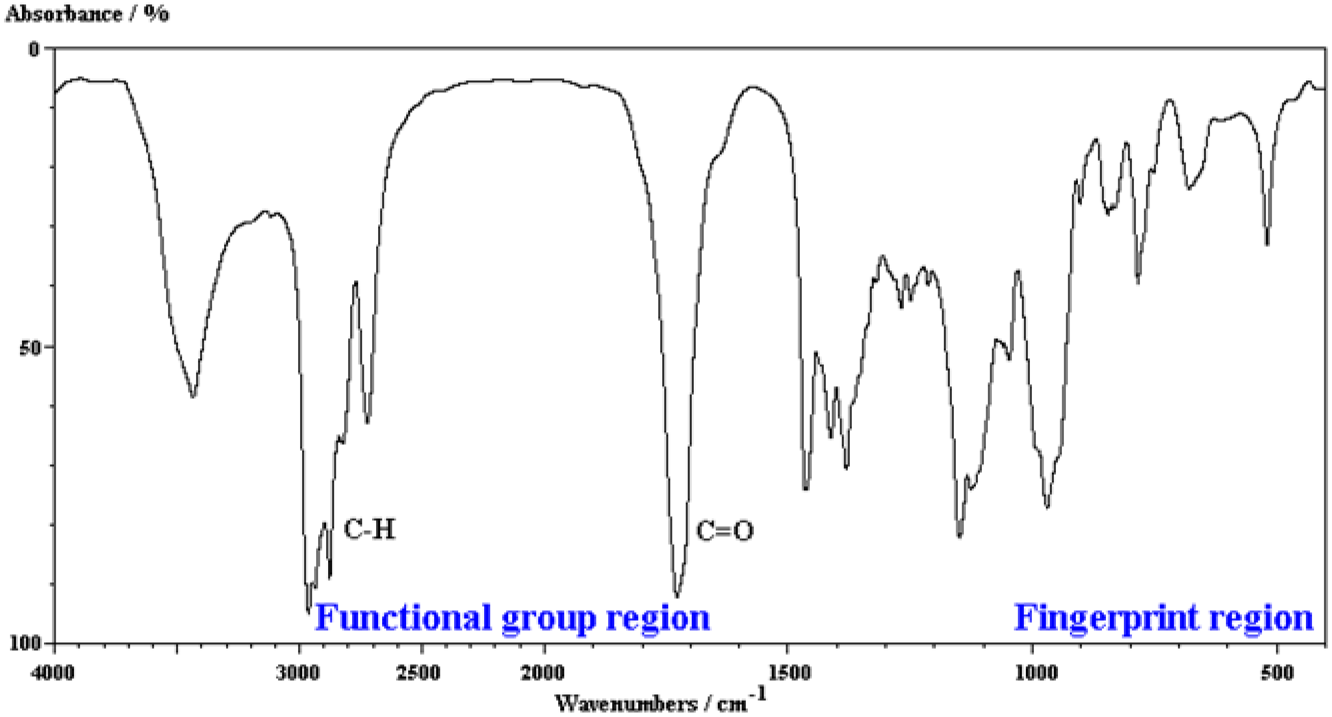
C=O (s): 1650-1700 $cm^{-1}$

N-H (s): 3400-3500 $cm^{-1}$ (can be broad due to hydrogen bonding)

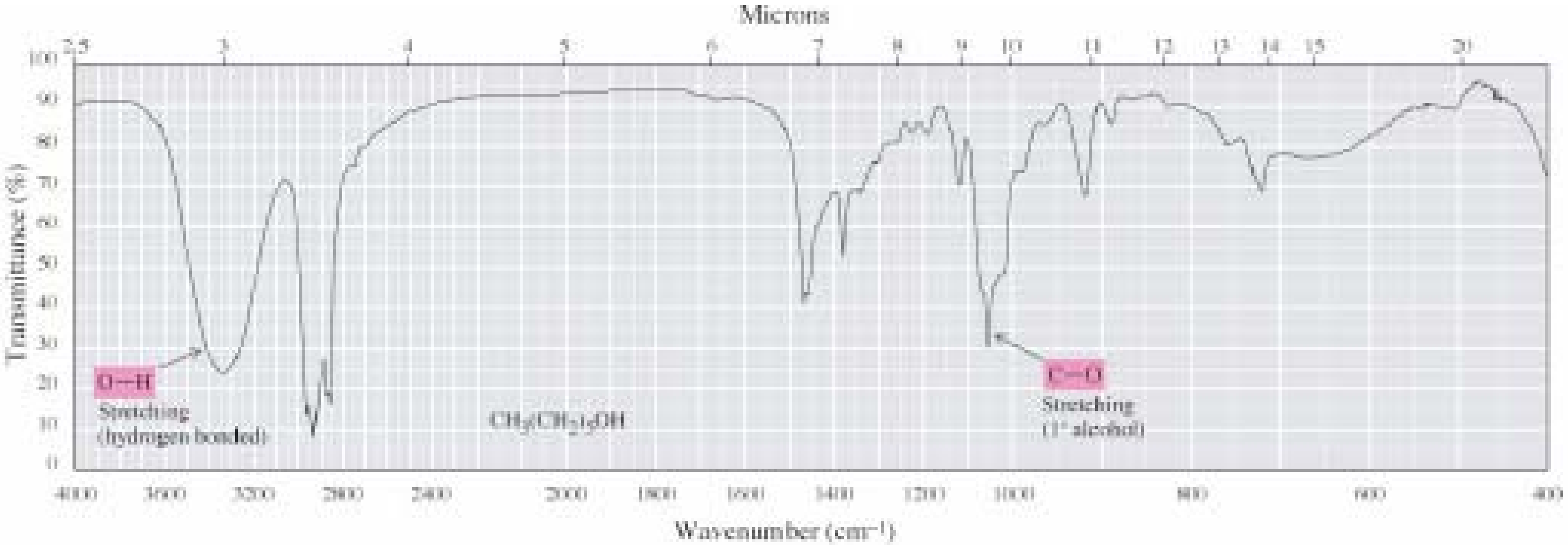
# IR Sample Preparation



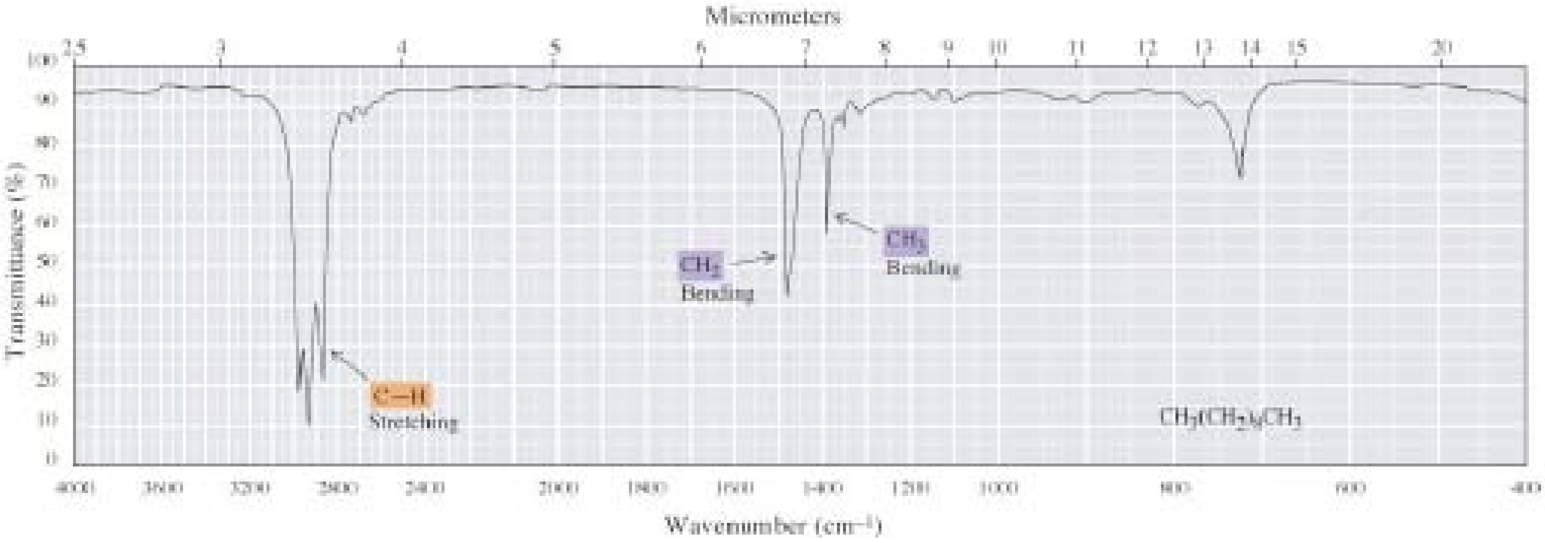
# IR Spectrum of Butanal



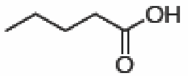
# IR Spectrum of 1-hexanol

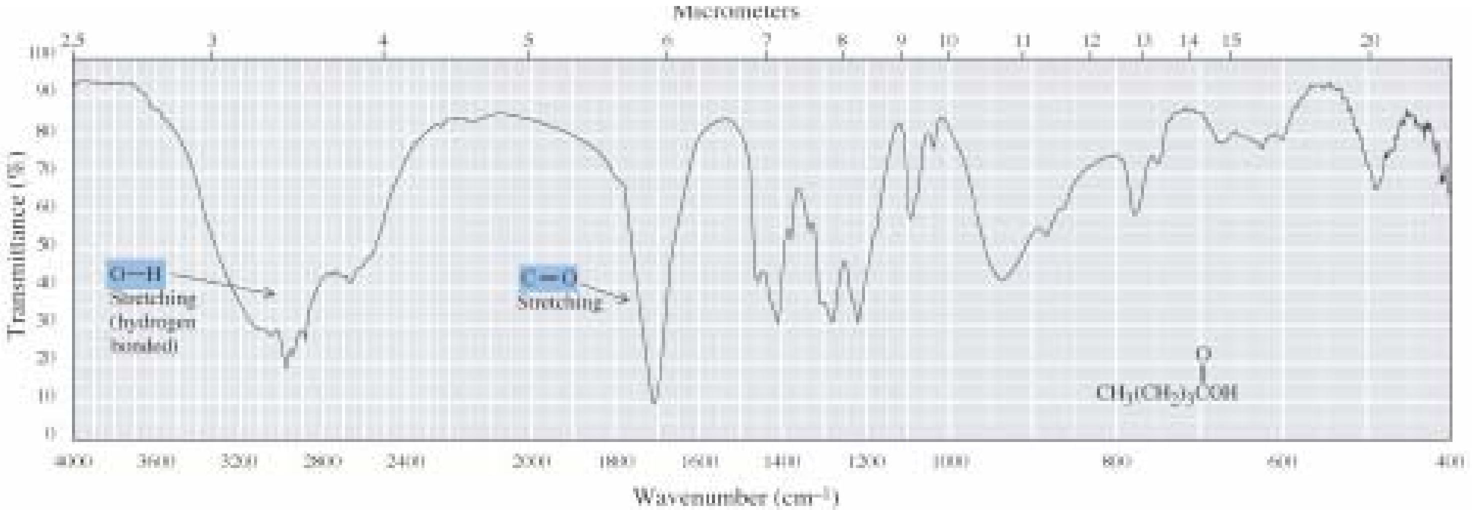


# IR Spectrum of Decane



## IR Spectrum of pentanoic acid



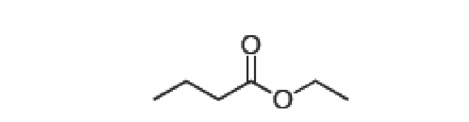


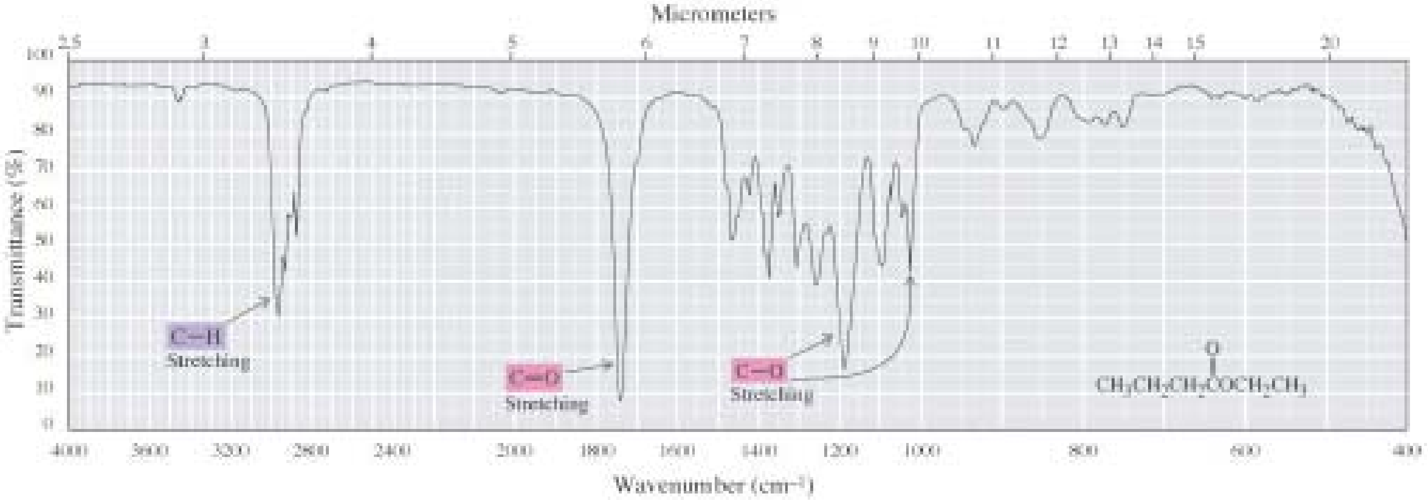
## Carbonyl Compounds

a. carboxylic acids

- strong C=O absorption in the range of 1700 to 1725 cm$^{-1}$
- very broad absorption band of the OH group from 2400 to 3400 cm$^{-1}$ (this band will usually overlap the alkane C-H stretch from 2850-3000 cm$^{-1}$)
- medium intensity C-O stretch (from C-OH bond) occurs in 1210-1320 cm$^{-1}$ range

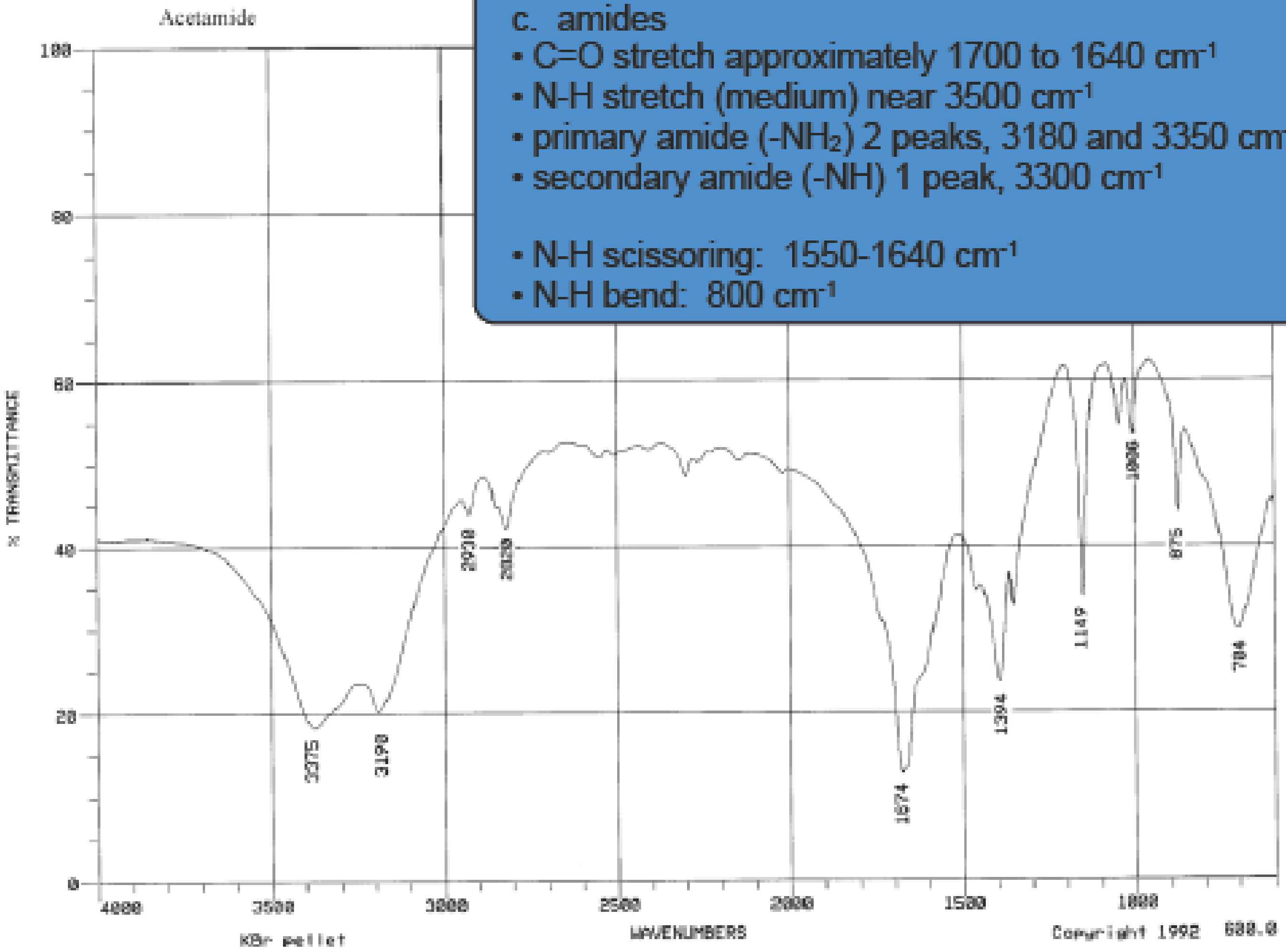
# IR Spectrum of ethyl butanoate





b. esters
- C=O stretch appears in the range of 1730 to 1750 $cm^{-1}$
- look for two or more C-O stretch bands, in range of 1100-1300 $cm^{-1}$

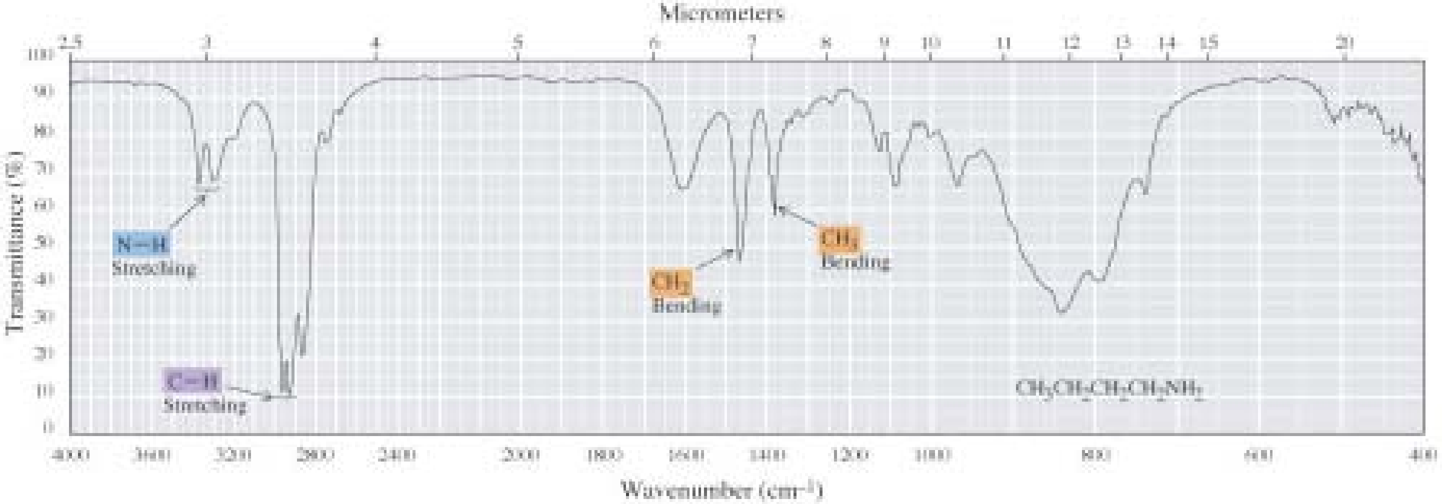
# IR Spectrum of Acetamide



c. amides
- C=O stretch approximately 1700 to 1640 cm$^{-1}$
- N-H stretch (medium) near 3500 cm$^{-1}$
- primary amide (-NH$_2$) 2 peaks, 3180 and 3350 cm$^{-1}$
- secondary amide (-NH) 1 peak, 3300 cm$^{-1}$

- N-H scissoring: 1550-1640 cm$^{-1}$
- N-H bend: 800 cm$^{-1}$

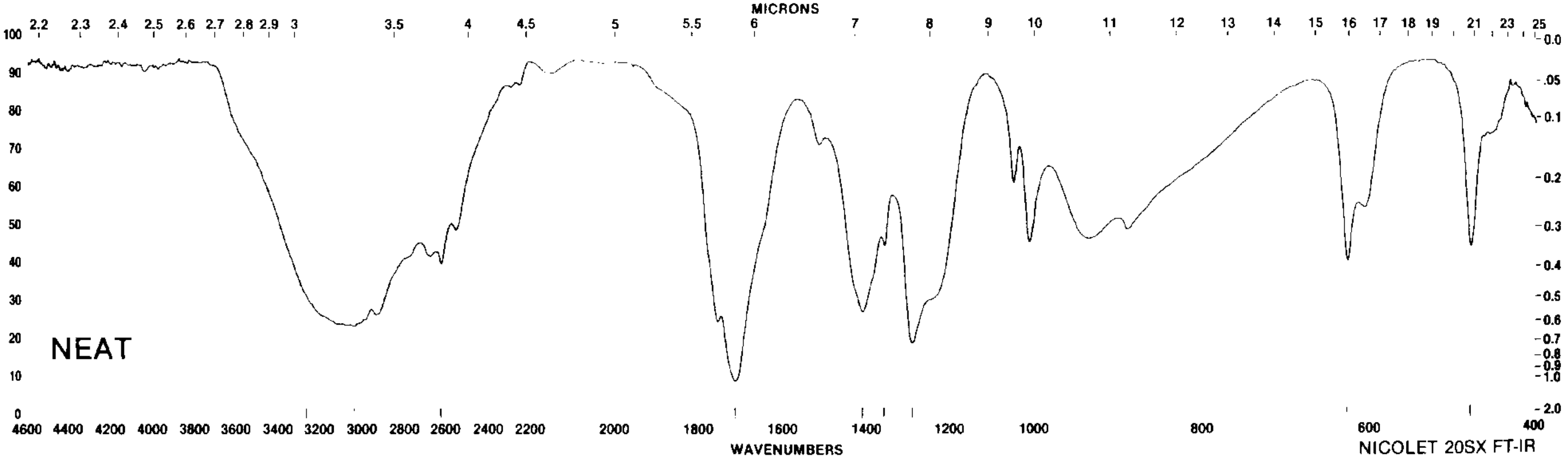
# IR Spectrum of 1-butylamine



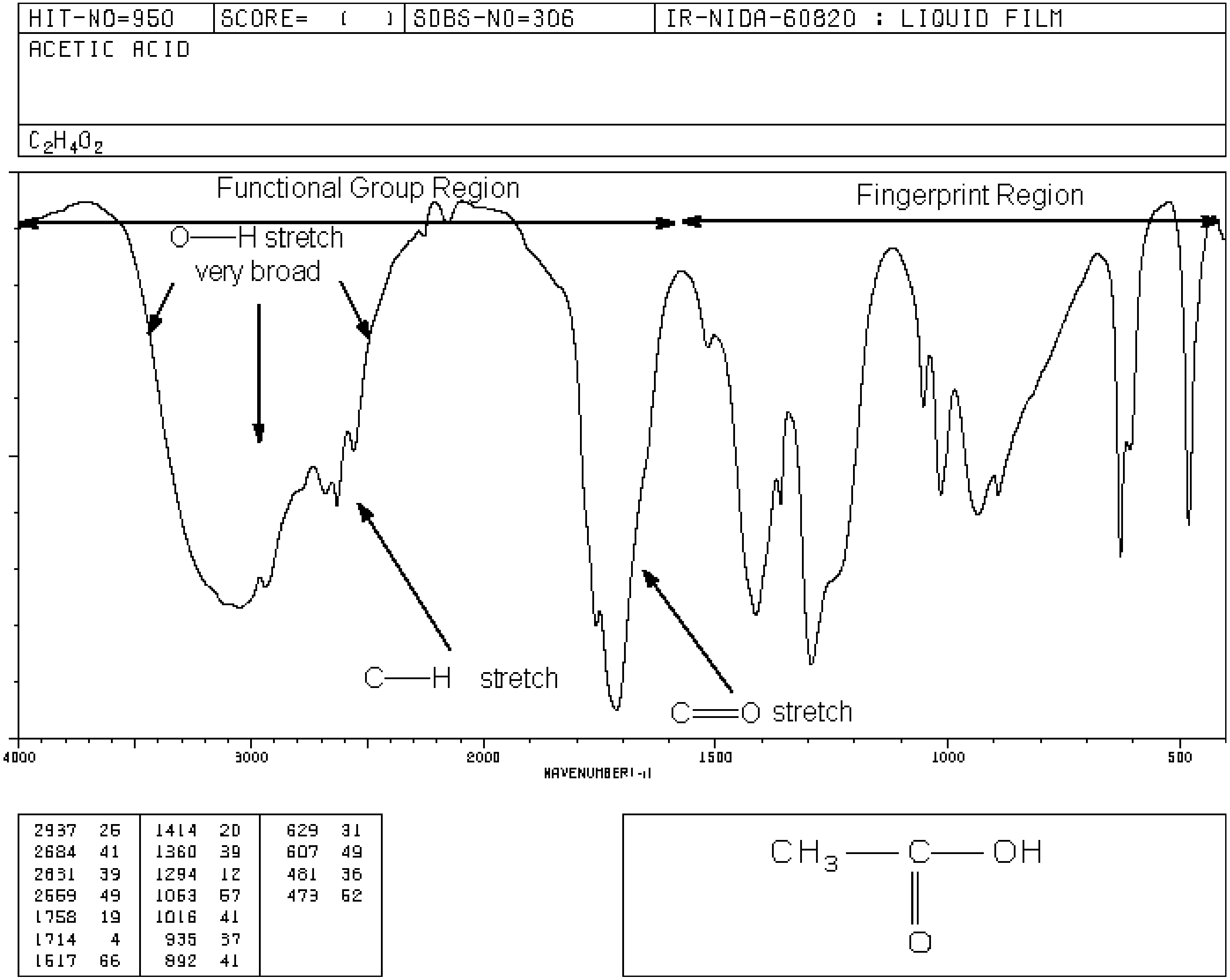
## Amines

- N-H stretch (medium) near 3500 cm$^{-1}$
  - primary (2 peaks)
  - secondary (1 peak)
  - tertiary (no peaks)
- N-H scissoring at 1560-1640 cm$^{-1}$
- N-H bend at 800 cm$^{-1}$

# IR Example, $C_2H_4O_2$



# Solution 1



HIT-NO=950 | SCORE= ( ) | SDBS-NO=306 | IR-NIDA-60820 : LIQUID FILM

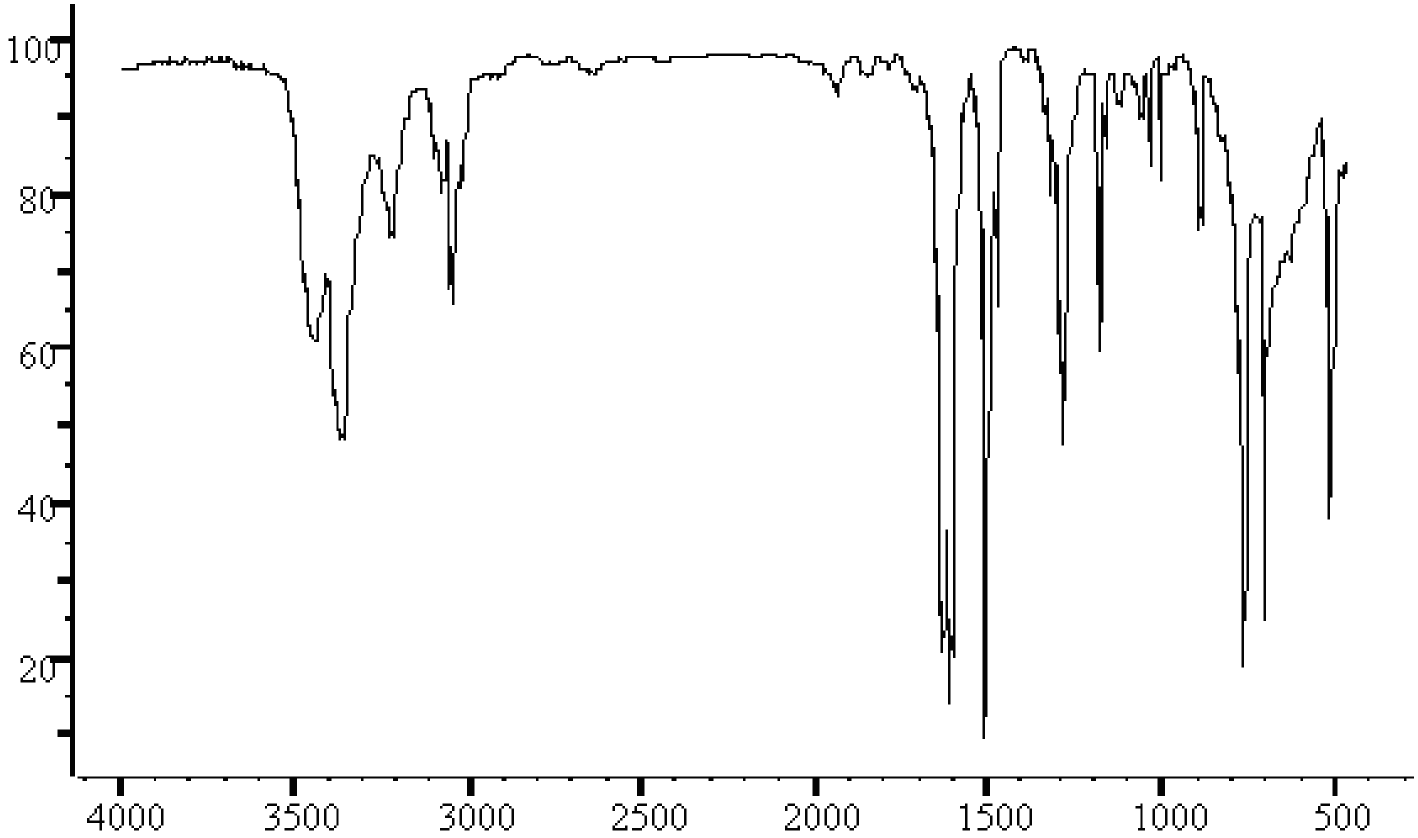
ACETIC ACID

$C_2H_4O_2$

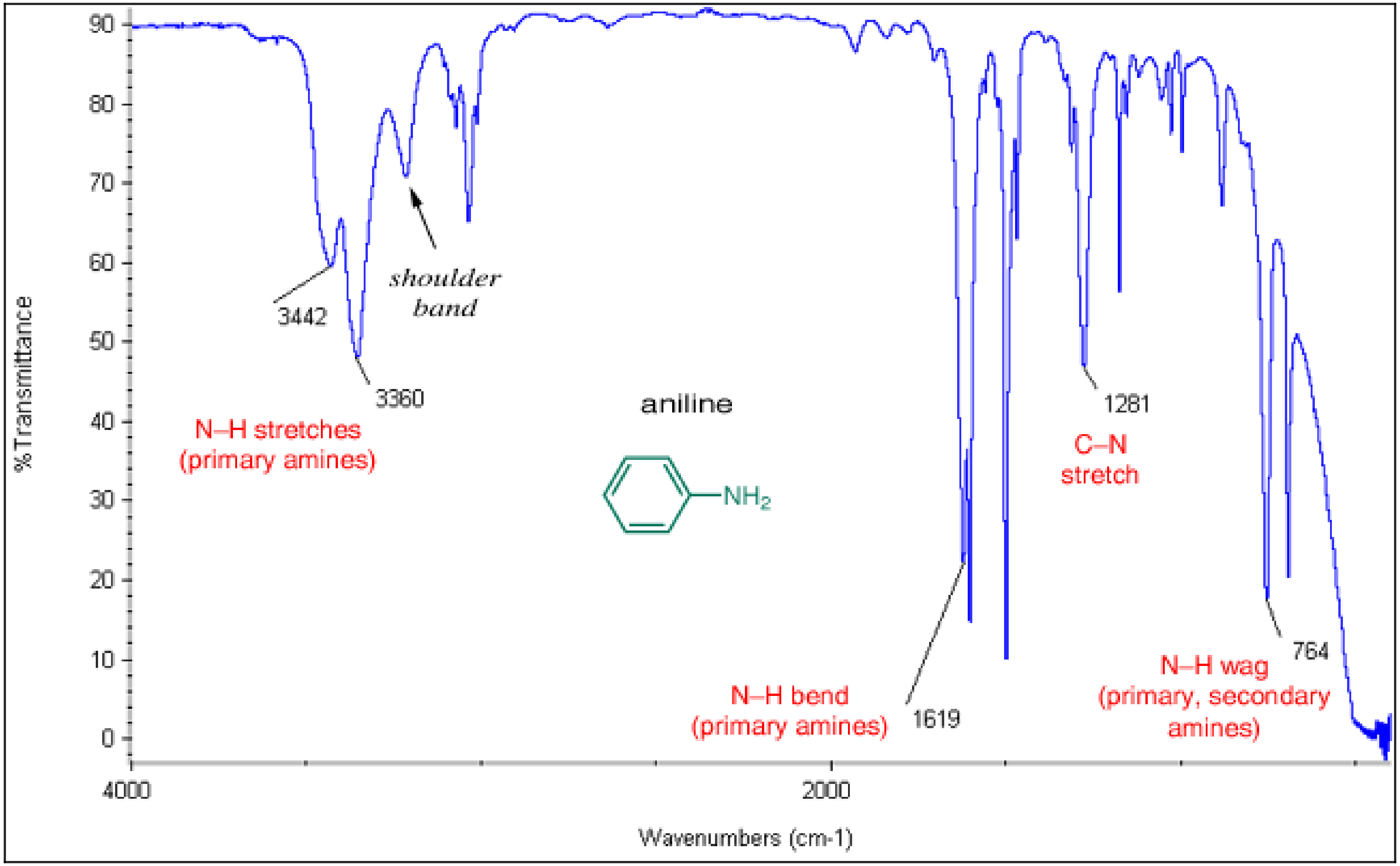
| | | | | | |
|---|---|---|---|---|---|
| 2937 | 26 | 1414 | 20 | 629 | 31 |
| 2684 | 41 | 1360 | 39 | 607 | 49 |
| 2831 | 39 | 1294 | 12 | 481 | 36 |
| 2669 | 49 | 1063 | 67 | 473 | 62 |
| 1758 | 19 | 1016 | 41 | | |
| 1714 | 4 | 935 | 37 | | |
| 1617 | 66 | 892 | 41 | | |

$CH_3—C(=O)—OH$

# IR Example 2, C6H7N



# Solution 2


90
80
70
60
50
40
30
20
10
0
% Transmittance
4000
2000
Wavenumbers (cm-1)
3442
3360
shoulder band
N–H stretches (primary amines)
aniline
NH2
1619
N–H bend (primary amines)
1281
C–N stretch
764
N–H wag (primary, secondary amines)

# IR Study Questions

1. Infrared spectroscopy provides valuable information about
molecular weight. melting point. conjugation. functional groups.

2. A strong signal at 3400 $cm^{-1}$ in an IR spectrum indicates the presence of which group?

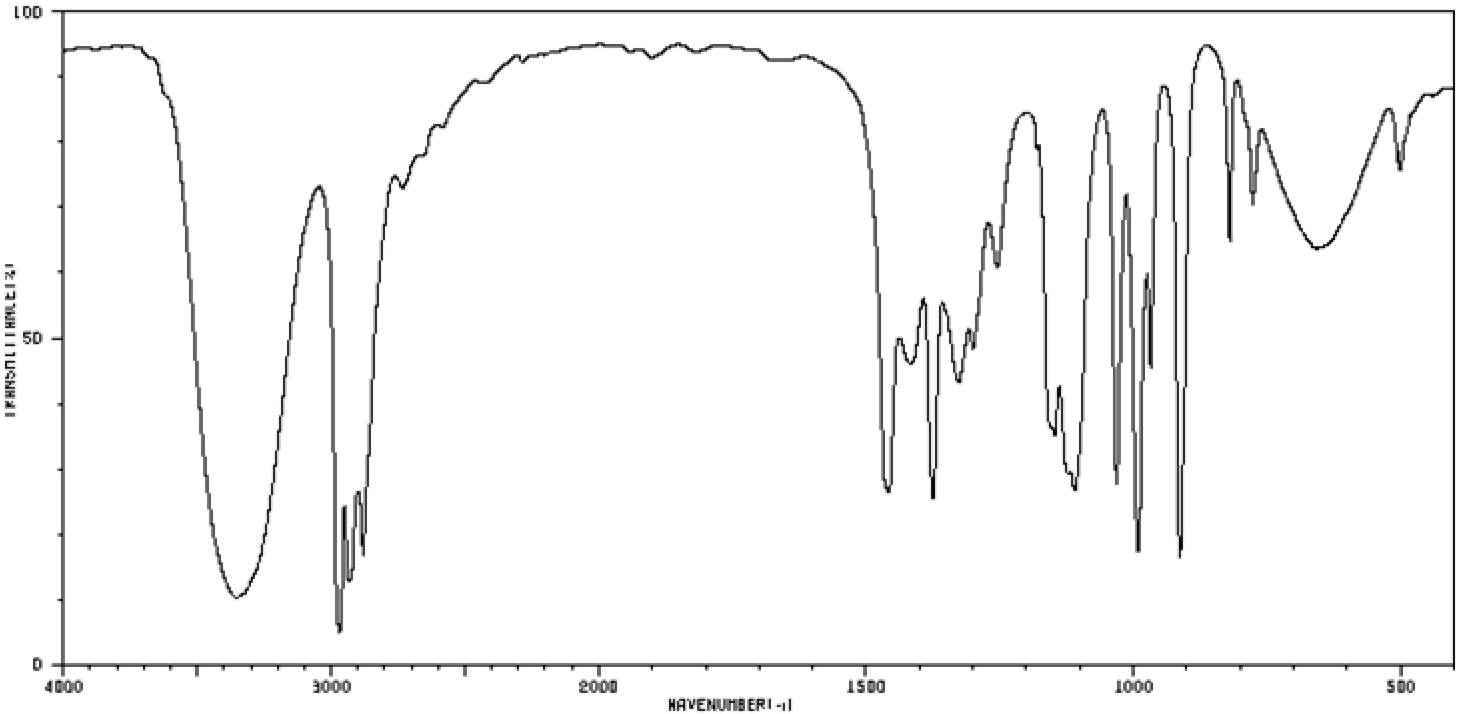
3. A strong signal at 1700 $cm^{-1}$ in an IR spectrum indicates the presence of which group?

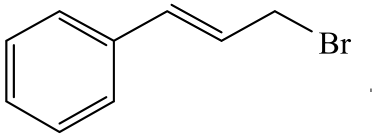
4. What is the relationship between wavelength and wavenumber ?

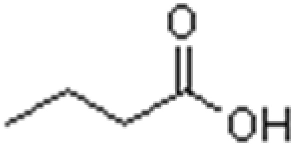
5. For a molecule to absorb IR, the molecule's vibrations should cause a change in ………… ………… of the molecule.

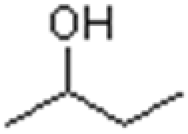
6. Vibration frequencies in IR spectroscopy depends on................. and .................

7. What is the identity of the compound responsible for the following infrared spectrum?









8. Is it possible to distinguish Ethanol and Propanol from each other by using Infrared spectroscopy?

9. Why is the oxygen-hydrogen absorption of $CH_3OH$ such a broad band in the infrared?