MOLECULAR SPECTROSCOPY:

-Molecular Absorption (UV-Vis and IR)

-Molecular Emission

An Introduction to Ultraviolet/Visible Molecular Absorption Spectrometry

13A Measurement Of Transmittance and Absorbance

- Absorption measurements based upon ultraviolet and visible radiation find widespread application for the quantitative determination of a large variety of species.
- Ordinarily, the concentration of an absorbing analyte is linearly related to absorbance as given by Beer's law:

–Beer's Law:

$$A = -\log T = \log P_0/P = \varepsilon bc$$

A = absorbance

ε = molar absorptivity [M^{-1} cm^{-1}]

c = concentration [M]

P_0 = incident power

P = transmitted power (after passing through sample)

TABLE 13-1 Important Terms and Symbols for Absorption Measurements

Term and Symbol*	Definition	Alternative Name and Symbol
Incident radiant power, P_0	Radiant power in watts incident on sample	Incident intensity, I_0
Transmitted radiant power, P	Radiant power transmitted by sample	Transmitted intensity, I
Absorbance, A	$\log(P_0/P)$	Optical density, D; extinction, E
Transmittance, T	P/P_0	Transmission, T
Path length of sample, b	Length over which attenuation occurs	l, d
Concentration of absorber, c	Concentration in specified units	
Absorptivity,† a	$A/(bc)$	Extinction coefficient, k
Molar absorptivity,‡ ε	$A/(bc)$	Molar extinction coefficient

Transmittance and absorbance, as defined in Table 13-1, cannot normally be measured in the laboratory because the analyte solution must be held in a transparent container or cell.

- Reflection occurs at the two air-wall interfaces as well as at the two wall-solution interfaces.
- The resulting beam attenuation is substantial, as we demonstrated in Example 6-2, where it was shown that about 8.5% of a beam of yellow light is lost by reflection in passing through a glass cell containing water.
- In addition, attenuation

of a beam may occur as a result of scattering by large molecules and sometimes from absorption by the container walls.

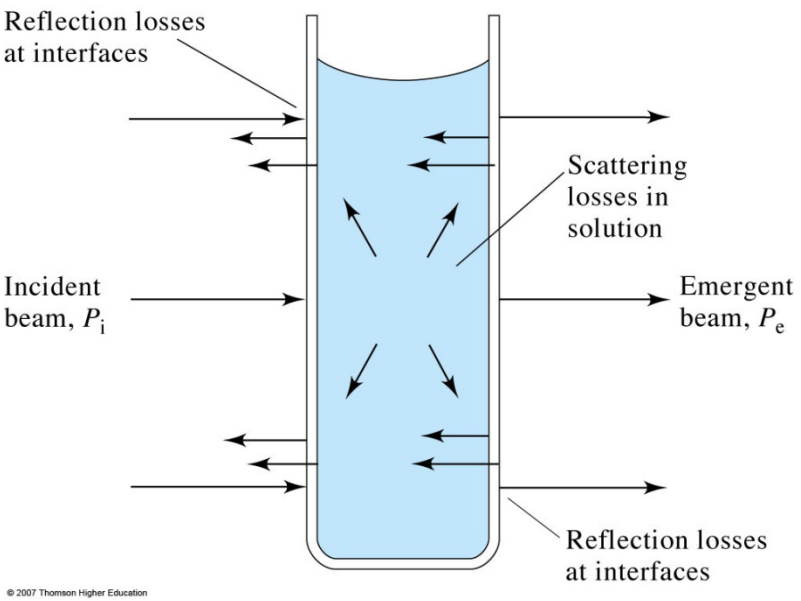


FIGURE 13-1 Reflection and scattering losses with a solution contained in a typical glass cell. Losses by reflection can occur at all the boundaries that separate the diffferent materials. In this example, the light passes through the air-glass, glass-solution, solution-glass, and glass-air interfaces.

Measurement of Transmittance and Absorbance:

The power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent. An experimental transmittance and absorbance are then obtained with the equations.

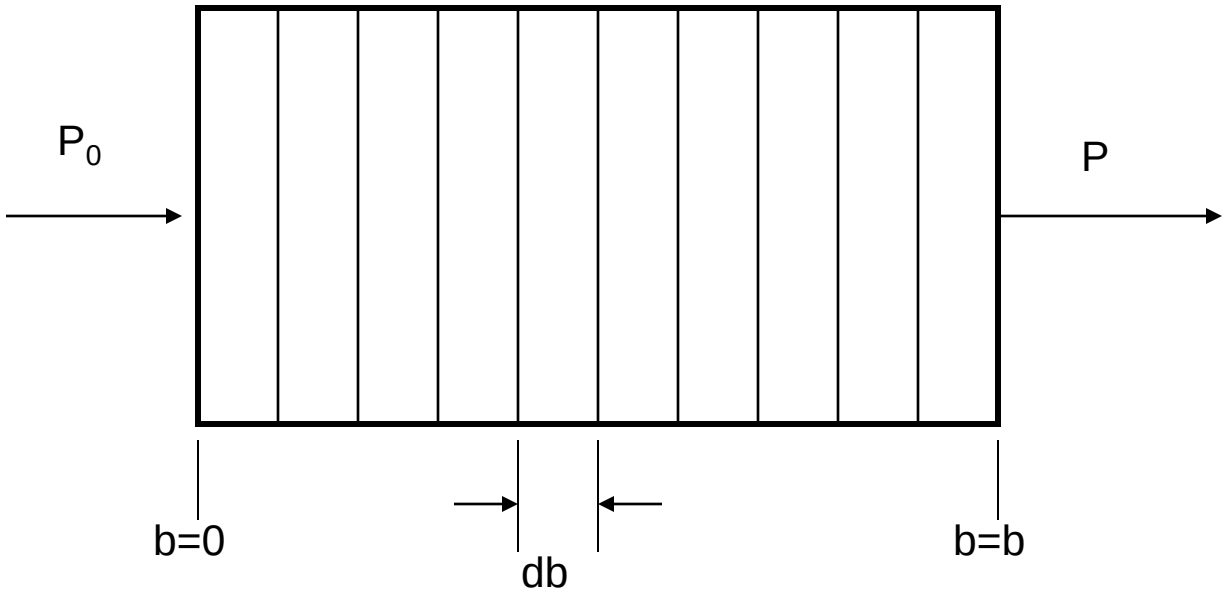
P_0 and P refers to the power of radiation after it has passed through the solvent and the analyte.

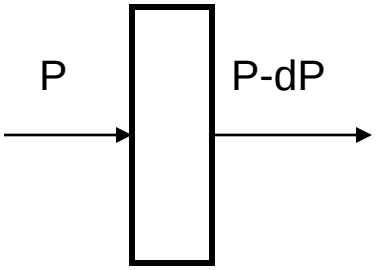
$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0}$$

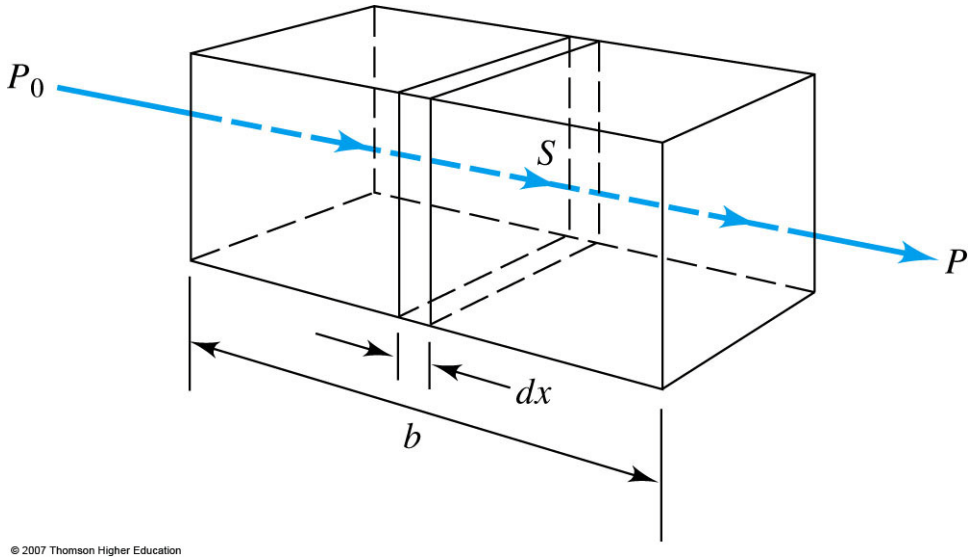
$$A = \log \frac{P_{solvent}}{P_{solution}} \approx \log \frac{P_0}{P}$$

- # Derivation of Beer's Law:

Sample cell with absorbing molecules







$dP \propto P$ Incremental power lost $\propto$ power in; i.e., increase power in, increase power absorbed

$dP \propto db$ Longer pathlength, greater number of molecules in incremental slice and more power absorbed

Therefore, $dP \propto Pdb$ $dP = -kPdb$

k = proportionality constant (function of λ, c)

negative sign: because power is lost (i.e., absorbed)

Rearrange:

$$\frac{dP}{P} = -kdb$$

Integrate: $\int_{P_0}^{P} \frac{1}{P} dP = -k \int_{0}^{b} db$

$$InP - InP_0 = -kb - (-k)(0)$$

$$In\frac{P}{P_0} = -kb$$

Factor out concentration part of k: k = k”c

$$In\frac{P}{P_0} = -k"bc$$

Convert fraction (remove –sign) and change In to log:

$$\log\frac{P_0}{P} = \frac{1}{2.303} k:"bc$$

$$A = \log\frac{P_0}{P} = \varepsilon bc$$

(1/2.303)k” = ε

13B-1 Application of Beer's Law to Mixtures

Beer's law applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by

$$A_{total} = A_1 + A_2 + \ldots + A_n$$
$$= \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \ldots + \varepsilon_n b c_n$$

where, the subscripts refer to absorbing components 1, 2, …, n.

Assumptions in derivation of Beer's Law

- incident radiation is Monochromatic(all molecules absorb light of one λ)
- Absorbing molecules act independently of one another i.e, low c
- Pathlength is uniform (all rays travel the same distance in sample)
- No scattering
- Absorbing medium is optically homogeneous
- Incident beam is not large enough to cause saturation
- All rays should be parallel to each other and perpendicular to surface of medium.

13B-2 Limitations (deviations) to Beer's Law

* ***Real Limitations***

  High concentration > 0.01 M

- the extent of solute-solvent interactions, solute-solute interactions, or hydrogen bonding can affect the analyte environment and its absorptivity.

* ***Chemical Deviations***

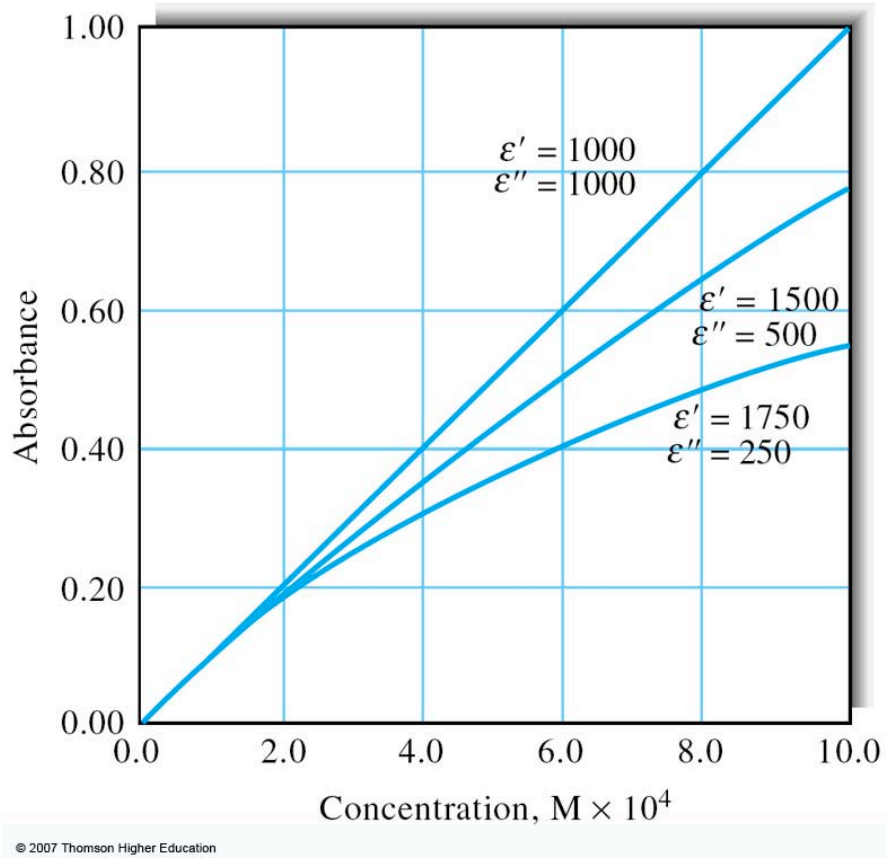
- Analyte dissociates, associates or reacts to give molecule with different absorption characteristics (e.g., pH-dependent indicators)
  - Example 13-1

* ***Instrumental Deviations***

  - Polychromatic radiation
  - Stray Radiation

Instrumental Deviations :

– In the presence of Polychromatic radiation (i.e., light of more than one λ)



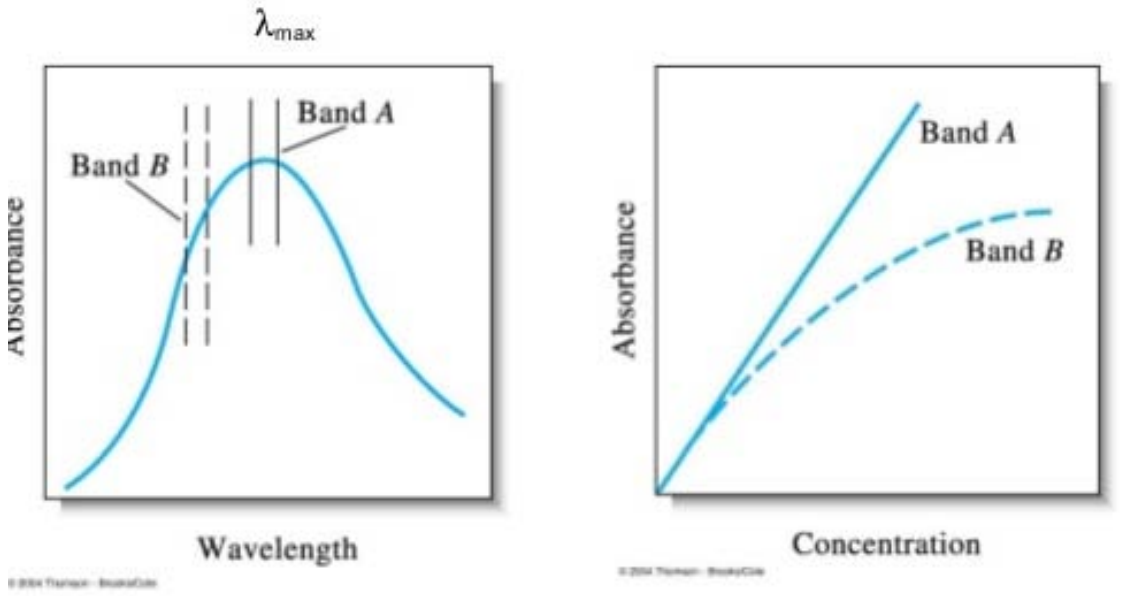
$$A_{meas} = \log \frac{\left(p'_0 + P''_0\right)}{\left(P' + P''\right)}$$

Where P' and P" are powers for λ' and λ", respectively

- Negative deviation = lower absorbance than predicted because higher transmittance
- Higher T because molecules don't absorb one λ as well as other

The absorber has the indicated molar absorptivities at the two wavelengths λ' and λ"

The effect of polychromatic radiation on Beer's law. In the spectrum on the left, the absorptivity of the analyte is nearly constant over band ***A*** from the source. Note in the Beer's law plot at the bottom that using band ***A*** *gives* a linear relationship. In the spectrum, band *B* corresponds to a region where the absorptivity shows substantial changes. In the lower plot, note the dramatic deviation from Beer's law that results.



To avoid deviations, it is advisable to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength

Instrumental Deviations :

–In the presence of Stray radiation

- the radiation exiting from a monochromator is usually contaminated with small amounts of scattered or stray radiation. This radiation, commonlv called *stray light, is defined as radiation from the instrument that is outside the nominal wavelength band chosen for the determination.*

- This *stray radiation often is the result of scattering and reflection off the surfaces of gratings, lenses or mirrors. filters, and windows*.

- The wavelength of stray radiation often differs greatly from that of the principal radiation and, in addition, the radiation may not have passed through the sample.

Instrumental Deviations :

–In the presence of Stray radiation

$$A' = \log \frac{\left(p'_0 + P_s\right)}{\left(P' + Ps\right)}$$

Ps = power from stray radiation

Extra light hits detector →

higher T; lower A

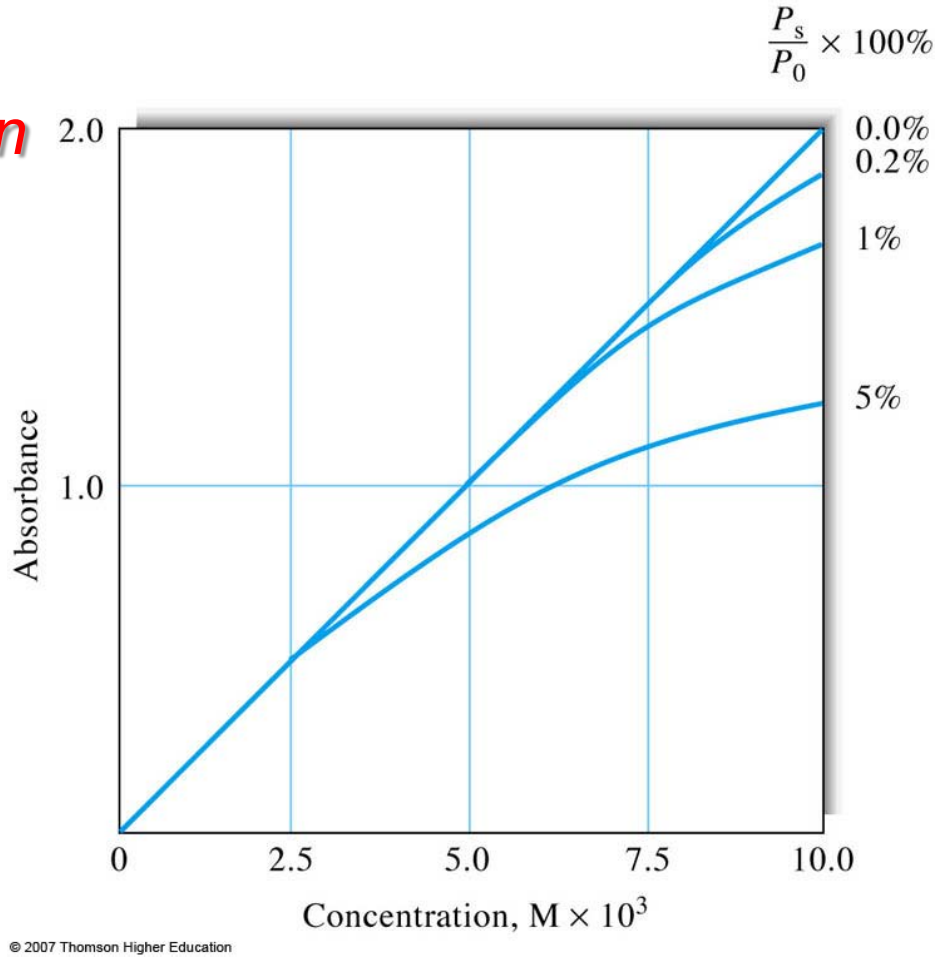
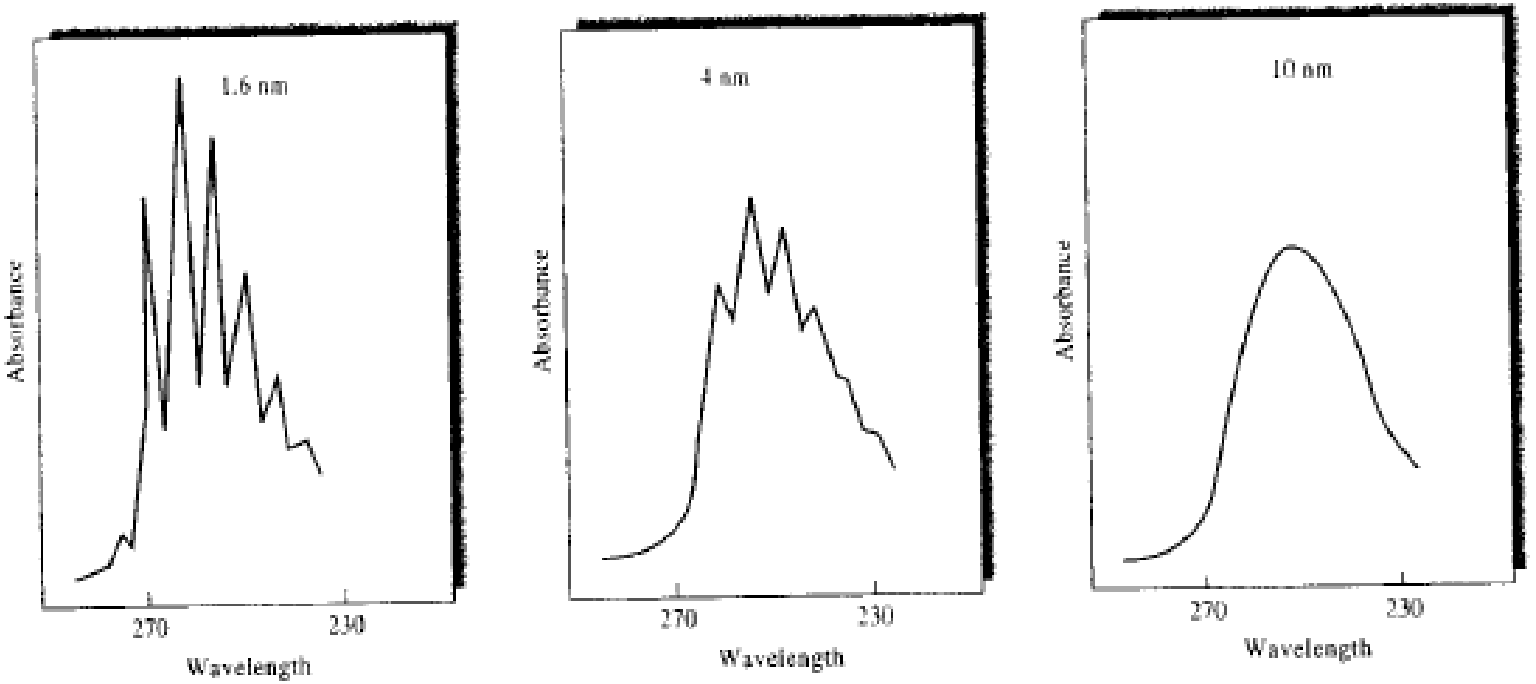


FIGURE 13-6 Apparent deviation from Beer's law brought about by various amounts of stray radiation. Note that the absorbance begins to level off with concentration at high
stray-light levels. Stray light always limitsthe maximum absorbance that can be obtained because when the absorbance is high, the radiant power transmitted through

13.C Effect of Slit Width on Absorbance Measurements



- Figure 13-8 illustrates the loss of detail that occurs when slit widths are increased from small values on the left to larger values in the middle and right. In this example, the absorption spectrum of benzene vapor was obtained at slit settings that provided effective bandwidths of 1.6, 4, and 10 nm.
- For qualitative studies, the loss of resolution that accompanies the use of wider slits is often important because the details of spectra are useful for identifying species. Narrow slits widths are required to resolve complex spectra

13.C Effect of Slit Width on Absorbance Measurements

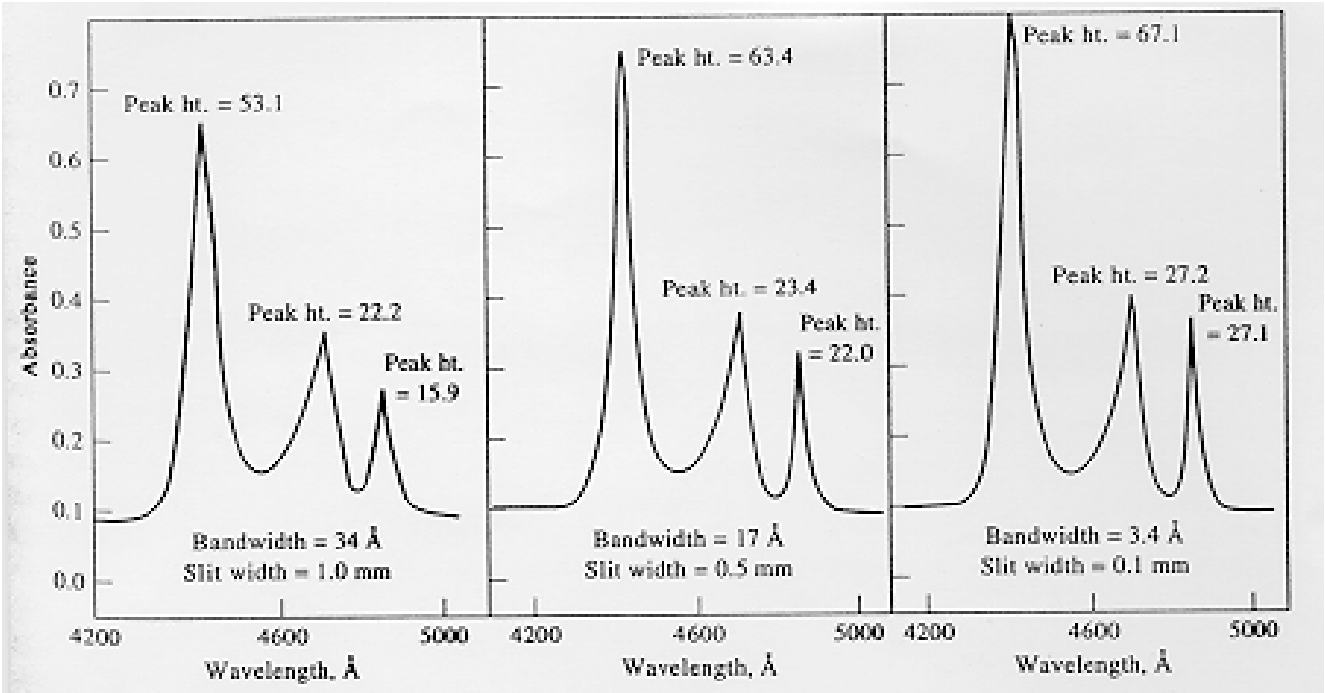


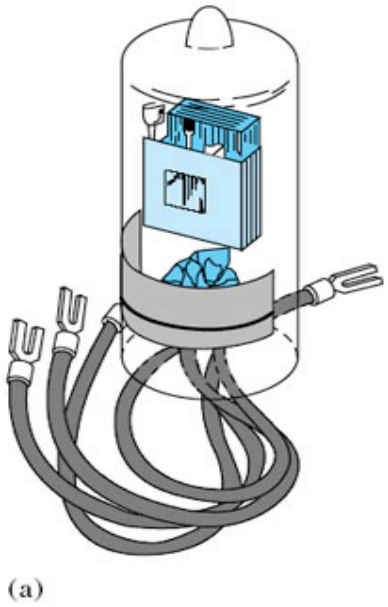
Figure 13-3 Effect of slit width and bandwidth on peak heights. Here, the sample was a solution of praseodymium chloride. *(From* Optimum Spectrophotometer Parameters, Application Report *AR 14-2, Cary Instruments: Monrovia, CA. With permission.)*

- quantitative measurement of narrow absorption bands requires using narrow slit widths or, alternatively, very reproducible slit-width settings. Unfortunately, a decrease in slit width by a factor of 10 reduces the radiant power by a factor of 100 because the radiant power is proportional to the square of the slit width.
- There is thus a trade-off between resolution and signal-to-noise ratio.
- For quantitative measurements, slit width needs to be kept large enough

Instrumentation

- Light source
- λ - selection
- Sample container
- Detector
- Signal processing
- Light Sources (commercial instruments)
  - D_2 lamp (UV: 160 – 375 nm)
  - W lamp (vis: 350 – 2500 nm)

Deuterium and Hydrogen Lamps



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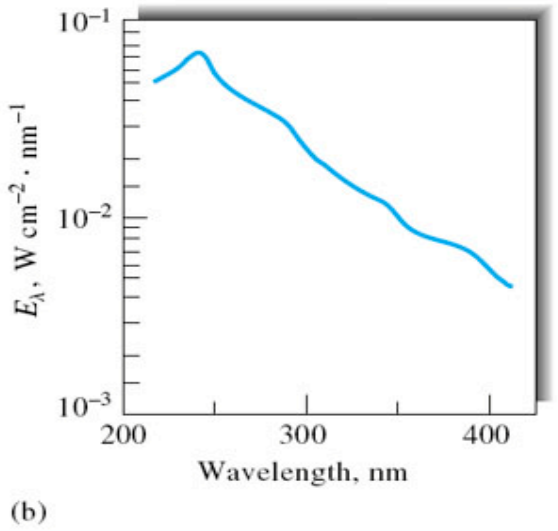

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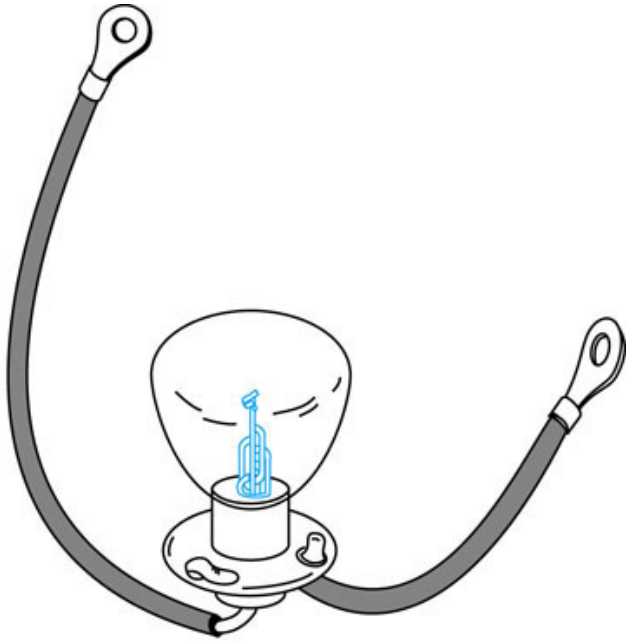
Tungsten Filament Lamps



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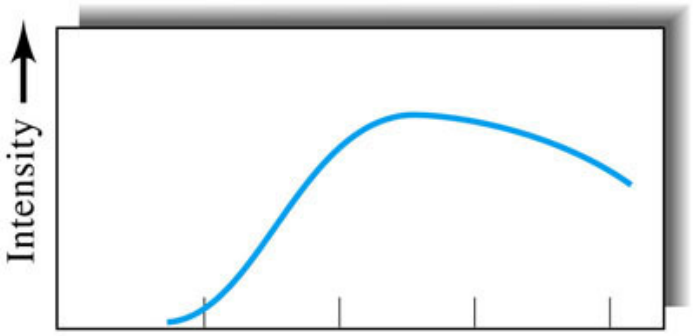
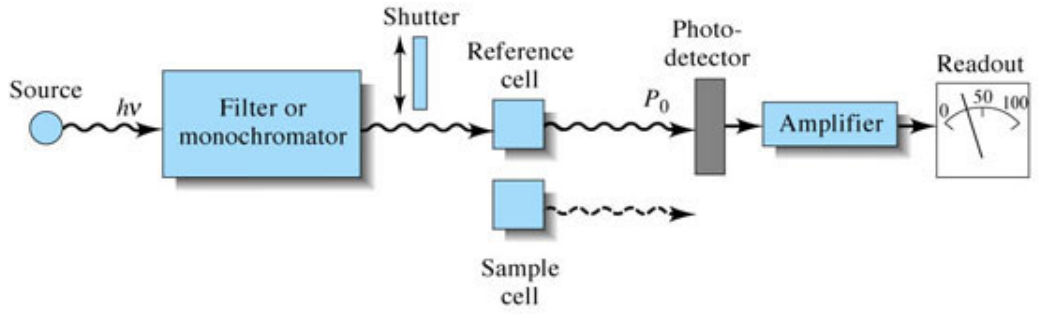


- λ - selection (monochromators)
- Sample holders
  - Cuvettes (b = 1 cm typically)
  - Glass (Vis)
  - Fused silica (UV 350 nm)
- Detectors
  - Photodiodes
  - PMTs

Types of Instruments

- *Single beam*
  - Place cuvette with blank (i.e., solvent) in instrument and take a reading → 100% T
  - Replace cuvette with sample and take reading → % T for analyte (from which absorbance is calc'd)

- Double beam (most commercial instruments)
  - Light is split and directed towards both reference cell (blank) and sample cell
  - Two detectors; electronics measure ratio (i.e., measure/calculate absorbance)
  - Advantages:
    - Compensates for fluctuations in source and drift in detector
    - Better design for continuous recording of spectra



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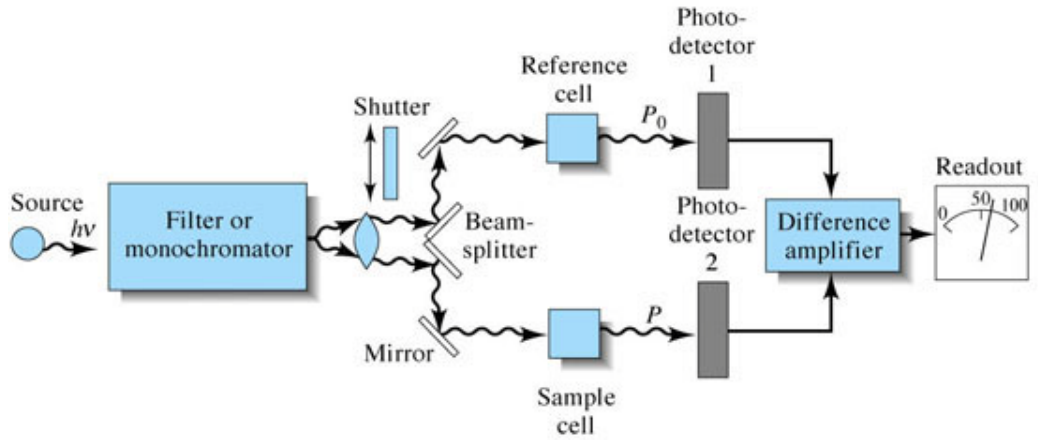

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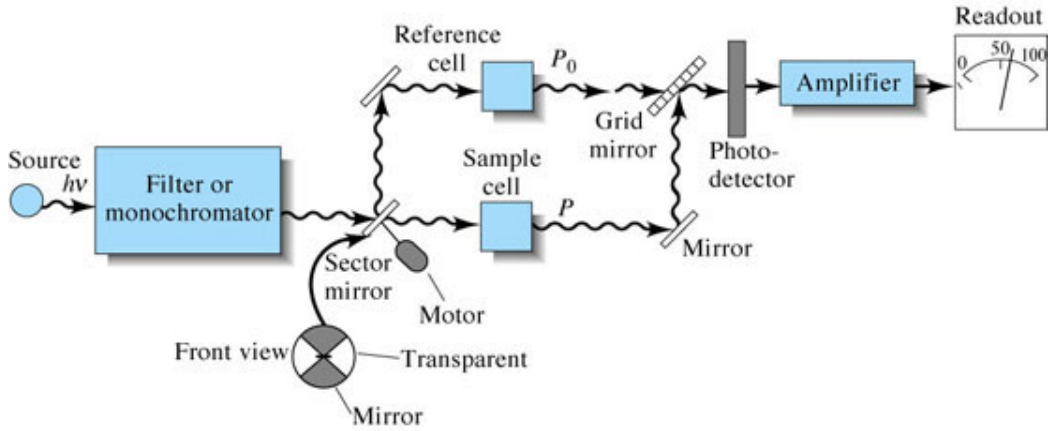


(c)

Single beam

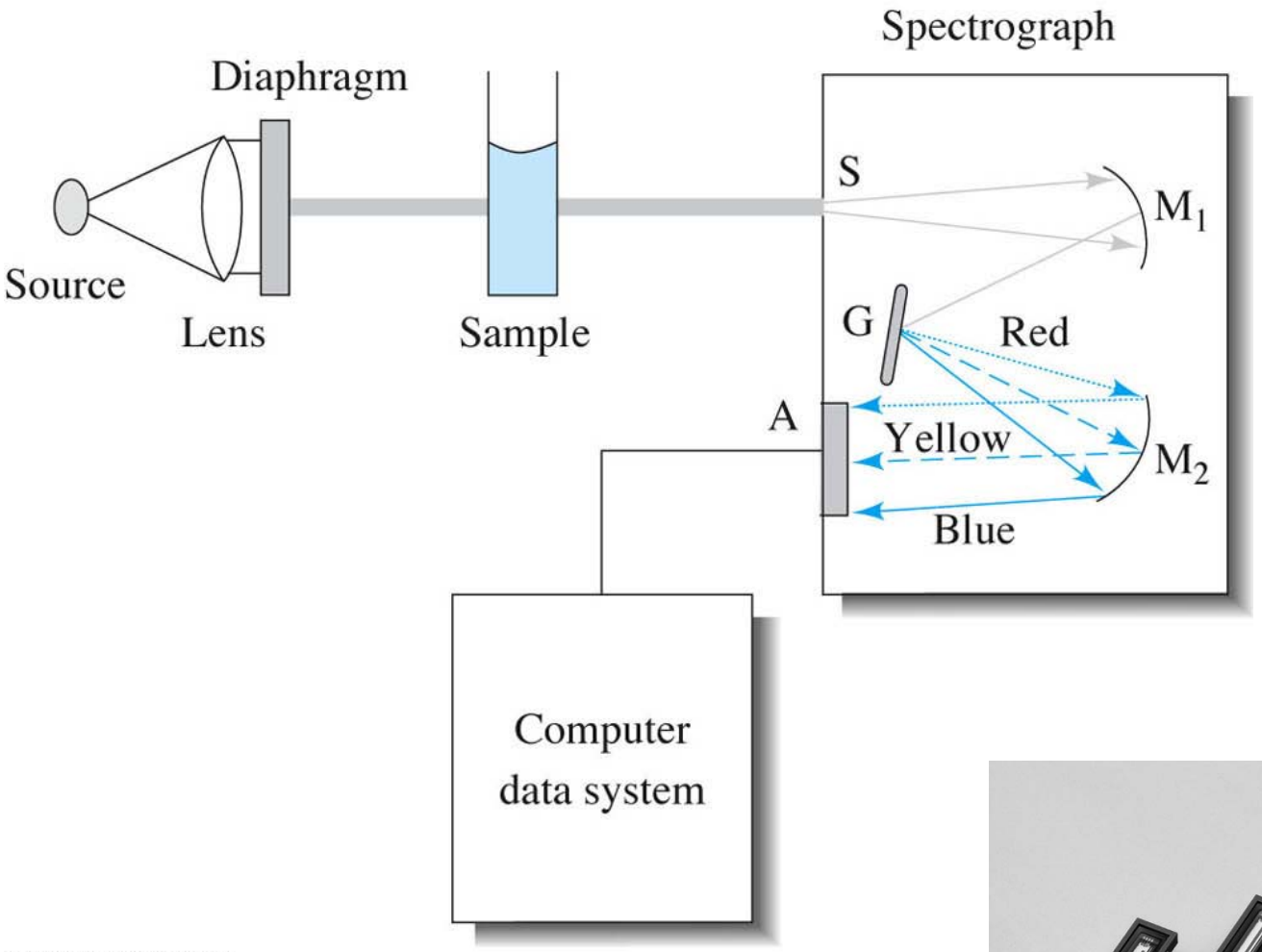
double beam in space

double beam in time

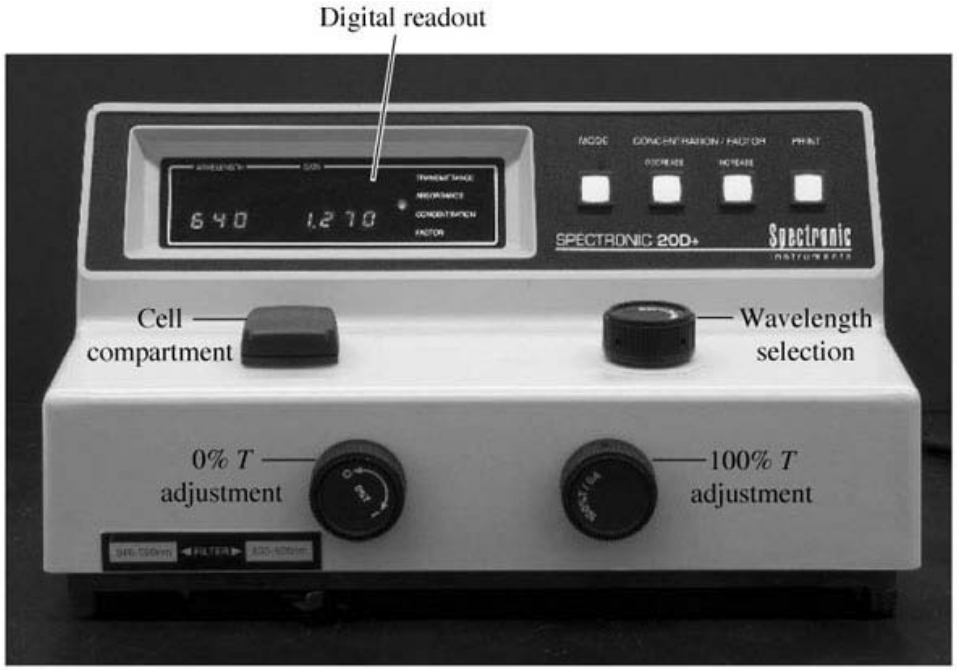
Multichannel Instruments

- Photodiode array detectors used (multichannel detector, can measure all wavelengths dispersed by grating simultaneously).
- Advantage: scan spectrum very quickly “snapshot” < 1 sec.
- Powerful tool for studies of transient intermediates in moderately fast reactions.
- Useful for kinetic studies.
- Useful for qualitative and quantitative determination of the components exiting from a liquid chromatographic column.

Multichannel Instruments



Instruments for the Visible Region.



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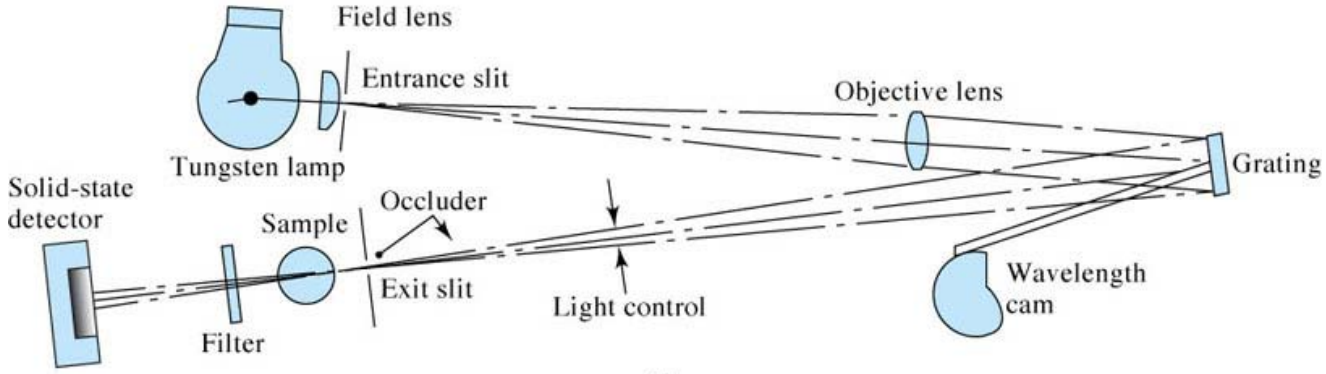

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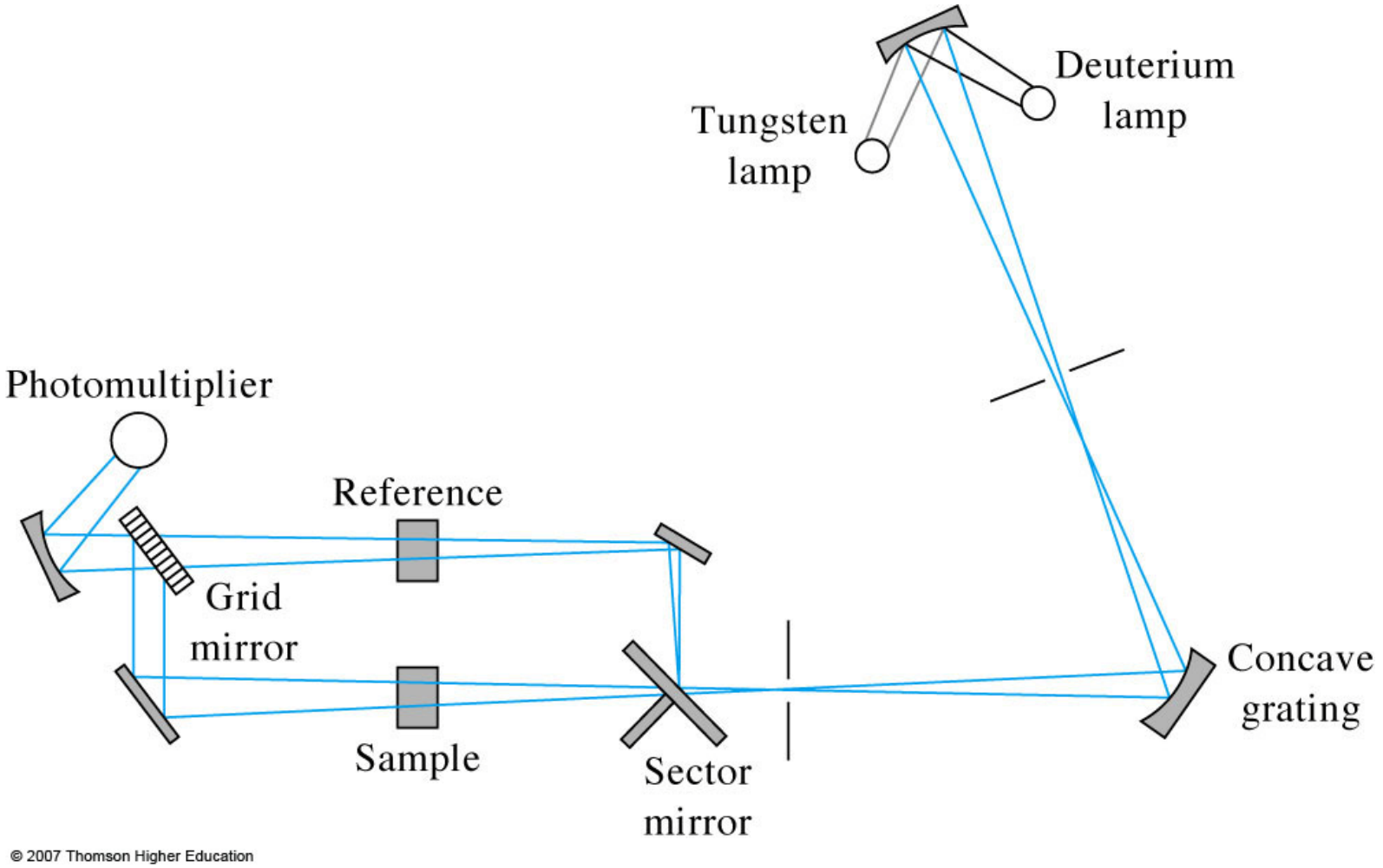


FIGURE 13·21 Schematic of a typical manual double-beam spectrophotometer for the UV-visible region.

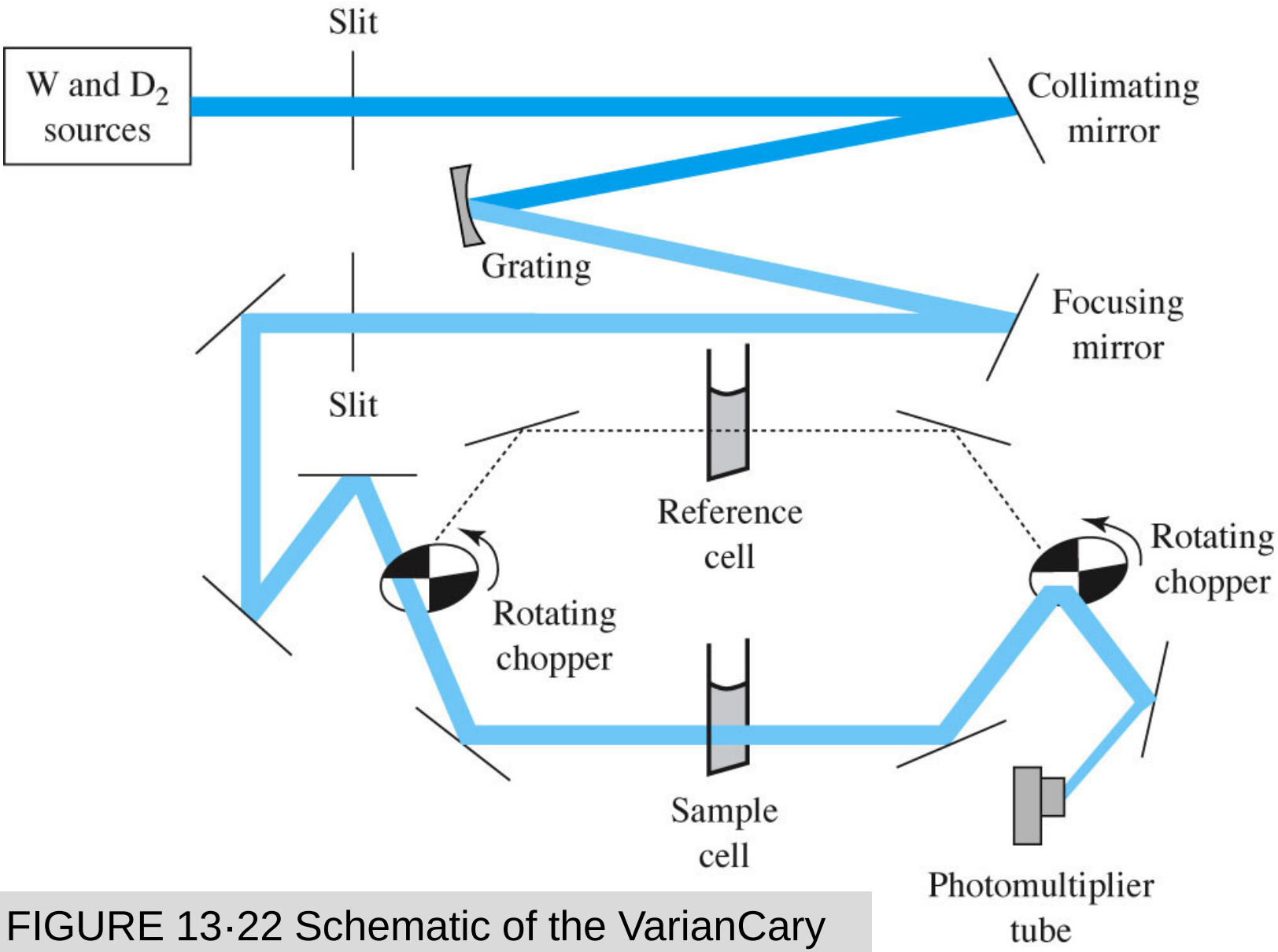


FIGURE 13·22 Schematic of the VarianCary 100 double-beam spectrophotometer for the UV-visible region

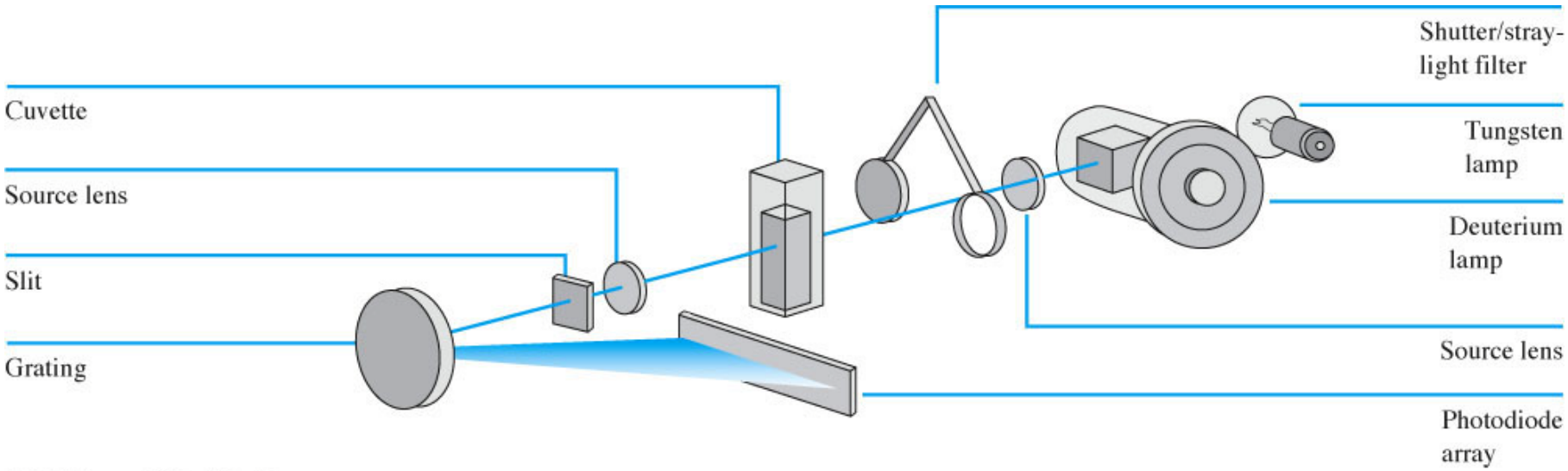


FIGURE 13-25 A multichannel diode-array spectrophotometer, the Agilent Technologies 8453.

Chapter 14: Applications of UV-Vis Molecular Absorption Spectrometry

Characteristics of UV/Vis Methods:

-Wide applicability to organic and inorganic systems

-Sensitivities to 10^{-4} to 10^{-7} *M*

-Moderate to high selectivity

-Good accuracy, about 1-3% relative uncertainty

-Ease and convenient data acquisition

UV/Vis Absorbance:

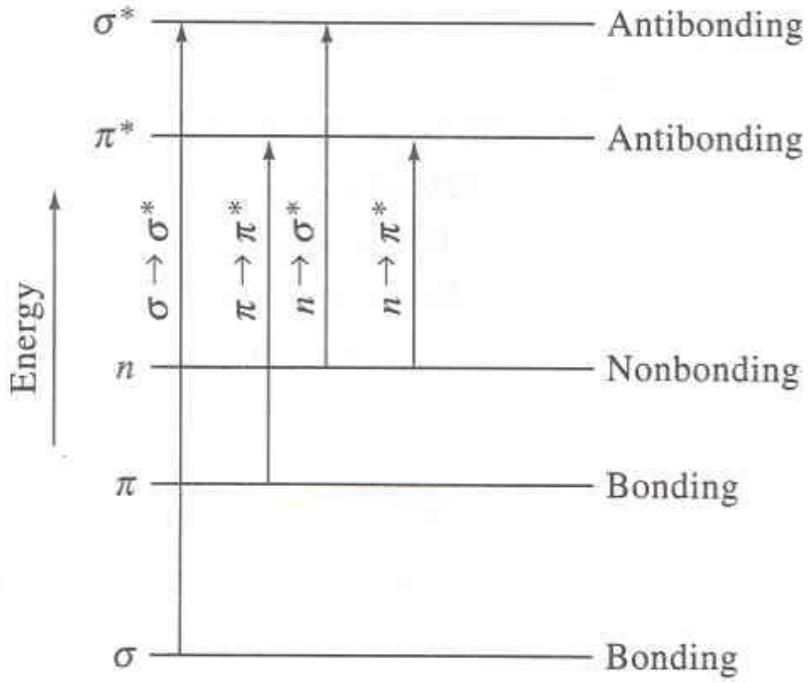
- Results from excitation of bonding electrons. So, can correlate wavelength of absorption peaks with types of bonds.
- Types of electronic transitions relative to UV/Vis absorbance:
  1) p, s, and *n* electrons
  2) *d* and *f* electrons
  3) Charge-transfer electrons

Absorbing Electrons:

Electrons that contribute to absorbance in organic molecules:

1) Those that directly participate in bond formation between atoms and are associated with more than one atom.

2) Nonbonding or unshared outer electrons largely localized about atoms like oxygen, sulfur, nitrogen, halogens.



- Most applications of absorption spectroscopy to organic molecules are based on *n* or p to p* transition which require unsaturated functional groups.

TABLE 14-2 Absorption Characteristics of Some Common Chromophores

Chromophore	Example	Solvent	λ_{max}(nm)	ϵ_{max}	Type of Transition
Alkene	$C_6H_{13}CH{=}CH_2$	*n*-Heptane	177	13,000	$\pi \rightarrow \pi^*$
Alkyne	$C_5H_{11}C{\equiv}C{-}CH_3$	*n*-Heptane	178	10,000	$\pi \rightarrow \pi^*$
			196	2,000	—
			225	160	—
Carbonyl	$CH_3\overset{\overset{O}{\|}}{C}CH_3$	*n*-Hexane	186	1,000	$n \rightarrow \sigma^*$
			280	16	$n \rightarrow \pi^*$
	$CH_3\overset{\overset{O}{\|}}{C}H$	*n*-Hexane	180	large	$n \rightarrow \sigma^*$
			293	12	$n \rightarrow \pi^*$
Carboxyl	$CH_3\overset{\overset{O}{\|}}{C}OH$	Ethanol	204	41	$n \rightarrow \pi^*$
Amido	$CH_3\overset{\overset{O}{\|}}{C}NH_2$	Water	214	60	$n \rightarrow \pi^*$
Azo	$CH_3N{=}NCH_3$	Ethanol	339	5	$n \rightarrow \pi^*$
Nitro	CH_3NO_2	Isooctane	280	22	$n \rightarrow \pi^*$
Nitroso	C_4H_9NO	Ethyl ether	300	100	—
			665	20	$n \rightarrow \pi^*$
Nitrate	$C_2H_5ONO_2$	Dioxane	270	12	$n \rightarrow \pi^*$

Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Effect of solvents in reducing fine structure in absorbance spectra

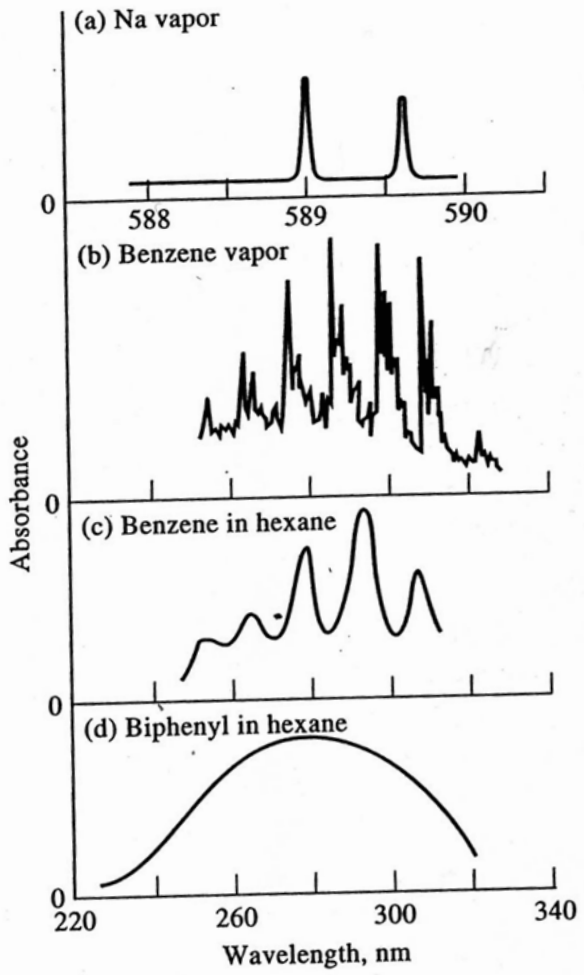
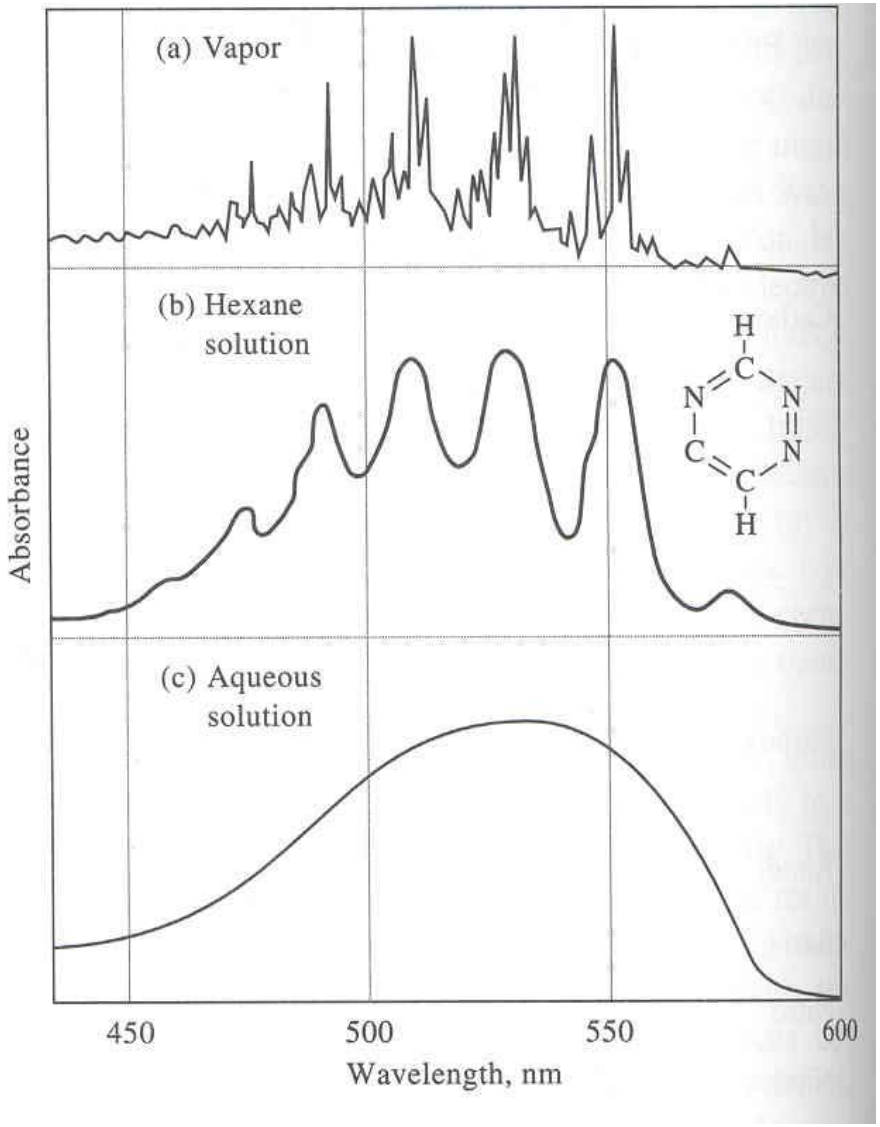


Figure 6-19 Some typical ultraviolet absorption spectra.



Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

- If chromophores are separated by more than one single bond, absorbance of multichromophores in a single organic molecule are approximately additive.

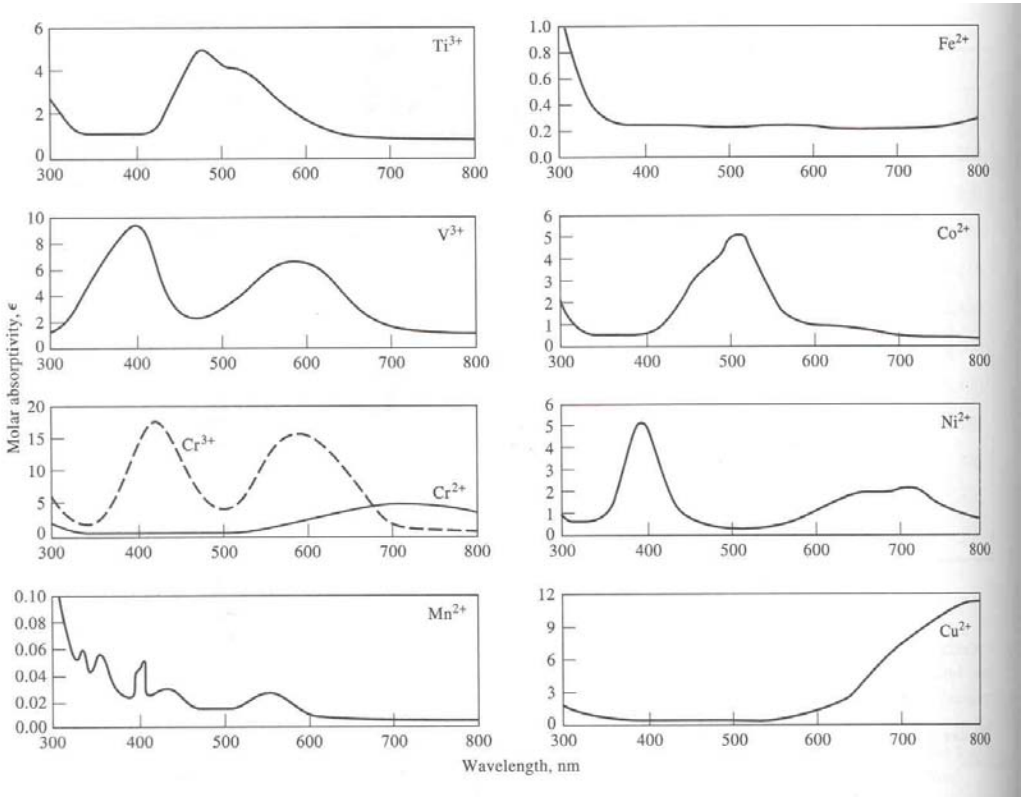
TABLE 14-3 Effect of Multichromophores on Absorption

Compound	Type	λ_{max}(nm)	ϵ_{max}
$CH_3CH_2CH_2CH{=}CH_2$	Olefin	184	~10,000
$CH_2{=}CHCH_2CH_2CH{=}CH_2$	Diolefin (unconjugated)	185	~20,000
$H_2C{=}CHCH{=}CH_2$	Diolefin (conjugated)	217	21,000
$H_2C{=}CHCH{=}CHCH{=}CH_2$	Triolefin (conjugated)	250	—
$CH_3CH_2CH_2CH_2\overset{\overset{O}{\|}}{C}CH_3$	Ketone	282	27
$CH_2{=}CHCH_2CH_2\overset{\overset{O}{\|}}{C}CH_3$	Unsaturated ketone (unconjugated)	278	30
$CH_2{=}CH\overset{\overset{O}{\|}}{C}CH_3$	α,β-Unsaturated ketone (conjugated)	324	24
		219	3,600

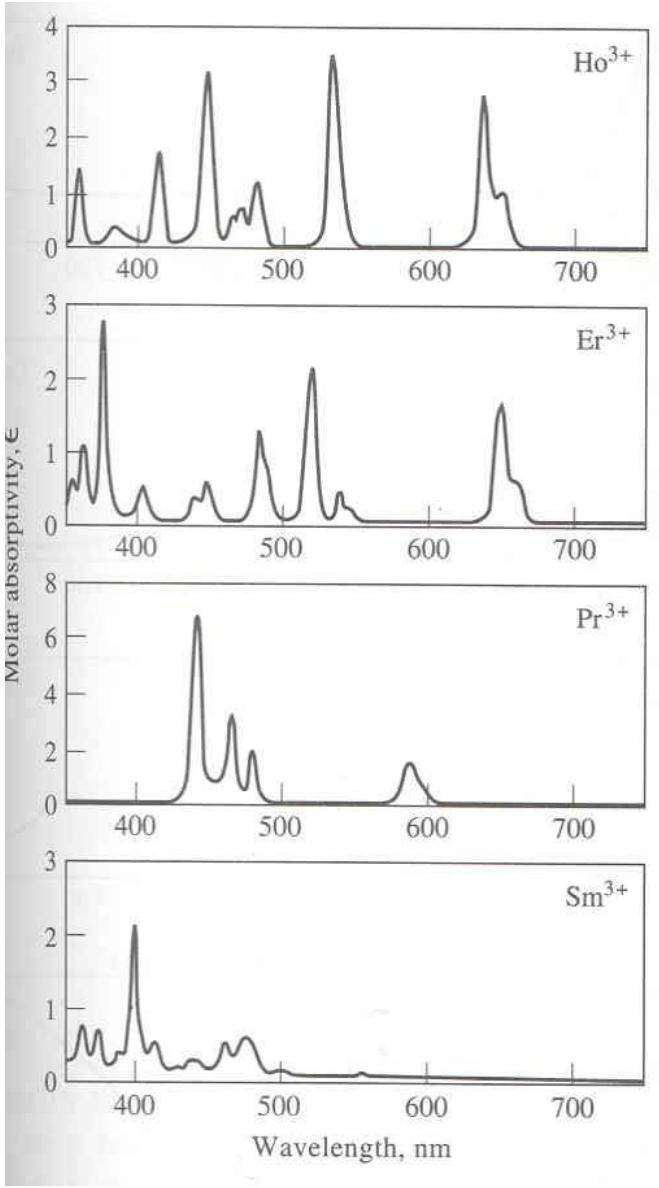
Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Absorption of d and f electrons:

-Most transition-metal ions absorb in UV/Vis.
-Lanthanides and actinides have narrow, well-defined, characteristic absorption peaks.
- Ions or complexes from first and second transition series tend to absorb in all oxidation states, tend to be broad bands that are dependent on chemical environment.



Sample spectra from transition-metal ions

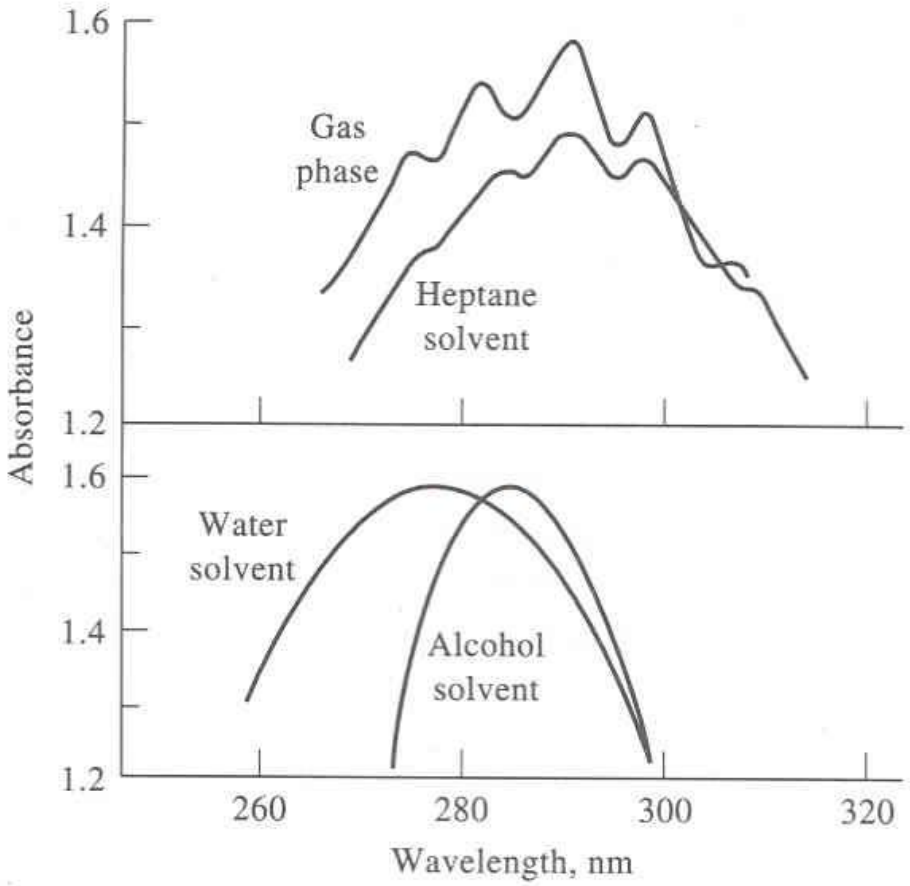


Sample spectra from lanthanide ions

Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Effect of Solvent on Absorption Spectra

- Polar solvents eliminate fine detail. Nonpolar solvent keep spectral details more similar to gas phase measurements.

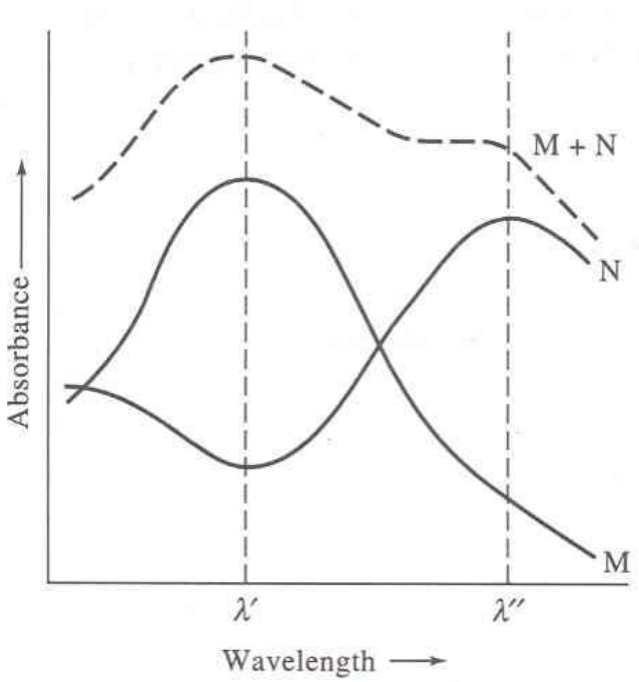


Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Absorbance Spectra of Mixtures:

- The absorbance of a solution at a given wavelength is the sum of the absorbances of the individual components.

$$A_1 = \varepsilon_{M,1} b c_M + \varepsilon_{N,1} b c_N$$
$$A_2 = \varepsilon_{M,2} b c_M + \varepsilon_{N,2} b c_N$$



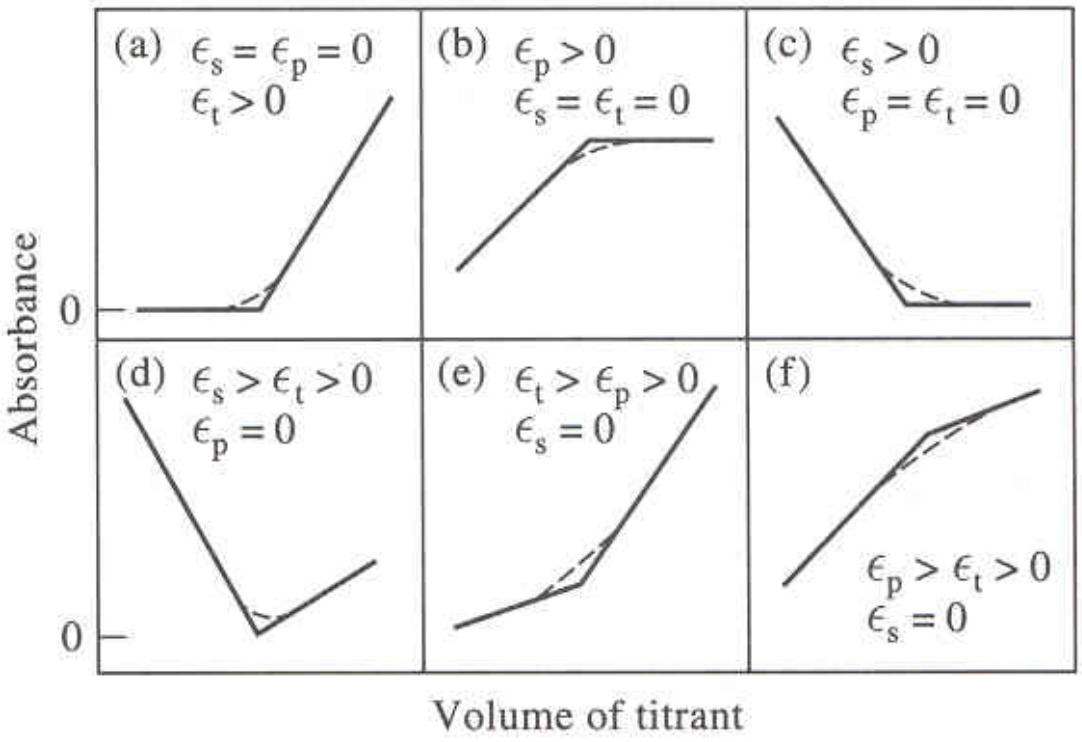
- To analyze the mixture, molar absorptivities for M and N are first determined at wavelengths λ1, and λ2 with sufficient concentrations of the two standard solutions to be sure that Beer's law is obeyed over an absorbance range that encompasses the absorbance of the sample.
- Note that the wavelengths selected are ones at which the molar absorptivities of the two components differ significantly. Thus, at λ1 the molar absorptivity of component M is much larger than that for component N.
- The greatest accuracy is obtained by choosing wavelengths at which the differences in molar absorptivities are large.

Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Photometric Titration:

-Plot absorbance (corrected for change in volume) as function of volume of titrant. Analyte, titrant, product, or indicator must absorb. High accuracy since multiple measurements are pooled.

- Photometric titrations are ordinarily performed with a spectrophotometer or a photometer that has been modified so that the titration vessel is held stationary in the light path



Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

Chapter 15
Molecular Luminescence Spectrometry

- Two types of Luminescence methods are:

  1**) Photoluminescence,** Light is directed onto a sample, where it is absorbed and imparts excess energy into the material in a process called "photo-excitation." One way this excess energy can be dissipated by the sample is through the emission of light, or luminescence.

  (i) fluorescence

  (ii) phosphorescence

  2) **Chemiluminescence,** based on an excited species formed by a chemical reaction. - no excitation source –

- In each, molecules of the analyte are excited to give a species whose emission spectrum provides information for qualitative or quantitative analysis.
- Luminescence methods are used as detectors for HPLC & CE.

Theory Of Fluorescence And Phosphorescence

Types of Fluorescence:

- Resonance Fluorescence
  - (emitted λ = excitation λ; e.g., AF)
- Stokes shift
  - (emitted λ > excitation λ; e.g., molecular fluorescence)

Electron spin and excited states:

Excited, paired = excited singlet state → fluorescence

Excited, unpaired = excited triplet state → phosphorescence

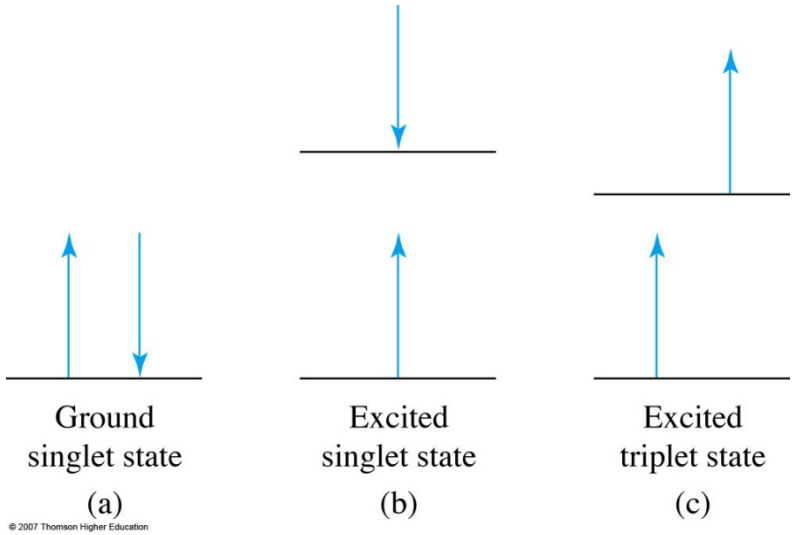


FIGURE 15-1 Electronic spin states of molecules. In (a) the ground electronic state is shown. In the lowest energy or ground state, the spins are always paired, and the state is said to be a singlet state.
In (b) and (c), excited electronic states are shown. If the spins remain paired in the excited state, the molecule is in an excited singlet state (b).
If the spins become unpaired, the molecule is in an excited triplet state (c).

Term Symbols

Example: Na ground state $1s^2\ 2s^2\ 2p^6\ 3s^1$

s=1/2, 2S+1=2, ground state doublet s electron written $3(^2S)$

Two spin states of equal energy (up/down)

→ Na 1st excited state $1s^2\ 2s^2\ 2p^6\ 3p^1$
Doublet written $3(^2P)$

BUT two spins states?

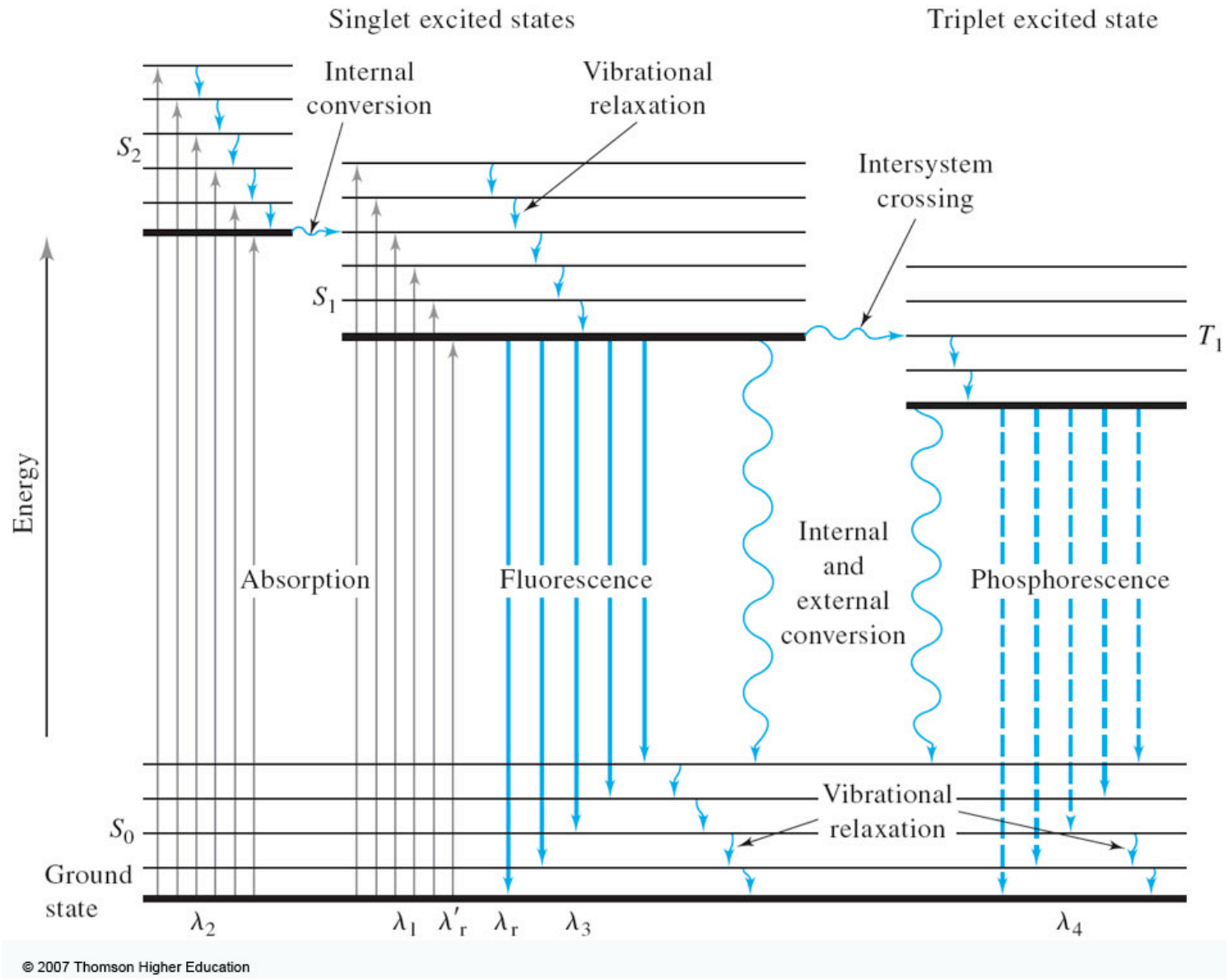
J (total ang. mom)=L+S or L-S

now $1s^2\ 2s^2\ 2p^6\ 3s^1 = 3(^2P_{1/2})$ and $3(^2P_{3/2})$

Term Symbol $^{2S+1}L_J$

Na 3p→3s fluorescence two lines : at 589.6 nm ($^2P_{3/2}$) and
at 589.0 nm ($^2P_{1/2}$)

Energy-level diagram for typical photoluminescent system



Deactivation Processes:

- **Fluorescence:** absorption of photon, short-lived excited state (singlet), emission of photon.
- **Phosphorescence:** absorption of photon, long-lived excited state (triplet), emission of photon.
- **Vibration Relaxation**
- **Internal Conversion**
- **External Conversion**
- **Intersystem Crossing**

Deactivation process by which an excited molecule returns to the ground state by minimizing lifetime of electronic state is preferred (i.e., the deactivation process with the faster rate constant will predominate)

Radiationless Deactivation

Without emission of a photon (i.e., without radiation)

Terms From Energy-level Diagram

Term: *Absorption* **Effect:** Excitation

Process: Analyte molecule absorbs photon (very fast ~ 10^{-14} – 10^{-15} s); electron is promoted to higher energy state. Slightly different wavelength → excitation into different vibrational energy levels.

Term: *Vibrational Relaxation* **Effect:** Radiationless Deactivation

Process: Collisions of excited state analyte molecules with other molecules → loss of excess vibrational energy and relaxation to lower vibrational levels (within the excited electronic state)

Term: *Internal conversion* **Effect:**RadiationlessDeactivation

Process: Molecule passes to a lower energy state – vibrational energy levels of the two electronic states overlap (see diagram) and molecules passes from one electronic state to the other.

Term: *Intersystem Crossing* **Effect:** Radiationless Deactivation

Process: Spin of electron is reversed leading to change from singlet to triplet state. Occurs more readily if vibrational levels of the two states overlap. Common in molecules with heavy atoms (e.g., I or Br)

Term: *External Conversion* **Effect:** Radiationless Deactivation

Process: Collisions of excited state analyte molecules with other molecules → molecule relaxes to the ground state without emission of a photon.

Term: *Fluorescence* **Effect:** Raditive Deactivation

Process: Emission of a photon via a singlet to singlet transition

(short – lived excited state ~10^{-5} – 10^{-10} s).

Term: *Phosphorescence* **Effect:** Radiative Deactivation

Process: Emission of a photon via a triplet to single transition

(long–lived excited state ~ 10^{-4} – 10^{1}s)

Quantum Efficiency or Quantum Yield:

- The quantum yield or quantum efficiency for fluorescence or phosphorescence is the ratio of the number of molecules that luminesce to the total number of excited molecule.
- It gives a measure of how efficient a fluorophore (i.e., fluorescing molecule) is.
- A quantum yield = 1 means that every excited molecules deactivates by emitting a photon – such a molecule is considered a very good fluorophore.
- We can express quantum yield as a function of rate constants

$$\text{Quantum Yield, } \phi = \frac{\text{total \# luminescing molecules}}{\text{total \# of excited molecules}}$$

$$\phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d} \quad [\text{k = rate constant}]$$

What Factors Affect fluorescence ?

1) Excitation wavelength

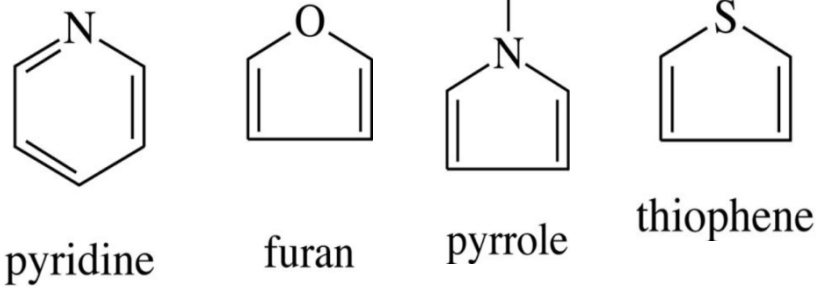
- Short λs break bonds, increase $k_{pre\text{-}dis}$ and k_{dis}
  - σ → σ* photochemical decomposition (seldom observed)
- Transitions mostly occur from
  - n → π* or Low–energy π → π* (aromatic, most intense fluorescence)

2) Molecular structure

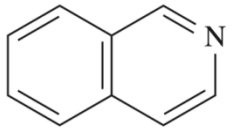
➢ Conjugated double bond structures exhibit fluorescence.

➢ Most unsubstituted aromatic hydrocarbons fluoresce in solution, the quantum efficiency usually increasing with the number of rings and their degree of condensation.

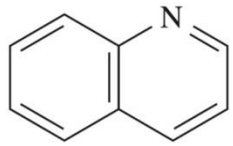
➢ The simple heterocyclics *such as pyridine, furan, thiophene, and pyrrole* do not fluoresce; heterocyclics fused to other rings fluoresce. Heteroatom increases ISC then ϕ_f decreases. (pyridine-quinoline)



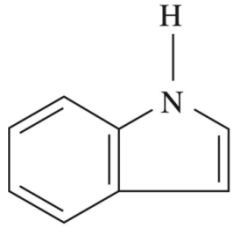
pyridine furan pyrrole thiophene



isoquinoline



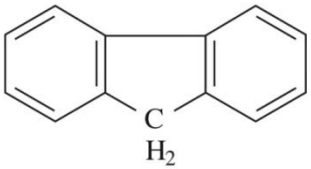
quinoline



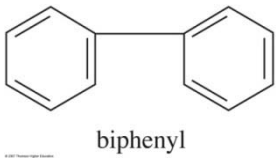
indole

3) Structural rigidity

- fluorescence is particularly favored in molecules with rigid structures.(e.g., fluorene vs biphenyl). If flexibility increases, ϕ_f decreases.



fluorene


biphenyl

- Lack of rigidity in a molecule probably causes an enhanced internal conversion rate and a consequent increase in the likelihood for radiationless deactivation.

4) Temperature and Solvent effect

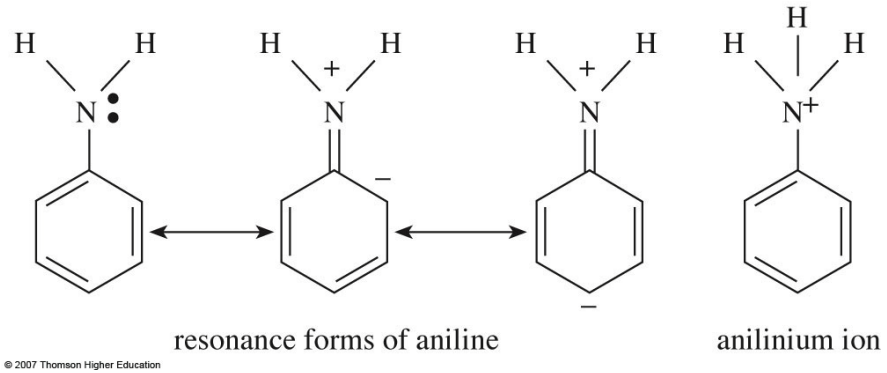
• The quantum efficiency *of fluorescence* in most molecules *decreases* with *increasing temperature* because the increased frequency of *collisions* at elevated temperatures improves the probability for deactivation by external conversion

• *increased fluorescence* with *increased viscosity* (decreased likelihood of external conversion – radiationless deactivation)

• The *fluorescence of a molecule is decreased* by solvents containing *heavy atoms* or other solutes with such atoms in their structure: Heavy atoms such as I, Br, Th increases ISC, as a consequence ϕ_f decreases. Compounds containing heavy atoms are frequently incorporated into solvents when enhanced phosphorescence is desired.

5) Effect of pH on Fluorescence

- The fluorescence of an aromatic compound with acidic or basic ring substituents is usually pH dependent.
- Both the wavelength and the emission intensity are likely to be different for the protonated and unprotonated forms of the compound.



Increased resonance structures (protonation or deprotonation) → stable excited state and greater quantum yield

• analytical procedures based on fluorescence frequently require close control of pH.

6) Dissolved oxygen;

• Presence of dissolved oxygen reduces fluorescence yield due to oxidation of the fluorescent specie. Also, paramagnetic properties of the oxygen promotes ISC and transition to triplet state.

Fluorescence Intensity And Concentration Of Analyte

- The power of fluorescence emission *F is proportional* to the radiant power of the excitation beam that is absorbed by the system.
- fluorescence intensity depends linearly on concentration. F = Kc
- Deviations occur at high concentrations
  - Self absorption: neighboring molecule absorbs emitted photon from other molecule – happens if there is overlap between the excitation and emission spectra
  - Quenching: collisions of excited state molecule with other excited state molecules → radiationless deactivation

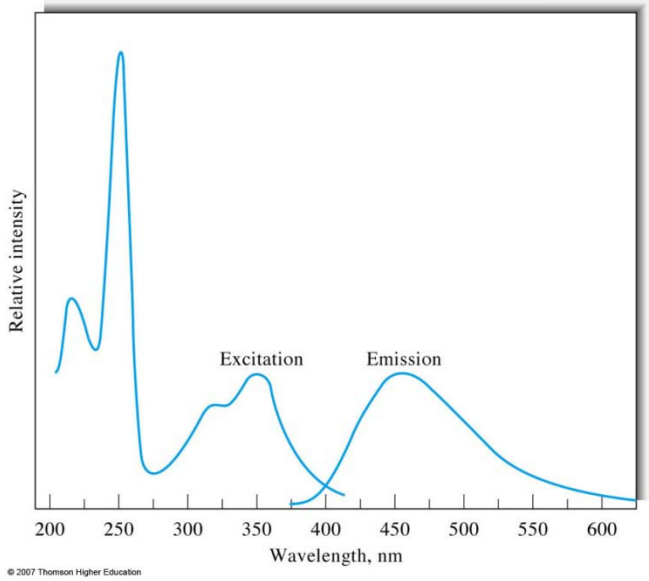
- Photobleaching, Photochemical Decomposition:

  Excited state molecule absorbs another photon and is destroyed → destroyed excited state molecule is not able to emit fluorescent photon

Excitation And Emission Spectra

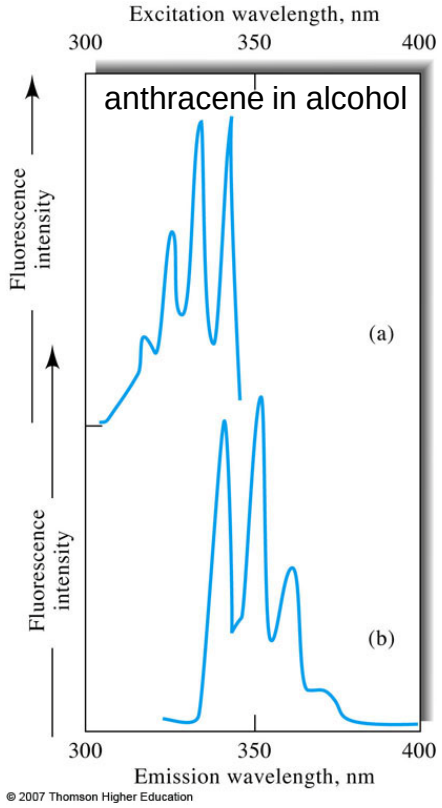
Excitation spectrum:

- Emission wavelength is fixed; excitation wavelength is scanned
- Monochromator or filters selected to allow only one λ of fluorescent light to pass through to the detector.
- Excitation wavelength is varied – at each excitation λ increment, fluorescent photons at the fixed emission λ are collected.
- The emission intensity (i.e., the number of fluorescent photons collected) at each λ increment varies as the excitation λ comes closer to or goes further from the λ of maximum absorption → this is why an excitation spectrum looks like an absorption spectrum.



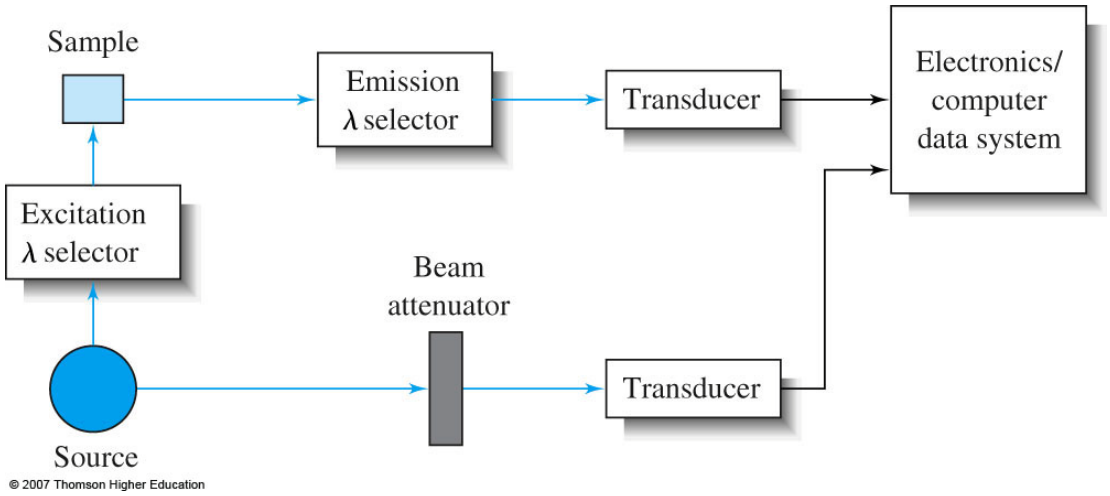
Emission spectrum:

- Excitation wavelength is fixed; emission wavelength is scanned
- Monochromator or filter is selected to allow only one λ of excitation light to pass onto the sample.
- Emission λ is varied → fluorescent photons are collected at each incremental emission λ.
- The emission intensity (i.e., the number of fluorescent photons collected) at each λ increment varies as the emission λ is changed.
- Spectrum shows at what λ the fluorescence intensity is a maximum for a given excitation λ.



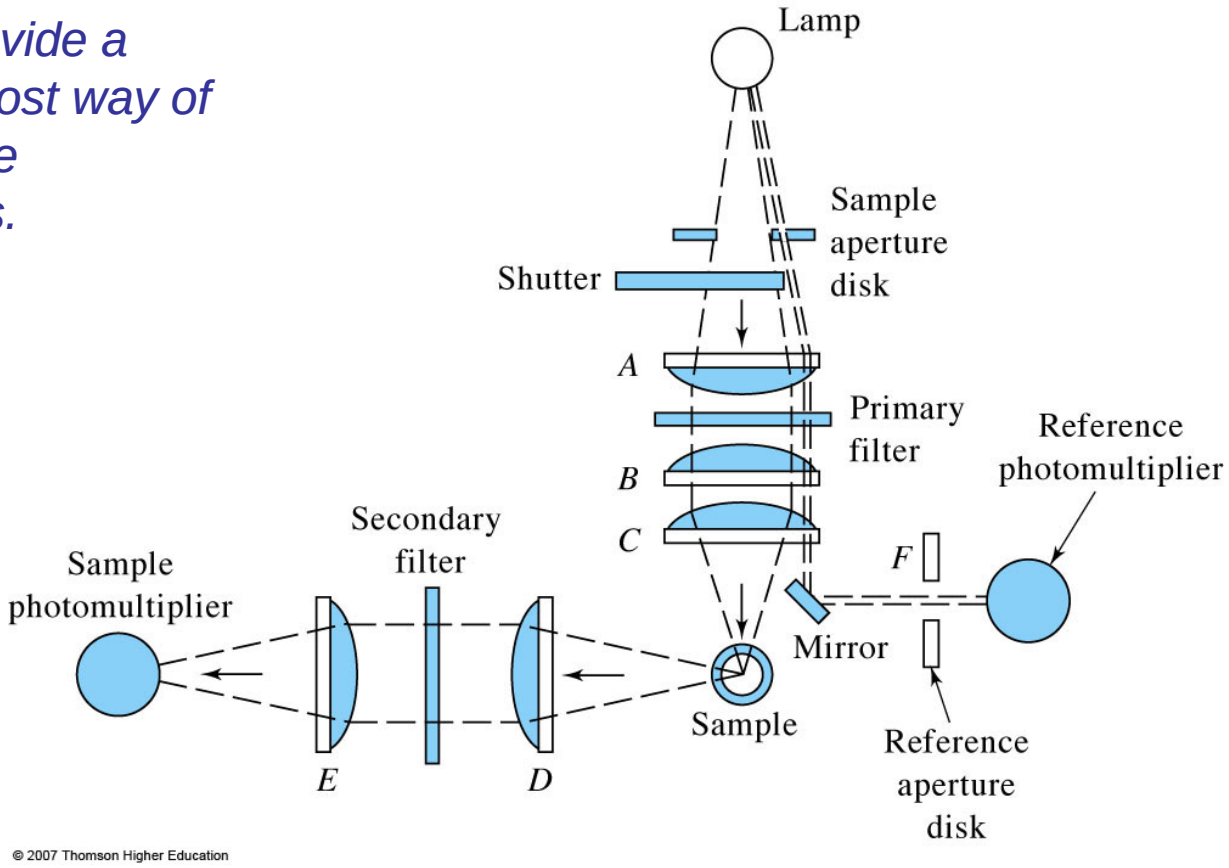
INSTRUMENTATION

- Sources
  - Low pressure Hg lamp (254, 302, 313, 546, 578 nm) line source
  - Xe lamp (300 – 1300 nm) continum source
  - Lasers (tunable dye lasers)
- Filter/monochromator
  - Isolate excitation λ
  - Scan excitation λ
  - Isolate emission λ from excitation λ
  - Scan emission λ
- Both cylindrical and rectangular cells fabricated of glass or silica are employed for fluorescence measurements. Care must be taken in the design of the cell compartment to reduce the amount of scattered radiation reaching the detector. Baffles are often introduced into the compartment for this purpose. Even more than in absorbance measurements, it is important to avoid fingerprints on cells because skin oils often fluoresce,
- Detector
  - Usually PMT: very low light levels are measured. Transducers are sometimes cooled to improve signal-to-noise ratios.
  - Charge-transfer devices such as charge-coupled devices (CCDs), are also used for spectrofluorometry. This type of transducer permits the rapid recording of both excitation and emission spectra and is particularly useful in chromatography and electrophoresis.



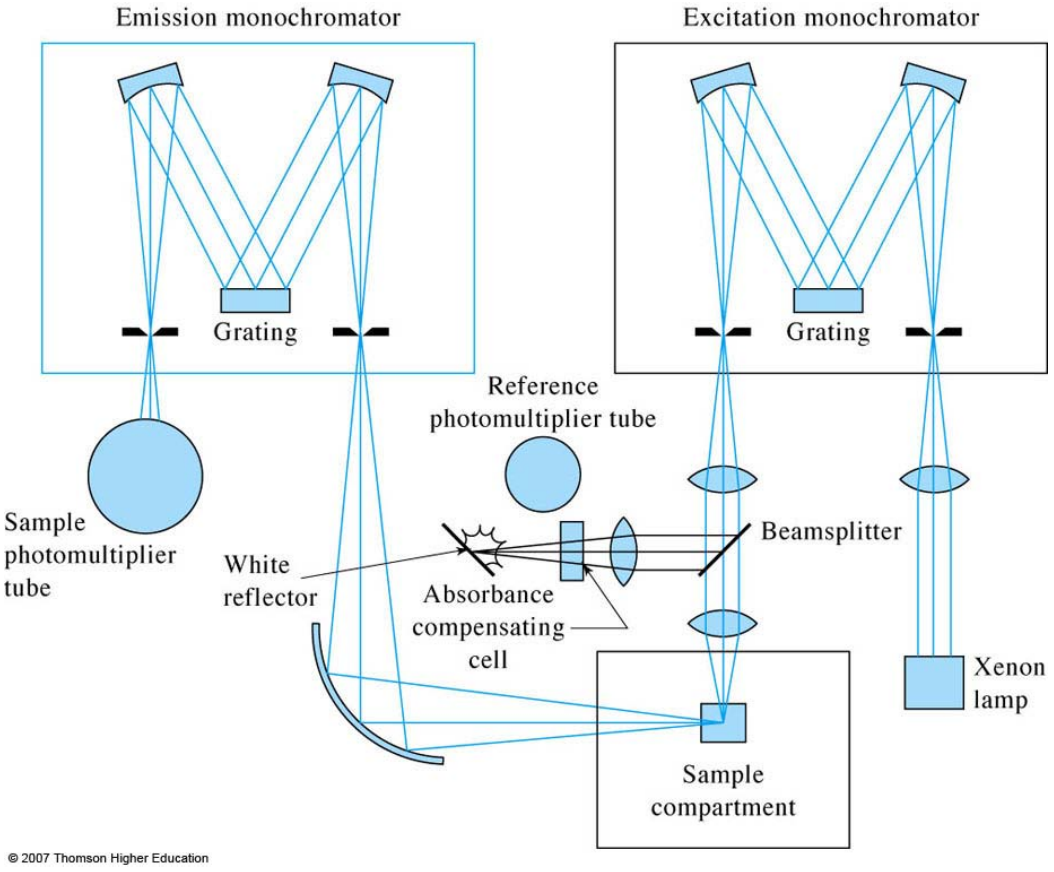
Fluorometer:

Filter fluorometers provide a relatively simple, lowcost way of performing quantitative fluorescence analyses.



Spectrofluorometer

- *Several instrument manufacturers offer spectrofluorometers capable of providing both excitation and emission spectra.*
- *The optical design of these instruments employs two grating monochromators.*
- *Radiation from the excitation monochromator is split, part passing to a reference photomultiplier and part to the sample.*
- *The resulting fluorescence radiation, after dispersion by the emission monochromator is detected by a second photomultiplier.*



Phosphorescence Instrumentation

• Similar in design to the fluorometers and spectrofluorometers except that two additional components are required.
• The first is a device that alternately irradiates the sample and, after a suitable time delay, measures the intensity of phosphorescence.
•The time delay is required to differentiate between long-lived phosphorescence emission and short-lived fluorescence emission, both of which would originate from the same sample.
• Both mechanical and electronic devices are used, and many commercial fluorescence instruments have accessories for phosphorescence measurements. Many of the current instruments use a gated scheme for the delay. A pulsed xenon arc lamp is often used to excite the sample. After a delay time, specified by the user, the data-acquisition system is activated to obtain the phosphorescence signal. Often, the signal is integrated during this period when the lamp is off and fluorescence has decayed to a very small value.
• The second new component is needed because phosphorescence measurements are usually performed at liquid nitrogen tcmperature in a rigid medium to minimize collisional deactivation of the long-lived triplet state.
• Usually, a Dewar flask with quartz windows, as shown in Figure 15-13, is a part of a phosphorimeter. At the temperature used, the analyte exists as a solute in a glass or solid solvent. A common solvent for this purpose is a mixture of diethylether, pentane, and ethanol.

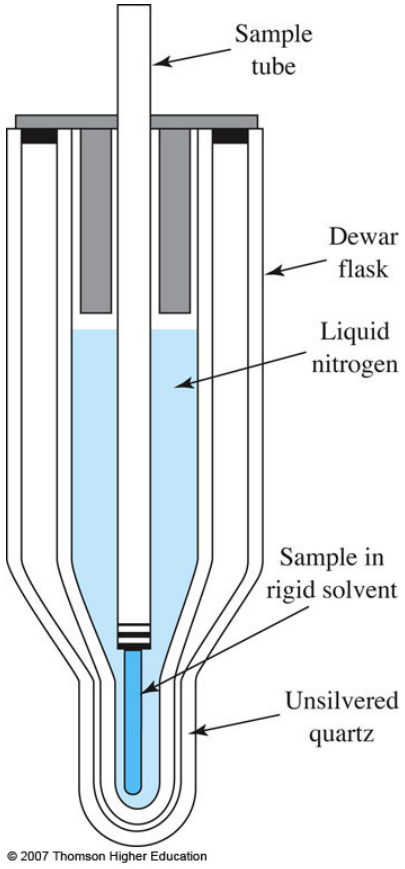
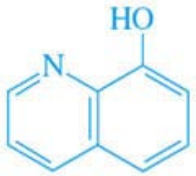
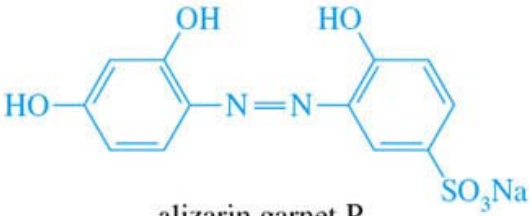


TABLE 15-2 Selected Fluorometric Methods for Inorganic Species

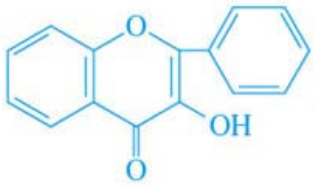
Ion	Reagent	Wavelength, nm		LOD, μg/mL	Interferences
		Absorption	Fluorescence		
Al^{3+}	Alizarin garnet R	470	500	0.007	Be, Co, Cr, Cu, F^-, NO_3^-, Ni, PO_4^{3-}, Th, Zr
F^-	Quenching of Al^{3+} complex of alizarin garnet R	470	500	0.001	Be, Co, Cr, Cu, Fe, Ni, PO_4^{3-}, Th, Zr
$B_4O_7^{2-}$	Benzoin	370	450	0.04	Be, Sb
Cd^{2+}	2-(*o*-Hydroxyphenyl)-benzoxazole	365	Blue	2	NH_3
Li^+	8-Hydroxyquinoline	370	580	0.2	Mg
Sn^{4+}	Flavanol	400	470	0.1	F^-, PO_4^{3-}, Zr
Zn^{2+}	Benzoin	—	Green	10	B, Be, Sb, colored ions



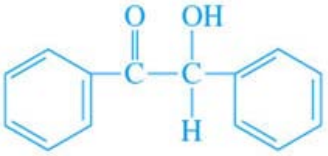
8-hydroxyquinoline
(reagent for Al, Be, and other metal ions)



alizarin garnet R
(reagent for Al, F^-)



flavanol
(reagent for Zr and Sn)



benzoin
(reagent for B, Zn, Ge, and Si)

Chapter 16-17
An Introduction and Application to Infrared Spectrometry

The infrared region of the spectrum encompasses radiation with wavenumbers ranging from about 12,800 to 10 cm^{-1} or wavelengths from 0.78 to 1000 μm. The infrared spectrum is divided into **near-, mid-,** and **far-** infrared radiation.

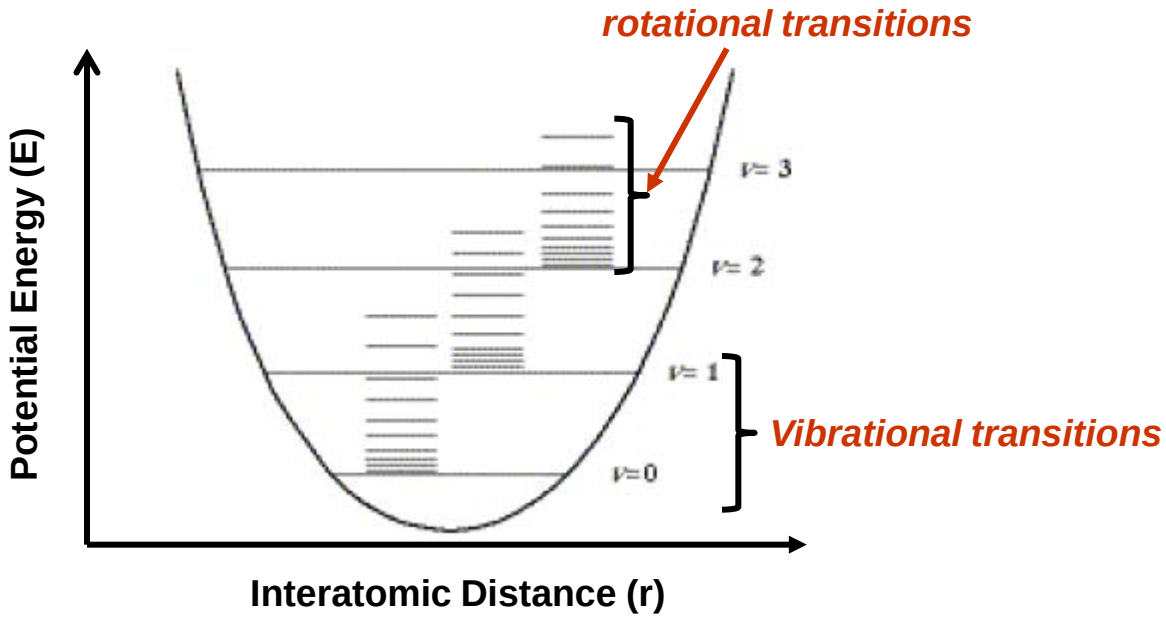
TABLE 16-1 IR Spectral Regions

Region	Wavelengths (λ), μm	Wavenumbers ($\bar{\nu}$), cm^{-1}	Frequencies (ν), Hz
Near	0.78 to 2.5	12800 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}

Infrared Spectroscopy

A) Introduction

A) 1.)Infrared (IR) spectroscopy: based on IR absorption by molecules as undergo vibrational and rotational transitions. Absorption of radiation in this region by a typical organic molecule results in the excitation of vibrational, rotational and bending modes, while the molecule itself remains in its electronic ground state.

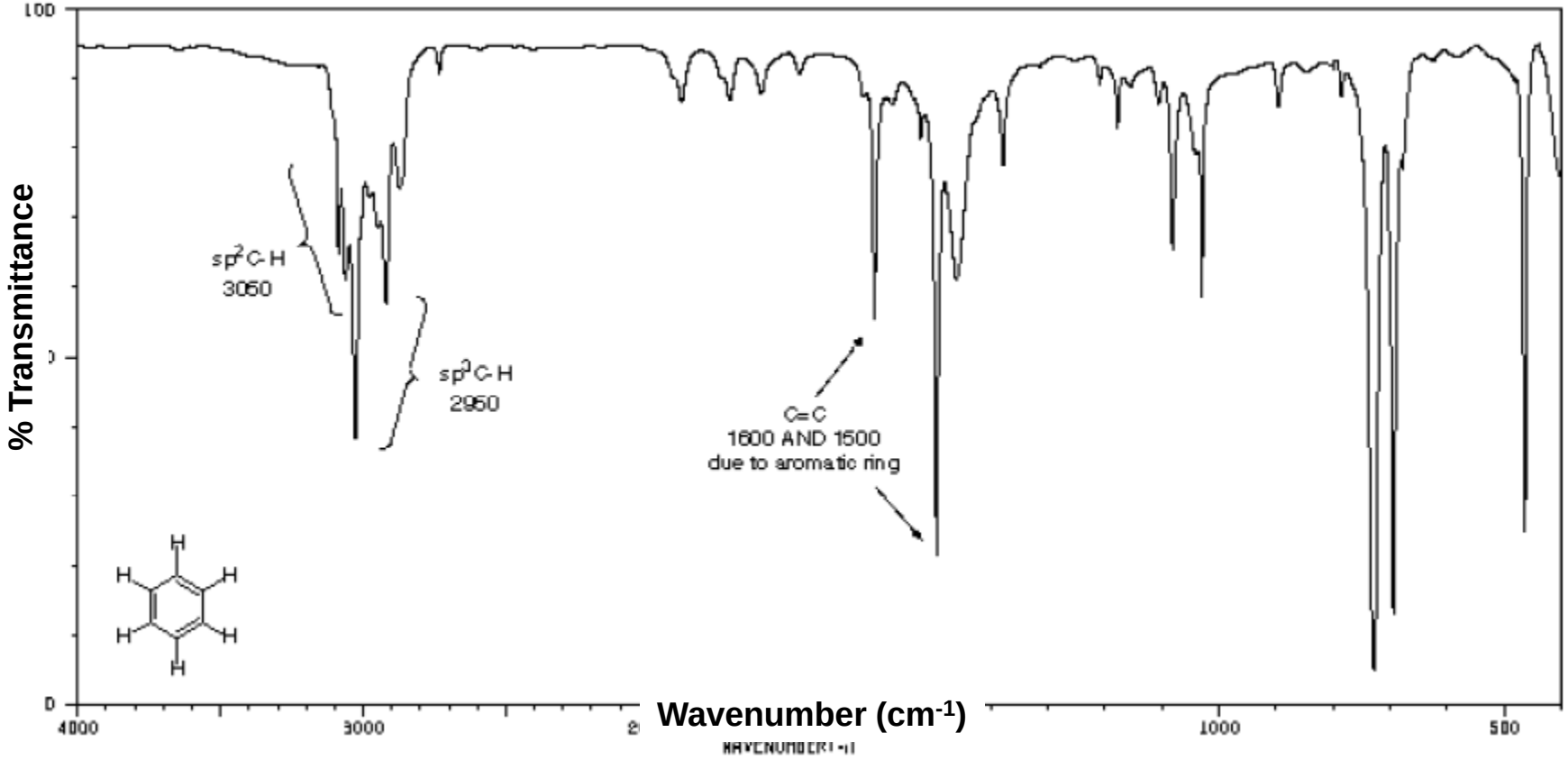


Potential energy resembles classic Harmonic Oscillator

2.) IR radiation is in the range of 12,800 – 10 cm^{-1} or λ = 0.78 – 1000 μm

- rotational transitions have small energy differences
  - ≤ 100 cm^{-1}, λ > 100 μm
- vibrational transitions occur at higher energies
- rotational and vibrational transitions often occur together

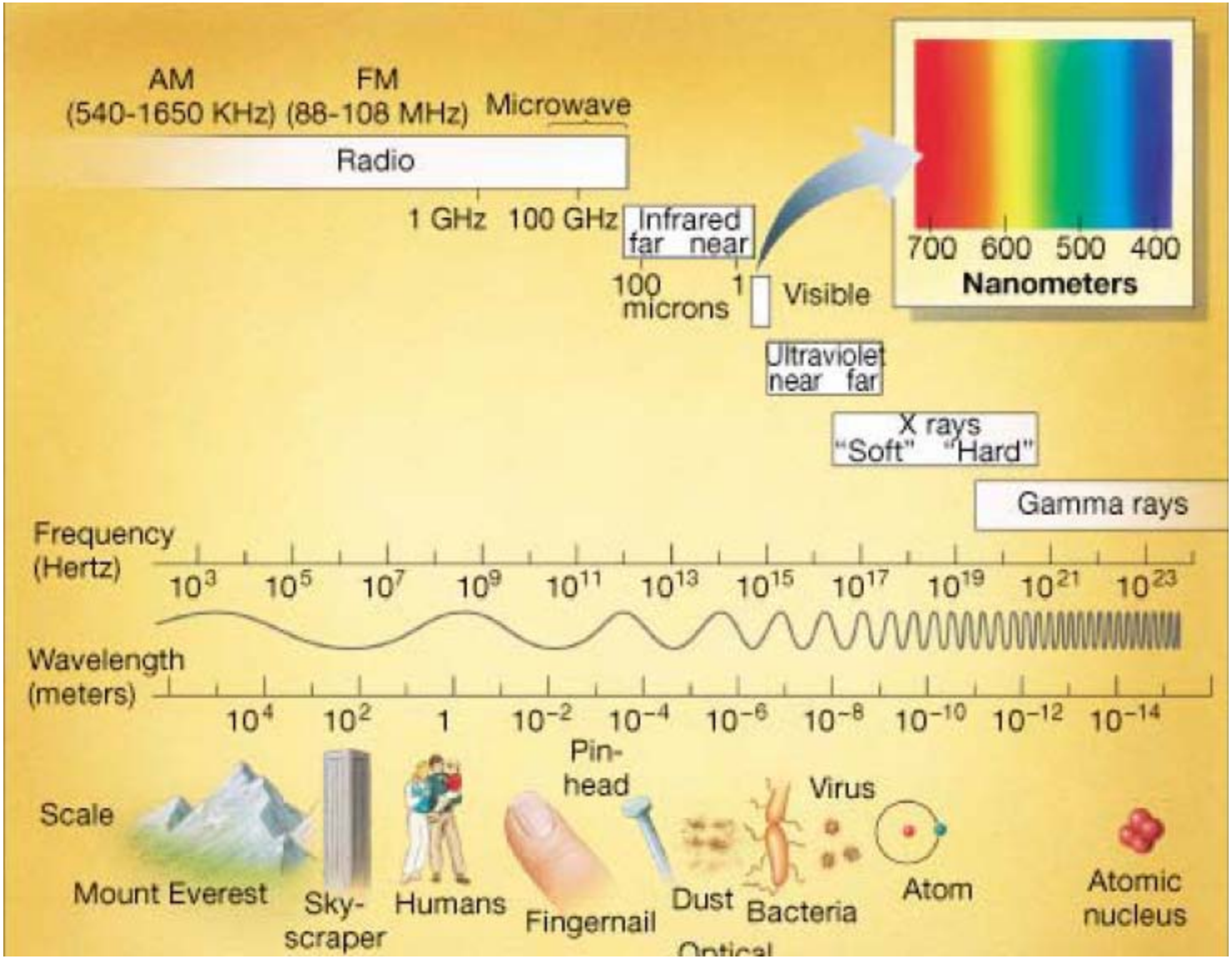
3.) Typical IR spectrum for Organic Molecule



Wide Range of Types of Electromagnetic Radiation in nature.

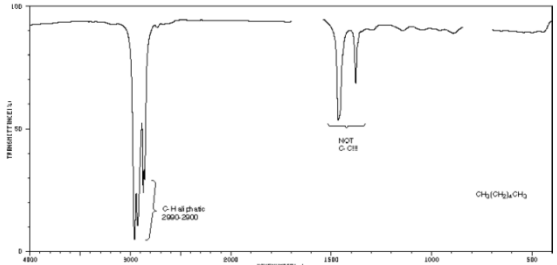
1. Only a small fraction (350-780 nM is visible light).
2. The complete variety of electromagnetic radiation is used throughout spectroscopy.
3. Different energies allow monitoring of different types of interactions with matter.

$$E=h\nu = hc/\lambda$$

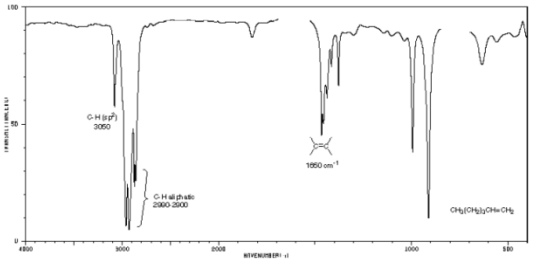


3.) Typical IR spectrum for Organic Molecule

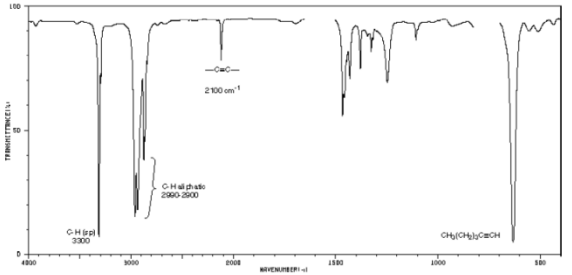
- many more bands then in UV-vis, fluorescence or phosphorescence
- bands are also much sharper
- pattern is distinct for given molecule
  - except for optical isomers
- good qualitative tool
  - can be used for compound identification
  - group analysis
- also quantitative tool
  - intensity of bands related to amount of compound present
- spectra usually shown as percent transmittance (instead of absorbance) vs. wavenumber (instead of λ) for convenience



Hexane



Hexene



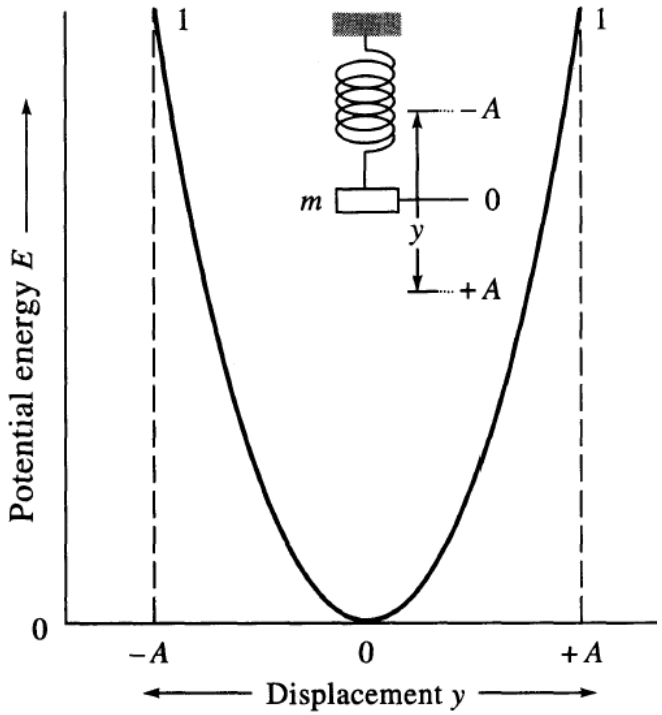
Hexyne

B) Theory of IR Absorption

1.) Molecular Vibrations

i.) Harmonic Oscillator Model:

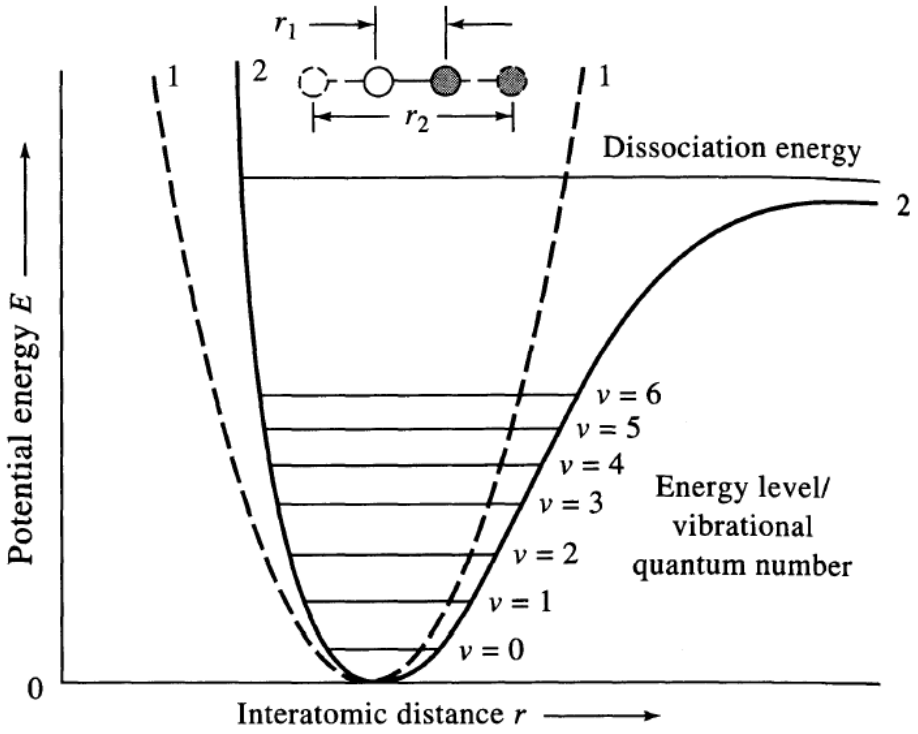
- approximate representation of atomic stretching
- two masses attached by a spring



$E = \frac{1}{2} ky^2$

where:

y is spring displacement
k is spring constant



Vibrational frequency given by:

$$\nu = 1/2\pi\sqrt{k/m}$$

where:
ν : frequency
k: force constant (measure of bond stiffness)
μ: reduced mass – m_1m_2/m_1+m_2

If know ν and atoms in bond, can get *k:*

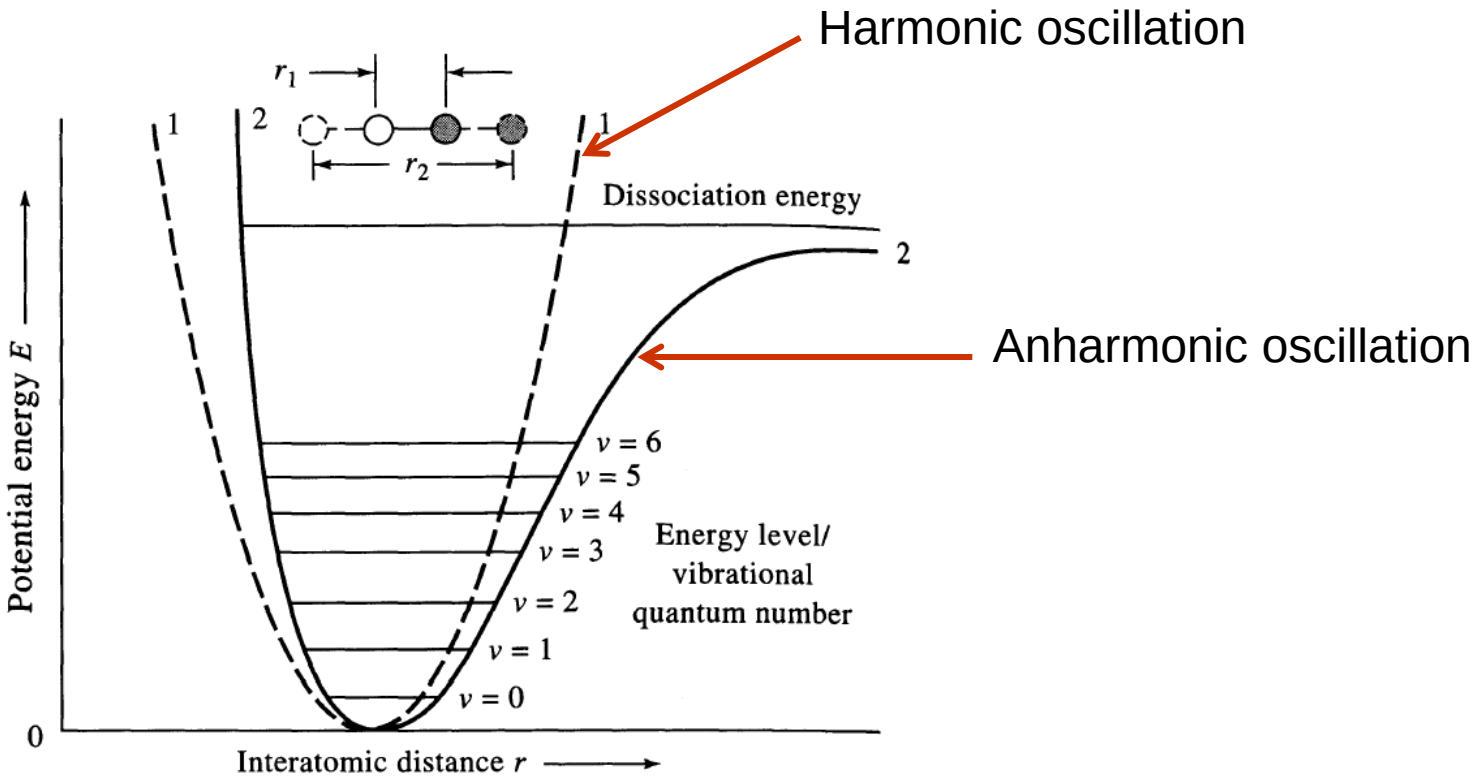
Single bonds:
k ~ 3x10^2 to 8 x10^2 N/m (Avg ~ 5x10^2)

double and triple bonds ~ 2x and 3x *k* for single bond.

$\nu \propto \sqrt{k}$ *So, vibration* ν *occur in order: single < double < triple*

ii.) Anharmonic oscillation:

- harmonic oscillation model good at low energy levels (ν_0, ν_1, ν_2, …)
- not good at high energy levels due to atomic repulsion & attraction
  - as atoms approach, coulombic repulsion force adds to the bond force making energy increase greater then harmonic
  - as atoms separate, approach dissociation energy and the harmonic function rises quicker

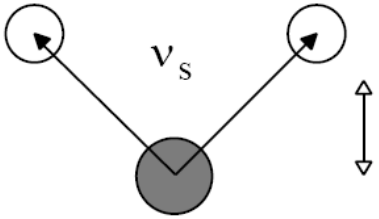


Because of anharmonics: at low ΔE, $\Delta\nu = \pm 2, \pm 3$ are observed which cause the appearance of overtone lines at frequencies at ~ 2-3 times the fundamental frequency. Normally $\Delta\nu = \pm 1$

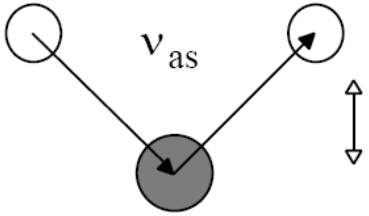
iii.) Types of Molecular Vibrations

Bond Stretching

symmetric

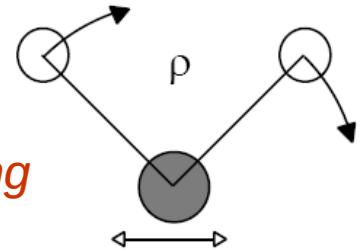


asymmetric

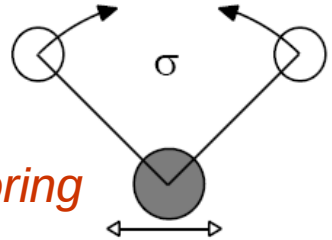


Bond Bending

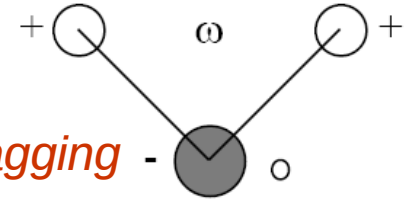
In-plane rocking



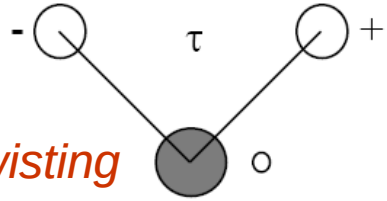
In-plane scissoring

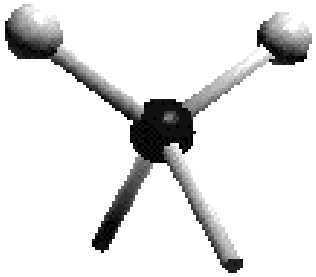


Out-of-plane wagging

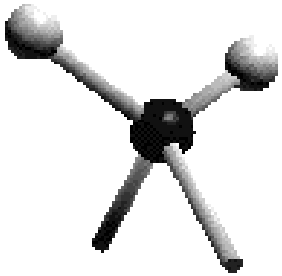


Out-of-plane twisting

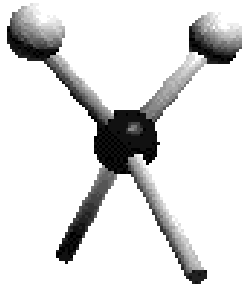




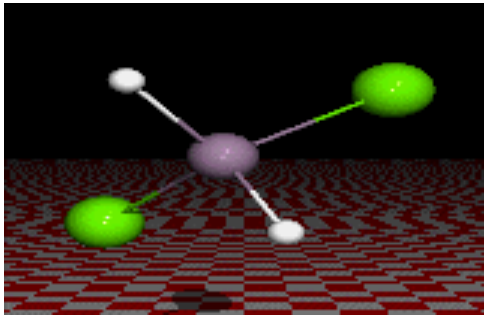
symmetric



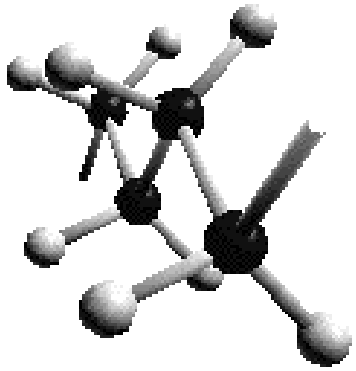
asymmetric



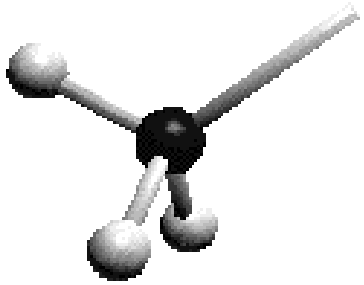
In-plane scissoring



Out-of-plane twisting



In-plane rocking



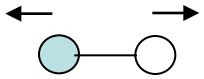
Out-of-plane wagging

iv.) Number of Vibrational Modes:

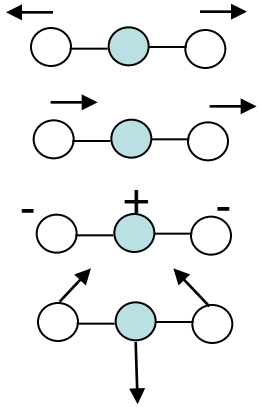
- for non-linear molecules, number of types of vibrations: 3N-6
- for linear molecules, number of types of vibrations: 3N-5
- why so many peaks in IR spectra
- observed vibration can be less then predicted because
  - symmetry (no change in dipole)
  - energies of vibration are identical
  - absorption intensity too low
  - frequency beyond range of instrument

Examples:

1) HCl: 3(2)-5 = 1 mode



2) CO_2: 3(3)-5 = 4 modes



moving in-out of plane

See web site for 3D animations of vibrational modes for a variety of molecules

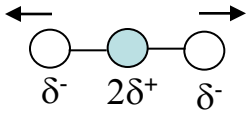
http://www.chem.purdue.edu/gchelp/vibs/co2.html

v.) IR Active Vibrations:

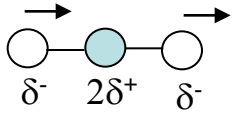
- In order for molecule to absorb IR radiation:
  - vibration at same frequency as in light
  - but also, must have a change in its *net dipole moment* as a result of the vibration

Examples:

1) CO_2: 3(3)-5 = 4 modes

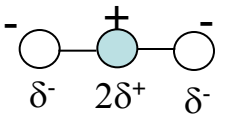


$\mu = 0$; IR inactive

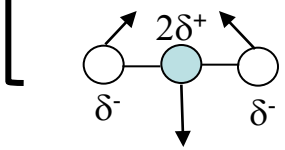


$\mu > 0$; IR active

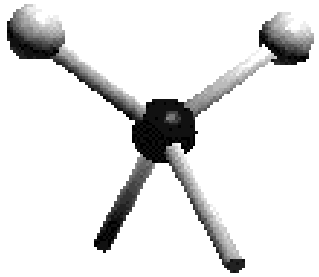
degenerate –identical energy single IR peak



$\mu > 0$; IR active



$\mu > 0$; IR active

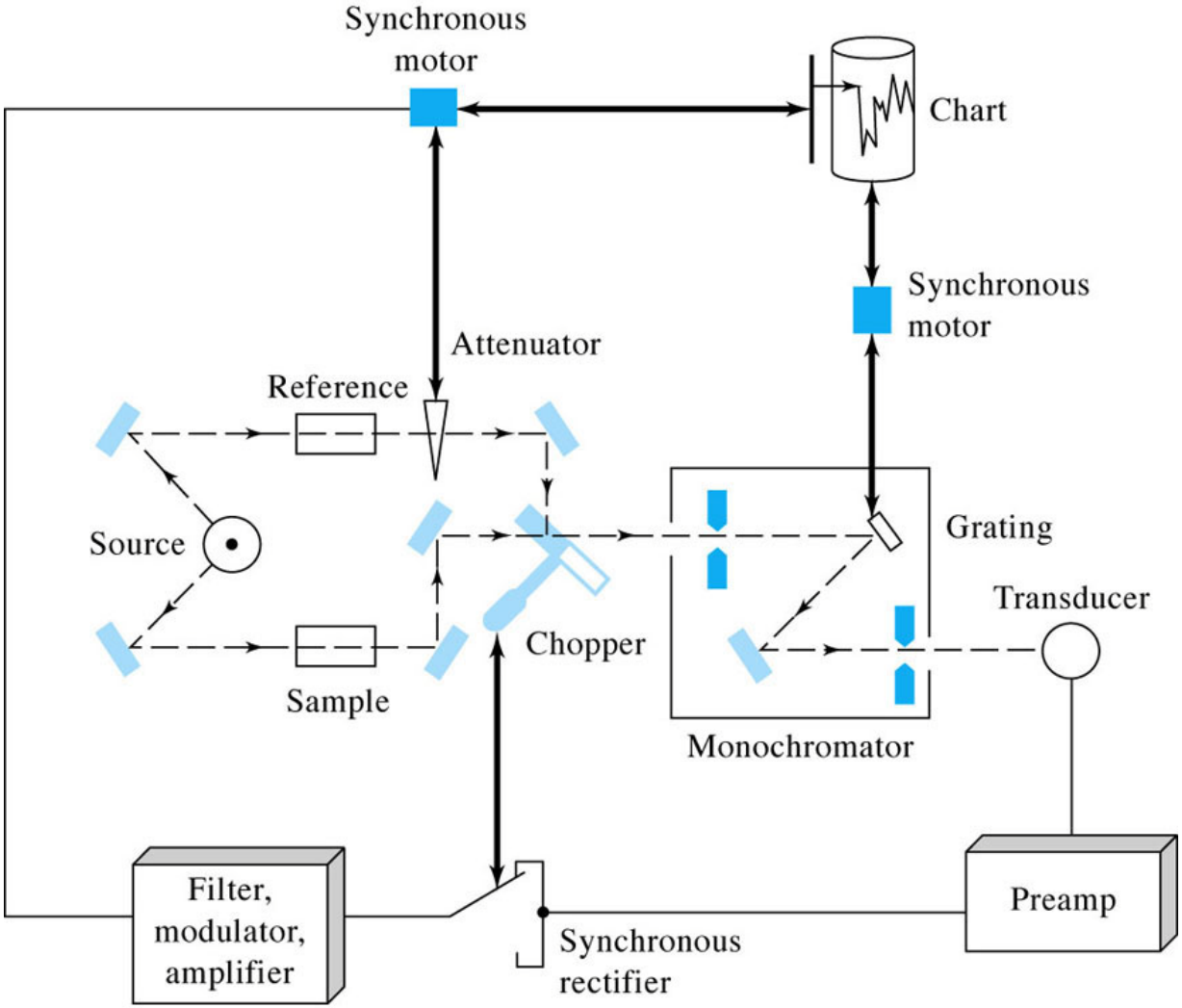


Example 8: Calculate the absorption frequency for the C-H stretch with a force constant of k = 5.0×10^2 N/m.

C) Instrumentation

1.) Basic Design

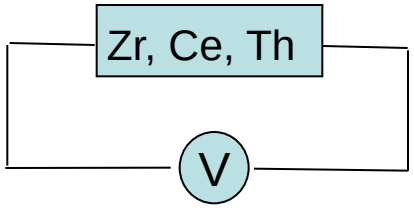
- normal IR instrument similar to UV-vis
- main differences are light source & detector



i.) Light Source:

- must produce IR radiation
- can't use glass since absorbs IR radiation
- several possible types

a) Nernst Glower



- rare earth metal oxides (Zr, Ce, Th) heated electrically
- apply current to cylinder, has resistance to current flow generates heat (1200^{o} – 2200^{o} C).
- causes light production similar to blackbody radiation
- range of use ~ 670 – 10,000cm^{-1}
- need good current control or overheats and damaged

b) Globar

- similar to Nernst Glower but uses silicon carbide rod instead of rare earth oxides
- similar usable range

c) Incandescent Wire Source

- tightly wound nichrome or rodium wire that is electrically heated
- same principal as Nernst Glower
- lower intensity then Nernst Glower or Globar, but longer lifetime

d) CO_2 Laser

- CO_2 laser gas mixture consists of 70% He, 15% CO_2, and 15% N_2
- a voltage is placed across the gas, exciting N_2 to lowest vibrational levels.
- the excited N_2 populate the asymmetric vibrational states in the CO_2 through collisions.
- infrared output of the laser is the result of transitions between rotational states of the CO_2 molecule of the first asymmetric vibrational mode to rotational states of both the first symmetric stretch mode and the second bending mode
- gives off band of ~ 100 cm^{-1}'s in range of 900-1100 cm^{-1}
- small range but can choose which band used & many compounds have IR absorbance in this region
- much more intense than Blackbody sources

e) Others

- mercury arc ($\lambda > 50$ μm) (far IR)
- tungsten lamp (4000 -12,800cm^{-1}) (near IR)

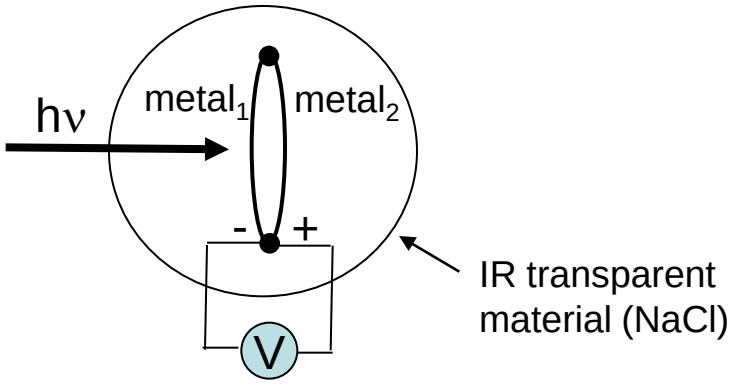
ii.) Detectors:

- two main types in common IR instruments

a) Thermal Detectors

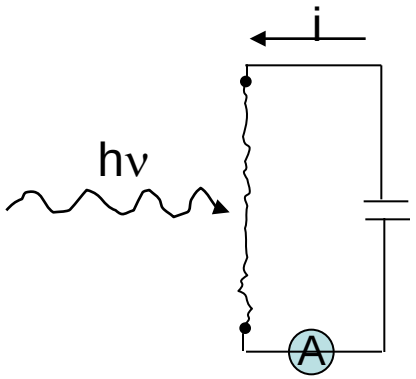
1.) Thermocouple

- two pieces of dissimilar metals fused together at the ends
- when heated, metals heat at different rates
- potential difference is created between two metals that varies with their difference in temperature
- usually made with blackened surface (to improve heat absorption)
- placed in evacuated tube with window transparent to IR (not glass or quartz)
- IR "hits" and heats one of the two wires.
- can use several thermocouples to increase sensitivity.



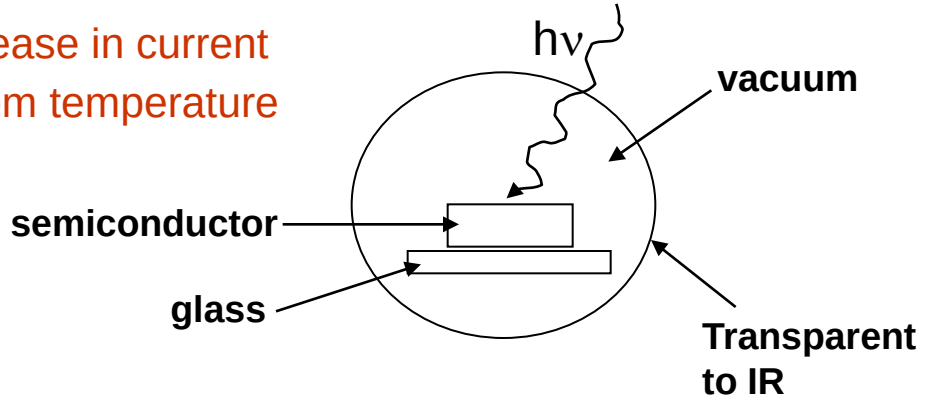
2.) Bolometer

- strips of metal (Pt, Ni) or semiconductor that has a large change in resistance to current with temperature.
- as light is absorbed by blackened surface, resistance increases and current decreases
- very sensitive



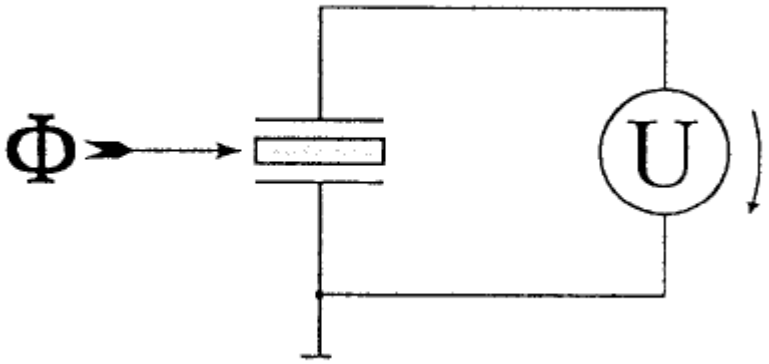
b) Photoconducting Detectors

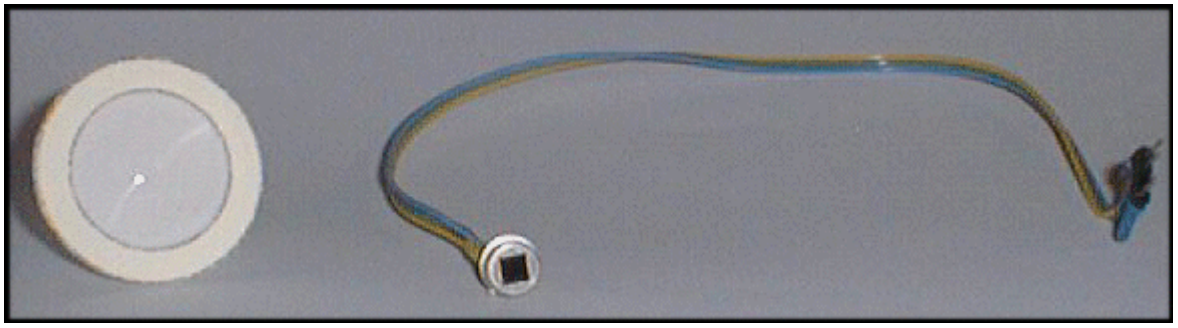
- thin film of semiconductor (ex. PbS) on a nonconducting glass surface and sealed in a vacuum.
- absorption of light by semiconductor moves from non-conducting to conducting state
- decrease in resistance → increase in current
- range: 10,000 -333 cm^{-1} at room temperature



c) Pyroelectric Detectors

- pyroelectric (ceramic, lithium tantalate) material get polarized (separation of (+) and (-) charges) in presence of electric field.
- temperature dependent polarization
- measure degree of polarization related to temperature of crystal
- fast response, good for FTIR

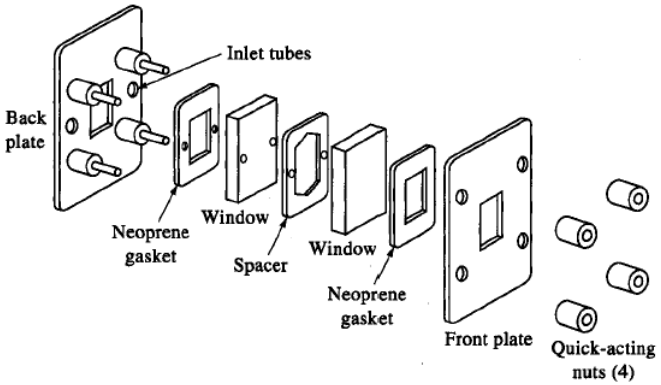




iii.) Other Components

a.) Sample Cell

- must be made of IR transparent material (KBr pellets or NaCl)

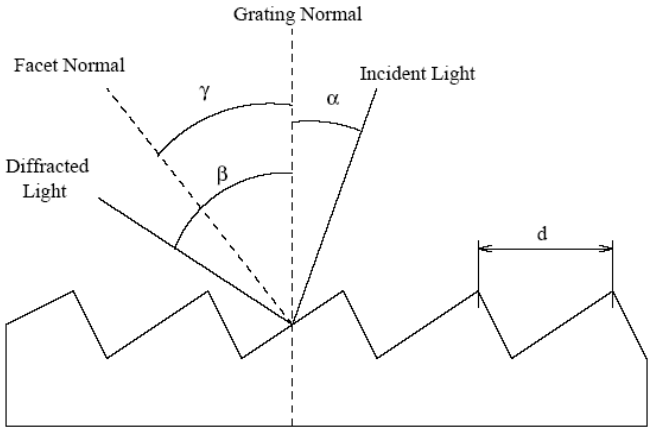


Liguid Sample Holder

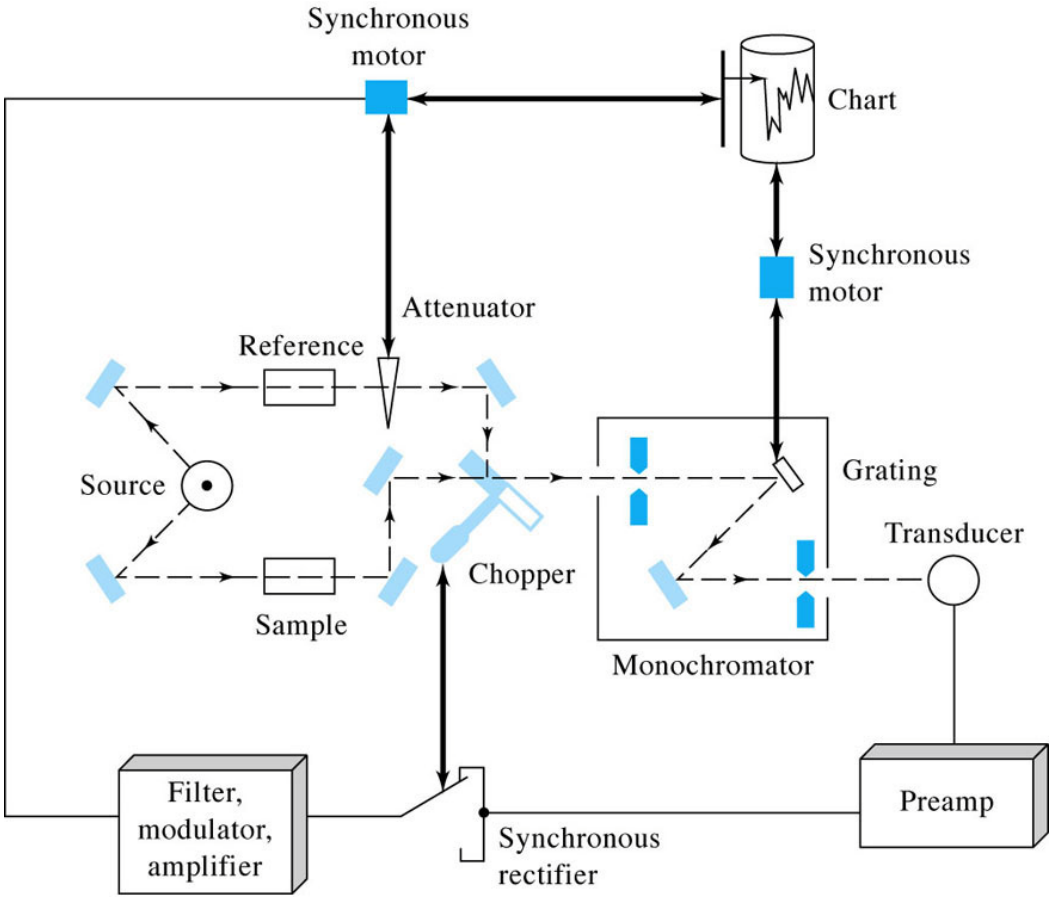
NaCl plates

b.) monochromator

- reflective grating is common
- can't use glass prism, since absorbs IR



iv.) Overall Instrument Design



-Need chopper to discriminate source light from background IR radiation

-Monochromator after sample cell

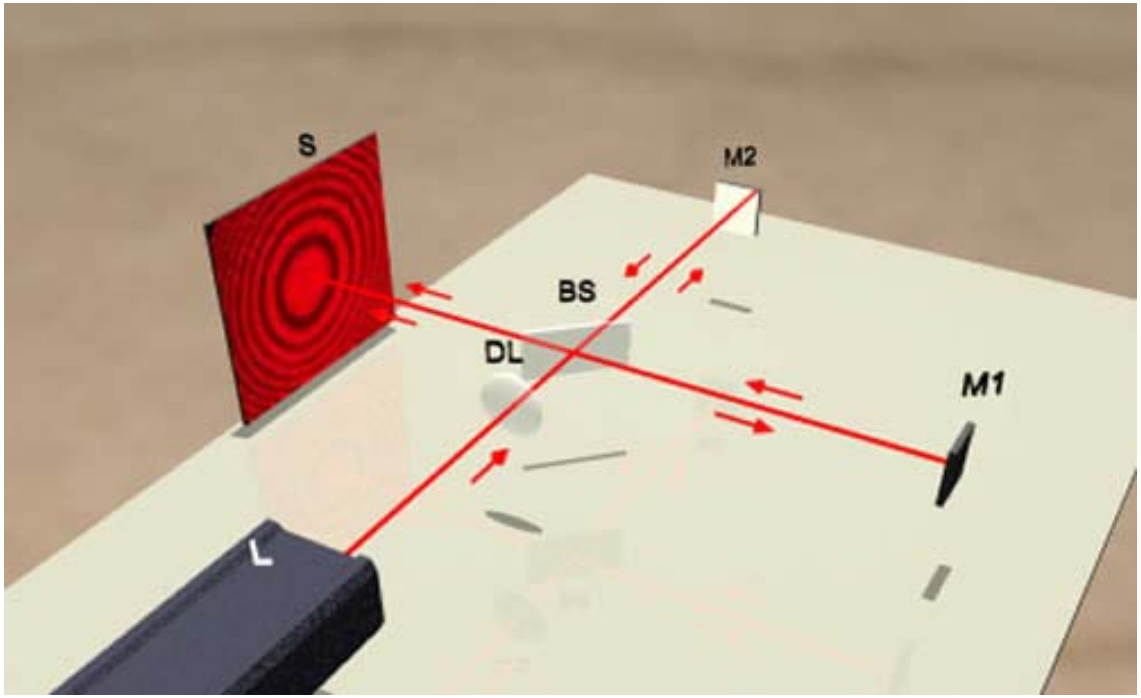
- -Not done in UV-Vis since letting in all hν to sample may cause photdegradation (too much energy)
- -IR lower energy
- -Advantage that allows monochromator to be used to screen out more background IR light

-Problems:

- -Source weak , need long scans
- -Detector response slow – rounded peaks

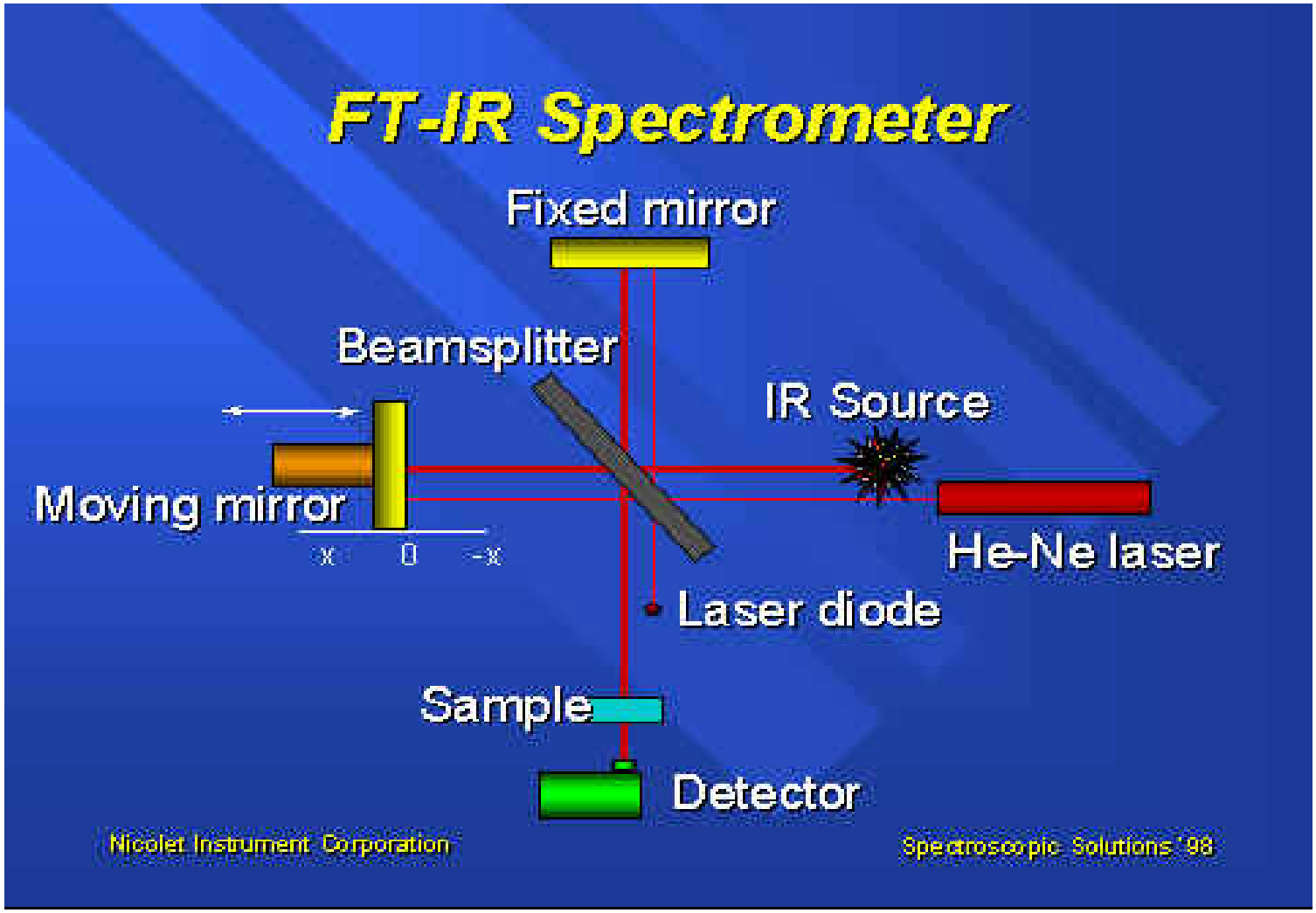
v.) Fourier Transfer IR (FTIR) – *alternative to Normal IR*

- Based on Michelson Interferometer



Principal:

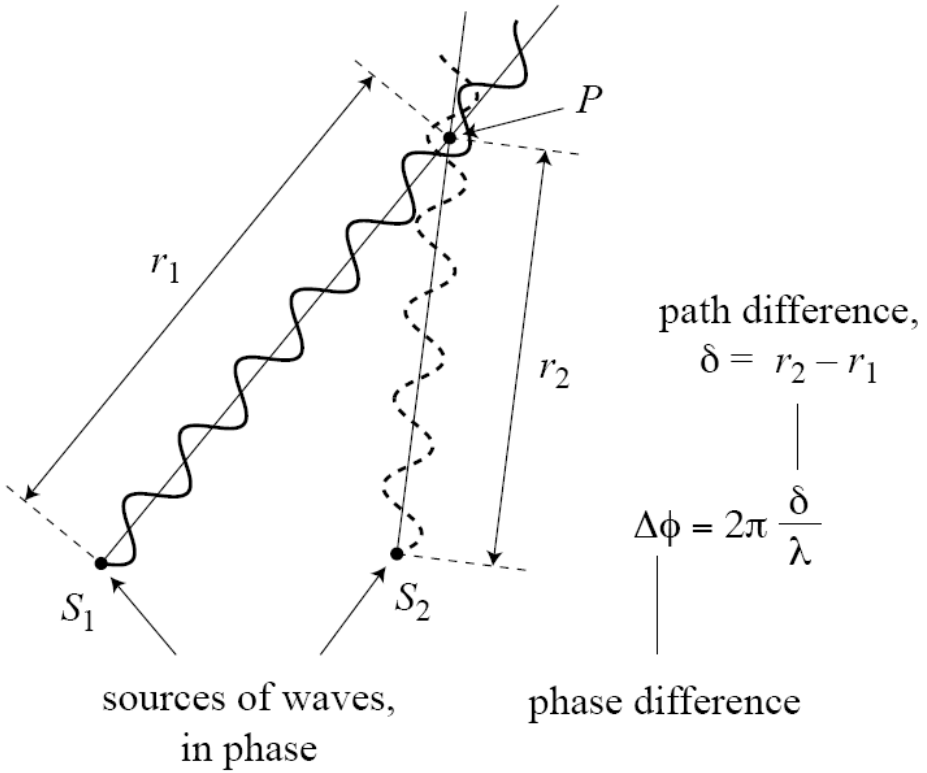
1) light from source is split by central mirror into 2 beams of equal intensity
2) beams go to two other mirrors, reflected by central mirror, recombine and pass through sample to detector
3) two side mirrors. One fixed and other movable
    a) move second mirror, light in two-paths travel different distances before recombined
    b) constructive & destructive interference
    c) as mirror is moved, get a change in signal



- **Light enters the spectrometer and is split by the beam splitter. The figure above shows what is referred to as the Michelson interferometer**

Remember

Destructive Interference can be created when two waves from the same source travel different paths to get to a point.



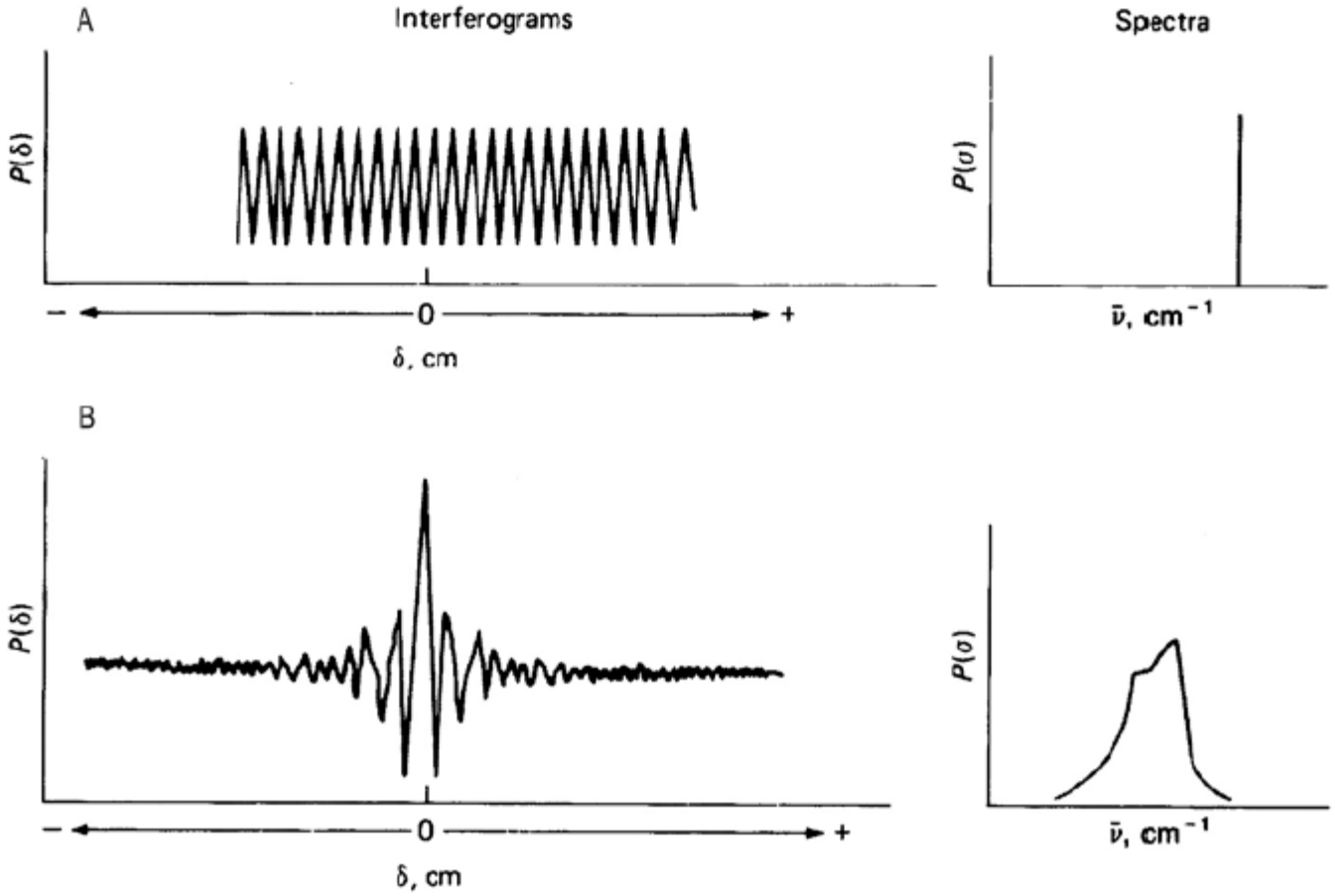
This may cause a difference in the phase between the two waves.

- *If the paths differ by an integer multiple of a wavelength, the waves will also be in phase.*
- *If the waves differ by an odd multiple of half a wave then the waves will be 180 degrees out of phase and cancel out.*

- observe a plot of Intensity vs. Distance (interferograms)
- convert to plot of Intensity vs. Frequency by doing a Fourier Transform

$$I(x) = \int_0^\infty B(\nu)(1 + \cos 2\pi\nu x)\, d\nu$$

- resolution $\Delta\nu = 1/\Delta\delta$ (interval of distance traveled by mirror)



Fourier transform pairs for (A) a monochromatic source and (B) a broadband source.

Advantages of FTIR compared to Normal IR:

1) much faster, seconds vs. minutes
2) use signal averaging to increase signal-to-noise (S/N)

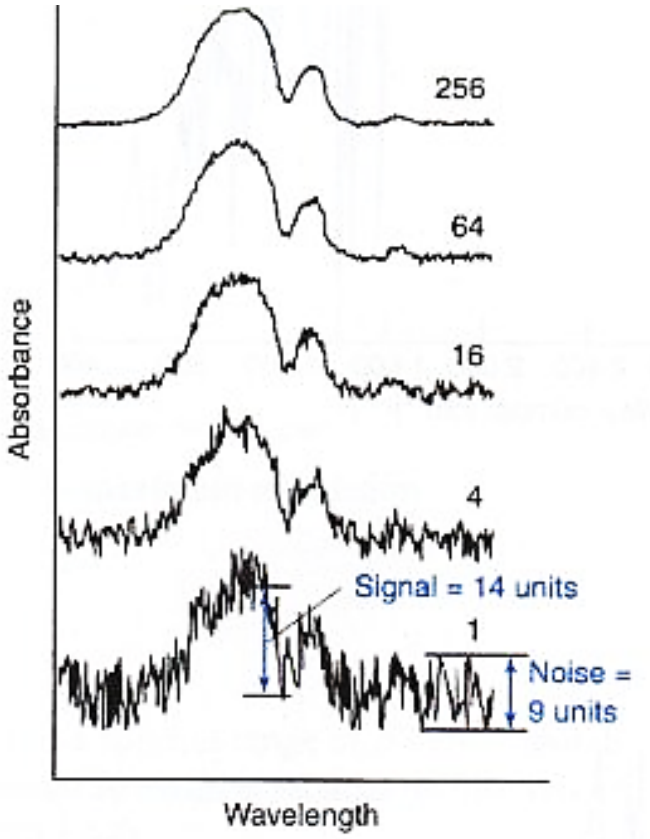
$$increase\ S/N \propto \sqrt{number\ scans}$$

3) higher inherent S/N – no slits, less optical equipment, higher light intensity
4) high resolution (<0.1 cm^{-1})

Disadvantages of FTIR compared to Normal IR:

1) single-beam, requires collecting blank
2) can't use thermal detectors – too slow

In normal IR, scan through frequency range. In FTIR collect all frequencies at once.



Advantages of FTIR:

Enhanced signal-to-noise

Rapid scanning

High resolution (<0.1 cm^{-1})

Accurate and reproducible frequency determinations

Larger energy throughput

Free from problems of stray radiation

D) Application of IR

1.) Qualitative Analysis (Compound Identification)

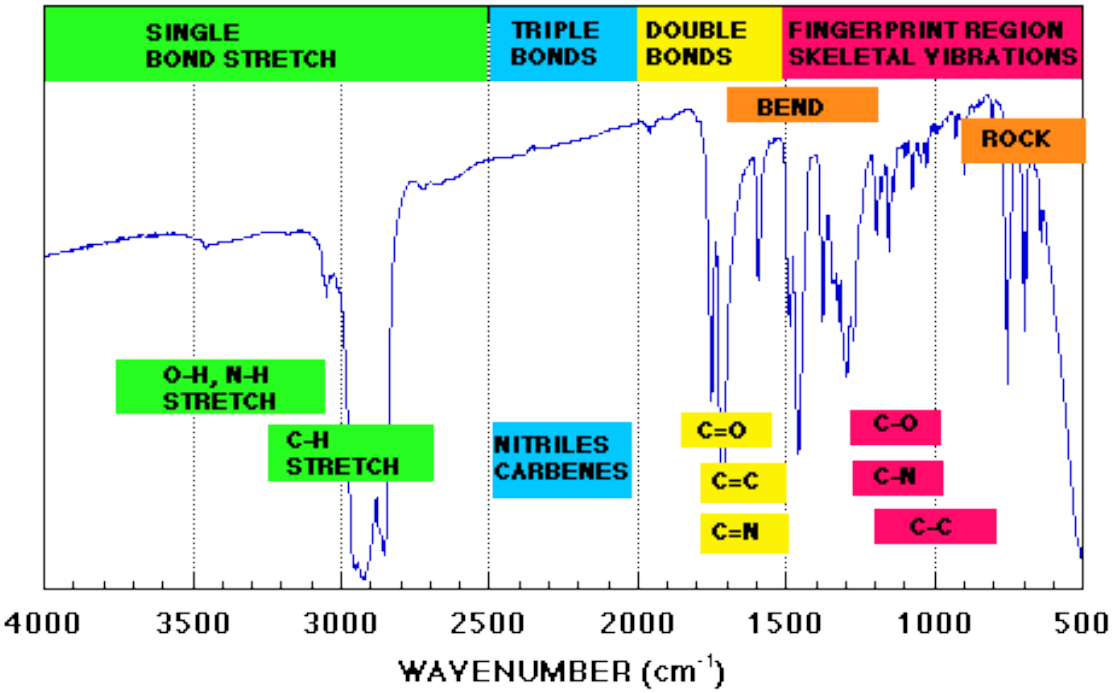
- main application
- Use of IR, with NMR and MS, in late 1950's revolutionized organic chemistry

► decreased the time to confirm compound identification 10-1000 fold

i.) General Scheme

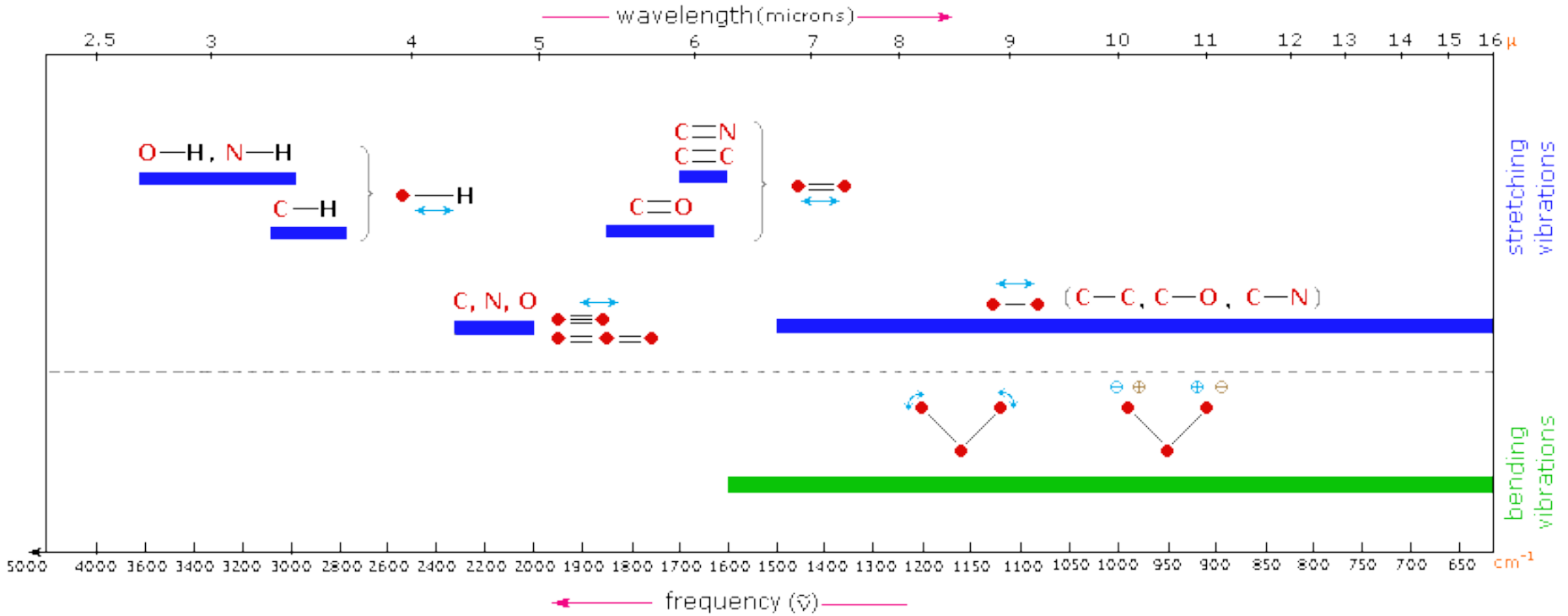
1) examine what functional groups are present by looking at group frequency region

- 3600 cm^{-1} to 1200 cm^{-1}

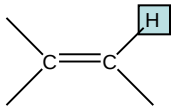
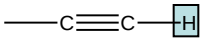


ii.) Group Frequency Region

- approximate frequency of many functional groups (C=O,C=C,C-H,O-H) can be calculated from atomic masses & force constants
- positions changes a little with neighboring atoms, ***but*** often in same general region
- serves as a good initial guide to compound identity, but not positive proof.

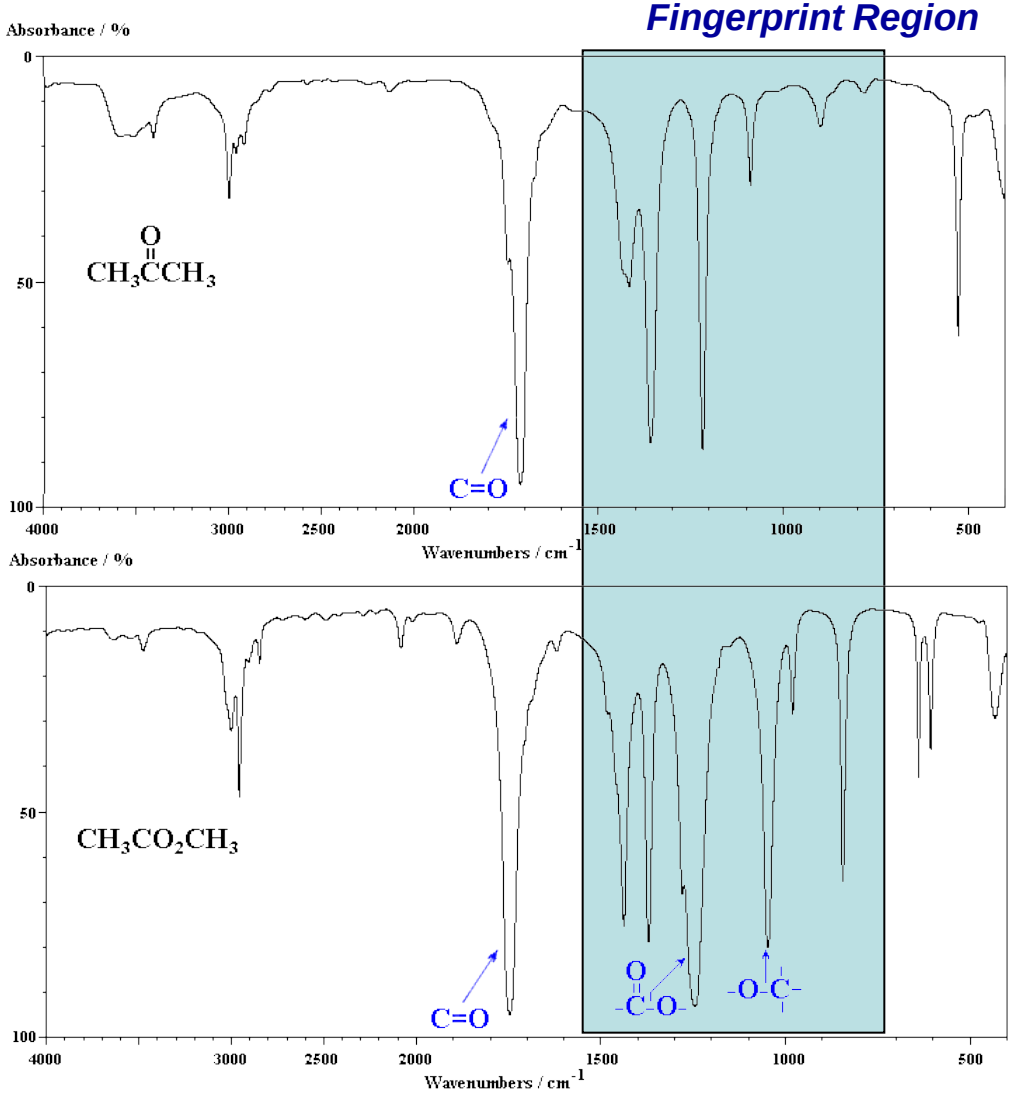


Abbreviated Table of Group Frequencies for Organic Groups

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C-H	Alkanes	2850-2970	Strong
C-H	Alkenes 	3010-3095 675-995	Medium strong
C-H	Alkynes 	3300	Strong
C-H	Aromatic rings	3010-3100 690-900	Medium strong
0-H	Monomeric alcohols, phenols Hydrogen-bonded alchohols, phenols Monomeric carboxylic acids Hydrogen-bonded carboxylic acids	3590-3650 3200-3600 3500-3650 2500-2700	Variable Variable, sometimes broad Medium broad
N-H	Amines, amides	3300-3500	medium
C=C	Alkenes	1610-1680	Variable
C=C	Aromatic rings	1500-1600	Variable
C≡C	Alkynes	2100-2260	Variable
C-N	Amines, amides	1180-1360	Strong
C≡N	Nitriles	2210-2280	Strong
C-O	Alcohols, ethers,carboxylic acids, esters	1050-1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690-1760	Strong
NO_2	Nitro compounds	1500-1570 1300-1370	Strong

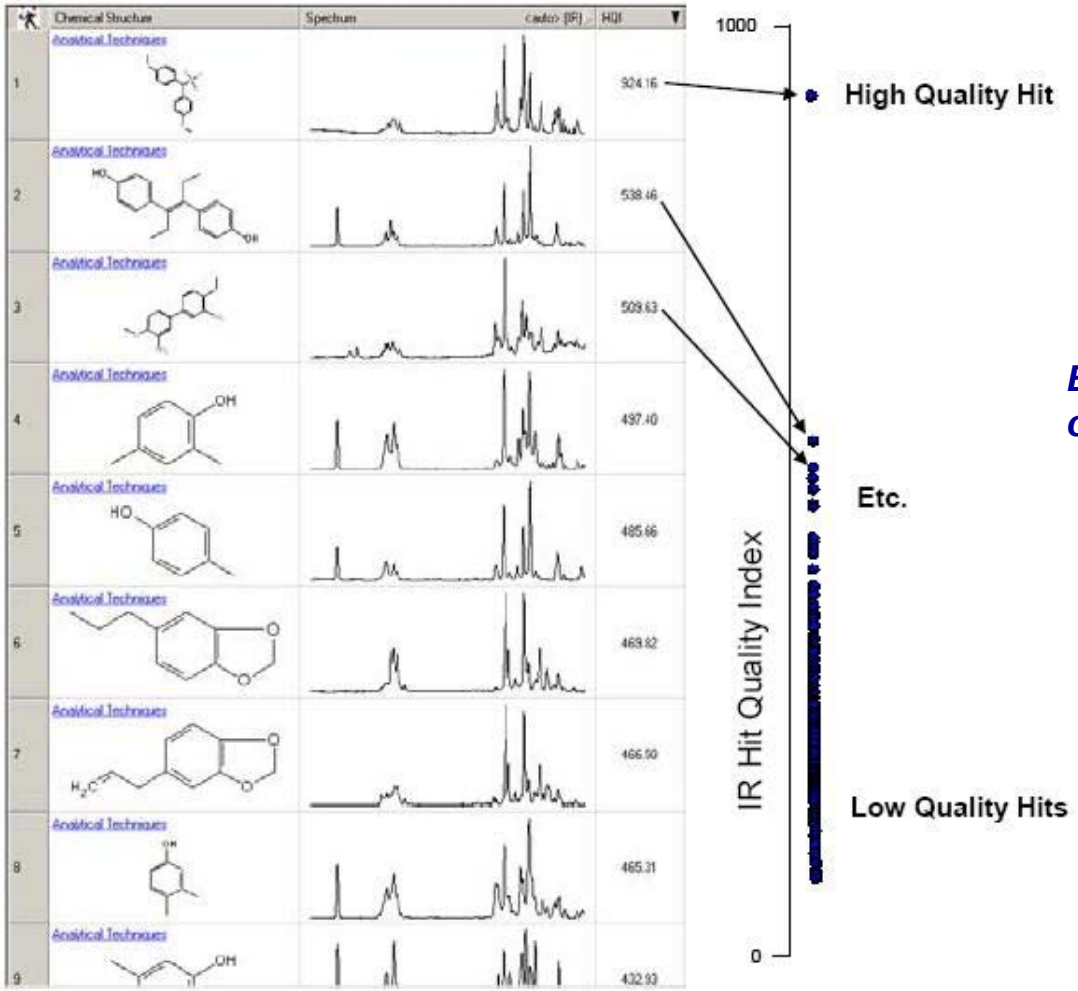
iii.) Fingerprint Region (1200-700 cm^{-1})

- region of most single bond signals
- many have similar frequencies, so affect each other & give pattern characteristics of overall skeletal structure of a compound
- exact interpretation of this region of spectra seldom possible because of complexity
- complexity → uniqueness



iv.) Computer Searches

- many modern instruments have reference IR spectra on file (~100,000 compounds)
- matches based on location of strongest band, then 2nd strongest band, etc
  overall skeletal structure of a compound
- exact interpretation of this region of spectra seldom possible because of complexity
- complexity → uniqueness



Bio-Rad SearchIT database of ~200,000 IR spectra

2.) Quantitative Analysis

- not as good as UV/Vis in terms of accuracy and precision
  - more complex spectra
  - narrower bands (Beer's Law deviation)
  - limitations of IR instruments (lower light throughput, weaker detectors)
  - high background IR
  - difficult to match reference and sample cells
  - changes in ε ($A=\varepsilon bc$) common
- potential advantage is good selectivity, since so many compounds have different IR spectra
  - one common application is determination of air contaminants.

Contaminants	Concn, ppm	Found, ppm	Relative error, %
Carbon Monoxide	50	49.1	1.8
Methylethyl ketone	100	98.3	1.7
Methyl alcohol	100	99.0	1.0
Ethylene oxide	50	49.9	0.2
chloroform	100	99.5	0.5

Example 9: The spectrum is for a substance with an empirical formula of C_3H_5N. What is the compound?