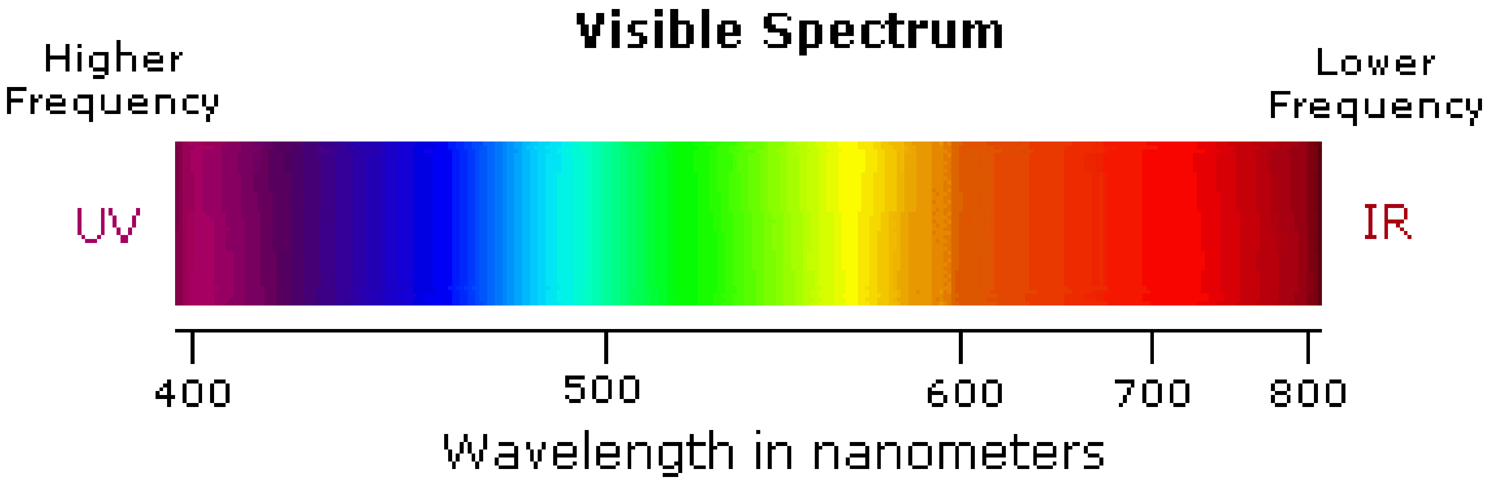
Molecular Spectroscopy

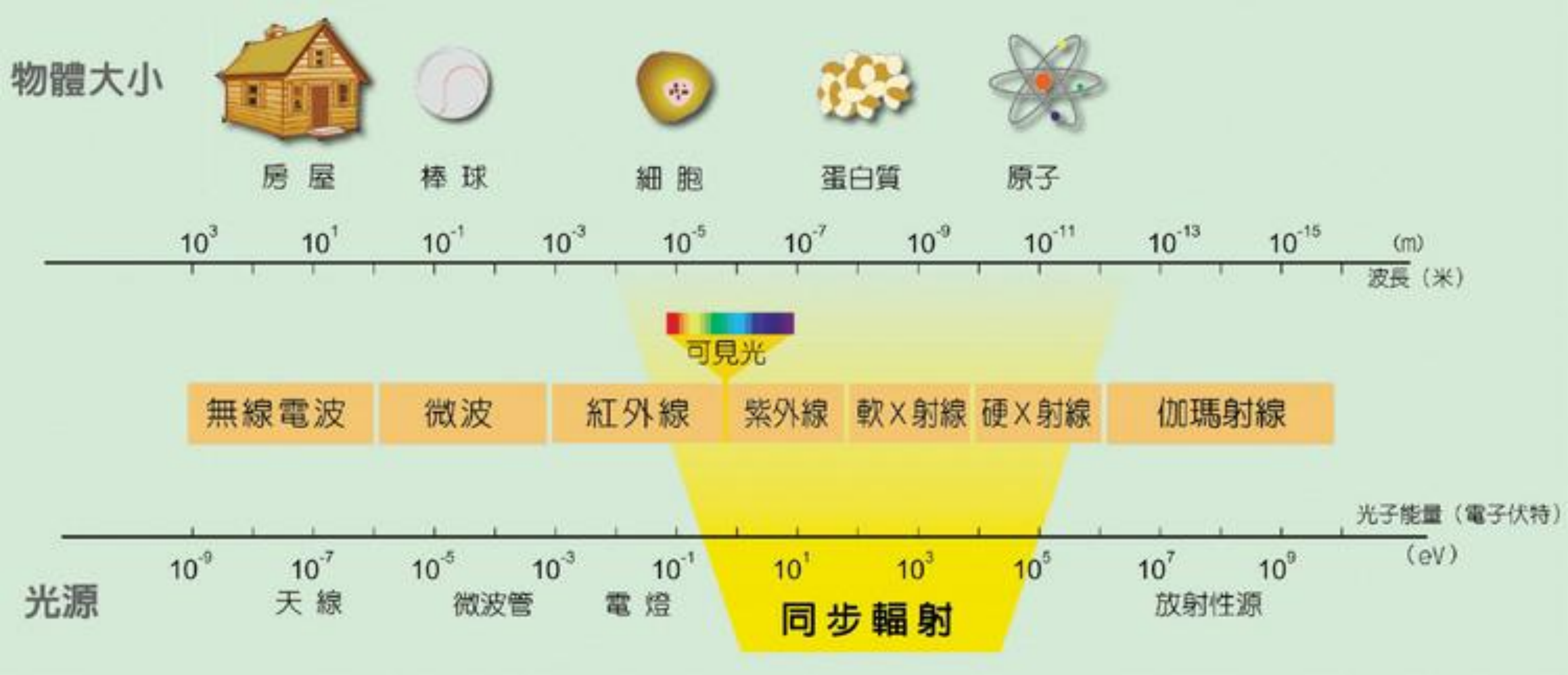
Visible and Ultraviolet Spectroscopy

- ***UV/VIS Spectroscopy***
- ***UV/VIS Spectrometer***
- ***Application for Quantitative Analysis***



- Ultraviolet: 190~400nm
- Violet: 400 - 420 nm
- Indigo: 420 - 440 nm
- Blue: 440 - 490 nm
- Green: 490 - 570 nm
- Yellow: 570 - 585 nm
- Orange: 585 - 620 nm
- Red: 620 - 780 nm

電磁波譜


物體大小
房 屋
棒 球
細 胞
蛋白質
原子
10^{3}
10^{1}
10^{-1}
10^{-3}
10^{-5}
10^{-7}
10^{-9}
10^{-11}
10^{-13}
10^{-15}
(m)
波長（米）
可見光
無線電波
微波
紅外線
紫外線
軟X射線
硬X射線
伽瑪射線
光子能量（電子伏特）
(eV)
10^{-9}
10^{-7}
10^{-5}
10^{-3}
10^{-1}
10^{1}
10^{3}
10^{5}
10^{7}
10^{9}
光源
天 線
微波管
電 燈
同步輻射
放射性源

Internal Energy of Molecules

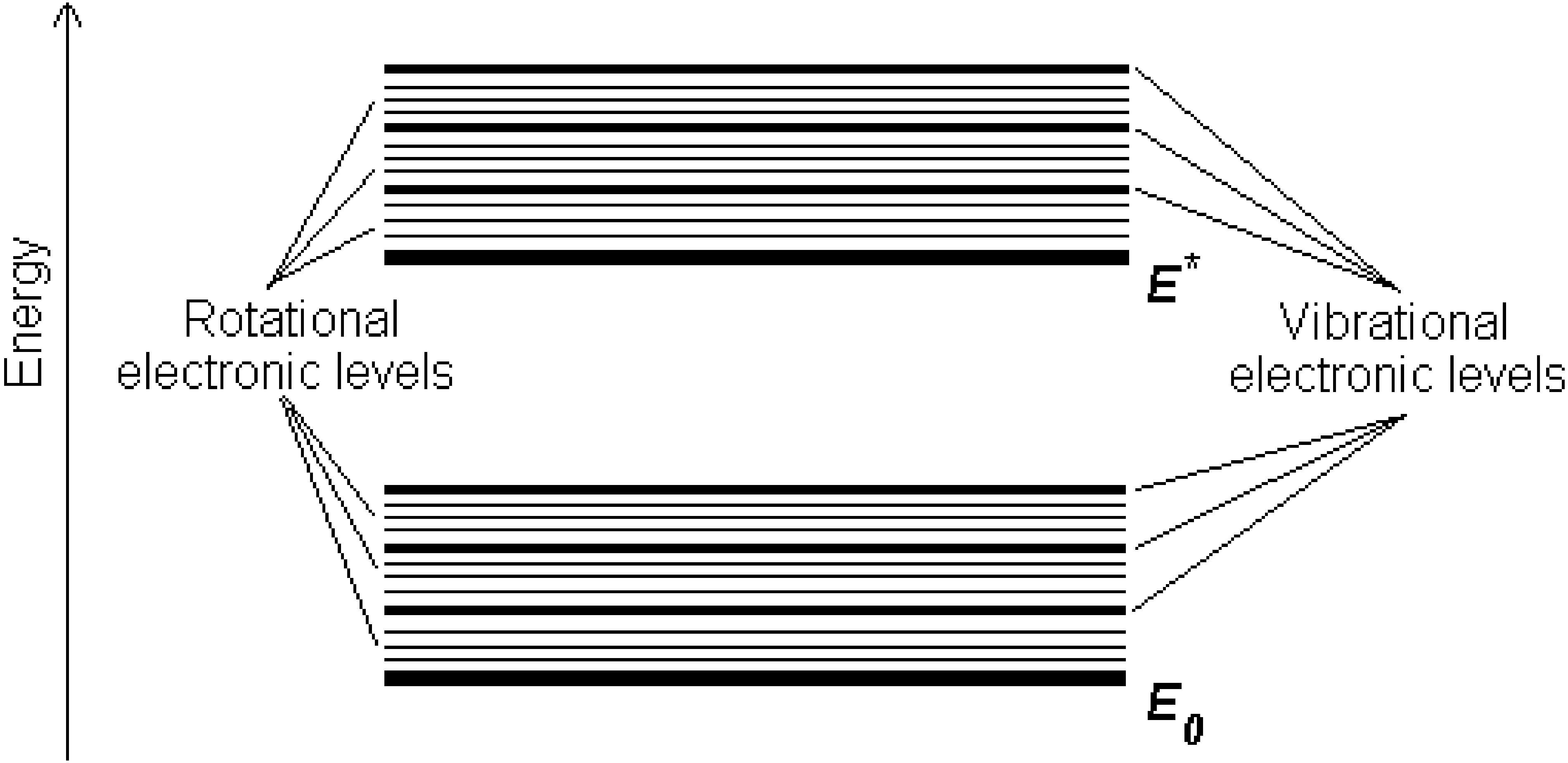
$E_{total}=E_{trans}+E_{elec}+E_{vib}+E_{rot}+E_{nucl}$

E_{elec}: electronic transitions (UV, X-ray)

E_{vib}: vibrational transitions (Infrared)

E_{rot}: rotational transitions (Microwave)

E_{nucl}: nucleus spin (nuclear magnetic resonance) or (MRI: magnetic resonance imaging)


Energy
Rotational electronic levels
E^*
Vibrational electronic levels
E_0

Electronic Spectroscopy

- Ultraviolet (UV) and visible (VIS) spectroscopy
- This is the earliest method of molecular spectroscopy.
- A phenomenon of interaction of molecules with ultraviolet and visible lights.
- Absorption of photon results in electronic transition of a molecule, and electrons are promoted from ground state to higher electronic states.

UV and Visible Spectroscopy

- **In structure determination : UV-VIS spectroscopy is used to detect the presence of chromophores like dienes, aromatics, polyenes, and conjugated ketones, etc.**

Electronic transitions

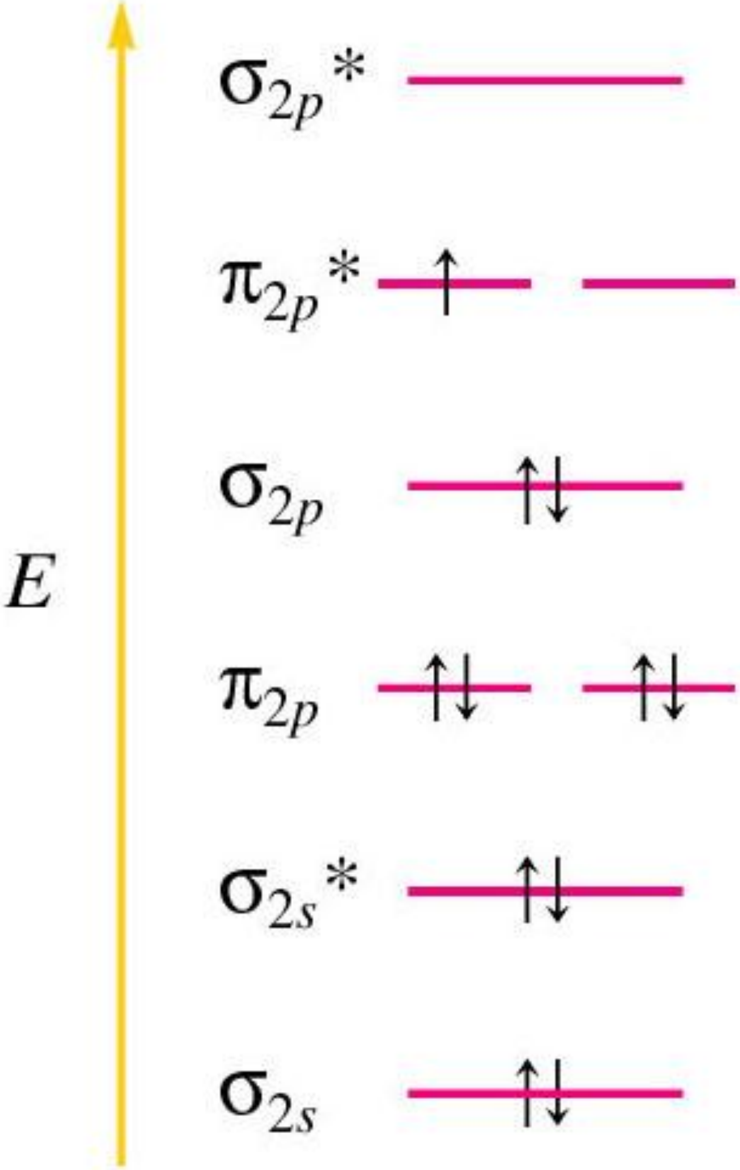
There are three types of electronic transition which can be considered;

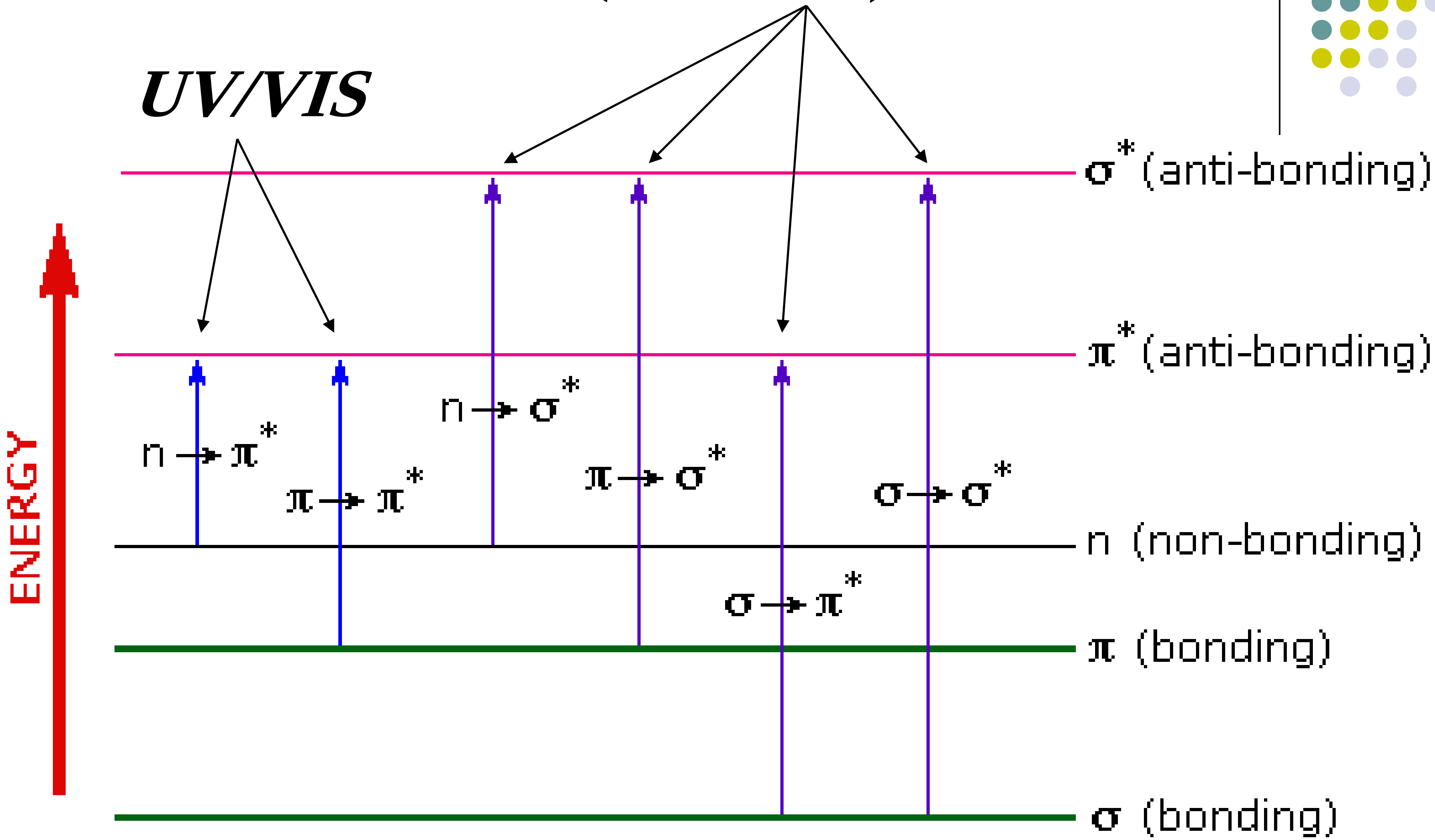
- Transitions involving p, s, and *n* electrons
- Transitions involving charge-transfer electrons
- Transitions involving *d* and *f* electrons

Absorbing species containing p, s, and *n* electrons

- Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (*chromophores*) that contain valence electrons of low excitation energy.

NO





Vacuum UV or Far UV

(λ<190 nm)

UV/VIS

ENERGY

σ^* (anti-bonding)

π^* (anti-bonding)

n (non-bonding)

π (bonding)

σ (bonding)

$n \rightarrow \pi^*$

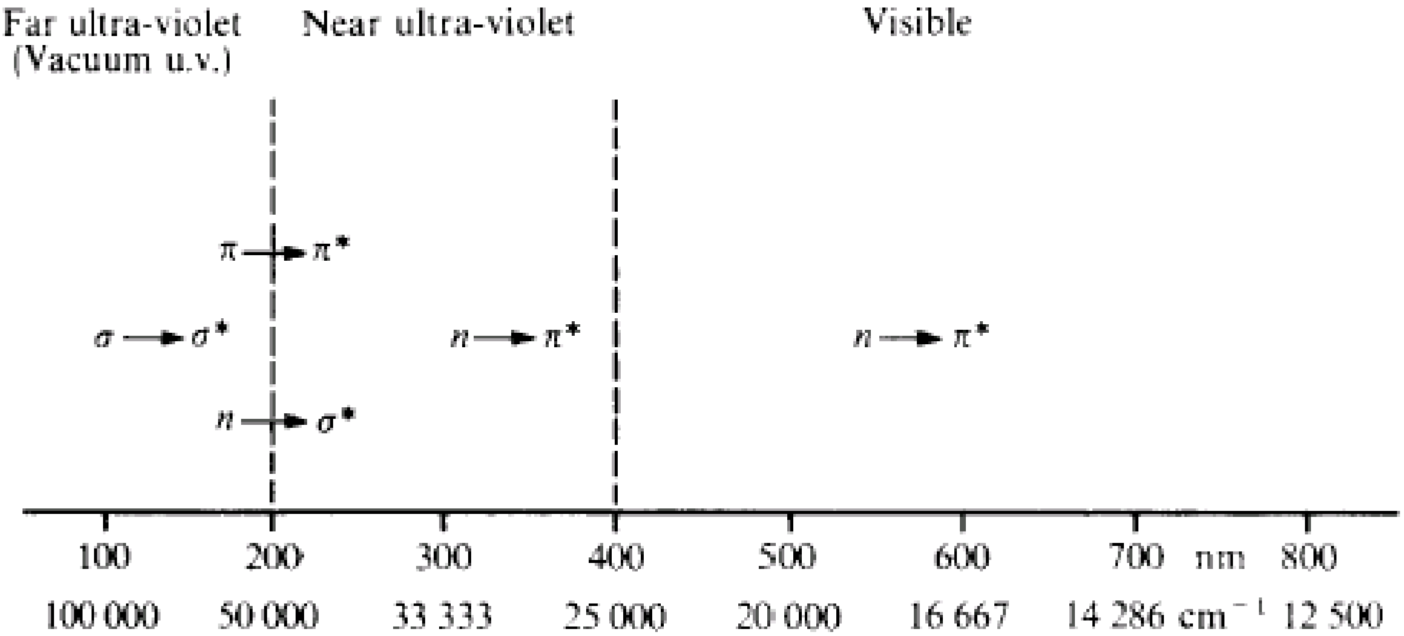
$\pi \rightarrow \pi^*$

$n \rightarrow \sigma^*$

$\pi \rightarrow \sigma^*$

$\sigma \rightarrow \sigma^*$

$\sigma \rightarrow \pi^*$



The regions of the electronic spectrum and the types of transitions that occur in each.

$\sigma \rightarrow \sigma^*$ Transitions

- An electron in a bonding s orbital is excited to the corresponding antibonding orbital. The energy required is large. For example, 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-VIS spectra (200 - 700 nm)

$n \rightarrow \sigma^*$ Transitions

- Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of $n \rightarrow \sigma^*$ transitions. These transitions usually need less energy than $\sigma \rightarrow \sigma^*$ transitions. 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.

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

- Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π^* excited state.
- 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 π electrons.

Chromophore	**Excitation**	**λ_{max}, nm**	**Solvent**
C=C	$\pi\rightarrow\pi^*$	171	hexane
C=O	$n\rightarrow\pi^*$ $\pi\rightarrow\pi^*$	290 180	hexane hexane
N=O	$n\rightarrow\pi^*$ $\pi\rightarrow\pi^*$	275 200	ethanol ethanol
C-X X=Br, I	$n\rightarrow\sigma^*$ $n\rightarrow\sigma^*$	205 255	hexane hexane

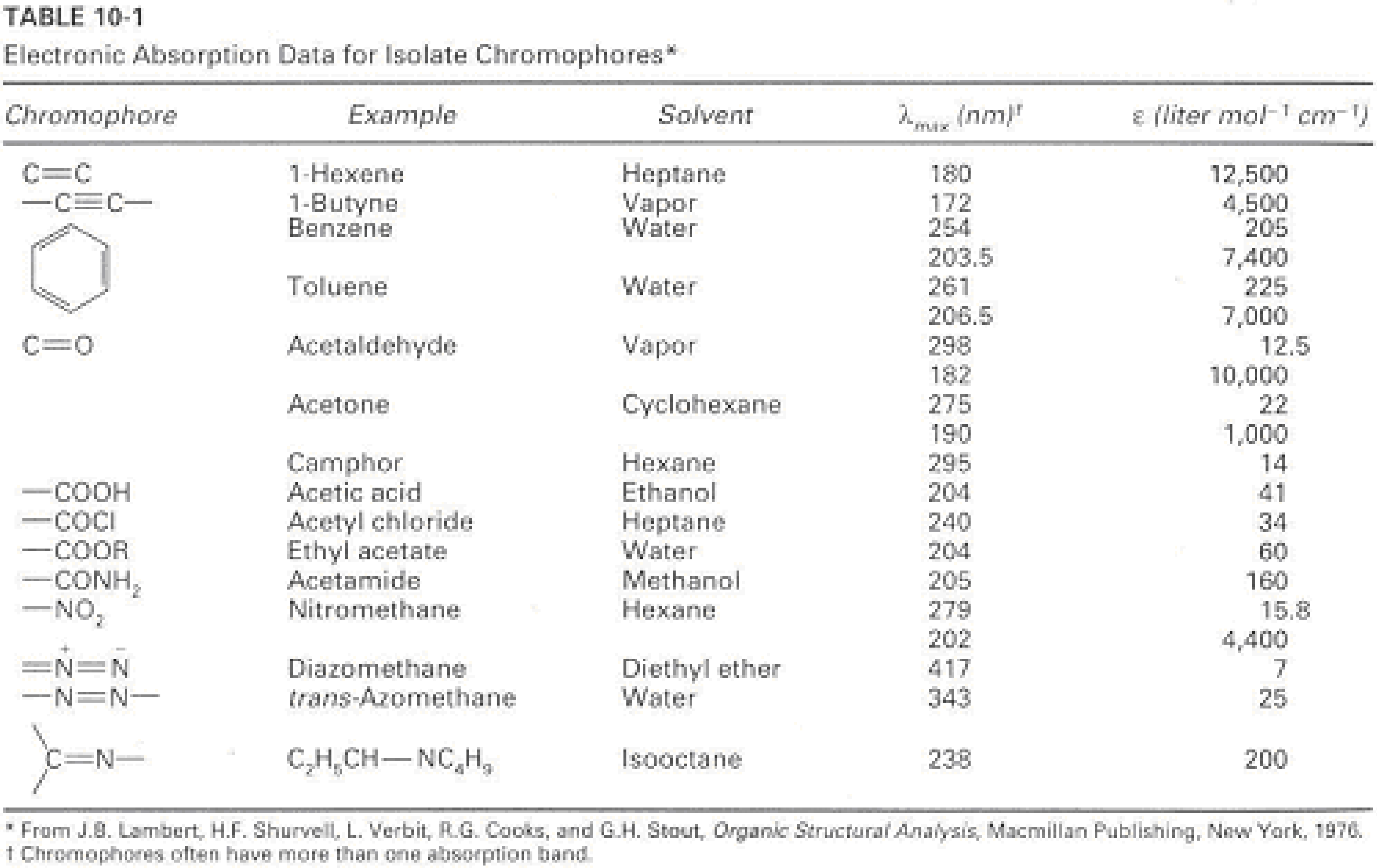


TABLE 10-1

Electronic Absorption Data for Isolate Chromophores*

Chromophore	*Example*	*Solvent*	λ_{max} *(nm)*†	ε *(liter mol⁻¹ cm⁻¹)*
C=C	1-Hexene	Heptane	180	12,500
—C≡C—	1-Butyne	Vapor	172	4,500
	Benzene	Water	254	205
			203.5	7,400
	Toluene	Water	261	225
			206.5	7,000
C=O	Acetaldehyde	Vapor	298	12.5
			182	10,000
	Acetone	Cyclohexane	275	22
			190	1,000
	Camphor	Hexane	295	14
—COOH	Acetic acid	Ethanol	204	41
—COCl	Acetyl chloride	Heptane	240	34
—COOR	Ethyl acetate	Water	204	60
—$CONH_2$	Acetamide	Methanol	205	160
—NO_2	Nitromethane	Hexane	279	15.8
			202	4,400
$=\overset{+}{N}=\overset{-}{N}$	Diazomethane	Diethyl ether	417	7
—N=N—	*trans*-Azomethane	Water	343	25
$>$C=N—	$C_2H_5CH—NC_4H_9$	Isooctane	238	200

* From J.B. Lambert, H.F. Shurvell, L. Verbit, R.G. Cooks, and G.H. Stout, *Organic Structural Analysis*, Macmillan Publishing, New York, 1976.

† Chromophores often have more than one absorption band.

Orbital Spin States

- **Singlet state** (S):Most molecules have ground state with all electron spin paired and most excited state also have electron spin all paired, even though they may be one electron each lying in two different orbital. Such states have zero total spin and spin multiplicities of 1, are called singlet (S) states.

Total Spin

$$\Delta S = +\frac{1}{2} - \frac{1}{2} = 0$$

Multiplicities

$$M = 2\Delta S + 1 = 2 \times 0 + 1 = 1$$

Orbital Spin States

- For some of the excited states, there are states with a pair of electrons having their spins parallel (in two orbitals), leading to total spin of 1 and multiplicities of 3.

Total Spin

$$\Delta S = +\frac{1}{2} + \frac{1}{2} = 1$$

Multiplicities

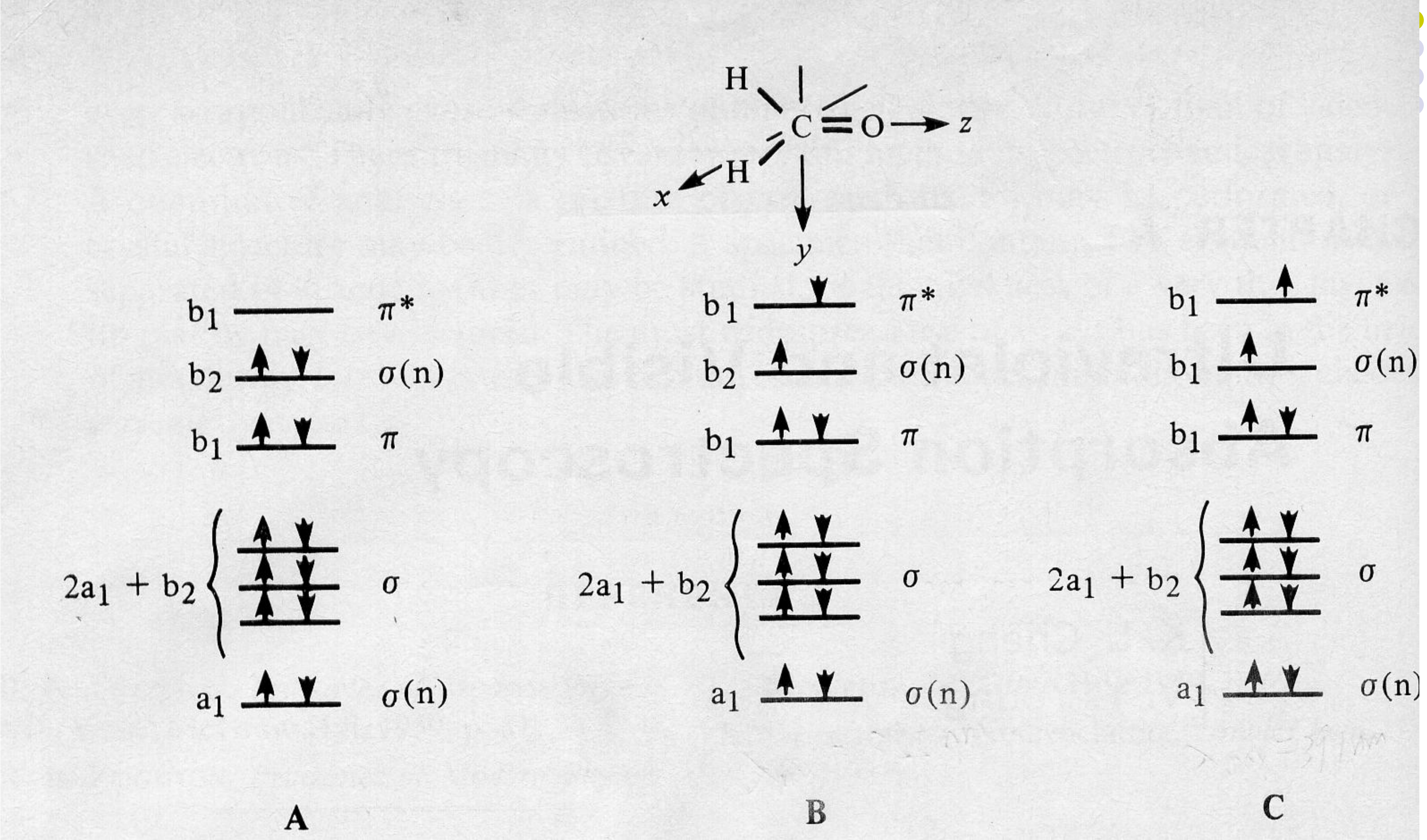
$$M = 2\Delta S + 1 = 2 \times 1 + 1 = 3$$

Orbital Spin States

- For triplet state: Under the influence of external field, there are three values (i.e. 3 energy states) of +1, 0, -1 times the angular momentum. Such states are called triplet states (T).
- According to the selection rule, S→S, T→T, are allowed transitions, but S→T, T→S, are forbidden transitions.

Selection Rules of electronic transition

- Electronic transitions may be classed as intense or weak according to the magnitude of ε_{max} that corresponds to *allowed* or *forbidden* transition as governed by the following **selection rules** of electronic transition:
- **Spin selection rule**: there should be no change in spin orientation or no spin inversion during these transitions. Thus, S→S, T→T, are allowed, but S→T, T→S, are forbidden. ($\triangle S=0$ *transition allowed*)


H
C
O
H
z
x
y
b1
π*
b2
σ(n)
b1
π
2a1 + b2
σ
a1
σ(n)
A
b1
π*
b2
σ(n)
b1
π
2a1 + b2
σ
a1
σ(n)
B
b1
π*
b1
σ(n)
b1
π
2a1 + b2
σ
a1
σ(n)
C

Terms describing UV absorptions

1. **Chromophores**: functional groups that give electronic transitions.
2. **Auxochromes**: substituents with unshared pair e's like OH, NH, SH ..., when attached to π chromophore they generally move the absorption max. to longer λ.
3. **Bathochromic shift**: shift to longer λ, also called red shift.
4. **Hysochromic shift**: shift to shorter λ, also called blue shift.
5. **Hyperchromism**: increase in ε of a band.
6. **Hypochromism**: decrease in ε of a band.

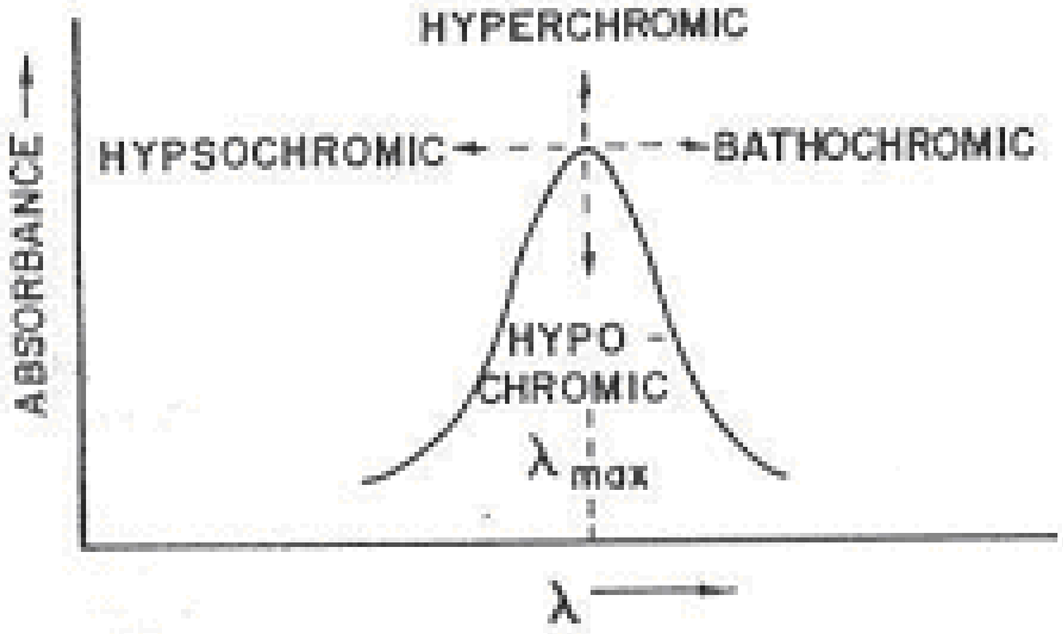
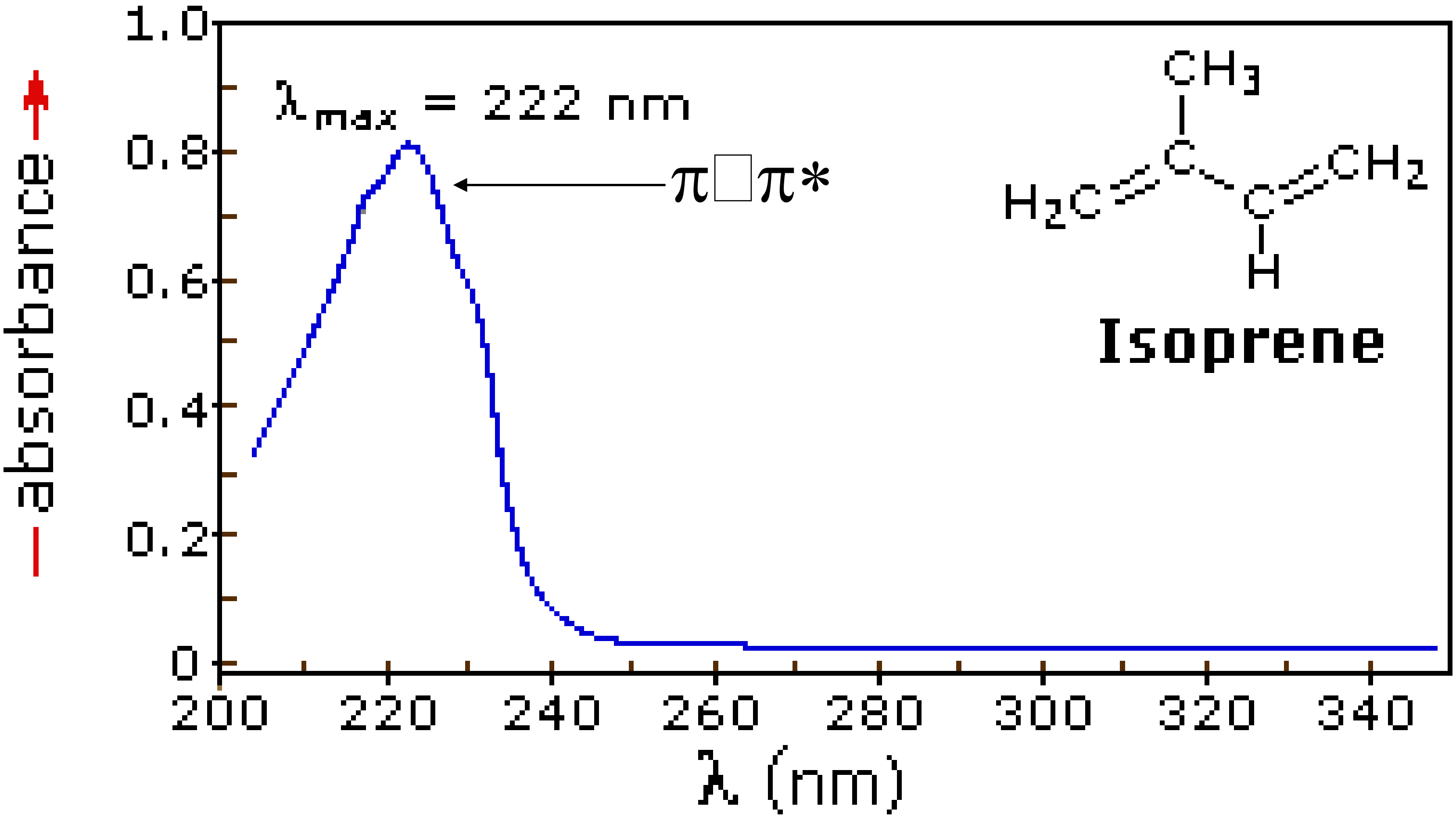
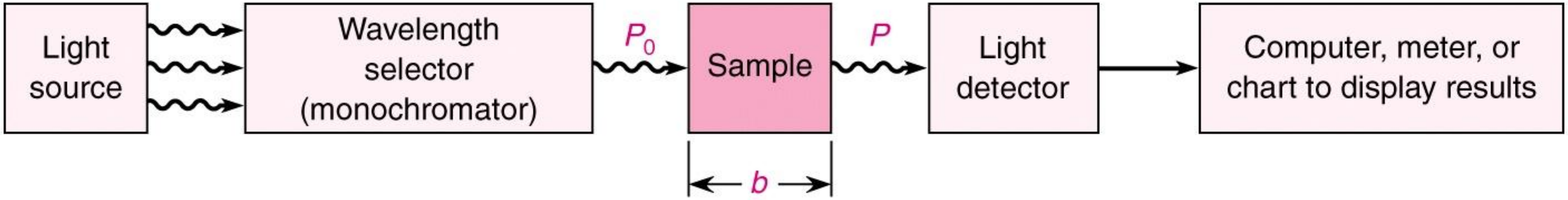


Figure 11-3 Terminology of shifts in the position of an absorption band.


λmax = 222 nm
π□π*
CH3
H2C
C
C
CH2
H
Isoprene
absorbance
1.0
0.8
0.6
0.4
0.2
0
200
220
240
260
280
300
320
340
λ (nm)

Instrumentation



Light source

Wavelength selector (monochromator)

P_0

Sample

P

Light detector

Computer, meter, or chart to display results

b

光源 分光器 樣品 偵測器 記錄器

Components of a Spectrophotometer

Light Source

- Deuterium Lamps — a truly continuous spectrum in the ultraviolet region is produced by electrical excitation of deuterium at low pressure. (160nm~375nm)
- Tungsten Filament Lamps — the most common source of visible and near infrared radiation.

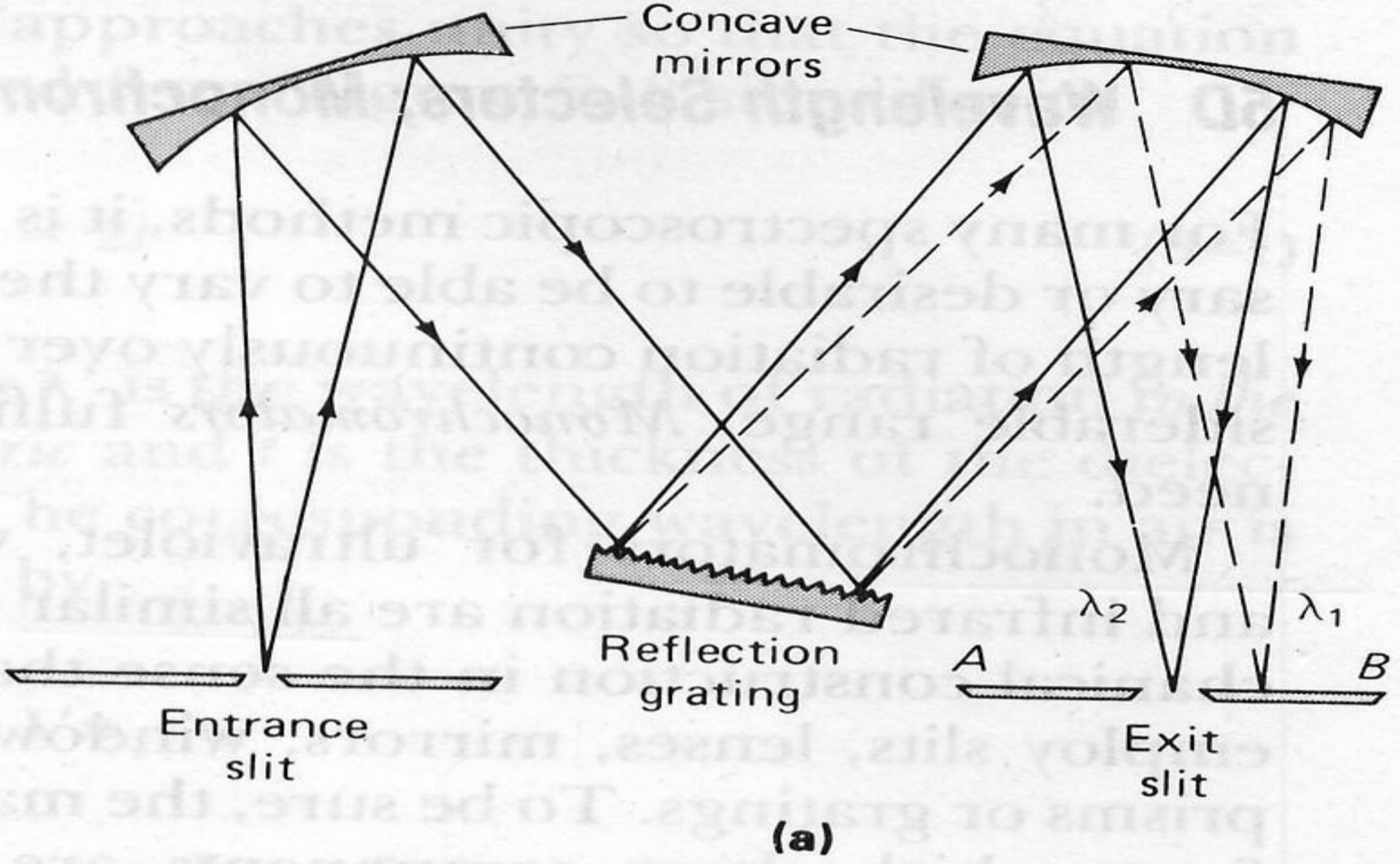
Components of a Spectrophotometer

Monochromator (分光器/單光器)

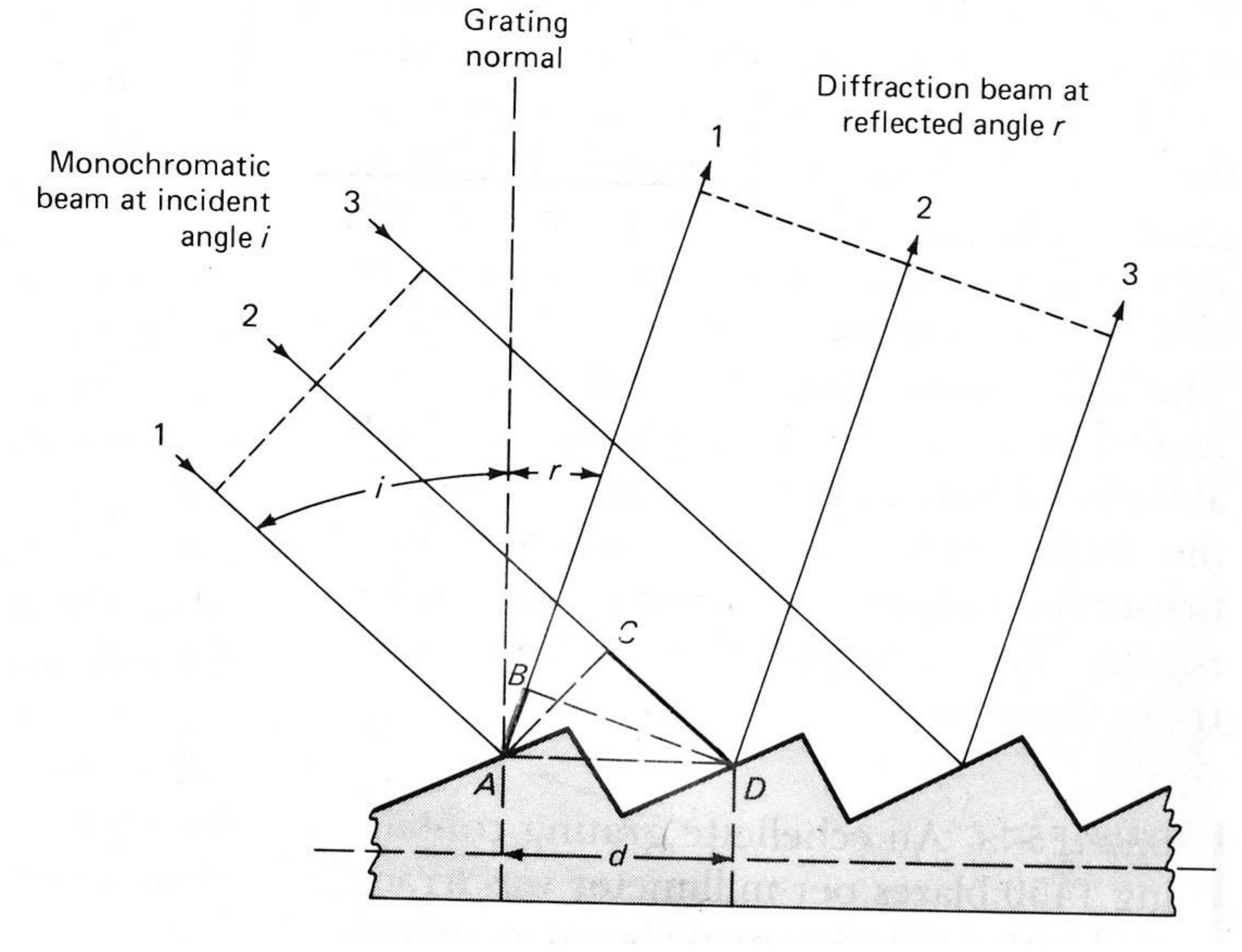
- Used as a filter: the monochromator will select a narrow portion of the spectrum (the bandpass) of a given source

- Used in analysis: the monochromator will sequentially select for the detector to record the different components (spectrum) of any source or sample emitting light.

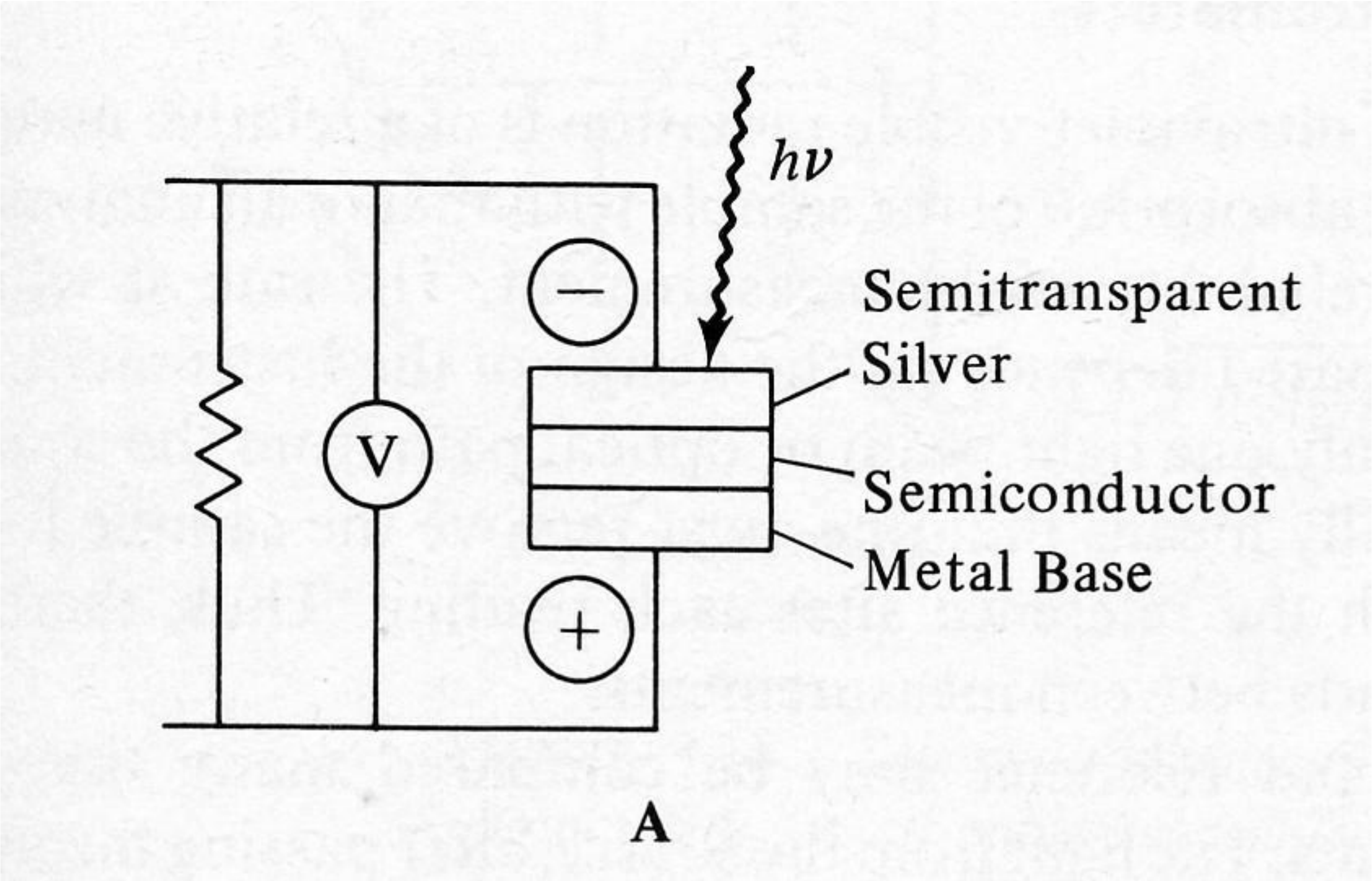
Monochromator Czerny-Turner design



Grating


Grating normal
Diffraction beam at reflected angle *r*
Monochromatic beam at incident angle *i*
1
2
3
3
2
1
i
r
B
C
A
D
d

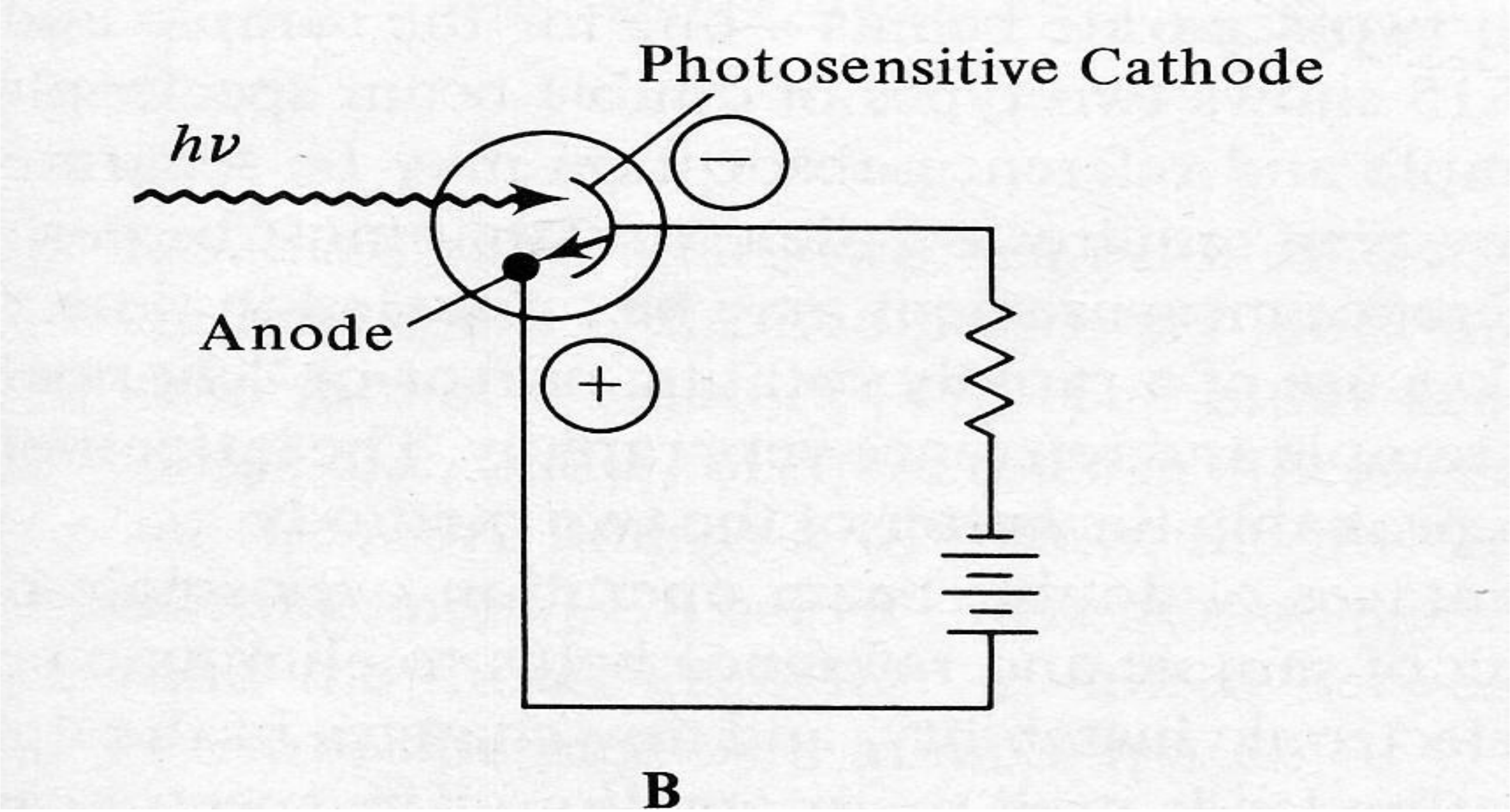
Detector
Barrier Layer/Photovoltaic



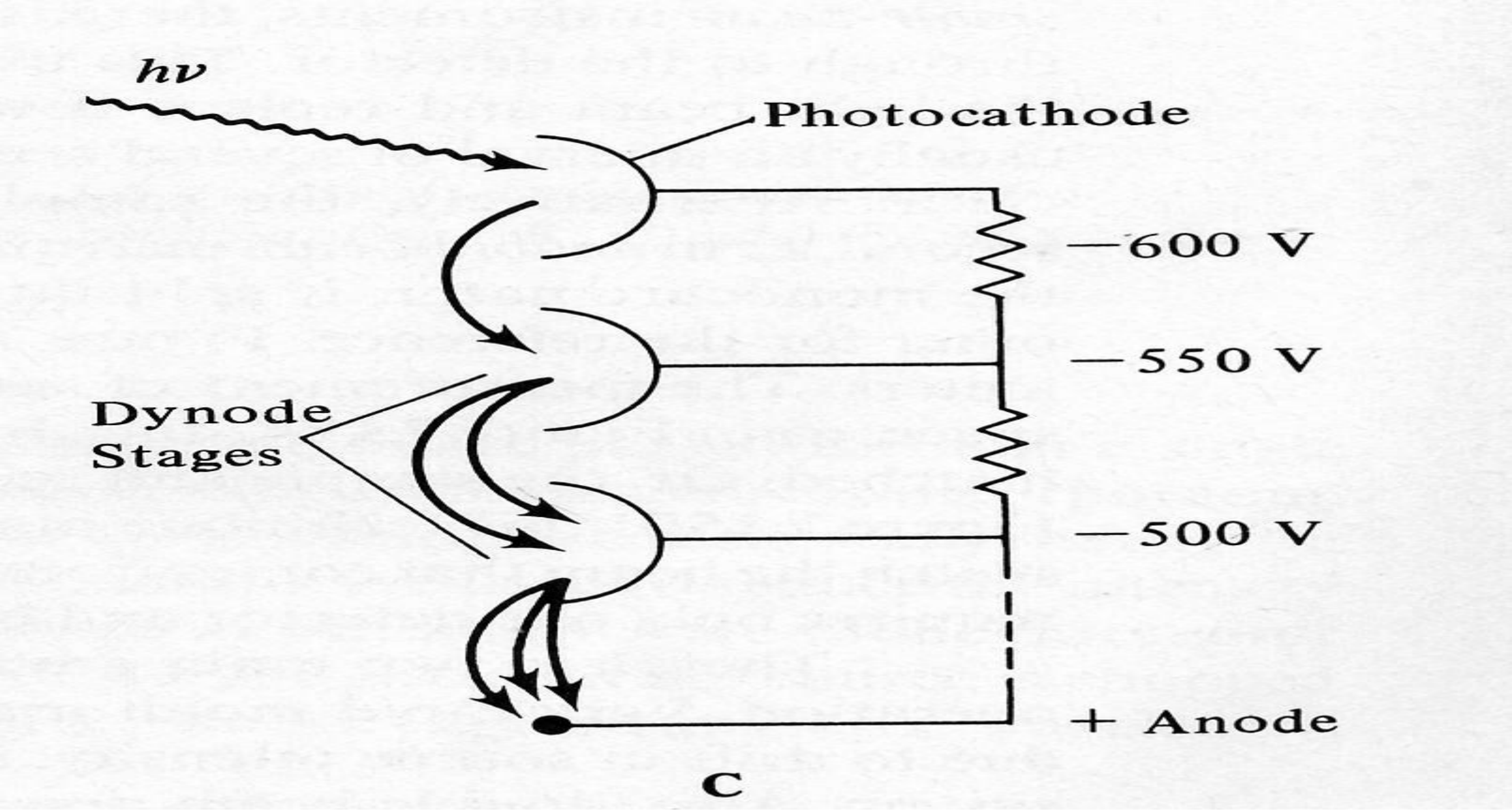
Principle of Barrier Layer/Photovoltaic Detector

- This device measures the intensity of photons by means of the voltage developed across the semiconductor layer.
- Electrons, ejected by photons from the semiconductor, are collected by the silver layer.
- The potential depends on the number of photons hitting the detector.

Detector
Phototube



Detector
Photomultiplier



Principle of Photomultiplier Detector

- The type is commonly used.
- The detector consists of a photoemissive cathode coupled with a series of electron-multiplying dynode stages, and usually called a photomultiplier.
- The primary electrons ejected from the photo-cathode are accelerated by an electric field so as to strike a small area on the first dynode.

Principle of Photomultiplier Detector

- The impinging electrons strike with enough energy to eject two to five secondary electrons, which are accelerated to the second dynode to eject still more electrons.
- A photomultiplier may have 9 to 16 stages, and overall gain of 10^6~10^9 electrons per incident photon.

Single and Double Beam Spectrometer

- Single-Beam: There is only one light beam or optical path from the source through to the detector.
- Double-Beam: The light from the source, after passing through the monochromator, is split into two separate beams-one for the sample and the other for the reference.

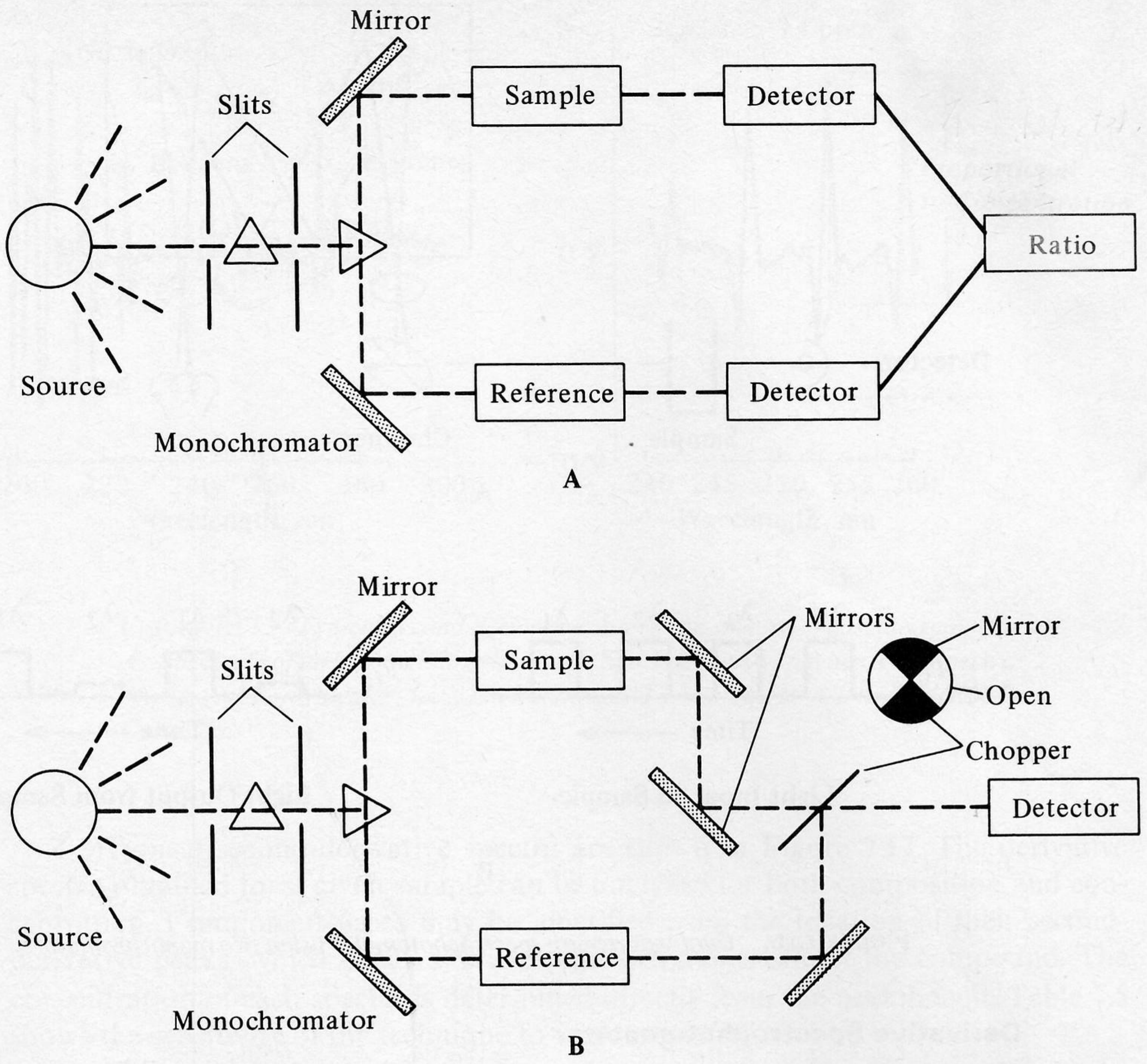
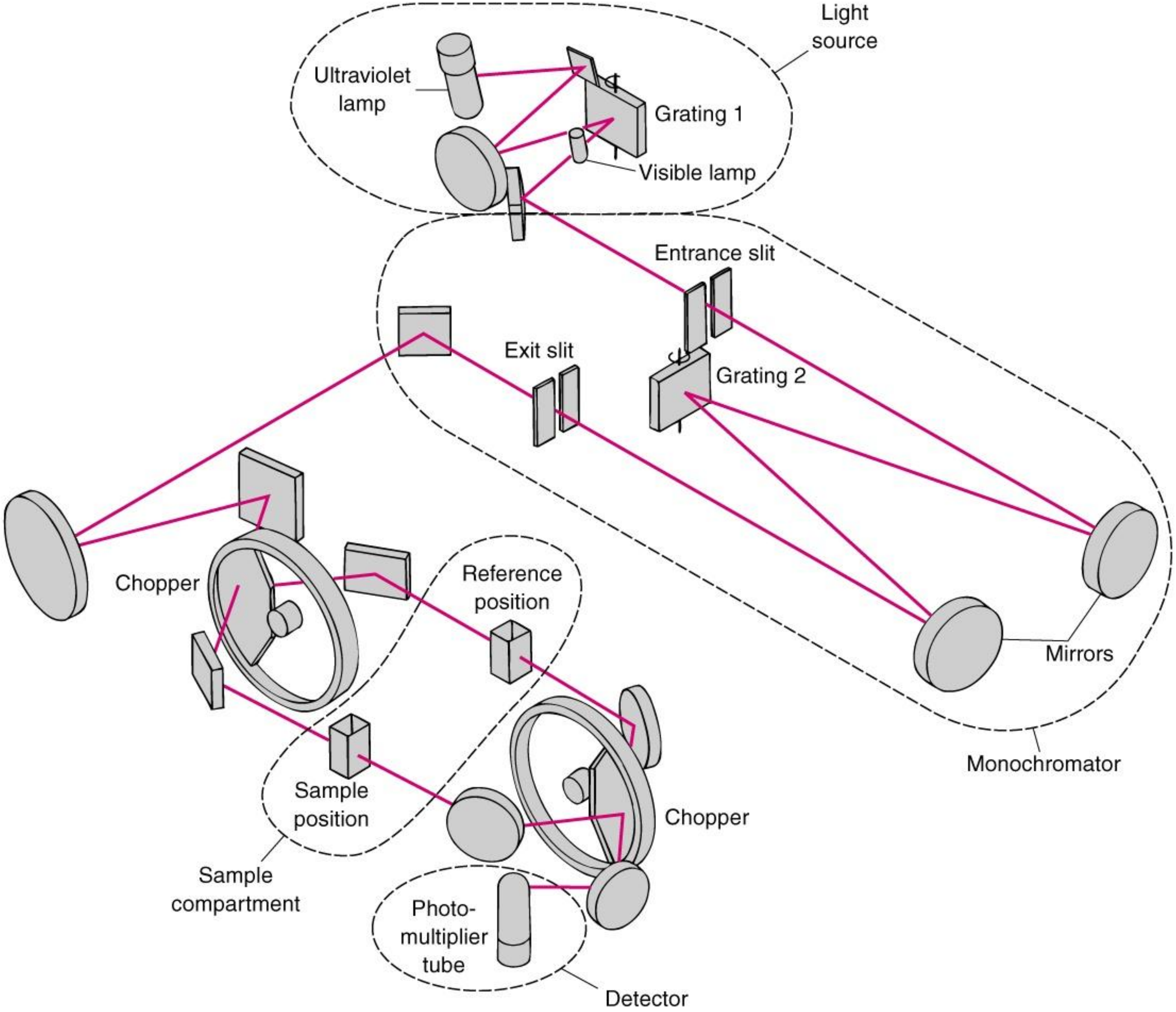


FIGURE 7.15. *Schematic diagram of two types of double-beam spectrophotometers. A: Double-beam-in-space configuration. B: Double-beam-in-time configuration.*


Light source
Ultraviolet lamp
Grating 1
Visible lamp
Entrance slit
Exit slit
Grating 2
Mirrors
Monochromator
Chopper
Reference position
Sample position
Sample compartment
Chopper
Photo-multiplier tube
Detector

(*b*)

Quantitative Analysis Beer's Law

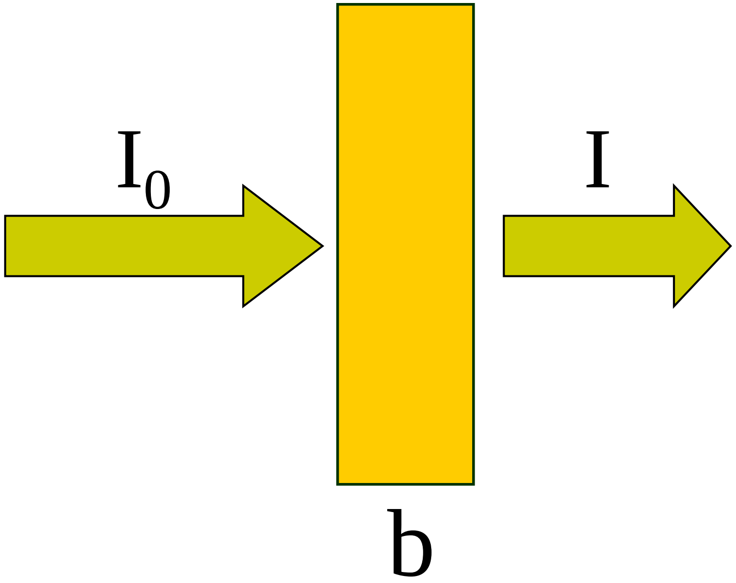
$A=\varepsilon bc$

ε: the molar absorptivity (L mol^{-1} cm^{-1})

b: the path length of the sample

c :the concentration of the compound in solution, expressed in mol L^{-1}

Transmittance



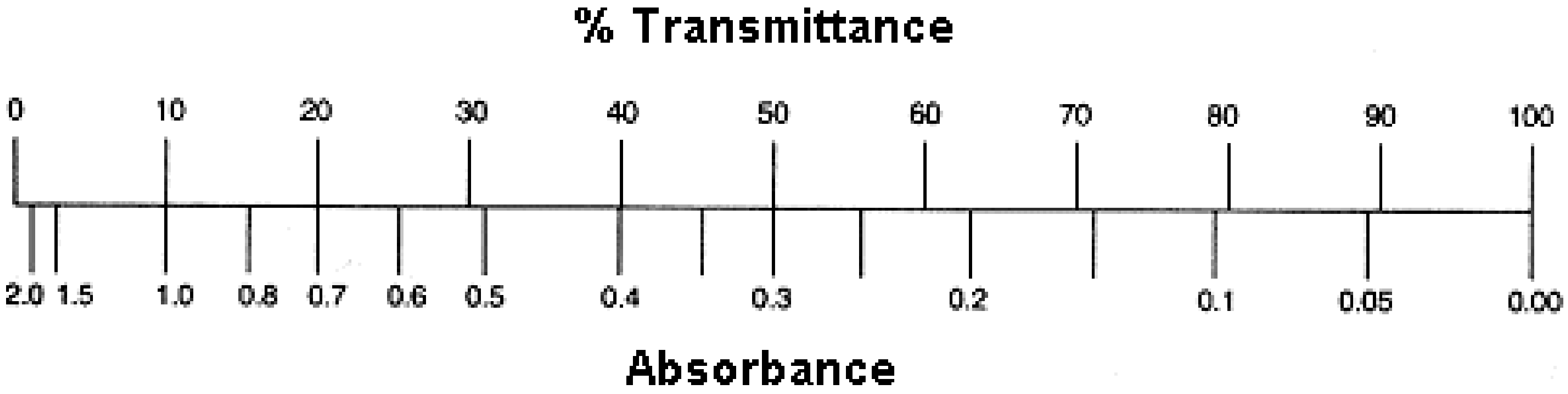
$$T = \frac{I}{I_0} \Rightarrow \frac{dI}{I_0} = kcdb$$

$$\int_{I_0}^{I} \frac{dI}{I_0} = -kc\int_0^b db$$

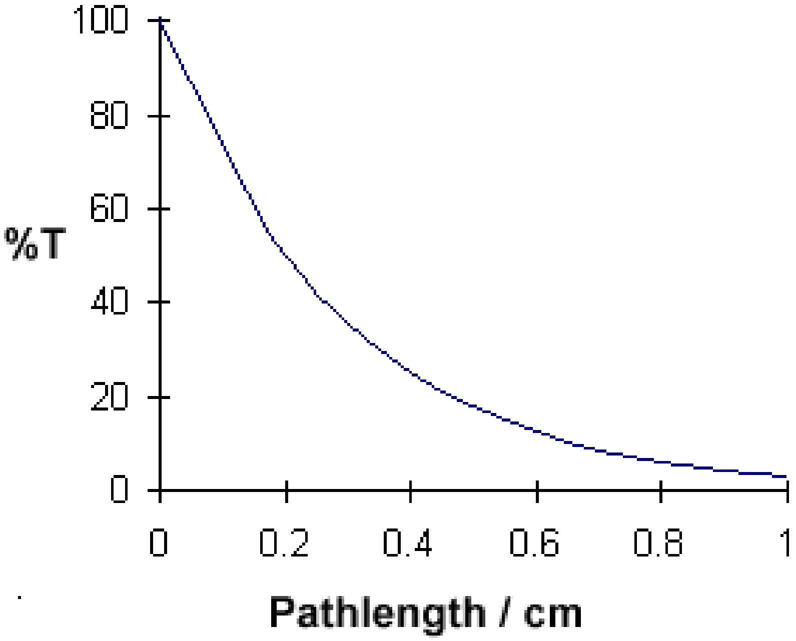
$$\Rightarrow \ln(\frac{I}{I_0}) = -kbc = 2.303\log(\frac{I}{I_0})$$

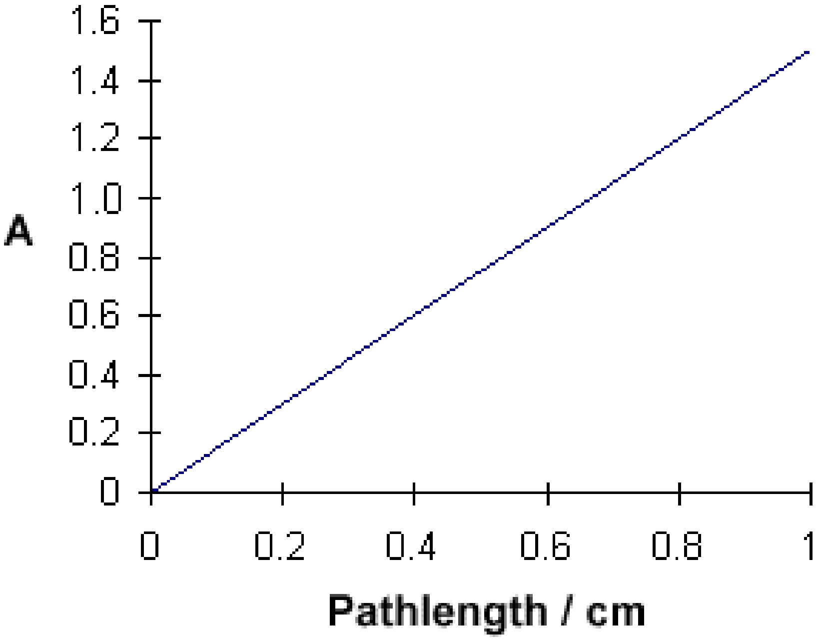
$$\Rightarrow -\log(\frac{I}{I_0}) = -\log T = A = \varepsilon bc$$

$$\varepsilon = \frac{k}{2.303}$$

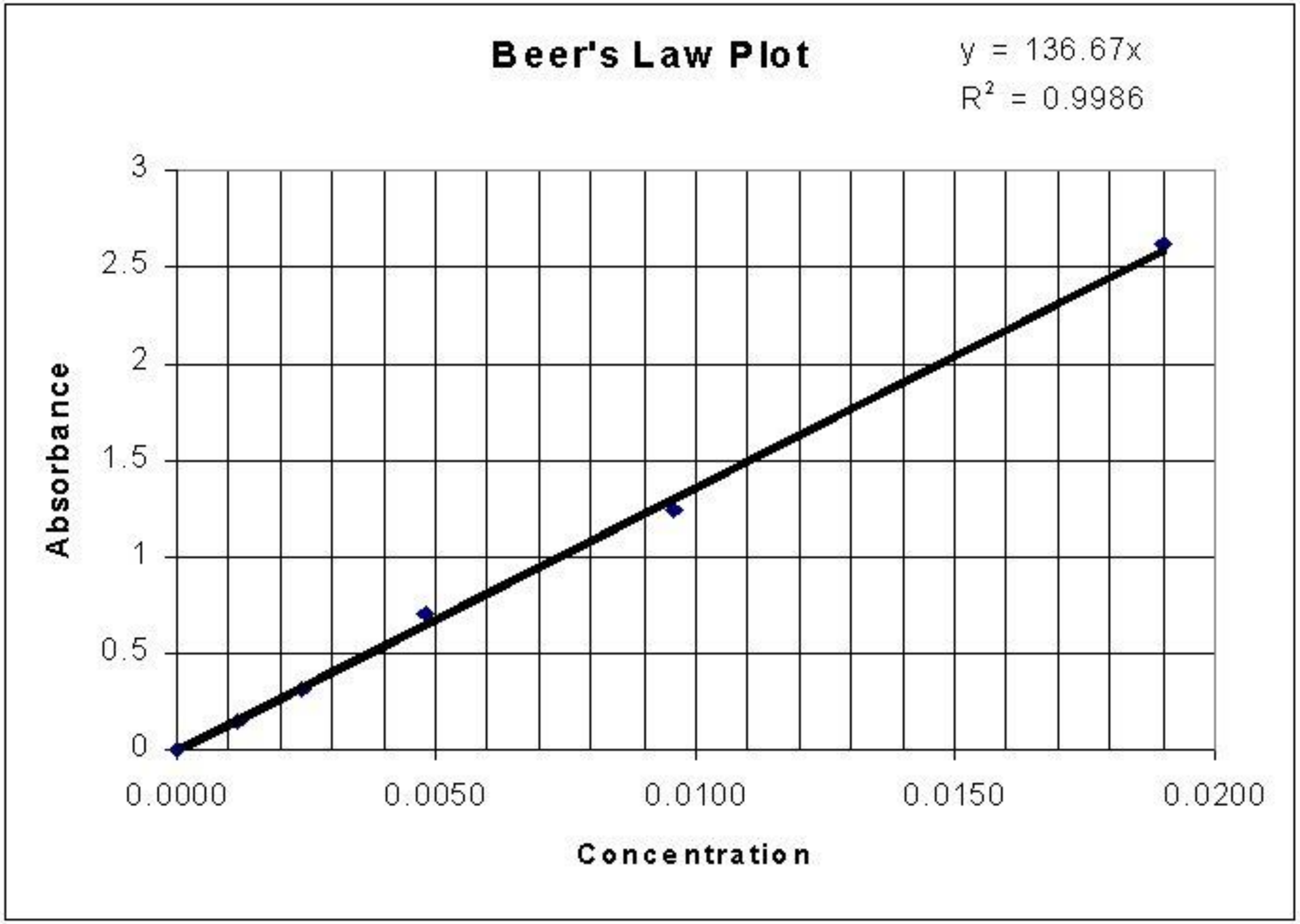

% Transmittance
0
10
20
30
40
50
60
70
80
90
100
2.0
1.5
1.0
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.05
0.00
Absorbance

Path length / cm	0	0.2	0.4	0.6	0.8	1.0
%T	100	50	25	12.5	6.25	3.125
Absorbance	0	0.3	0.6	0.9	1.2	1.5





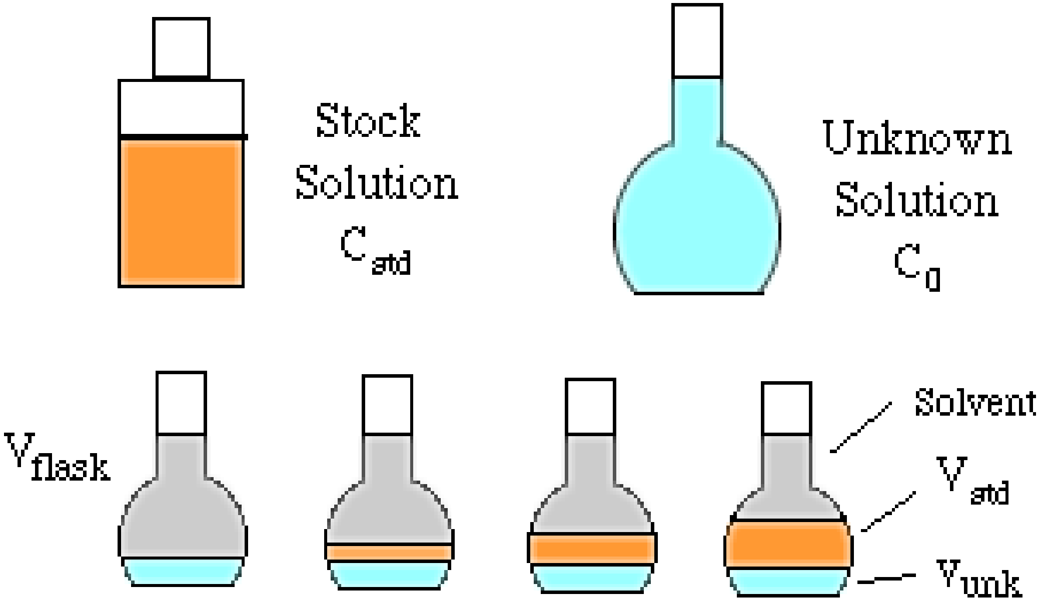
External Standard and the Calibration Curve



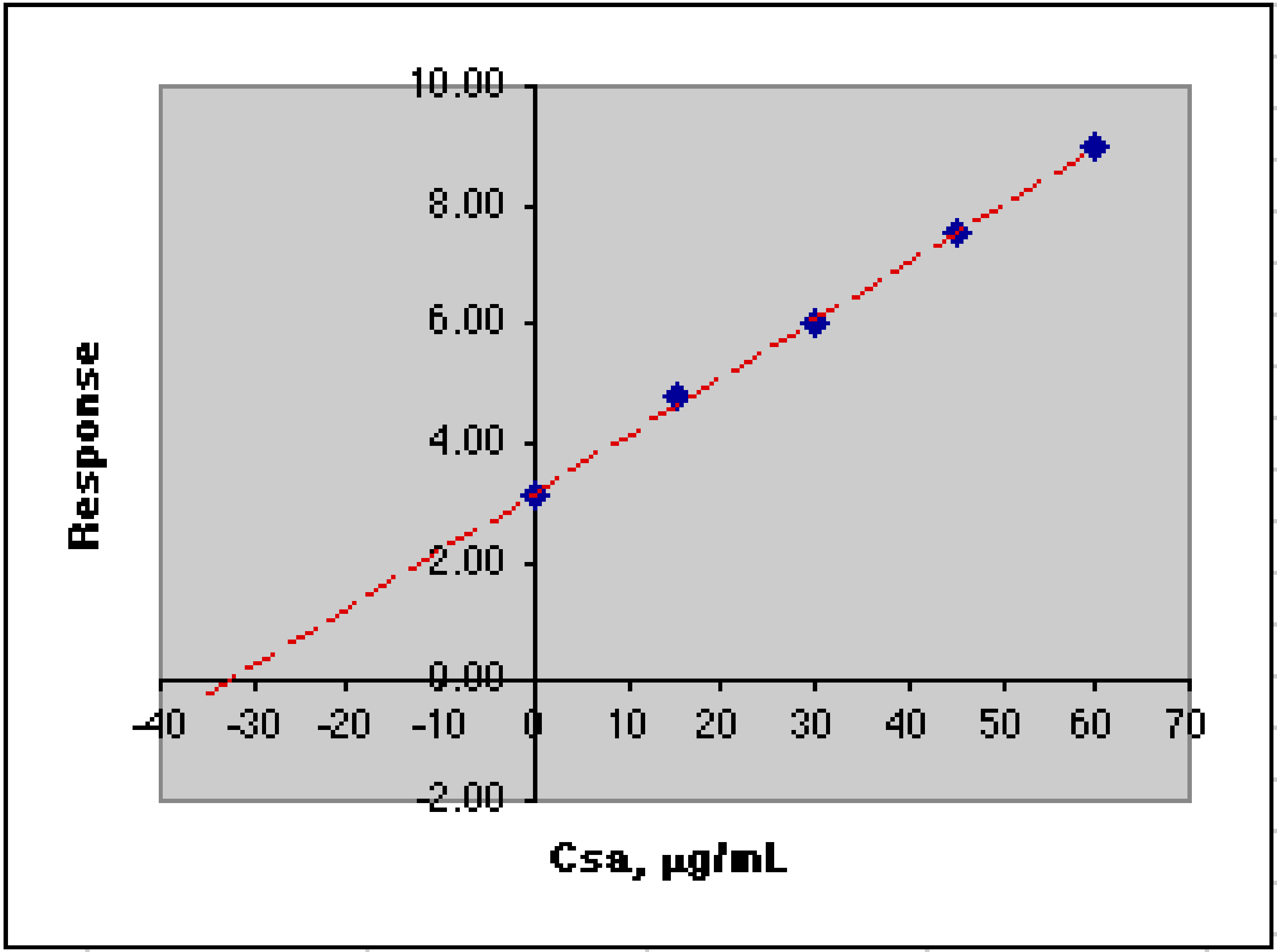
Standard Addition Method

- Standard addition must be used whenever the matrix of a sample changes the analytical sensitivity of the method. In other words, the slope of the working curve for standards made with distilled water is different from the same working curve.

Prepare the Standards



The concentration and volume of the stock solution added should be chosen to increase the concentration of the unknown by about 30% in each succeeding flask.



10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-40
-30
-20
-10
0
10
20
30
40
50
60
70
Response
Csa, µg/mL

$$A = \varepsilon bC$$

$$A = \frac{\varepsilon bV_x C_x}{V_t} + \frac{\varepsilon bV_s C_s}{V_t} = kV_x C_x + kV_s C_s$$

$$y = b + ax \;\; (a = kV_s,\, x = C_s,\, b = kV_x C_x)$$

$$A = 0 \Rightarrow kV_x C_x = -kV_s C_s \Rightarrow C_x = -\frac{V_s C_s}{V_x}$$

C_x: *unknown concentration*

Limits to Beer's Law

- Chemical Deviations
  - absorbing undergo association, dissociation or reaction with the solvent
- Instrumental Deviations
  - non-monochromatic radiation
  - stray light

Limits to Beer's Law

Chemical Deviations

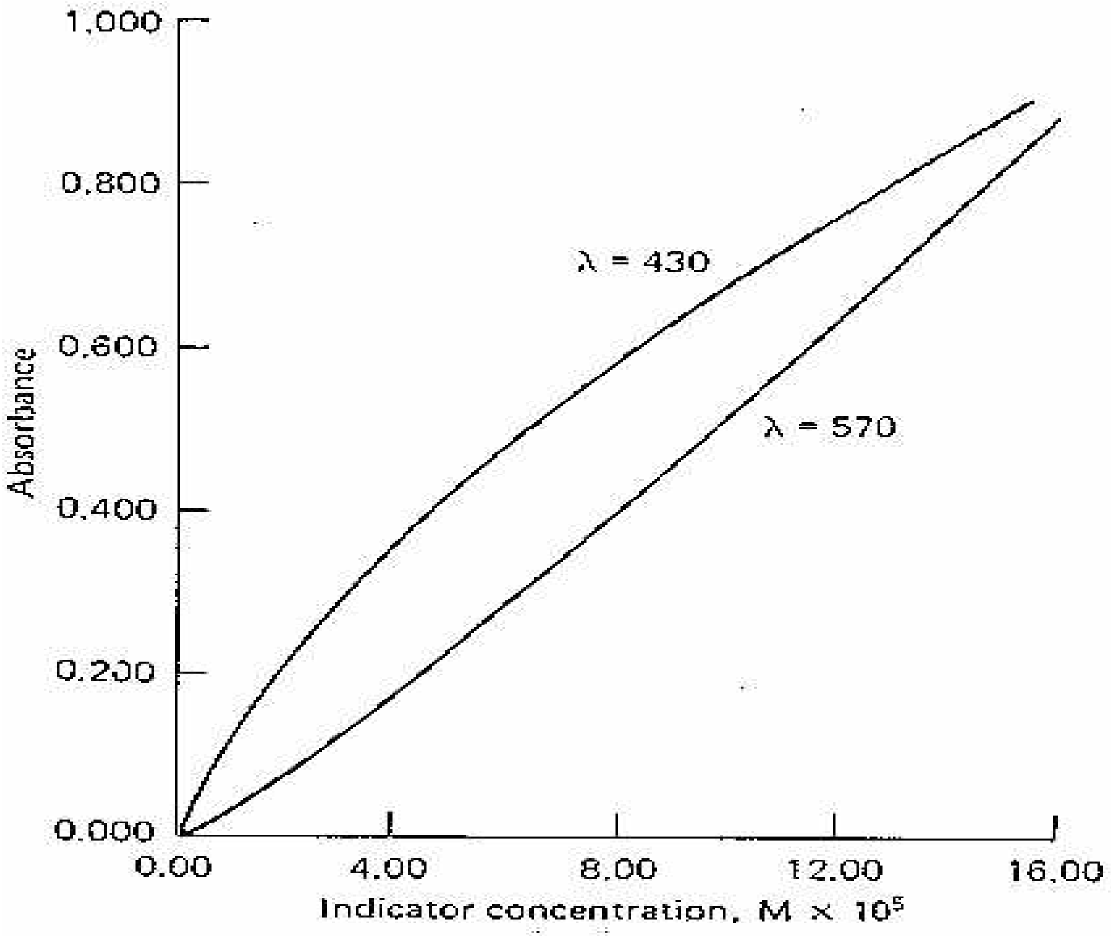
- high concentration – particles too close
- Average distance between ions and molecules are diminished to the point.
- Affect the charge distribution and extent of absorption.
- Cause deviations from linear relationship.

Limits to Beer's Law

Chemical Deviations

- chemical interactions — monomer-dimer equilibria, metal complexation equilibria, acid/base equilibria and solvent-analyte association equilibria

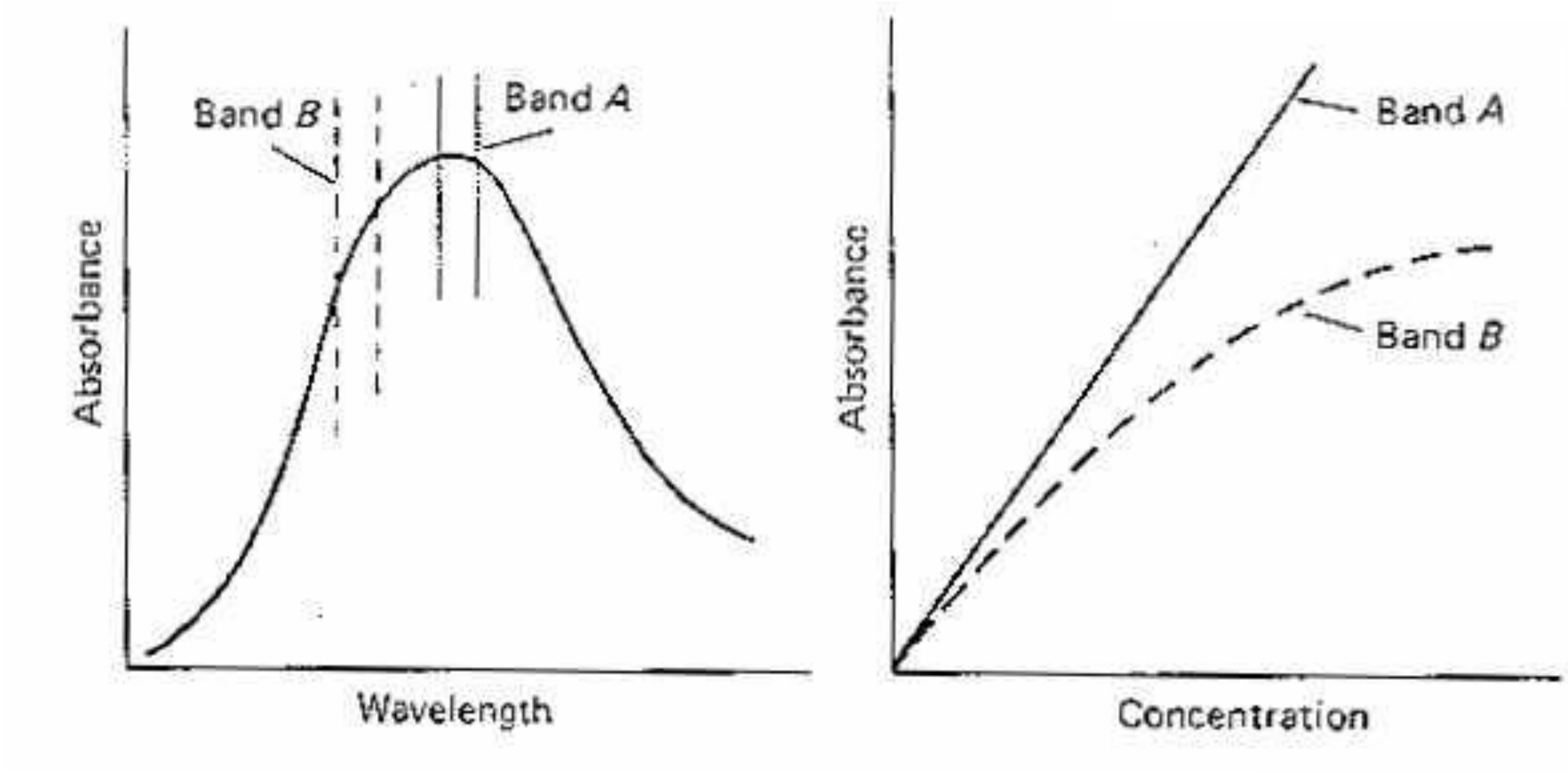
  The extent of such departure can be predicted from molar absorptivities and equilibrium constant. (see p561 ex 21-3)


1.000
0.800
0.600
0.400
0.200
0.000
Absorbance
λ = 430
λ = 570
0.00
4.00
8.00
12.00
16.00
Indicator concentration, M × 10⁵

Limits to Beer's Law

Instrumental Deviations

- non-monochromatic radiation

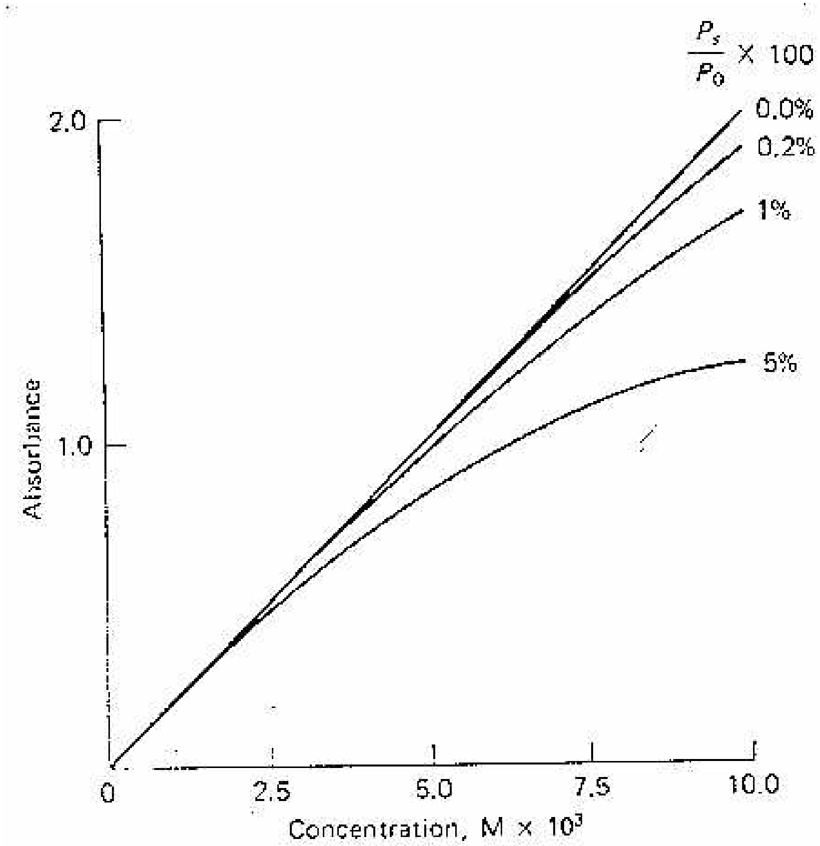


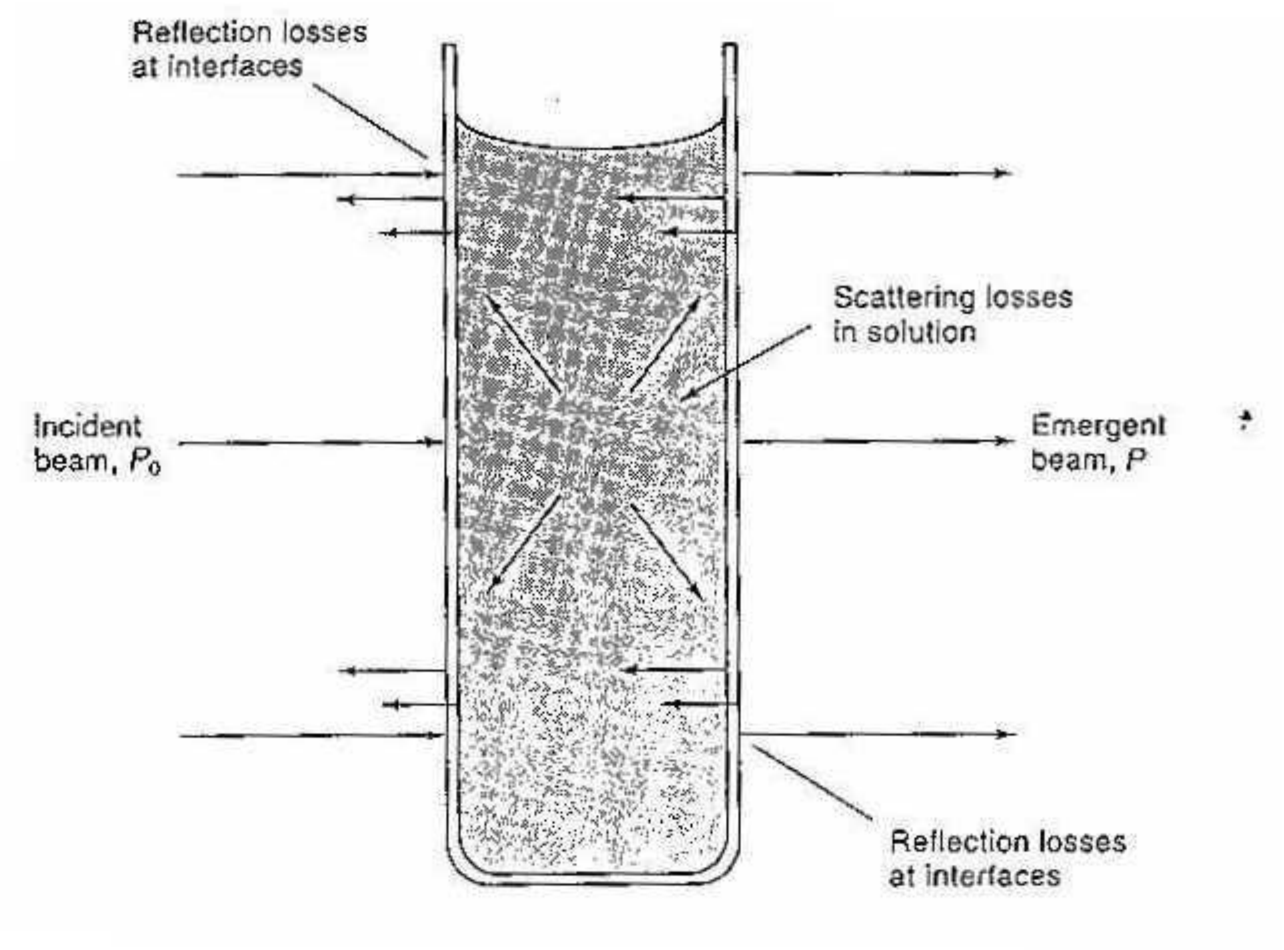
Limits to Beer's Law

Instrumental Deviations

- Stray light

$$A_m = \log \frac{(P_o' + P_o'')}{(P' + P'')}$$




Reflection losses at interfaces
Scattering losses in solution
Incident beam, P_0
Emergent beam, P
Reflection losses at interfaces