

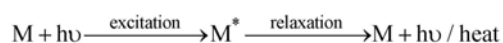
Applying UV-vis Spectrophotometry

Qualitative

- 1) The UV-vis absorption spectrum is related to molecular structure, therefore it provides qualitative information as to structure both from ϵ and λ . In order to take advantage of this it is desirable to have a scanning instrument to measure the entire spectrum (either a double beam, direct reading instrument or a single beam unit with computer interface).

By itself an electronic spectrum is not sufficient to identify a compound (while an IR spectrum is)

Applications of UV-Vis Spectrometry:



How probable?

ϵ ranges 0 to ~100,000 L/mol·cm

Which electrons get excited?

In UV-Vis, photon provides enough energy to move **outer valence (bonding) electrons**

Arrange in terms of **energy**:

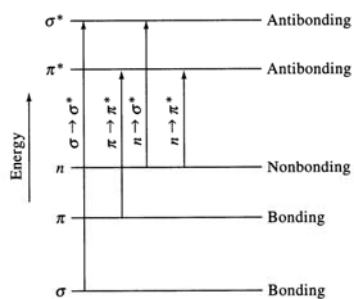


Fig 14-3

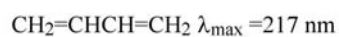
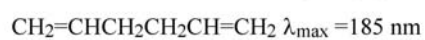
$\sigma \rightarrow \sigma^*$ ΔE large ($\lambda < 150$ nm) $\epsilon = 10-10,000$ L/mol·cm

$n \rightarrow \sigma^*$ (halogens, N, O, S) ΔE smaller ($\lambda = 150-250$ nm)
 $\epsilon = 200-2000$ L/mol·cm

$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ ΔE small ($\lambda = 200-700$ nm) $\epsilon = 10-10,000$ L/mol·cm

Ideal for UV-Vis spectrometry of organic chromophore

- **Red shift** of λ_{\max} with increasing **conjugation**

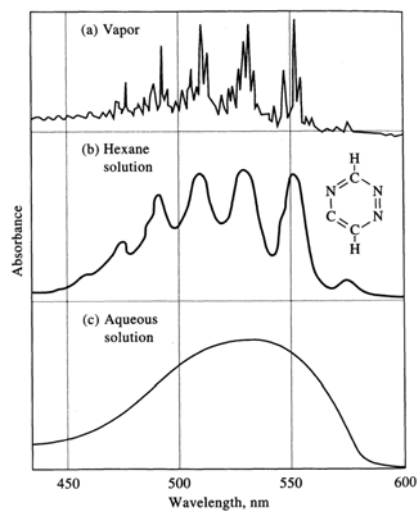


- **Red shift** of λ_{\max} with **# of rings**



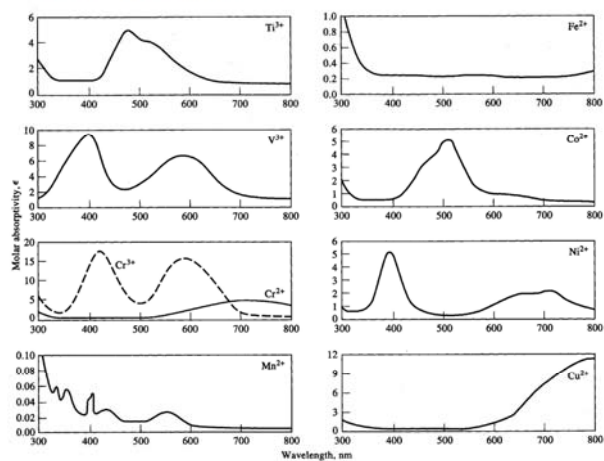
- Blurred with **solvent**

- Blurred with solvent



Inorganic Ions

Most transition metal ions are colored (absorb in UV-vis) due to $d \rightarrow d$ electronic transitions (Fig 14-7)

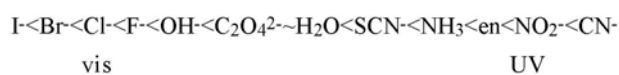


Remember:

- Solution absorbs red appears blue-green
- Solution absorbs blue-green appears red

Ligand Field Strengths:

	λ_{\max} for complex (nm)				
	Increasing Ligand Field Strength \rightarrow				
	6Cl ⁻	6H ₂ O	6NH ₃	3en	6CN ⁻
Cr(III)	736	573	462	456	380



Solvent Effects:

(i) Solvent transparency in UV (Table 14-6)

Solvent	Approximate* Transparency Minimum (nm)
Water	190
Ethanol	210
<i>n</i> -Hexane	195
Cyclohexane	210
Benzene	280
Diethyl ether	210
Acetone	330
1,4-Dioxane	220

*For 1-cm cells.

(ii) Polar solvents "blur" vibrational features more than nonpolar

(iii) Polar solvents more likely to shift absorption maxima

Shifts of λ_{\max} with solvent polarity

$n \rightarrow \pi^*$ hypsochromic/blue shift

$\pi \rightarrow \pi^*$ bathochromic/red shift

Quantitative

- II) The UV-vis region of the spectrum is particularly well suited to quantitative analysis for several reasons (both chemical & spectroscopic reasons):
- 1) Large values for ϵ make it possible to measure low concentrations
 - 2) Many compounds (both organic & inorganic) absorb in the UV-vis region, but most common solvents do not. This is a limiting factor in IR.

- 3) Absorption bands are broad making it easy to achieve conditions where there are no instrumental deviations from Beer's Law
- 4) Good instrumentation is available, i.e. good sources, detectors & monochromators

- III) UV-vis can be used for studying chemical processes that involve conversion of a non-absorber to an absorber or vice versa

Beer's Law:

$$A = \epsilon bc = -\log T = -\log \frac{I}{I_0} = \log \frac{I_0}{I}$$

Absorbance is **additive**

$$\begin{aligned} A_{\text{total}} &= A_1 + A_2 \dots \\ &= \epsilon_1 bc_1 + \epsilon_2 bc_2 \dots \end{aligned}$$

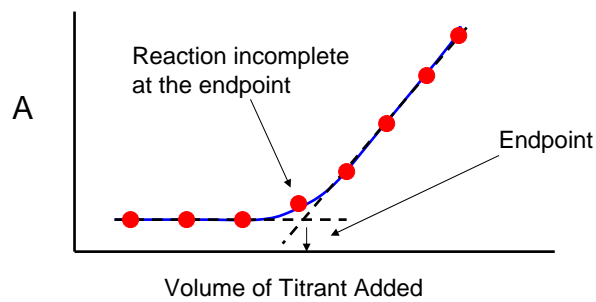
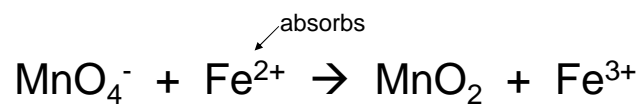
in a 2 component mixture

$$A_{\lambda 1} = \epsilon_{1,\lambda 1} \cdot b \cdot c_1 + \epsilon_{2,\lambda 1} \cdot b \cdot c_2$$

$$A_{\lambda 2} = \epsilon_{1,\lambda 2} \cdot b \cdot c_1 + \epsilon_{2,\lambda 2} \cdot b \cdot c_2$$

1) Spectrophotometric Titrations
Example where titrant absorbs

Sample + Titrant \rightarrow Product



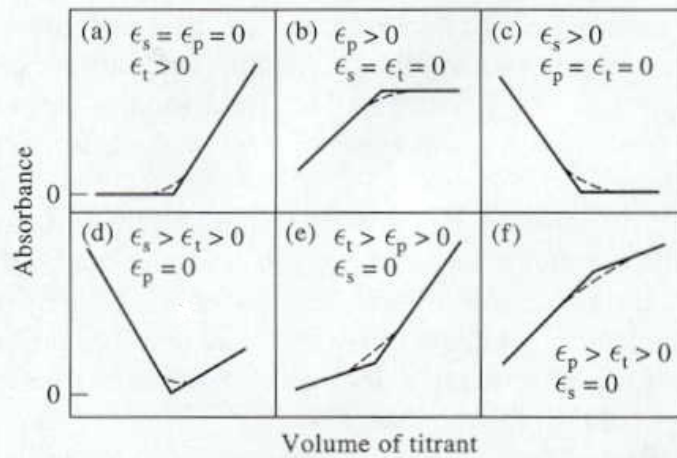
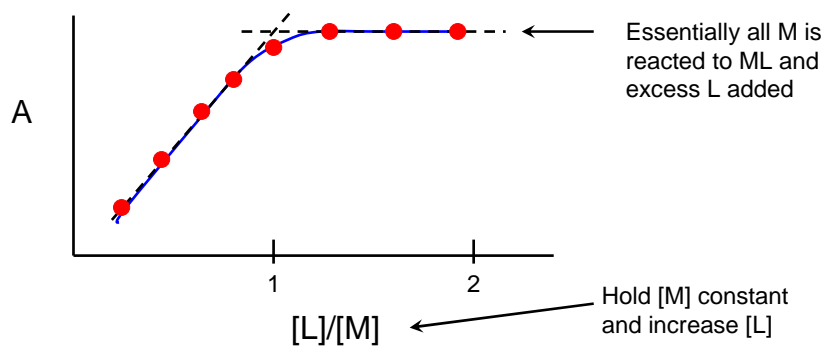
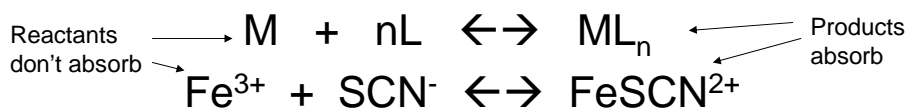


Figure 14-18 Typical photometric titration curves. Molar absorptivities of the substance titrated, the product, and the titrant are given by ϵ_s , ϵ_p , ϵ_t , respectively.

2) Study Equilibria (acid-base, complex formation, redox, etc.)



Can calculate ϵ_{ML} from A at excess [L]

Get n from extrapolated curve

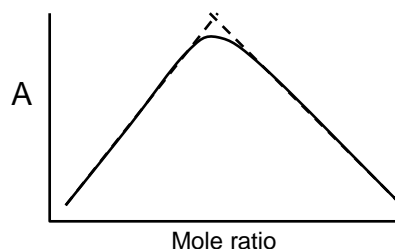
Can calculate equilibrium constant (K) from

$$K = \frac{[ML_n]}{[M][L]^n}$$

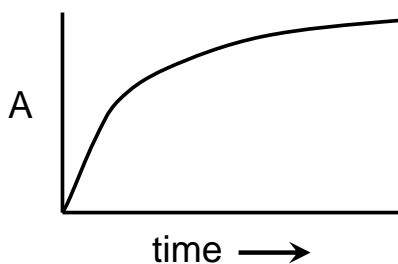
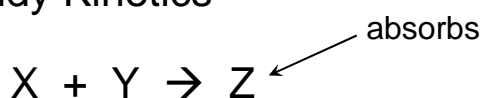
and mass balances

e.g. $C_M = [M] + [ML_n]$

Other calculation methods can be used involving graphical or computer curve fitting procedures, e.g. Job's Method or the Method of Continuous Variation



3) Study Kinetics



Measure A vs time after mixing

For quantitative applications, we usually want to measure at one particular λ . Must first scan spectrum to determine the most suitable λ for measurements.

Errors in Photometric Analysis

- 1) Deviations from Beer's Law - either chemical or instrumental can be handled, in general, by preparing a calibration curve

Important – the calibration curve should include standards both at higher & lower concentrations than the sample

- 2) Technique errors – cells must be carefully cleaned & kept free of scratches, fingerprints, etc. Clean in concentrated HNO_3 . Rinse several times with water. Rinse with sample before use.

Round cells, e.g. Spectronic 20 cells should be positioned the same way for each measurement because of imperfections in glass (square cells too)

- 3) Source drift – in single beam instruments.
Must: a) Allow source sufficient time to warm up
b) Check 0 and 100 % T settings frequently

4) Stray Radiation – strikes detector without going through sample

$$A_{\text{true}} = \log \frac{I_0}{I}$$

$$A_{\text{measured}} = \log \frac{I_0 + I_s}{I + I_s} \quad \begin{array}{l} \text{where } I_s \text{ is intensity} \\ \text{of stray radiation} \end{array}$$

Stray Light Specification – important characteristic of monochromators

Stray Light Specification

e.g. Cary 14 $I_s = < 10^{-6} I_0$ at all λ 's

Spectronic 505 $I_s = < 10^{-3} I_0$

Stray light more serious at high concentrations (i.e. high A) → makes it difficult to measure high A (i.e. A = 3 or 4) Some instruments are capable of measuring large absorbance

Spec 505

for $A_{\text{true}} = 2$

$$= \log \frac{I_0}{I} = \log \frac{100}{1} = 2$$

$$A_{\text{measured}} = \log \frac{100 + 0.1}{1 + 0.1} = 1.96$$

$$I_s = 10^{-3} \times 100 = 0.1$$

5) Reading Error – random error involved in reading A value; today with digital readouts this usually involves:

- 1) Not allowing instrument to stabilize before taking the reading
- 2) Assuming greater precision (or accuracy) than the instrument is capable of
- 3) Biasing the result

e.g. 0.019 ± 0.002 and 0.021 ± 0.002

Are these 2 readings different from one another?

Answer is NO! What about if s.d. is 0.001?

Scale Expansion – sometimes reading error can be improved upon by expanding scale

Normally use a blank solution to set 100% T & set 0% T with shutter closed (i.e. no light)

Expand scale by using a standard that has (for example) 50% T & set instrument to 100% T → this expands lower end of scale by a factor of 2 & allows readings in the 0% T to 50% T range to be made with greater accuracy & precision

There is a limit to scale expansion imposed by instrument stability, so you can only expand scale to a point, then source and detector noise become limiting factors

Can expand both ends of the scale although it is usually preferable to dilute

Often reading error is not significant so it doesn't help to expand the scale

- HW4
- 14-1
- 14-8
- 14-21

Luminescence Spectroscopy (Chapter 15)

- fluorescence, phosphorescence, chemiluminescence
- all follow electronic excitation

Luminescence – light emission accompanying a transition from higher to lower energy levels

- 1) Phosphorescence – example of photoluminescence (excited state generated by photons) often exhibited by **solids** like glow in the dark key chains, television screens (CRTs) & “fluorescent” lights change in electronic spin. Longer time(seconds to minutes)
- 2) Fluorescence – also photoluminescence which is usually observed in **solution** like quinine that is added to tonic water many analytical applications no change in electronic spin. Short lived (10^{-5} s)

- 3) Bioluminescence – excited state induced biologically (enzymatic process) exhibited by fireflies, some algae & fish
- 4) Chemiluminescence – excited state induced chemically by bond breakage, often oxidation & used for light sticks & some analytical applications
- 5) Triboluminescence – excited state induced mechanically → bite down on hard candy, also Curad bandaids
- 6) Electroluminescence – electrical excitation found in some polymers & electric pickle

Photoluminescence is the most useful kind of luminescence for analysis because:

- 1) Many compounds exhibit this phenomenon
- 2) The effect can be readily produced
- 3) Several variables can be used to control the process (e.g. excitation λ , emission λ , pulsing or modulating excitation source, gating or synchronizing detector response)
- 4) Inherent sensitivity, DL 3 orders lower than absorption
- 5) Larger linear concentration range

Chemiluminescence can be used for analysis also, but it is harder to control

Bioluminescence is chemiluminescence

Others are basically useless analytically

Excited Electronic States:

Each electron has unique set of *quantum numbers* (Pauli Exclusion Principle)

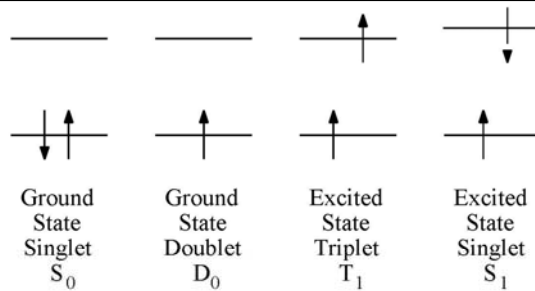
- n principal (1s, 3p...)
- l angular momentum (l=0=s, l=1=p...)
- s spin
- m magnetic

Any two electrons in same orbital (n, l, m) must have **different spins**

$$s = +\frac{1}{2} \text{ or } -\frac{1}{2}$$

$$S = \sum |s_i|$$

Multiplicity: $2S+1$ (either 1, 2, 3...)



Multiplicities:

S_0 - **common**, diamagnetic (not affected by B fields)

D_0 - unpaired electron, many radicals, two equal energy states

T_1 - **rare**, paramagnetic (affected by B fields)

(T_0 - doesn't exist, not ground state)

$$\text{Energy}(S_1) > \text{Energy}(T_1)$$

(difference is energy required to flip electron spin)

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 $1s^2 2s^2 2p^6 3p^1$

D_1 written $3(2P)$

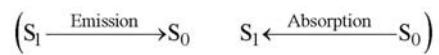
BUT two spins states?

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 $3p \rightarrow 3s$ fluorescence two lines at **589.6 nm** ($2P_{3/2}$) and **589.0 nm** ($2P_{1/2}$)



What about Lifetimes?

- **Absorption:**

$S_1 \leftarrow S_0$ very fast 10^{-15} - 10^{-13} s

- **Relaxation:**

Resonant emission $S_1 \rightarrow S_0$ fast 10^{-9} - 10^{-5} s (**fluorescence**)

common in atoms

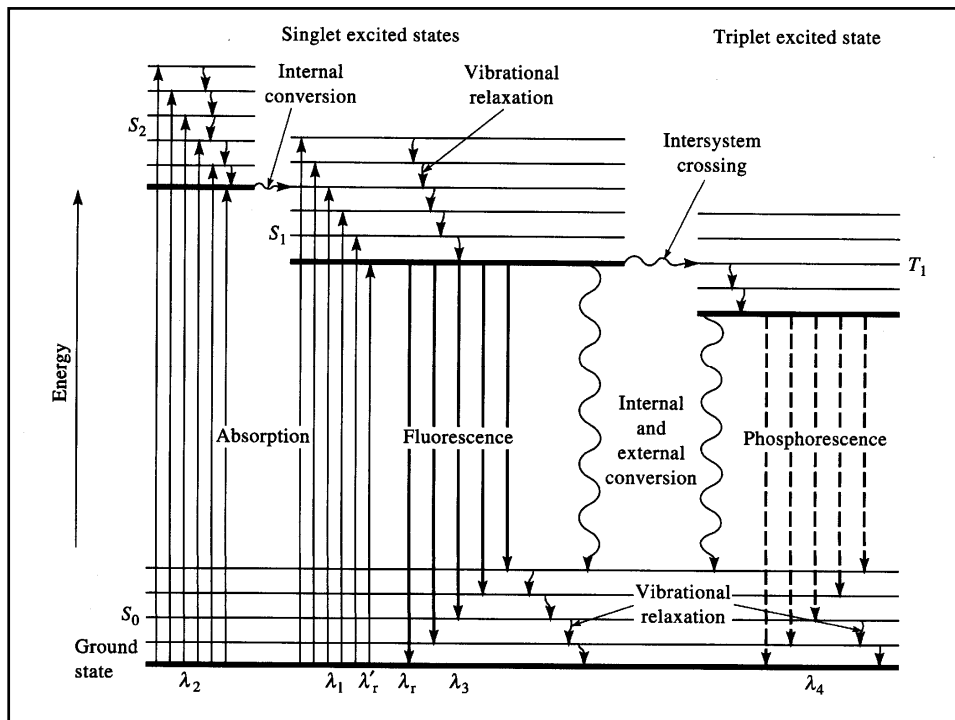
strong absorber \Rightarrow shorter lifetime

Non-resonant emission $S_1 \rightarrow S_0$ fast 10^{-9} - 10^{-5} s (**fluorescence**)

common in molecules

v. fast vibrational relaxation

red shifted emission (**Stokes shift**)



Internal Conversion: radiationless transition to lower state when vibrational energy levels "match"

External Conversion: radiationless transition to lower state by collisional deactivation

Intersystem Crossing: transition with spin change (e.g. S to T)

Fluorescence: emission not involving spin change (e.g. $S \rightarrow S$, $T \rightarrow T$), efficient, short-lived $< 10^{-5}$ s

Phosphorescence: emission involving spin change ($T \rightarrow S$), improbable, long-lived $> 10^{-5}$ s

Dissociation: excitation to vibrational state with enough energy to break bond

Predissociation: relaxation to state with enough energy to break bond

When absorption of a photon occurs (process #1), several things can happen:

Vibrational Relaxation (VR) - is a non-radiative process (#2) by which the upper vibrational levels lose energy & go to the lowest vibrational level in a given electronic energy state (very fast $\sim 10^{-12}$ s)

Internal Conversion (IC) - non-radiative process (#4) where excited state couples to upper vibrational level of lower electronic energy level followed by VR

Because of Vibrational Relaxation (VR) all absorbed photons result in the promoted electron ending up in the lowest vibrational level of the **first excited singlet state**

This level can then do one of three things:

1) **Fluoresce** → make transition to one of the vibrational levels of the ground state giving up energy as a photon (process #3)

- 2) **Radiationless Deactivation** (#4) – return to ground state giving up energy as heat, by internal conversion or some sort of collisional deactivation
- 3) **Intersystem Crossing** (#5) – lowest vibrational level of first excited singlet couples to upper vibrational level of triplet state followed by vibrational relaxation
Once formed the triplet state can go to ground state radiationlessly or by emitting a photon = **phosphorescence** (#6)

The transition $T_1 \rightarrow S_0$ (G) with emission of a photon is spin forbidden, has a low probability and a slow rate

Fluorescence involves a spin allowed transition \rightarrow very probable \rightarrow fast rate & short lifetime (typically 1-20 nsec)

Phosphorescence involves spin forbidden transition \rightarrow not so probable \rightarrow slower rate & longer lifetime (from 10^4 – 10 sec)

Moderately interesting website showing an animated Jablonski Diagram for absorption, VR, fluorescence, phosphorescence, etc.

<http://micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/>

Fluorescence Quantum Yield - ratio of number of molecules fluorescing to number excited

$$\Phi_{\text{fluor}} = \frac{\# \text{ photons fluor}}{\# \text{ species excited}} \quad (\Phi_{\text{fluor}} = 0.0 \text{ to } 1.0)$$

$$= \frac{k_{\text{fluor}}}{k_{\text{fluor}} + k_{\text{int con}} + k_{\text{ext con}} + k_{\text{ISC}} + k_{\text{pre dis}} + k_{\text{dis}}}$$

What Factors Affect Φ_{fluor} ?

(1) **Excitation λ**

Short λ 's break bonds increase $k_{\text{pre-dis}}$ and k_{dis}
 rarely observed most common
 $\sigma^* \rightarrow \sigma$ $\pi^* \rightarrow n$ $\pi^* \rightarrow \pi$
 emission usually from *lowest lying* excited state

(2) **Lifetime of state**

Transition probability measured by ϵ
 Large ϵ implies short lifetime
 Largest fluorescence from short lifetime/high ϵ state
 $\pi^* \rightarrow \pi > \pi^* \rightarrow n$ (10^{-9} - 10^{-7} s $>$ 10^{-7} - 10^{-5} s)

(3) **Structure**

Few conjugated aliphatics fluoresce

Many aromatics fluoresce

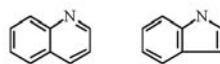
Desire short lifetime S_1 , no/slowly accessible T_1

Fluorescence increased by # fused rings and substitution on/in ring



Pyridine

Pyrrole



Quinoline

Indole

(4) **Rigidity**

Rigid structures fluoresce

Increase in fluorescence with chelation



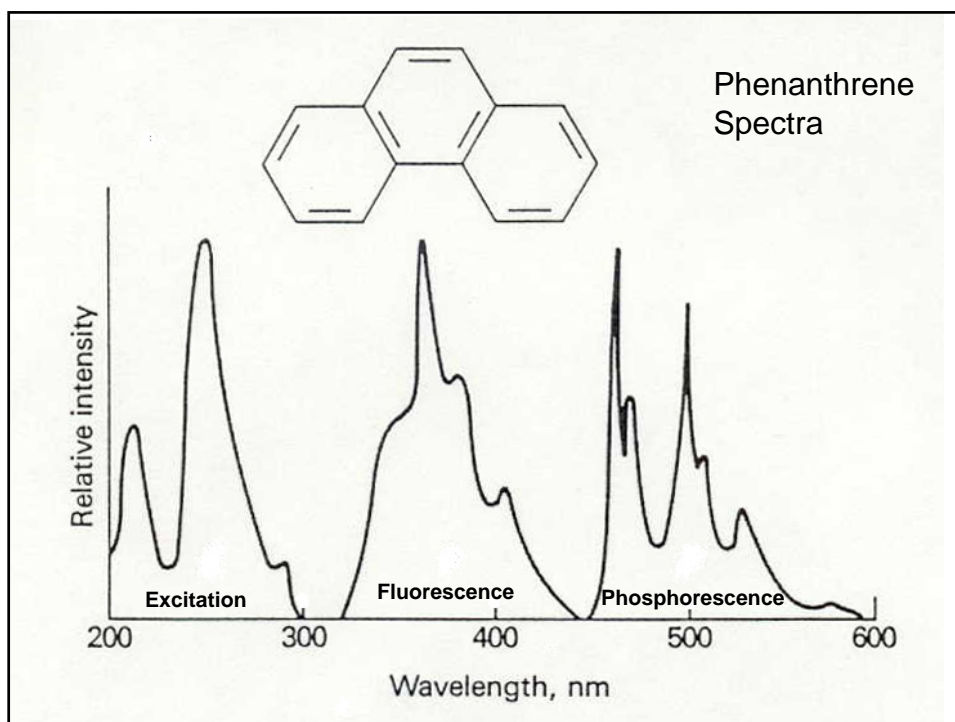
Fluorene

Biphenyl

(5) **Temperature, pH, solvent** (p 363-364)

Spectra

- 1) Excitation Spectrum – fluorescence or phosphorescence intensity (at fixed λ) as a function of excitation λ or absorption λ
- 2) Fluorescence Emission Spectrum - fluorescence emission intensity vs. λ for a fixed excitation λ (= absorption λ , max.)
- 3) Phosphorescence Emission Spectrum – phosphorescence emission intensity vs. λ for a fixed excitation λ (= absorption λ , max.)



Fluorescence and Phosphorescence spectra are at longer λ 's because the transition Δ from $S_1 \rightarrow S_0$ or G is smaller than the absorption Δ from S_0 (G) to some upper vibrational level of S_1

Degree of structure in fluorescence spectrum is due to the degree of solvation as discussed for absorption

The rigidity of the phenanthrene molecule and its inherent lack of interaction with the solvent also plays a role in spectrum

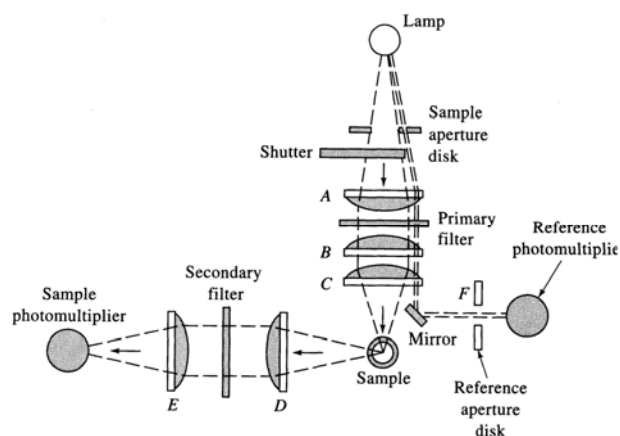
Quantitative Luminescence Spectrophotometry:

$$\begin{aligned} \text{Fluorescence} \\ \vec{F} &= K' \overbrace{(I_0 - I)}^{\text{Absorbed}} \\ &= K' \cdot 2.303 \epsilon b c \cdot I_0 \\ &= K \cdot c \end{aligned}$$

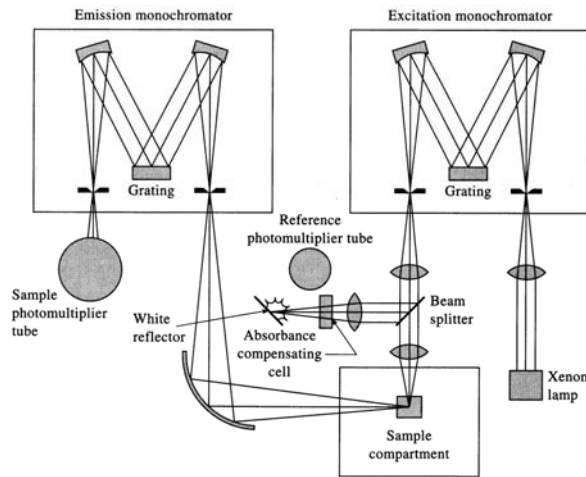
Only works at low A (<0.05)

- (i) self quenching (collisions between excited states)
- (ii) self absorption (when absorption and fluorescence band overlap)

Fluorometer - filters to isolate excitation and fluorescence wavelengths (but no scanning)

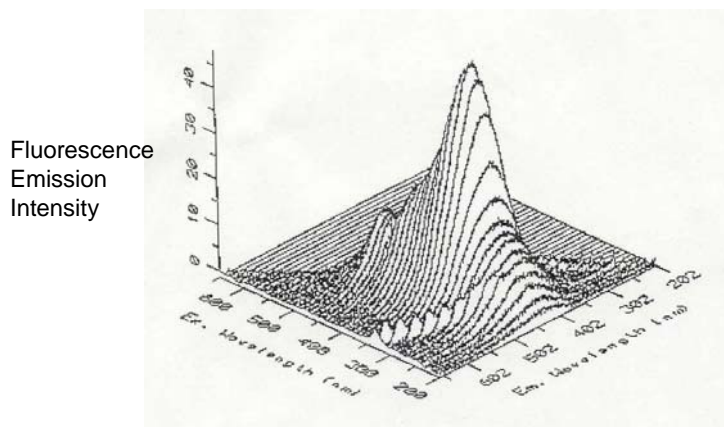


Spectrofluorometer - two monochromators for excitation scanning or fluorescence scanning

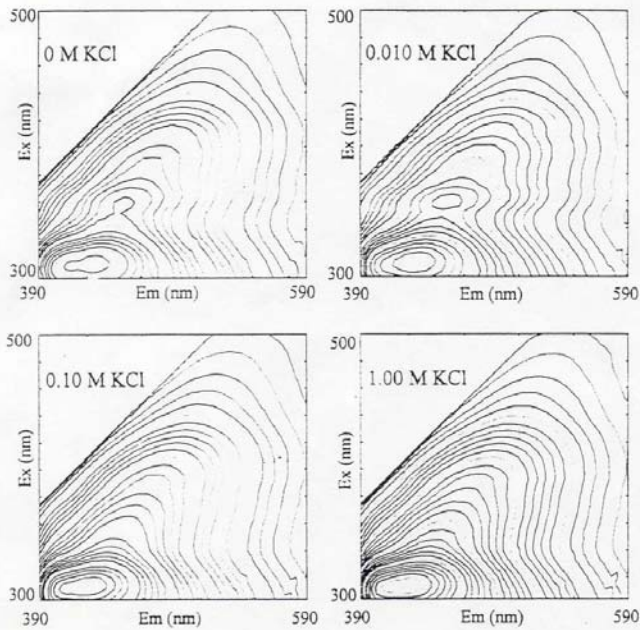
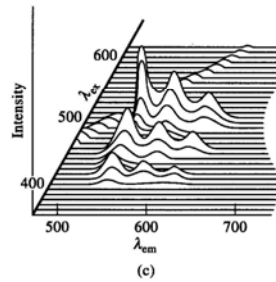
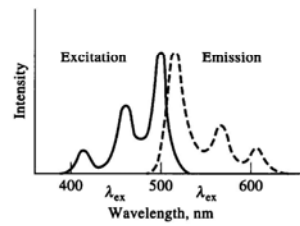
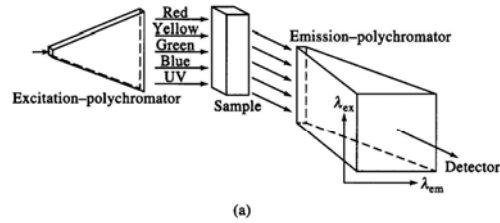


Spectra

Excitation Emission Matrix (EEM) or Total Luminescence Spectrum – 3D plot of emission for all possible combinations of excitation λ and emission λ



Total Luminescence Measurements:

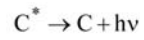
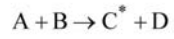


EEMs represented as contour plots

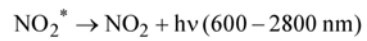
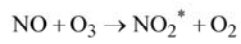
FIGURE 3. Absorbance-corrected EEMs of PFAR (50 mg/L, pH 6.0) at different ionic strengths.

Chemiluminescence: (see www article)

Reaction produces molecule in electronically excited state



Example:



Used to detect NO from 1 ppb to 10 ppt

Intensity depends on **rate** of reaction of production of C^*

$$I_{\text{CL}} = \phi_{\text{CL}} \frac{d[C^*]}{dt}$$

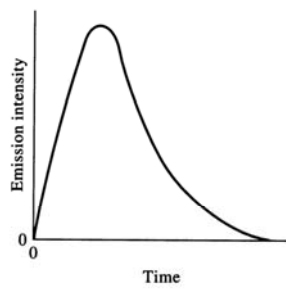


Fig. 15-11

Advantages: (i) simple instrumentation (no excitation $h\nu$) (ii) high sensitivity (ppm to <ppb)

Disadvantages: (i) few reactions