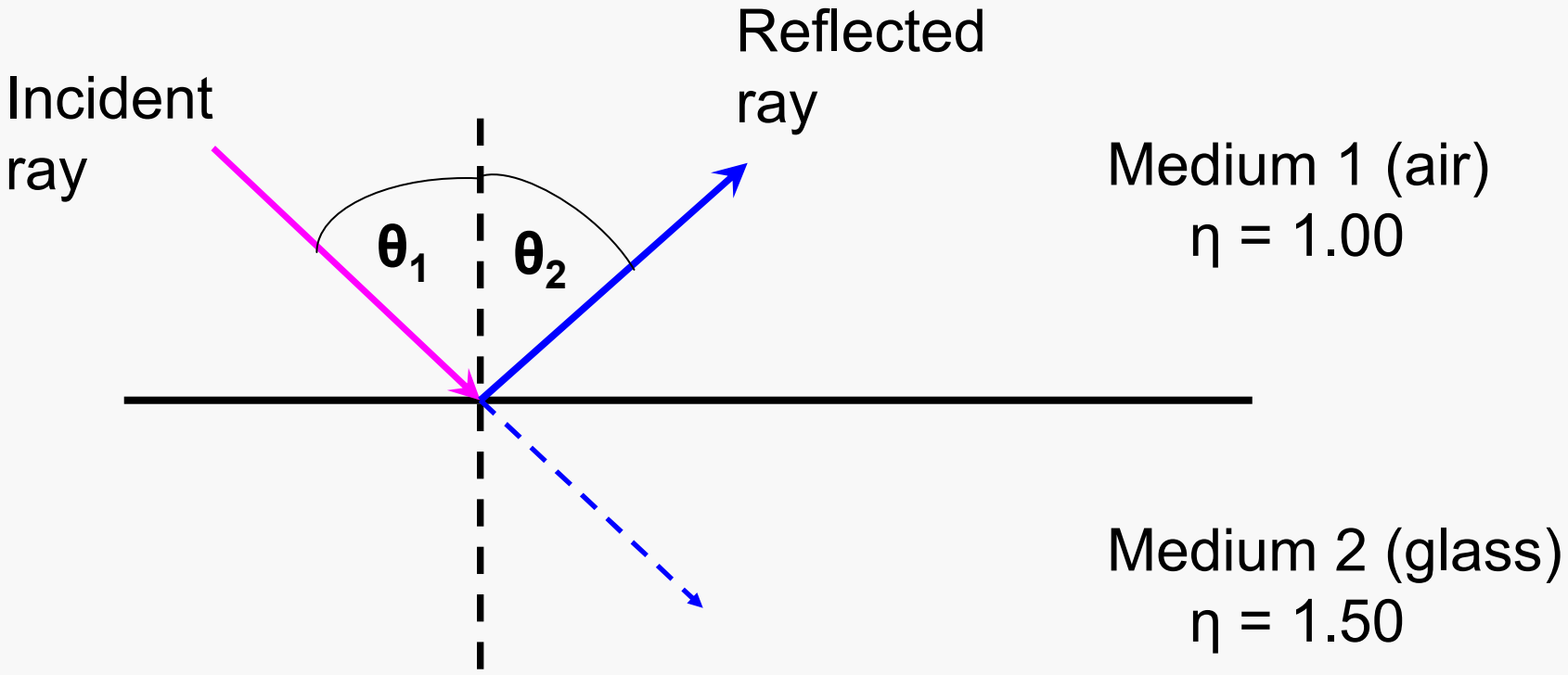
Reflection = EM strikes a boundary between two media differing in η and bounces back



Specular reflection = situation where angle of incidence (θ_i) equals angle of reflection (θ_r)

$$\text{Reflectance} = R = \frac{I_r}{I_i} = \frac{(\eta_2 - \eta_1)^2}{(\eta_2 + \eta_1)^2}$$

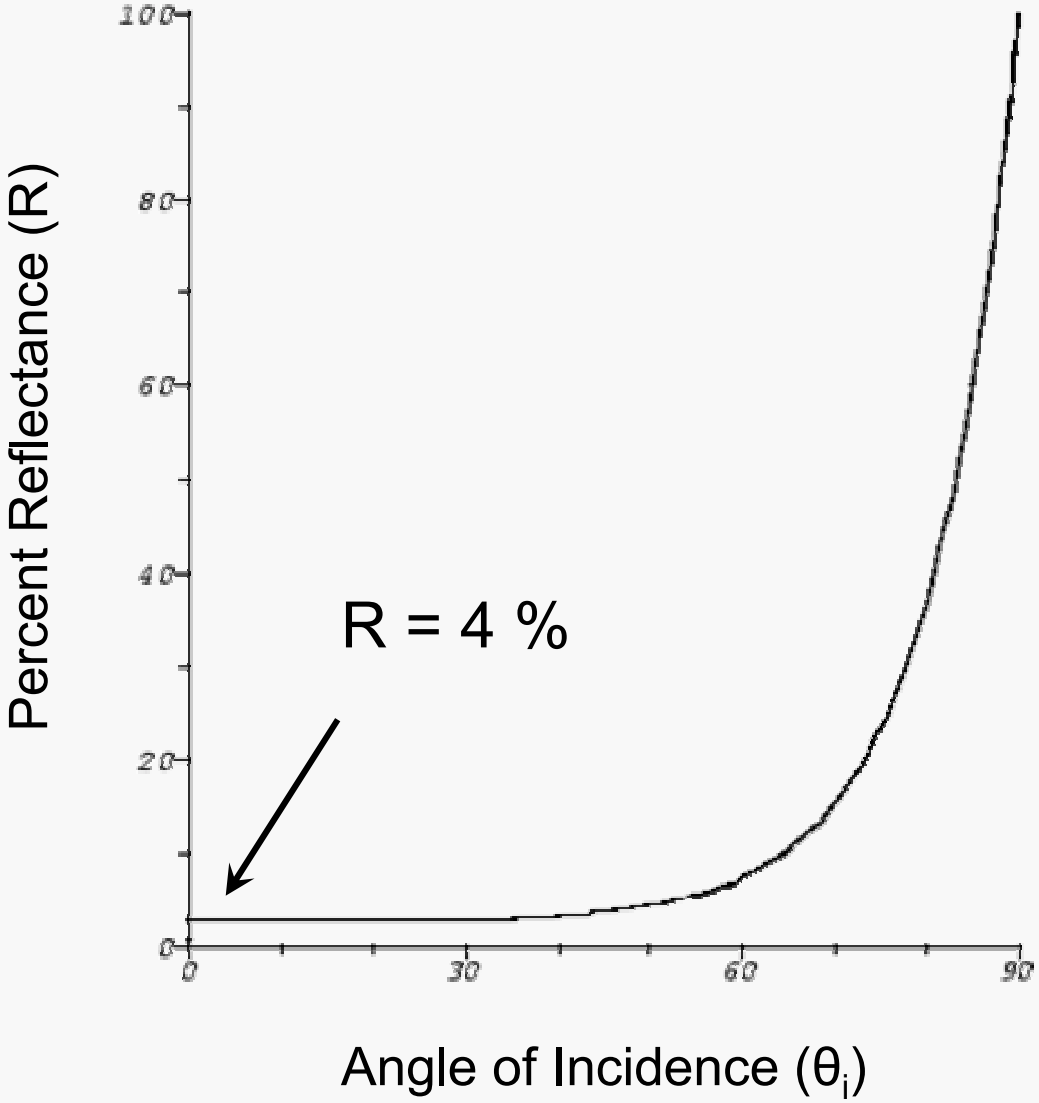
Where I_i and I_r = incident & reflected intensity

For radiation going from air ($\eta = 1.00$) to glass ($\eta = 1.50$) as shown in previous slide

$$R = 0.04 = 4\ \%$$

Many surfaces at 4 % each (i.e., many lenses) can cause serious light losses in a spectrometer. This generates **stray radiation** or **stray light**.

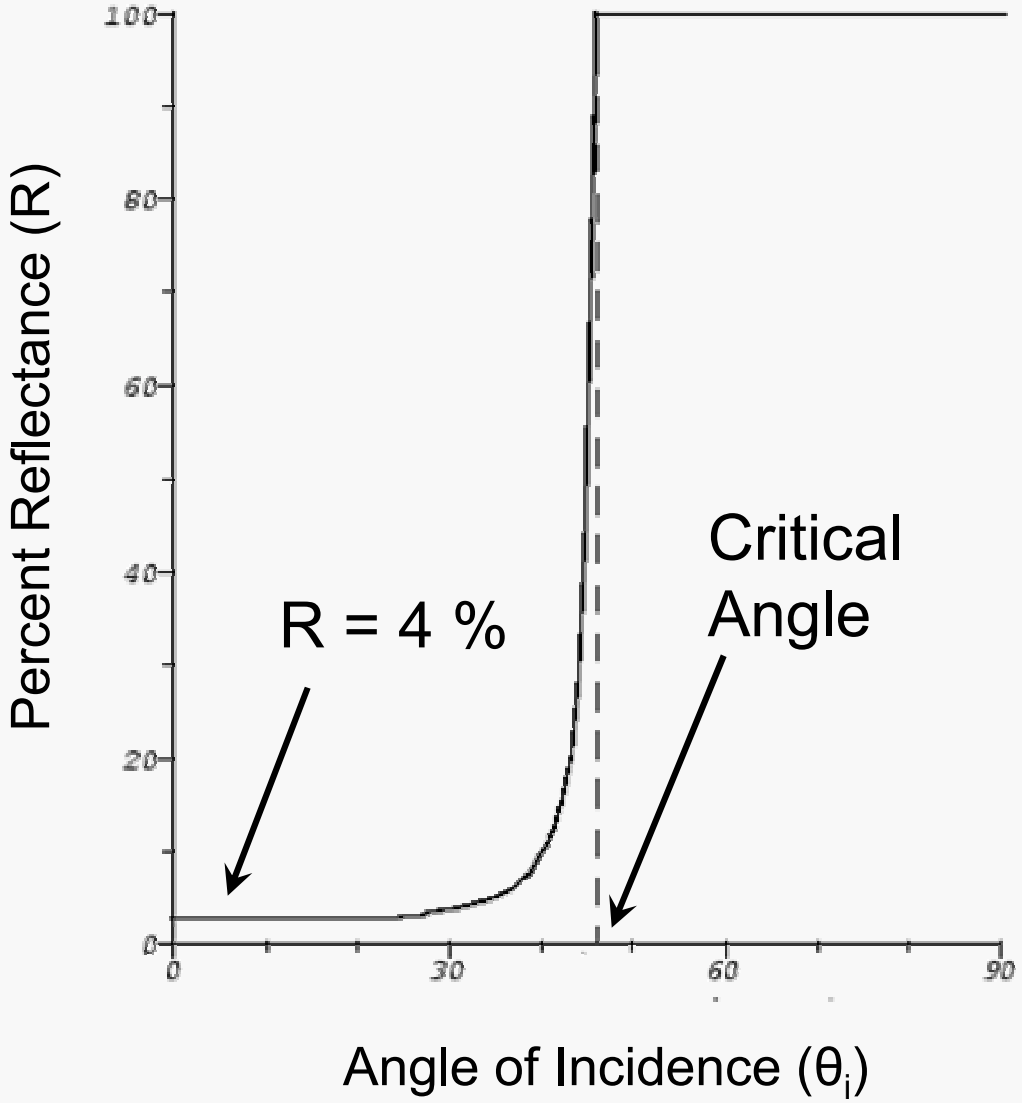
Reflectance varies with the angle of incidence



EM going from air ($\eta = 1.00$) to glass ($\eta = 1.50$)

For monochromatic radiation, as incident angle deviates from the normal, the R tends to increase

EM going from glass (η = 1.50) to air (η = 1.00)

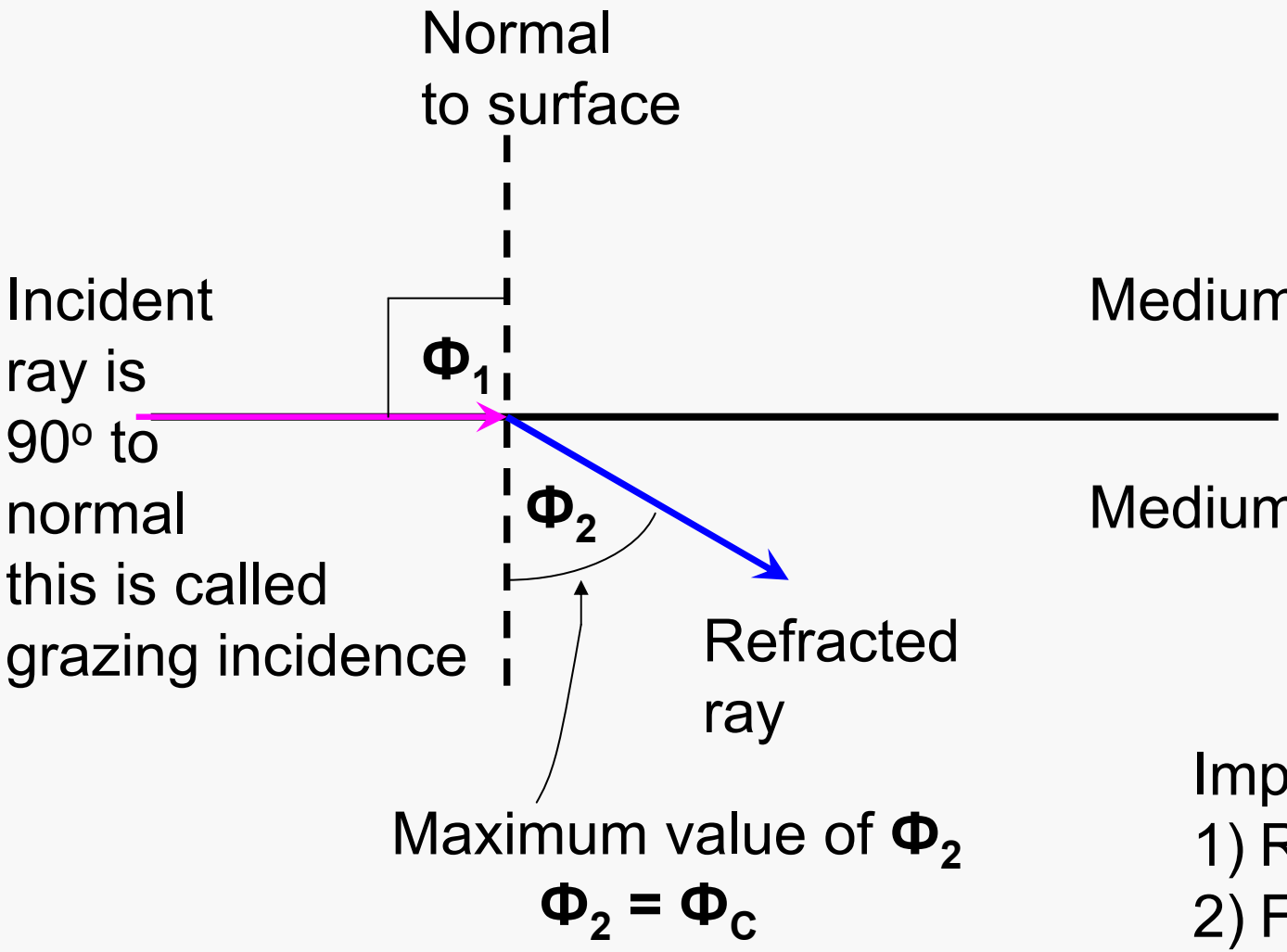


EM going from a medium of higher η to a medium of lower η, the angle of incidence can only increase to the critical angle before all of the light is completely reflected back

Critical Angle (Φ_C)

At 90° incidence sin $\Phi_1 = 1.0$

$$\sin \Phi_c = \frac{\eta_1}{\eta_2}$$

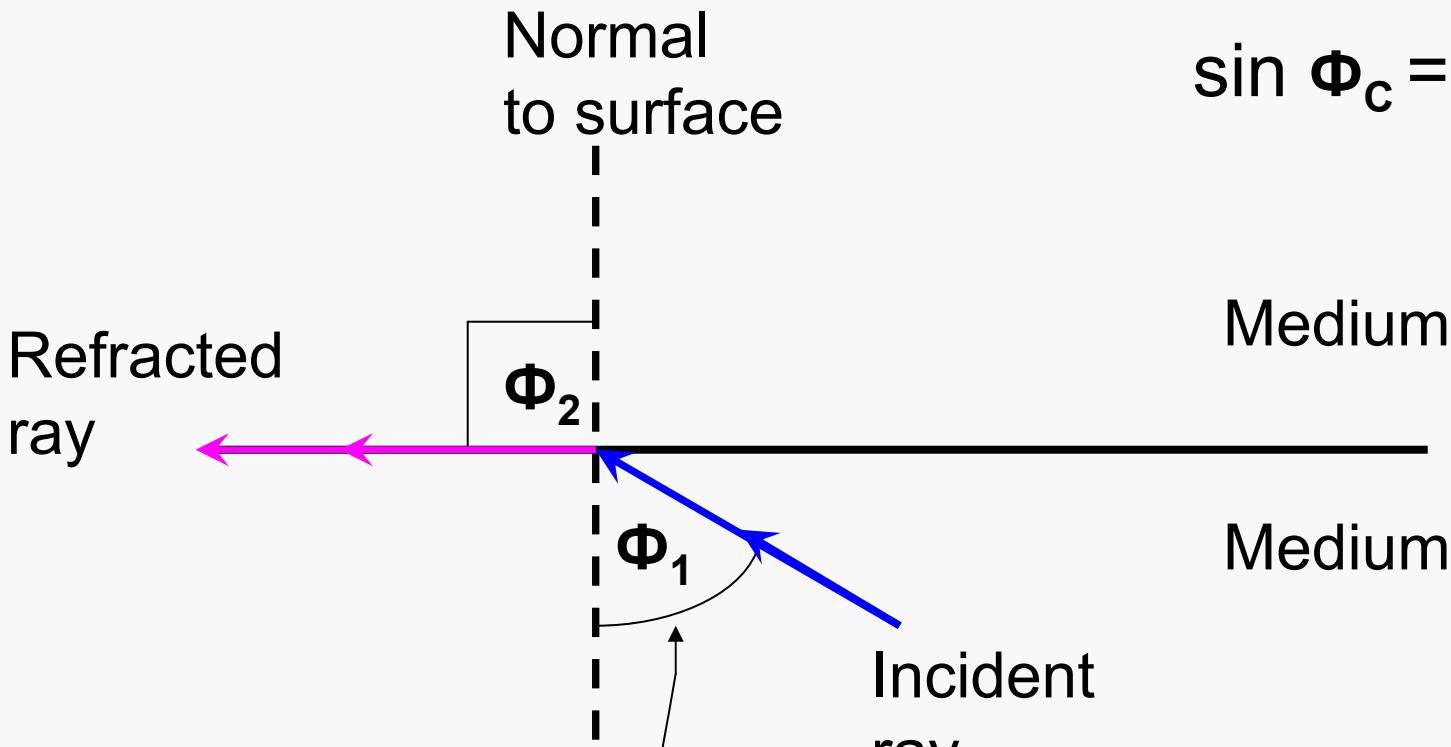


Important for:
1) Refractometers
2) Fiber Optics

Critical Angle (Φ_c)

When incidence is at the Critical angle, refraction is at 90°

$$\sin \Phi_c = \frac{\eta_2}{\eta_1}$$

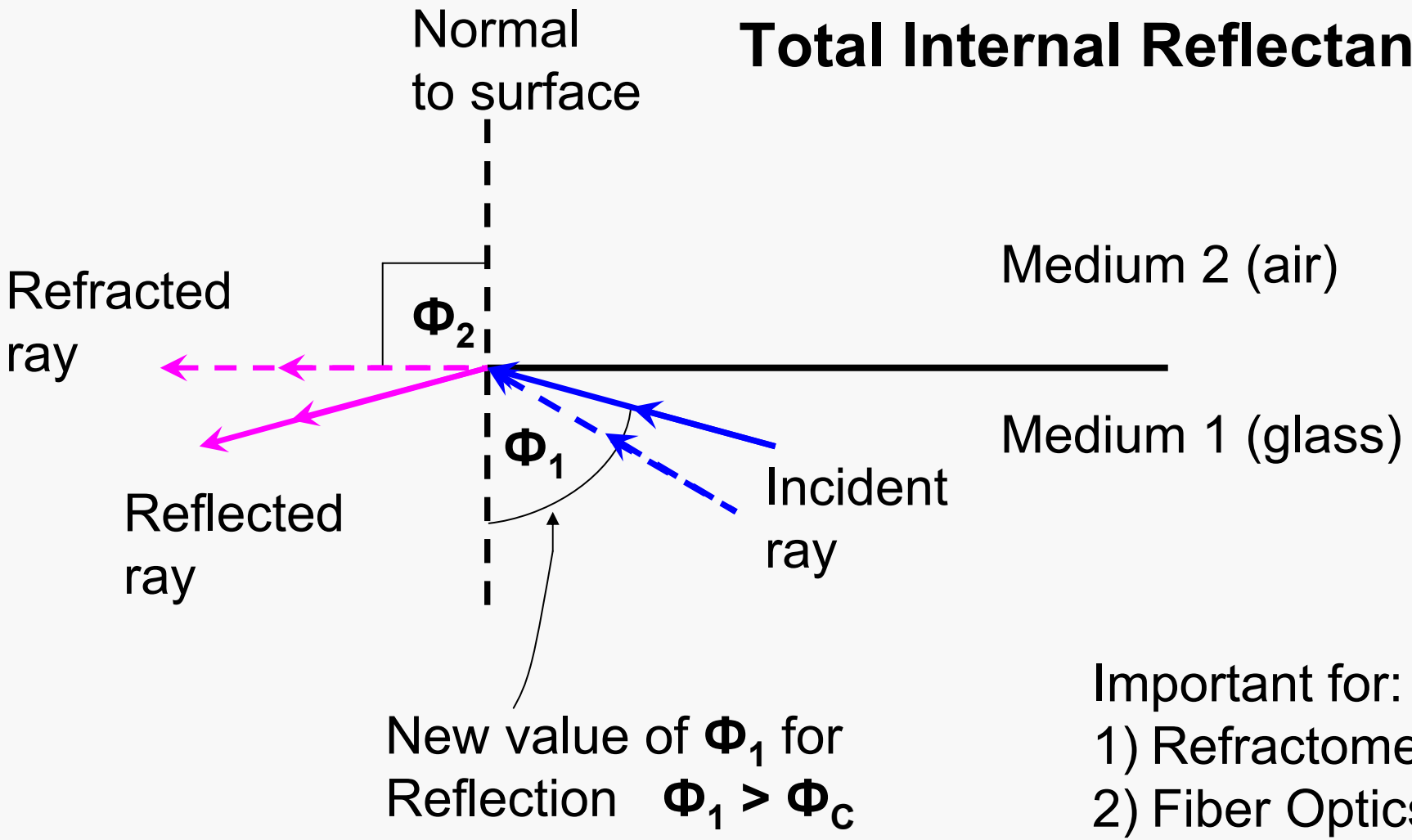


Maximum value of Φ_1 for Refraction $\Phi_1 = \Phi_c$

Important for:
1) Refractometers
2) Fiber Optics

Critical Angle (Φ_C)

At angles greater than the Critical angle, 100 % reflection occurs or **Total Internal Reflectance**



Important for:
1) Refractometers
2) Fiber Optics

Applying UV-vis Spectrophotometry

Qualitative (as discussed)

I) 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)

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

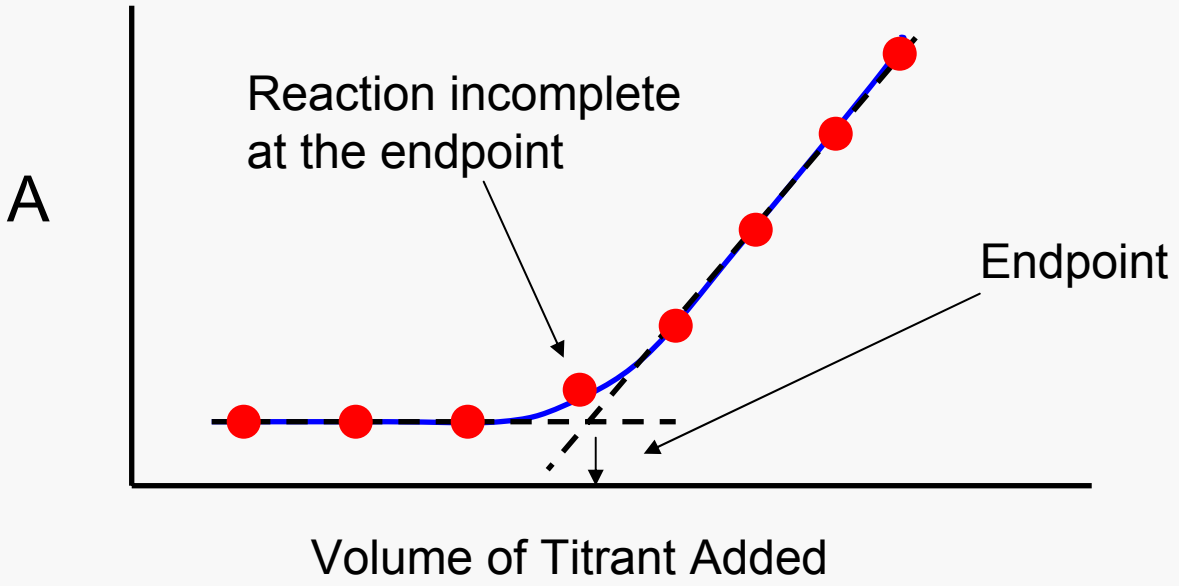
1) Spectrophotometric Titrations

Example where titrant absorbs

Sample + Titrant → Product

absorbs

MnO_4^- + Fe^{2+} → MnO_2 + Fe^{3+}



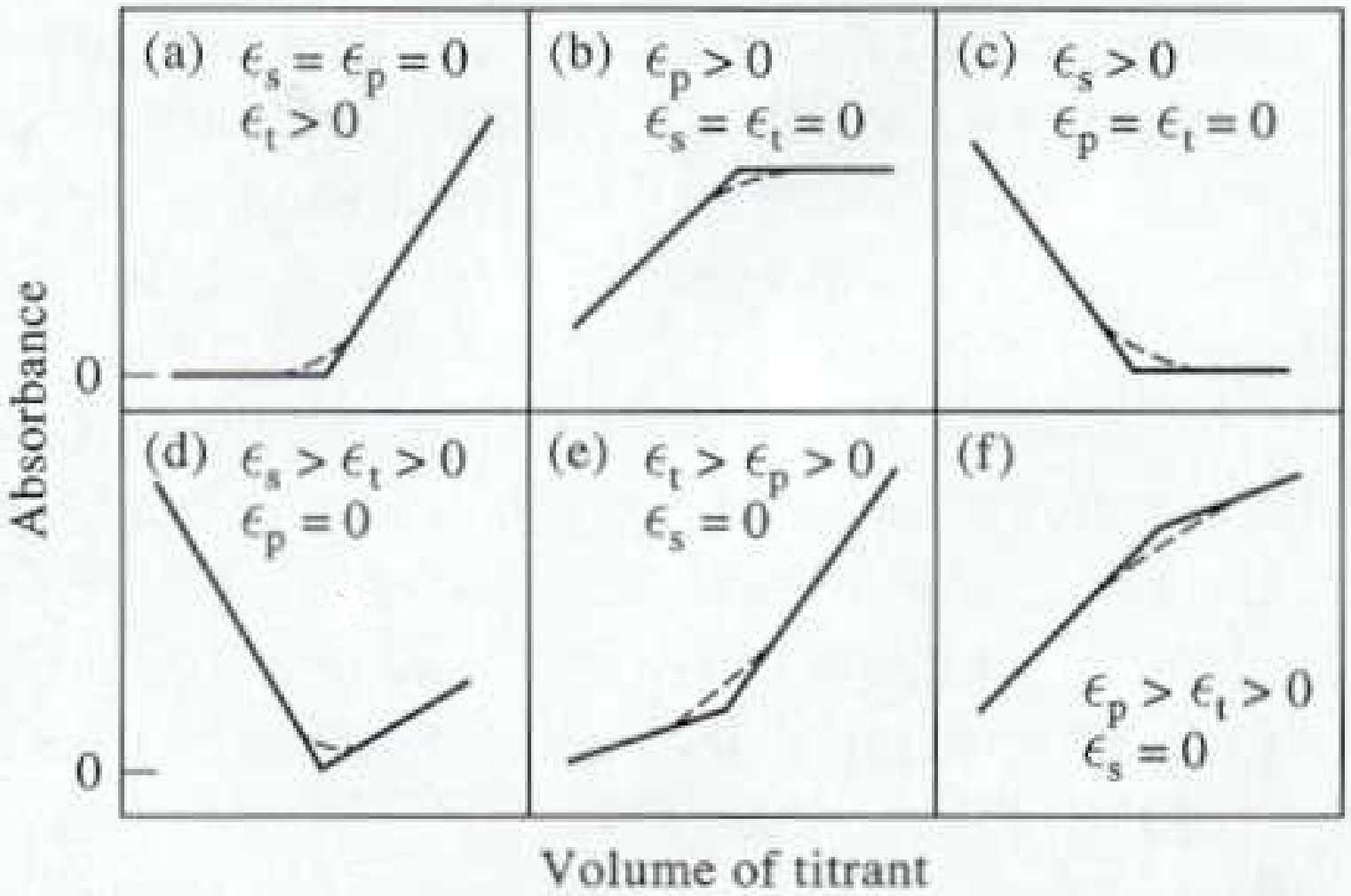


Figure 14-18 Typical photometric titration curves. Molar absorptivities of the substance titrated, the product, and the titrant are given by ϵ_s, ϵ_p, ϵ_t, respectively.

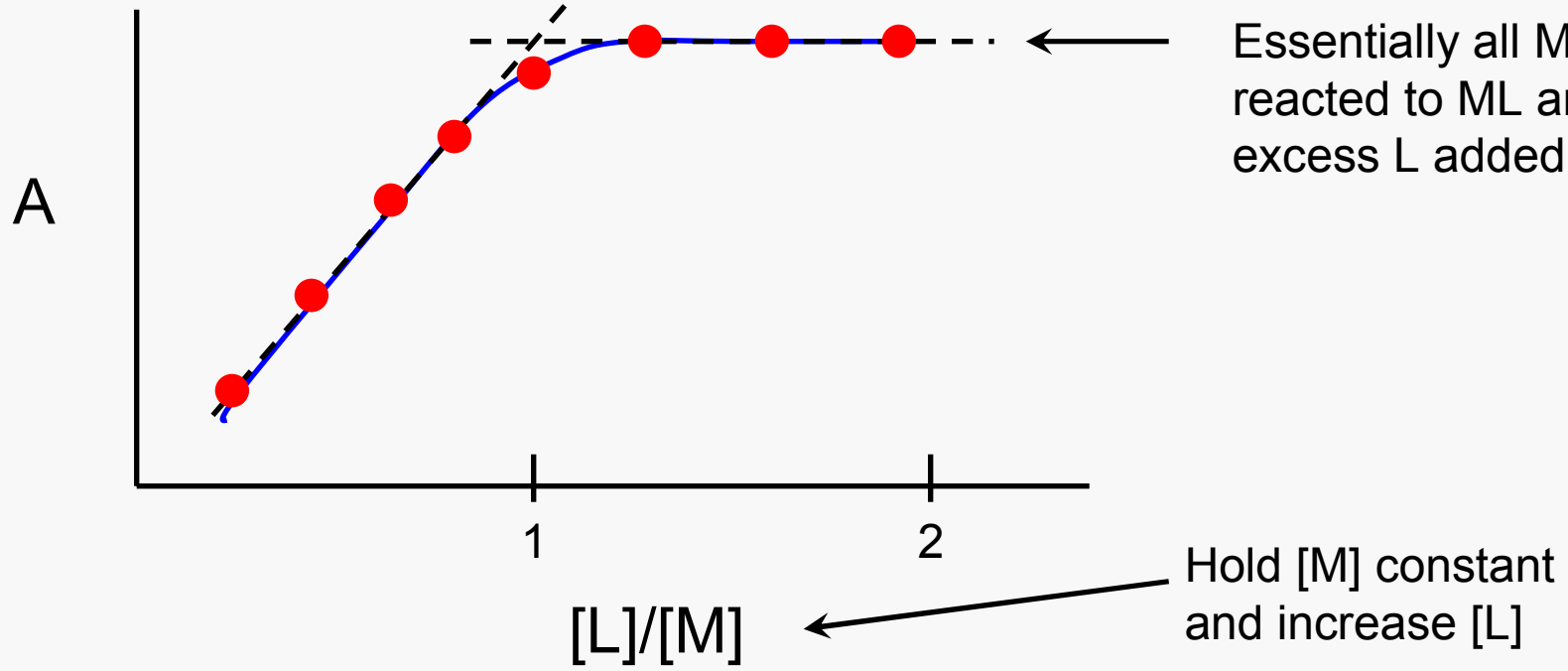
2) Study Equilibria (acid-base, complex formation, redox, etc.)

Reactants don't absorb

$$M + nL \leftrightarrow ML_n$$

Products absorb

$$Fe^{3+} + SCN^{-} \leftrightarrow FeSCN^{2+}$$



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

Get n from extrapolated curve

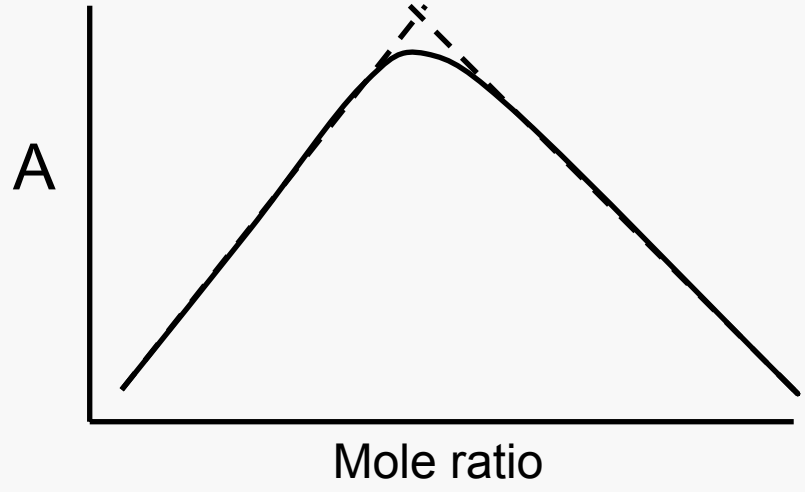
Can calculate equilibrium constant (K) from

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

and mass balances

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

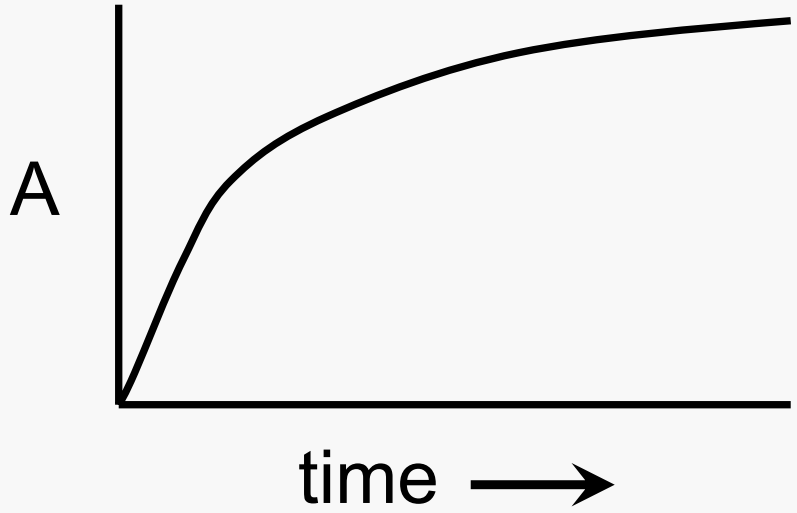
Other calculation methods can be used involving graphical or computer curve fitting procedures, e.g. Job's Method or the Method of Continuous Variation



3) Study Kinetics

absorbs

$X + Y \rightarrow Z$



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

$$A_{true} = \log \frac{I_o}{I}$$

$$A_{measured} = \log \frac{I_o + I_s}{I + I_s}$$

where I_s is intensity of stray radiation

Stray Light Specification – important characteristic of monochromators

Stray Light Specification

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

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

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 $A_{true} = 2$

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

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

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

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 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

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
2) Fluorescence – also photoluminescence which is usually observed in solution like quinine that is added to tonic water many analytical applications

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

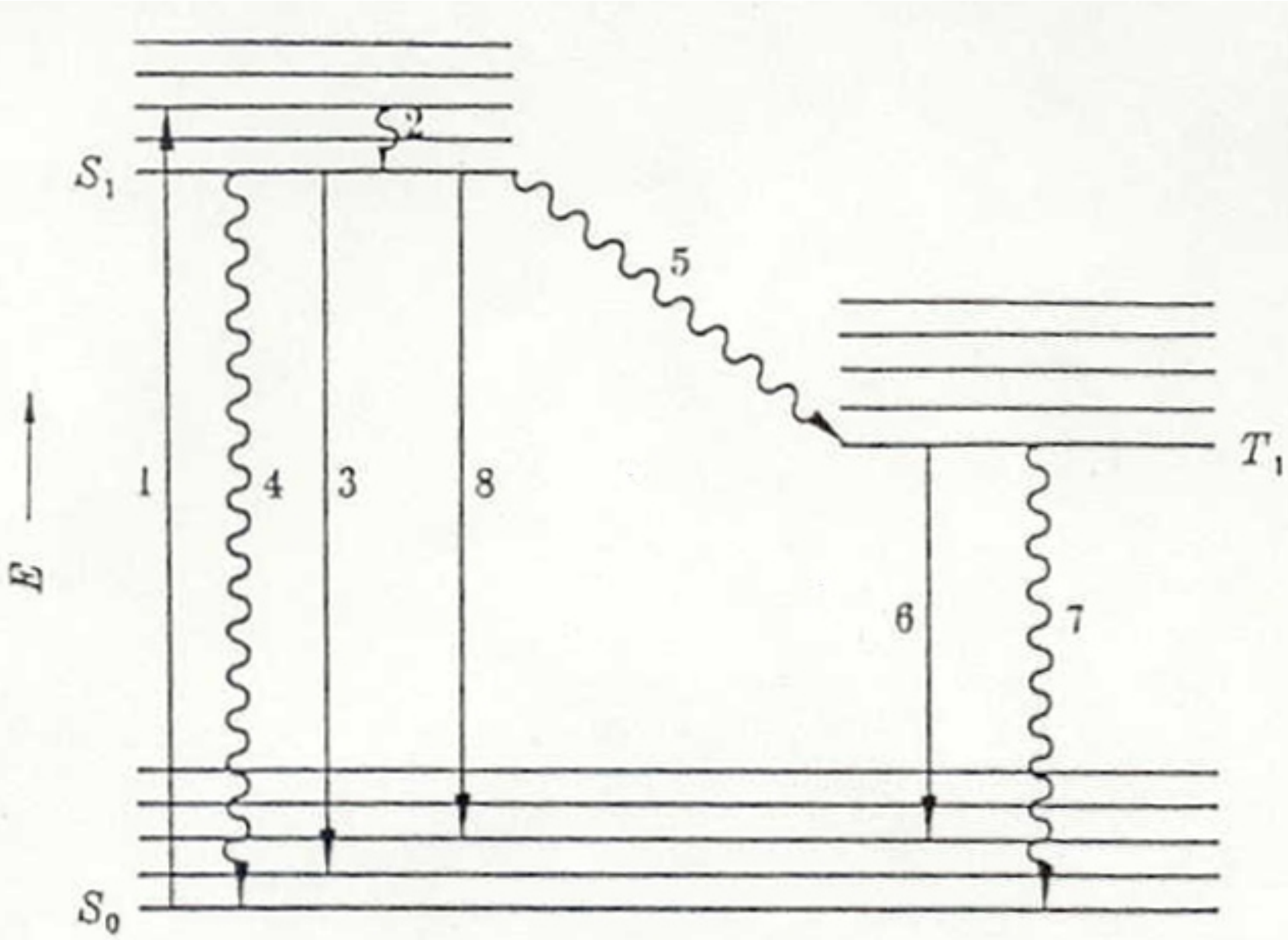
Photoluminescence is the most useful kind of luminescence for analysis because:

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)

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

Bioluminescence is chemiluminescence

Others are basically useless analytically



1 = absorption, 2 = vibrational relaxation, 3 = fluorescence, 4 & 7 = radiationless deactivation, 5 = intersystem crossing, 6 = phosphorescnce

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 ~ 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

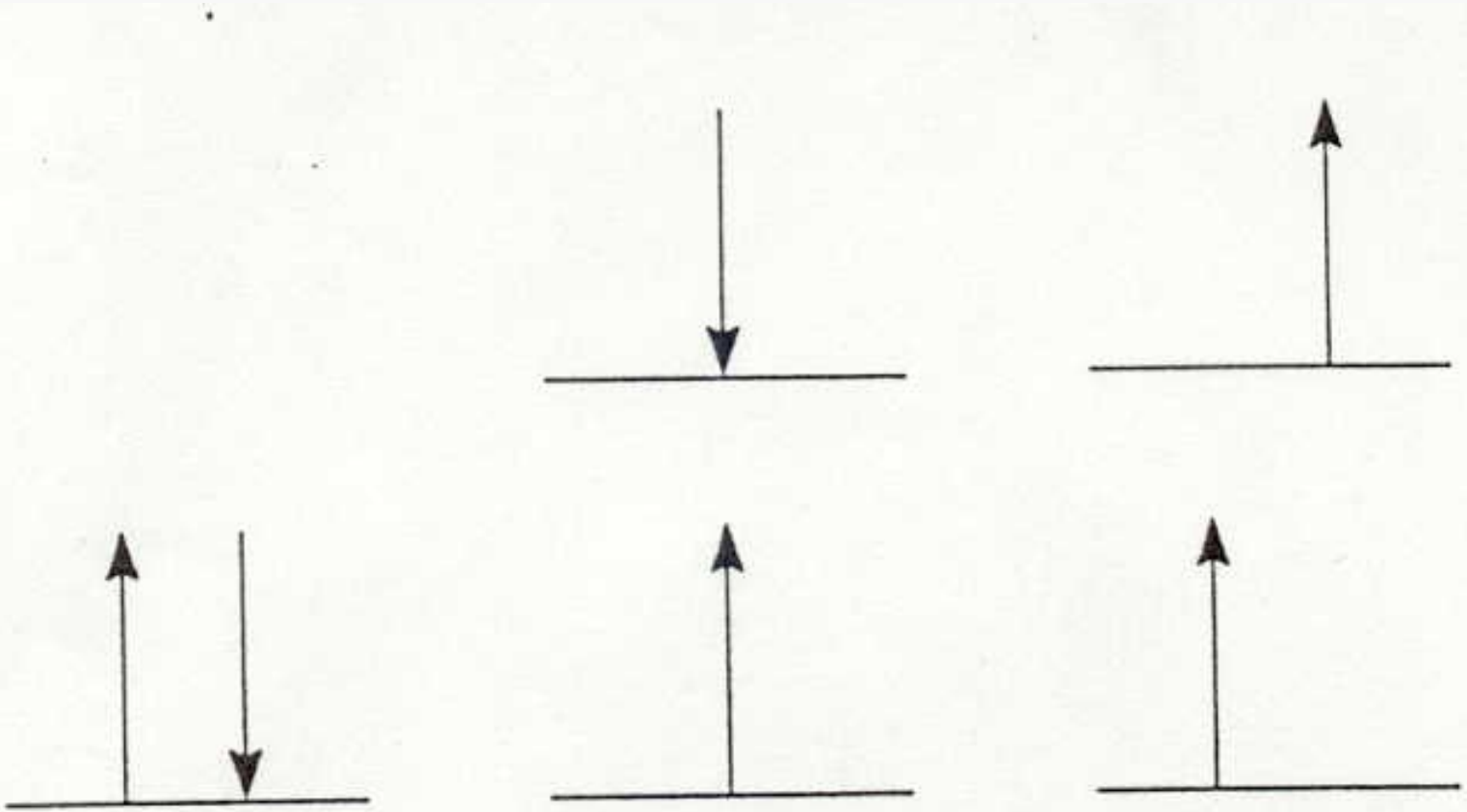
3) **Intersystem Crossing** (#5) – lowest vibrational level of first excited singlet couples to upper vibrational level of triplet state followed by vibrational relaxation

   Once formed the triplet state can go to ground state radiationlessly or by emitting a photon = **phosphorescence** (#6)

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

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

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


ground
singlet state
excited
singlet state
excited
triplet state

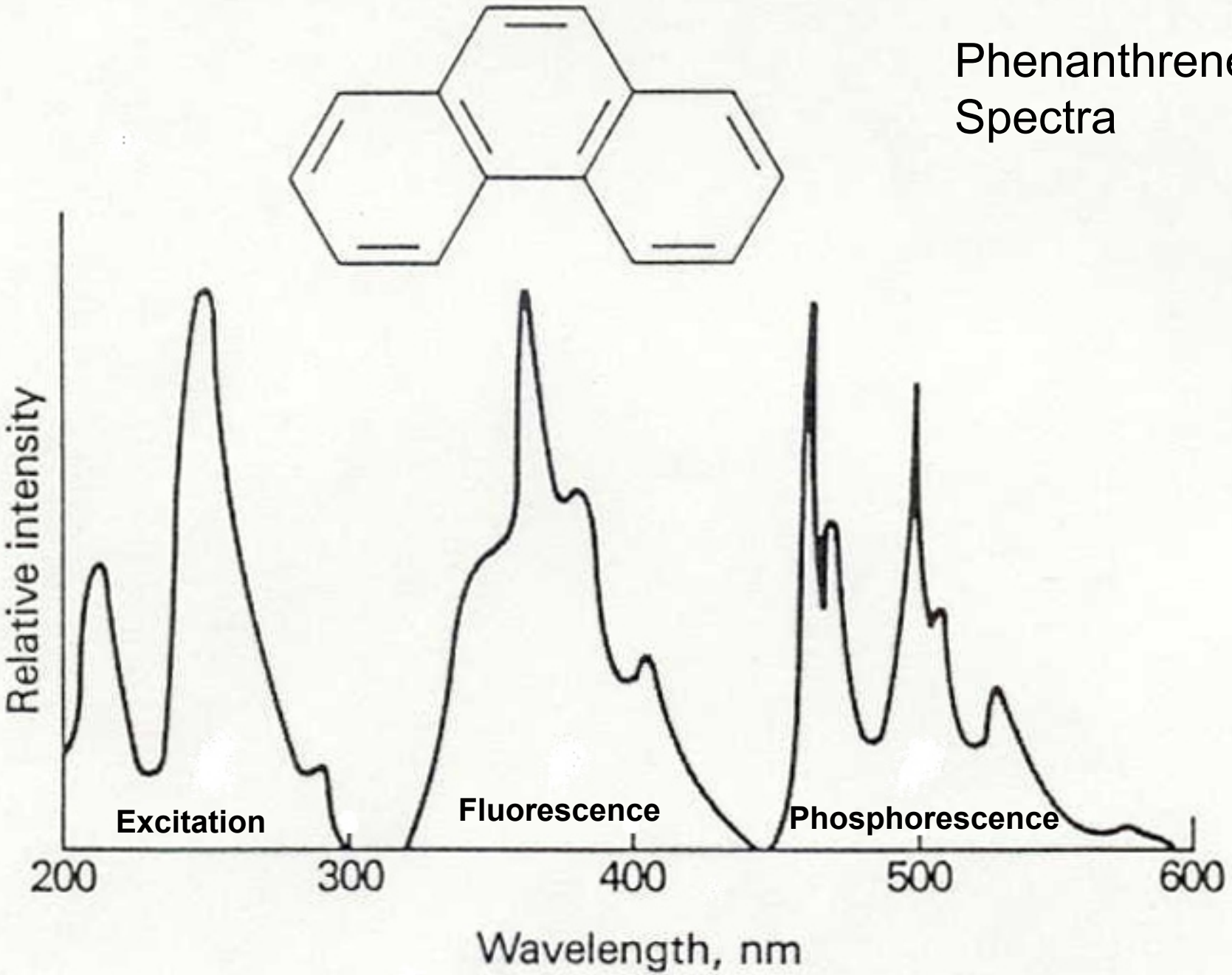
Moderately interesting website showing an animated Jablonski Diagram for absorption, VR, fluorescence, phosphorescence, etc.

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

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.)

Phenanthrene Spectra



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

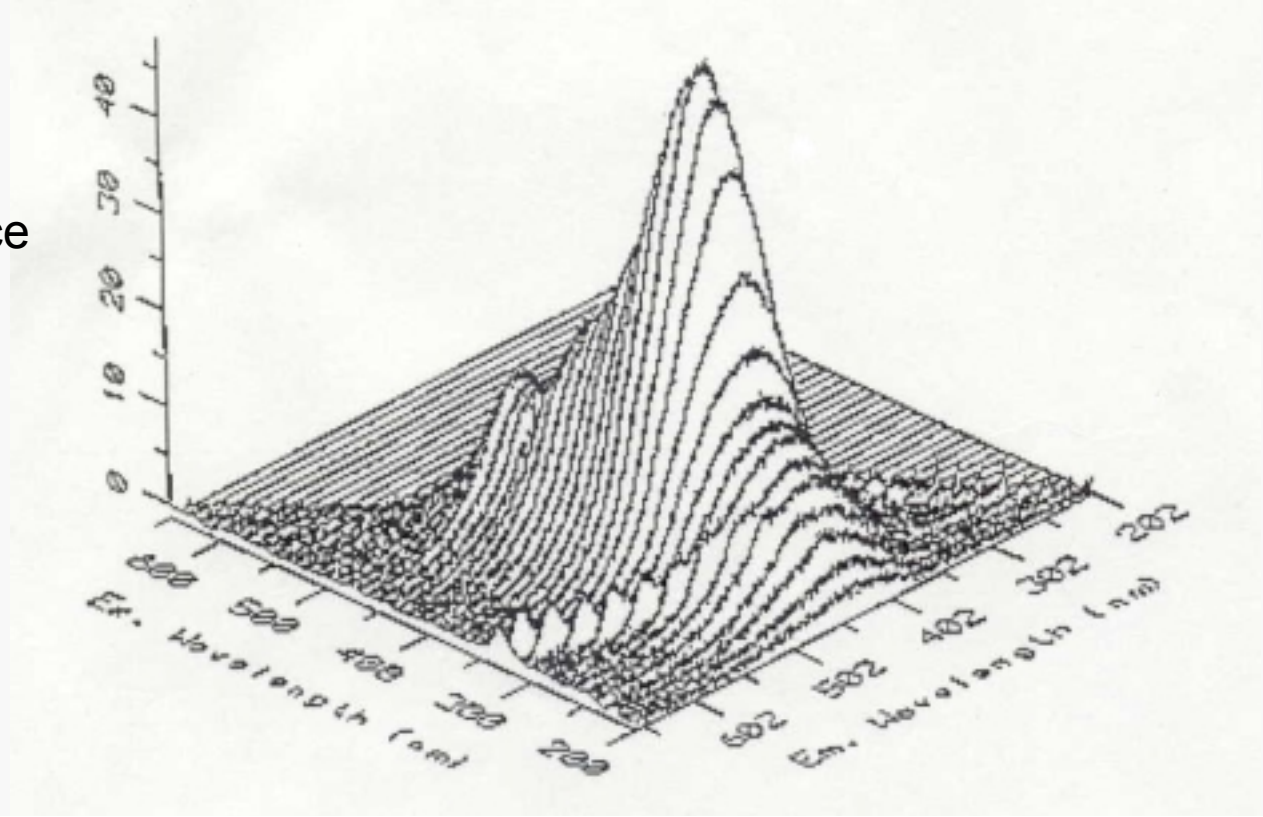
Degree of structure in fluorescence spectrum is due to the degree of solvation as discussed for absorption

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

Spectra

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

Fluorescence Emission Intensity



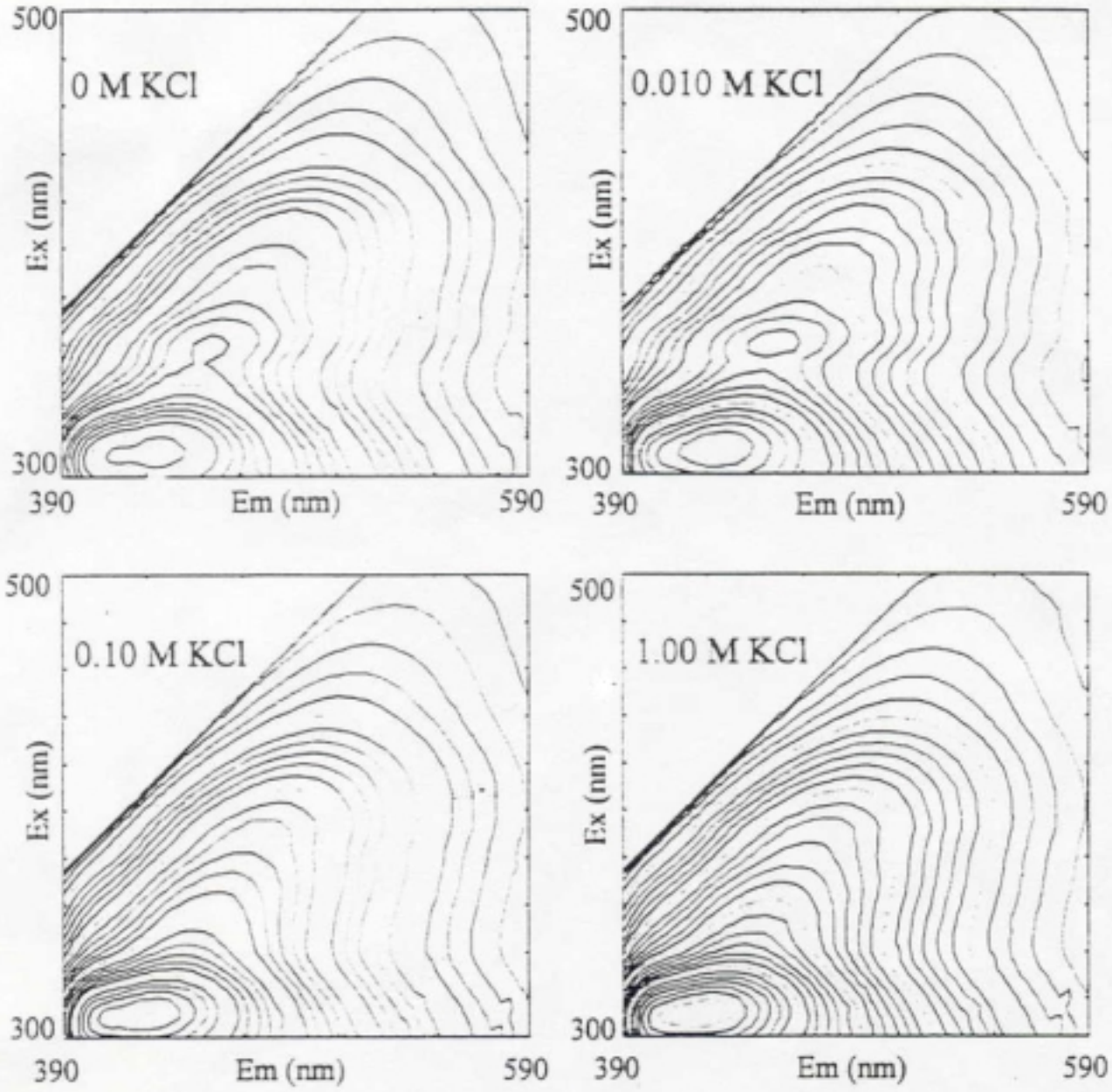


FIGURE 3. Absorbance-corrected EEMs of PFAR (50 mg/L, pH 6.0) at different ionic strengths.

EEMs represented as contour plots