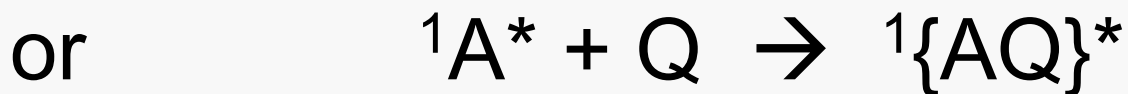**Excited State Chemistry** – it should be recognized that an electronic excited state is a distinct chemical entity with its own chemical properties

Sometimes fluorescence & phosphorescence emission are complicated by things that the excited states do, e.g.:

1) Chemical Reactions – the excited state can undergo chemical reactions that the ground state can't, e.g. dyes fade in sunlight → this is photochemistry →

In chemical analysis, photodecomposition is a serious problem – rate of photo-decomposition is proportional to number of excited states, i.e. source intensity

2) <u>Acid-Base Chemistry</u> – K_a for excited singlet state can differ by as much as 10^6 from K_a for ground state

e.g. Phenol   ground state $pK_a = 10.0$

                    excited singlet $pK_a = 4.00$

At pH's between 4 & 10 you see fluorescence characteristics of phenolate anion = good way to study excited state chemistry

Effects of other solutes on excited state emission → can promote radiationless return to ground state = quenching

1) Energy Transfer

$$^1A^* + Q \rightarrow A + {}^1Q^*$$

or $\quad ^1A^* + Q \rightarrow {}^1\{AQ\}^*$

Occurs by

Where A = Analyte (species of interest) which absorbs and Q = quencher $^1A^*$ & $^1Q^*$ designate first excited singlet & $^1\{AQ\}^*$ = complex

    a)   Q absorbs the emission of A

    b)   Collisional non-radiative transfer of energy → obeys <u>Stern-Volmer Law</u>

Stern-Volmer Equation

Quenching constant

Fluorescence Quantum Yield in the absence of quencher

Concentration of Quencher

$$\frac{\Phi_F^o}{\Phi_F} = 1 + K_Q \tau_F^o [Q]$$

With quencher

Fluorescence Lifetime of A (no Q)

Two types of quenching are covered by the Stern-Volmer Equation:

1) Static quenching – where A & Q form a complex that doesn't emit (=dark complex)

2) Dynamic quenching – collisional

Both represented as $^1A^* + Q \rightarrow {}^1\{AQ\}^*$

(increased rate of radiationless deactivation K_{IC})

Fluorescence Quenching of Fulvic Acid, Q = Cu^{2+}

Emission scans of 15 mg/L Fulvic Acid with varying $[Cu^{2+}]$ and excitation at 340 nm

Hays, 1996
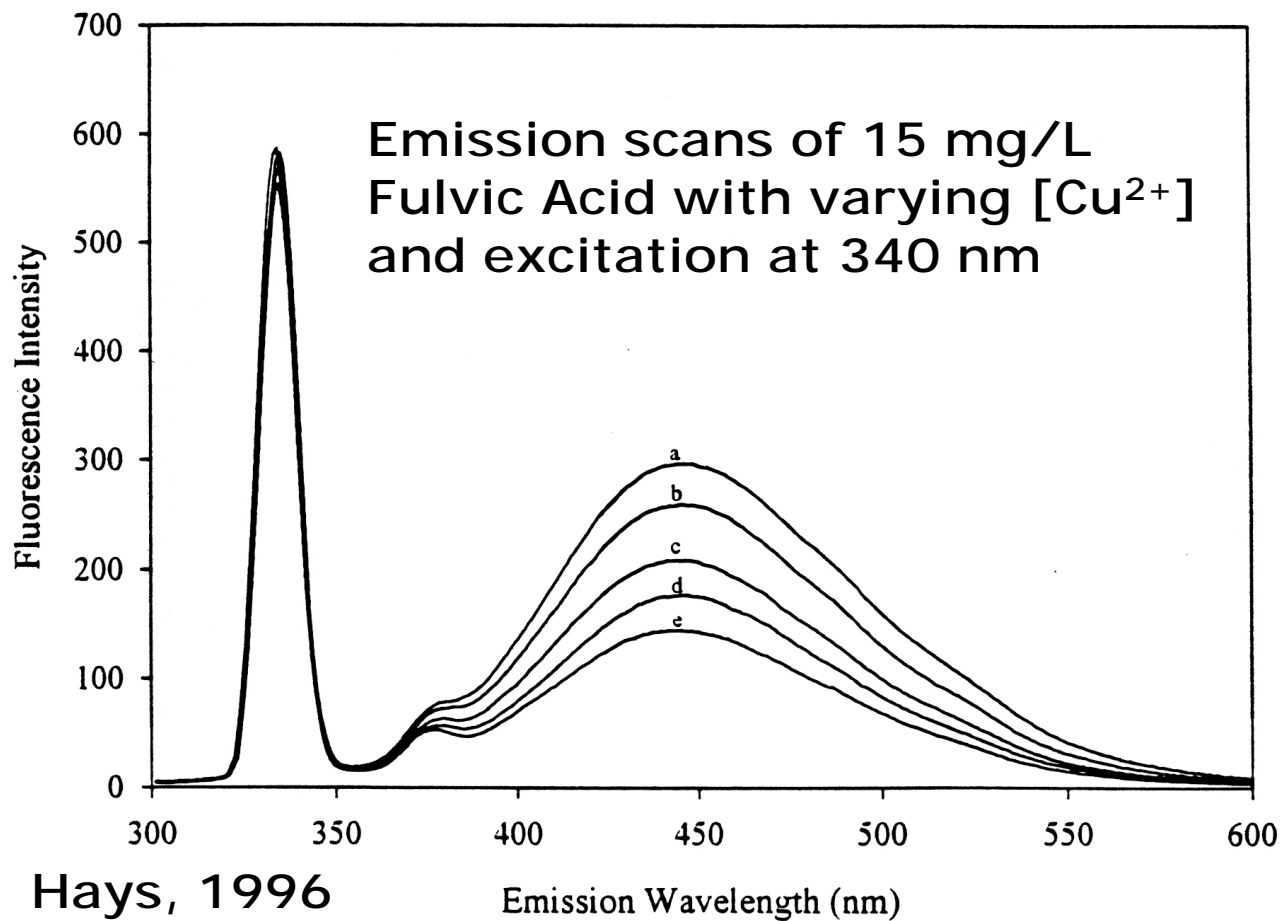
Stern Volmer Equation

$$\frac{I_0 - I}{I} = K[M]$$

The Ryan Equation

$$\frac{[ML]}{C_L} = \frac{I_0 - I}{I_0 - I_{RES}}$$

1:1 Complex Formation

M + L = ML

$$K = \frac{[ML]}{[M]\,[L]}$$

Where M = metal ion; L = ligand; ML = complex

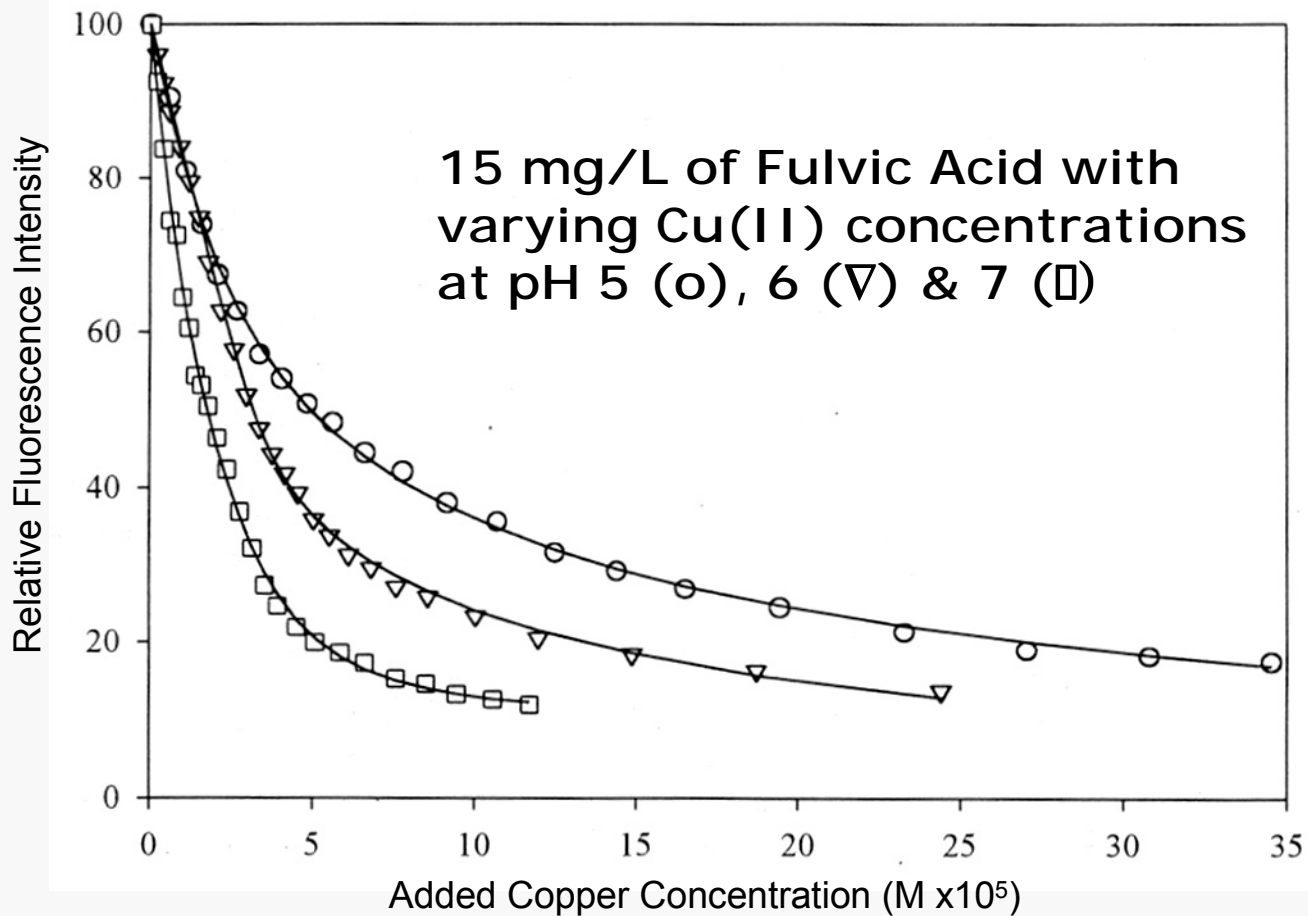# Equations for Fitting Data

Equation for One Site Binding

$$I=[200+2KI_{RES}C_M-I_{RES}[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_MC_L)^{0.5}]]$$
$$/[2+2KC_M-[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_LC_M)^{0.5}]]$$
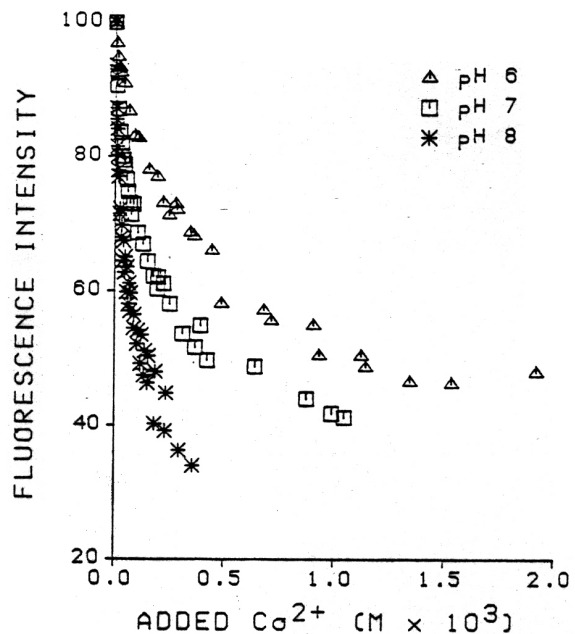
Equations for Two Site Binding

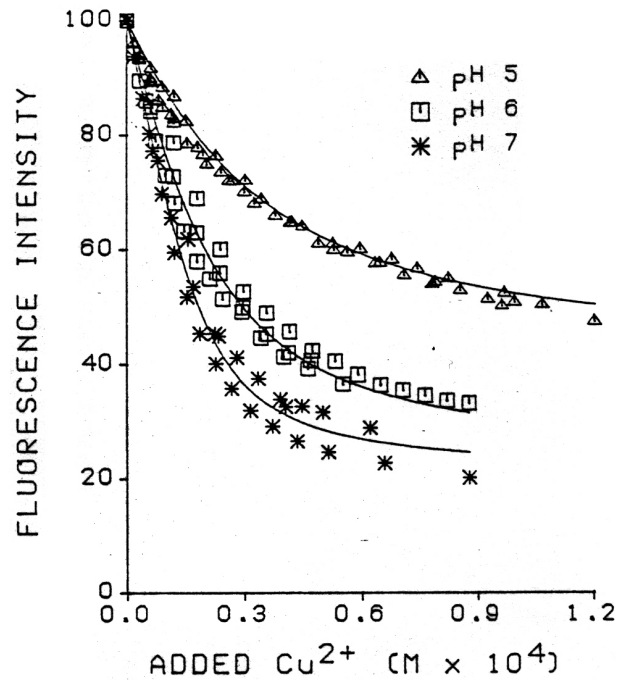$$C_M = [M] + (K_1C_{L1}[M]/K_1[M] + 1)+(K_2C_{L2}[M]/K_2[M] + 1)+....$$
$$+K_nC_{Ln}[M]/K_n[M] + 1$$

$$K_1K_2[M]^3 + \{K_1K_2(C_{L1}+C_{L2}-C_M) + K_1 + K_2\}[M]^2 +$$
$$\{C_{L1}K_1+K_2C_{L2}-C_M(K_1 + K_2+1\}[M] - C_M = 0$$

Fluorescence Quenching Curves

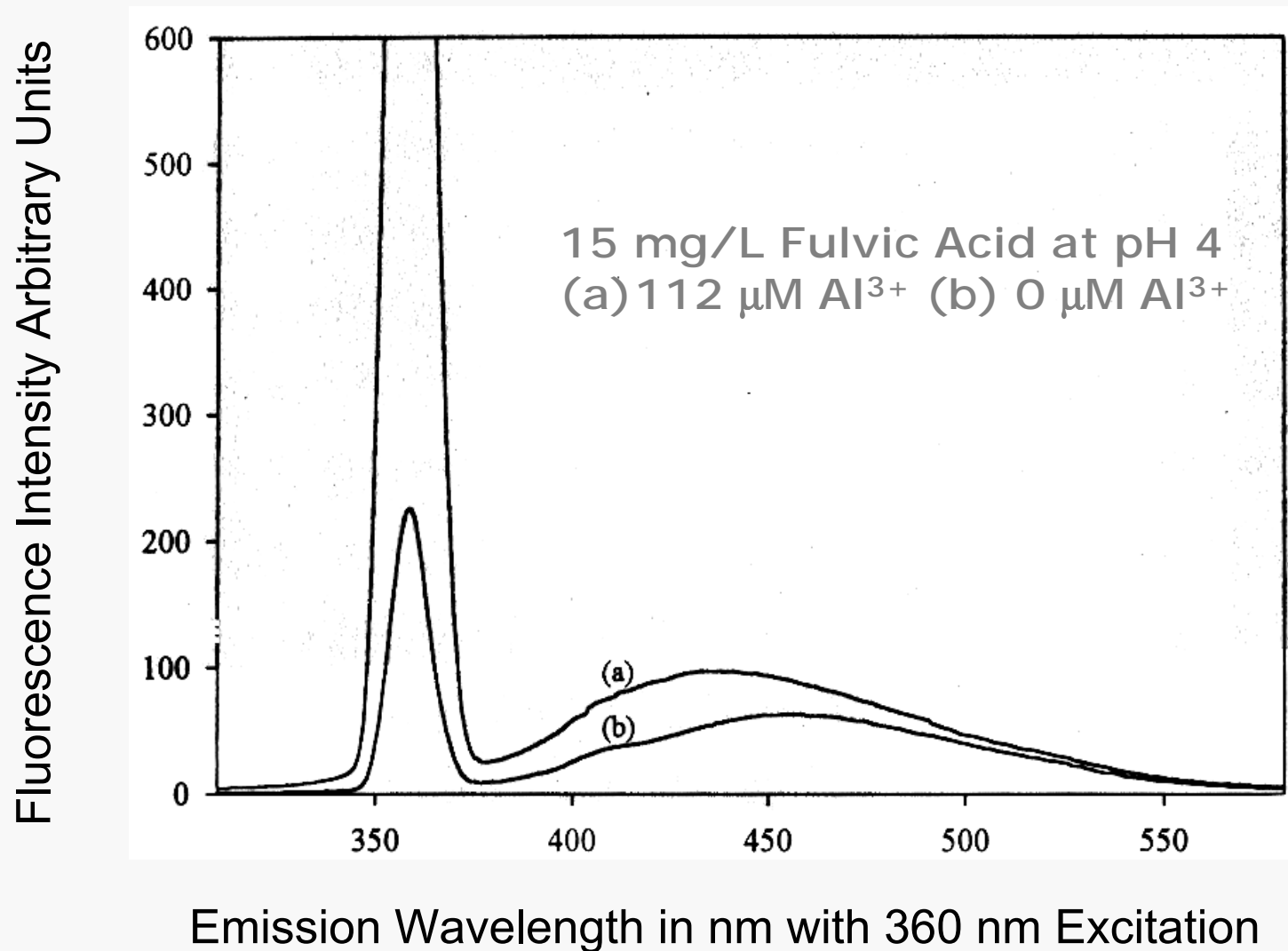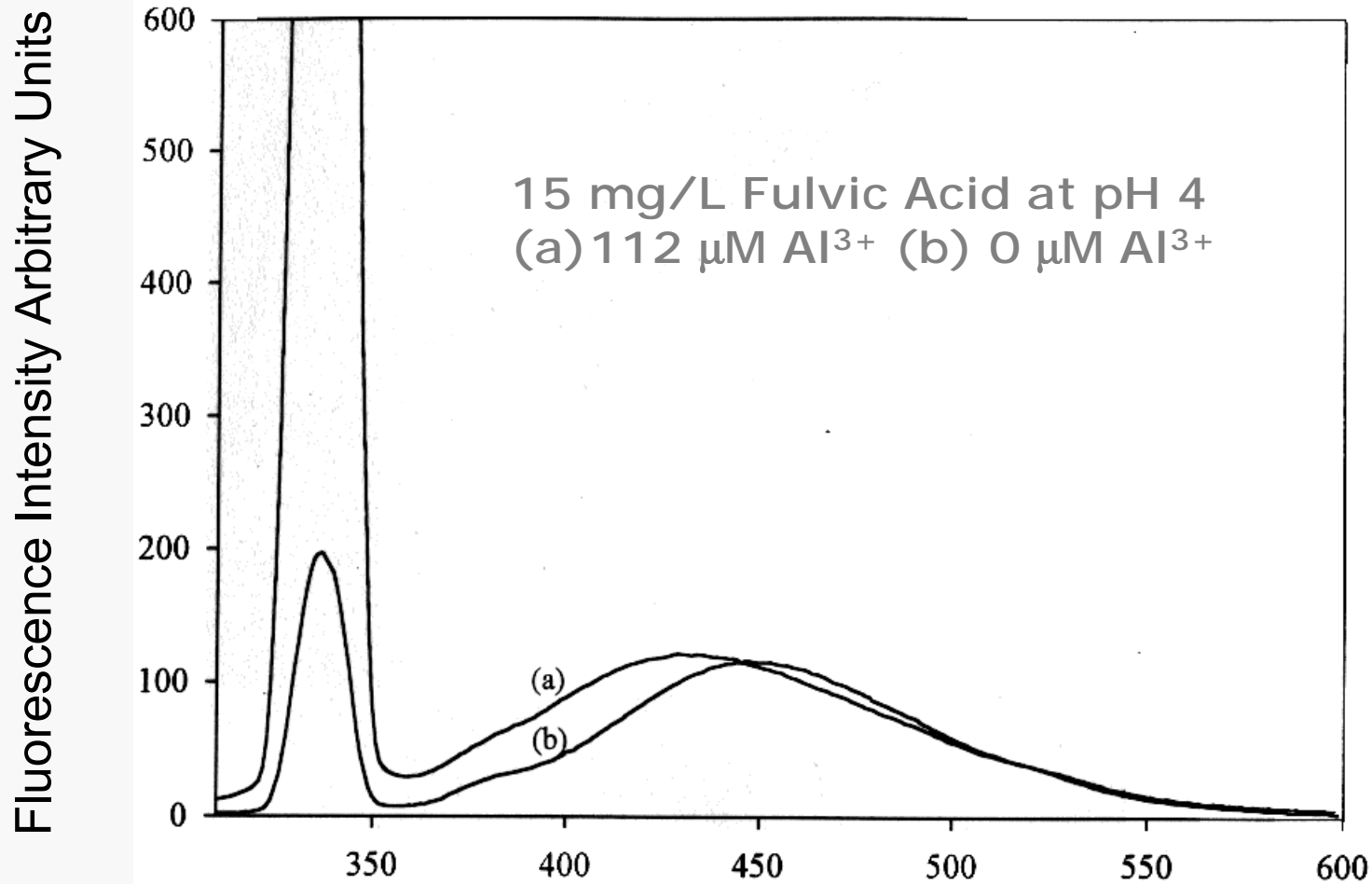15 mg/L of Fulvic Acid with varying Cu(II) concentrations at pH 5 (o), 6 (∇) & 7 (□)

Relative Fluorescence Intensity

Added Copper Concentration (M x10^5)

Hays, 1996
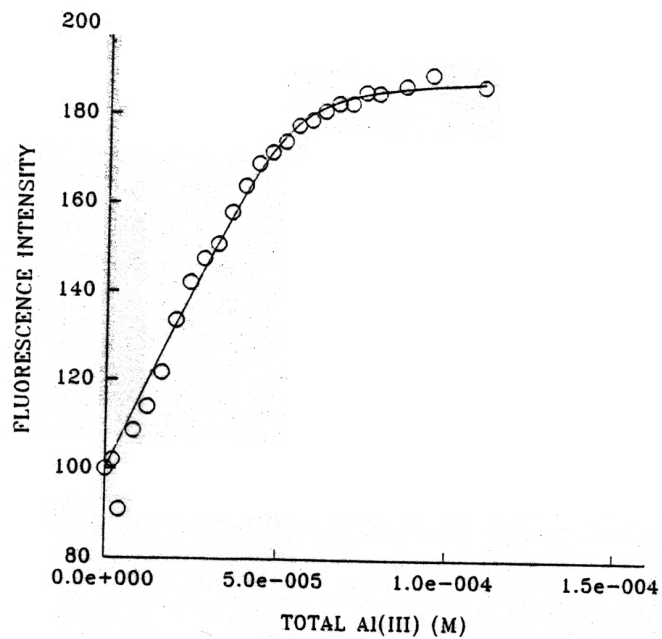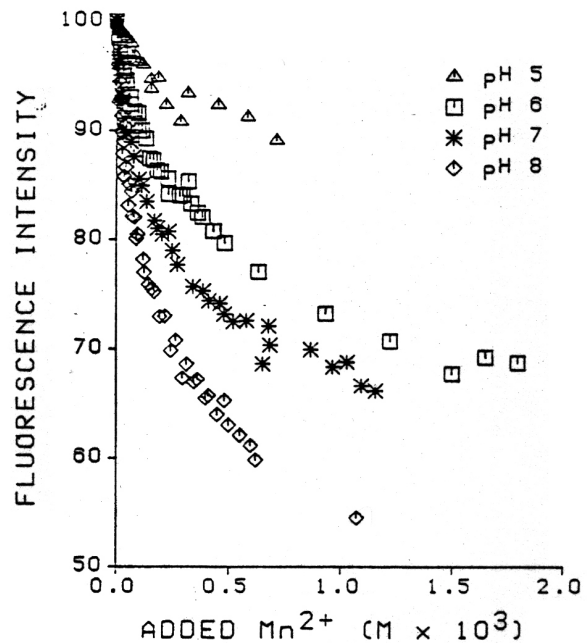
Fluorescence binding curves for Cu, Co, Mn & Al at pH values of 5-8

Fluorescence Enhancement

15 mg/L Fulvic Acid at pH 4
(a) 112 μM Al^{3+} (b) 0 μM Al^{3+}

Fluorescence Intensity Arbitrary Units

Emission Wavelength in nm with 360 nm Excitation

Fluorescence Enhancement

15 mg/L Fulvic Acid at pH 4
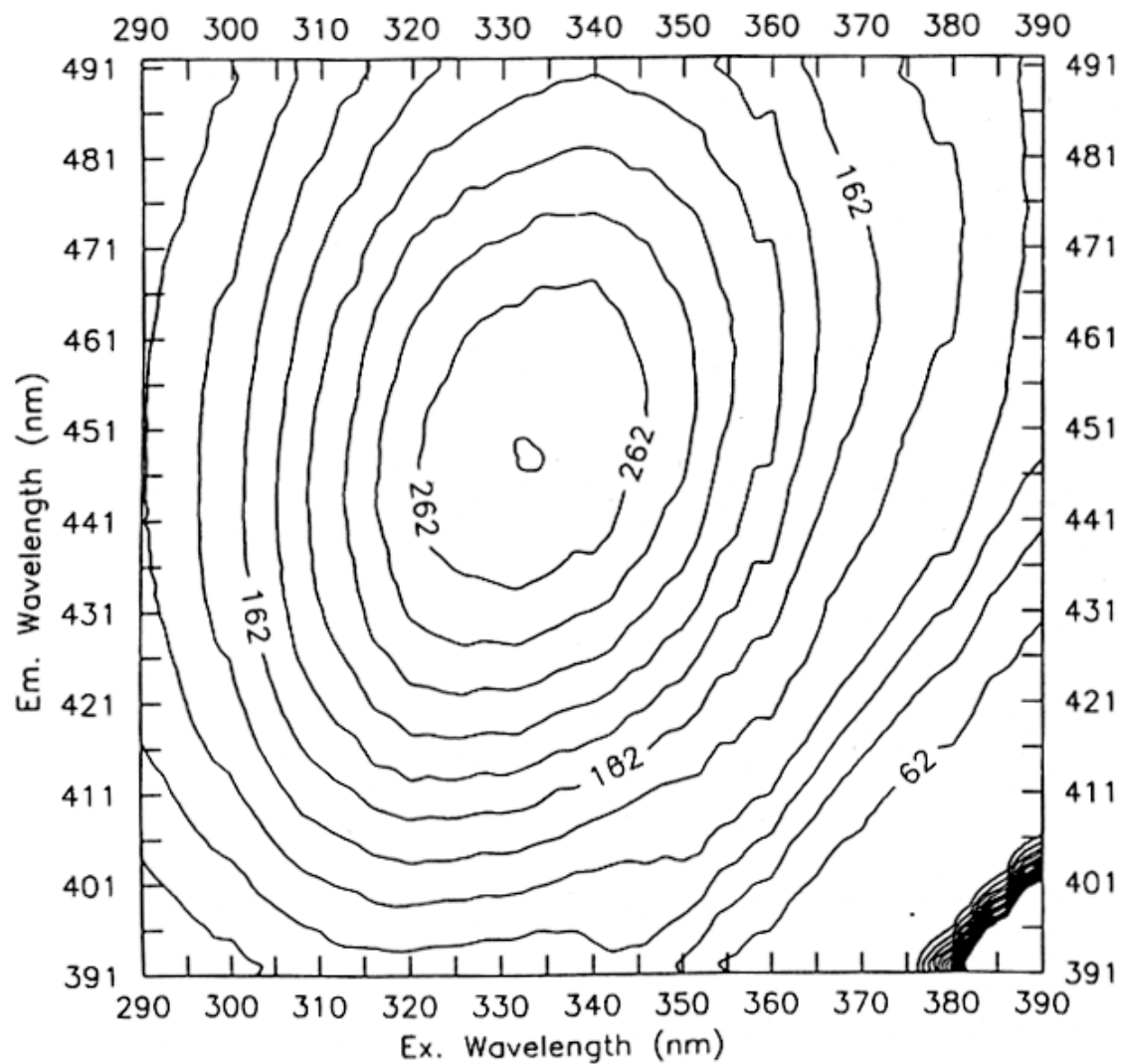(a) 112 µM Al^{3+} (b) 0 µM Al^{3+}

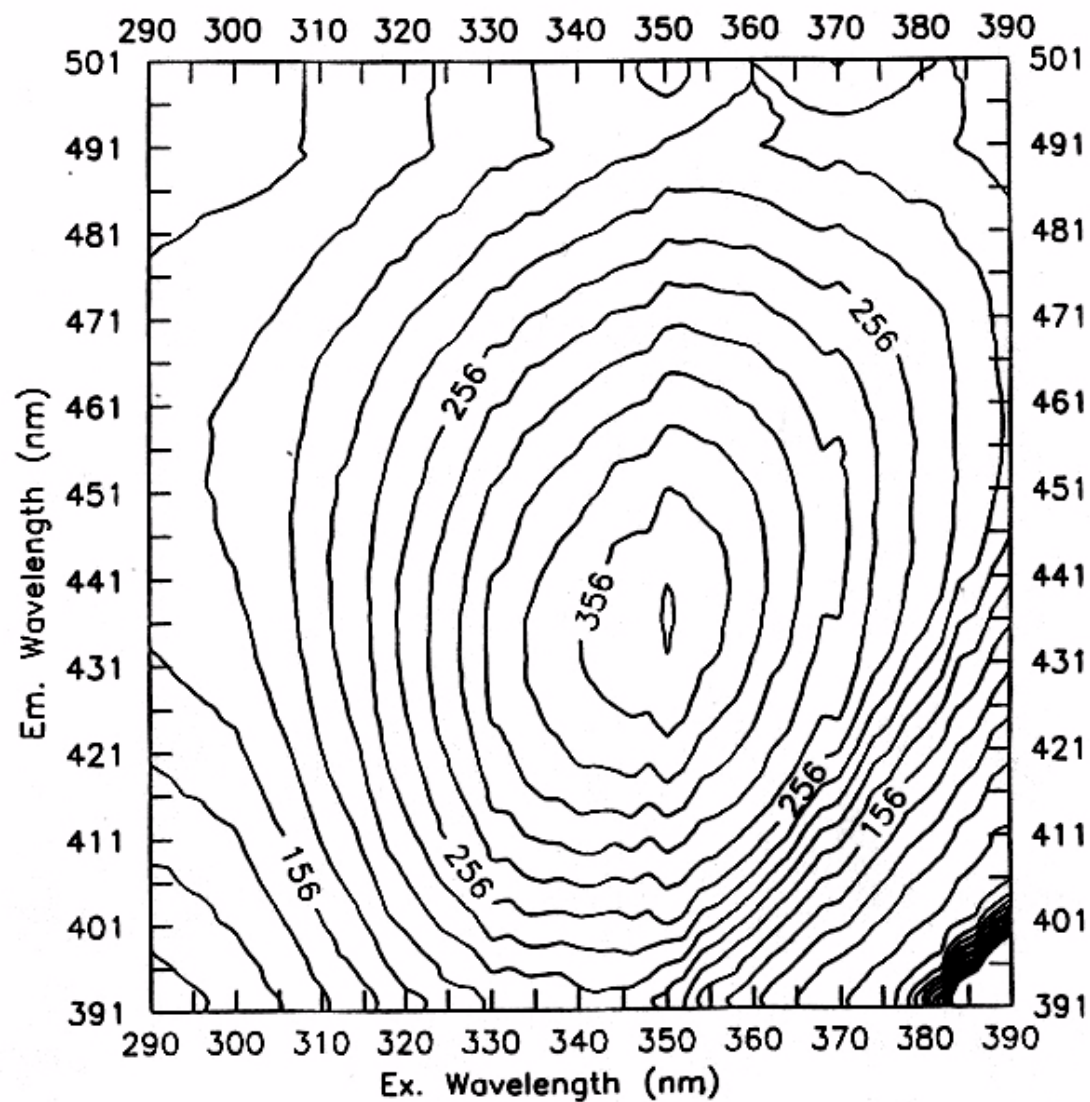Fluorescence Intensity Arbitrary Units

Emission Wavelength in nm with 340 nm Excitation
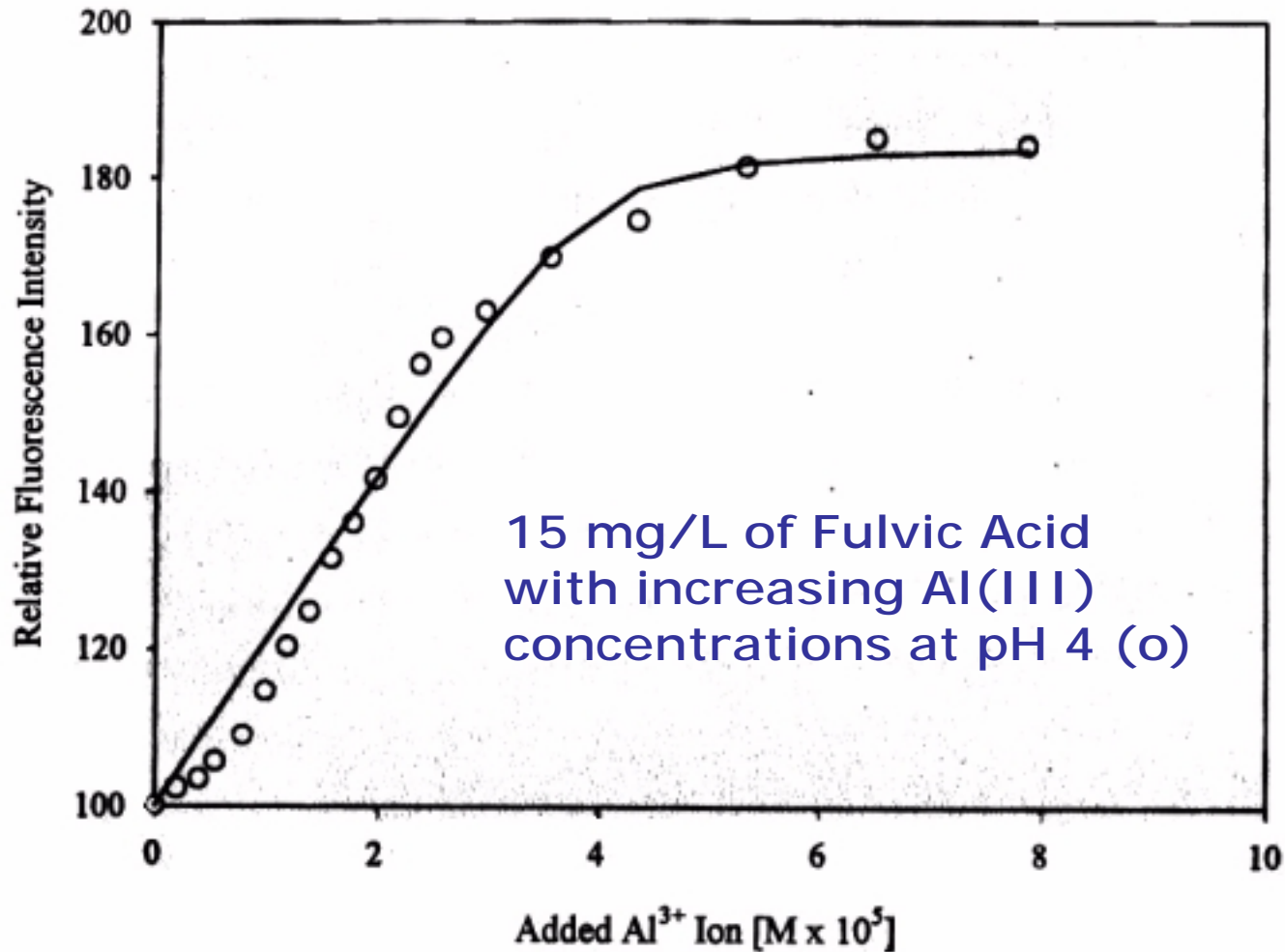
Excitation Emission Matrix

15 mg/L Fulvic Acid at pH 4 with no Al(III)
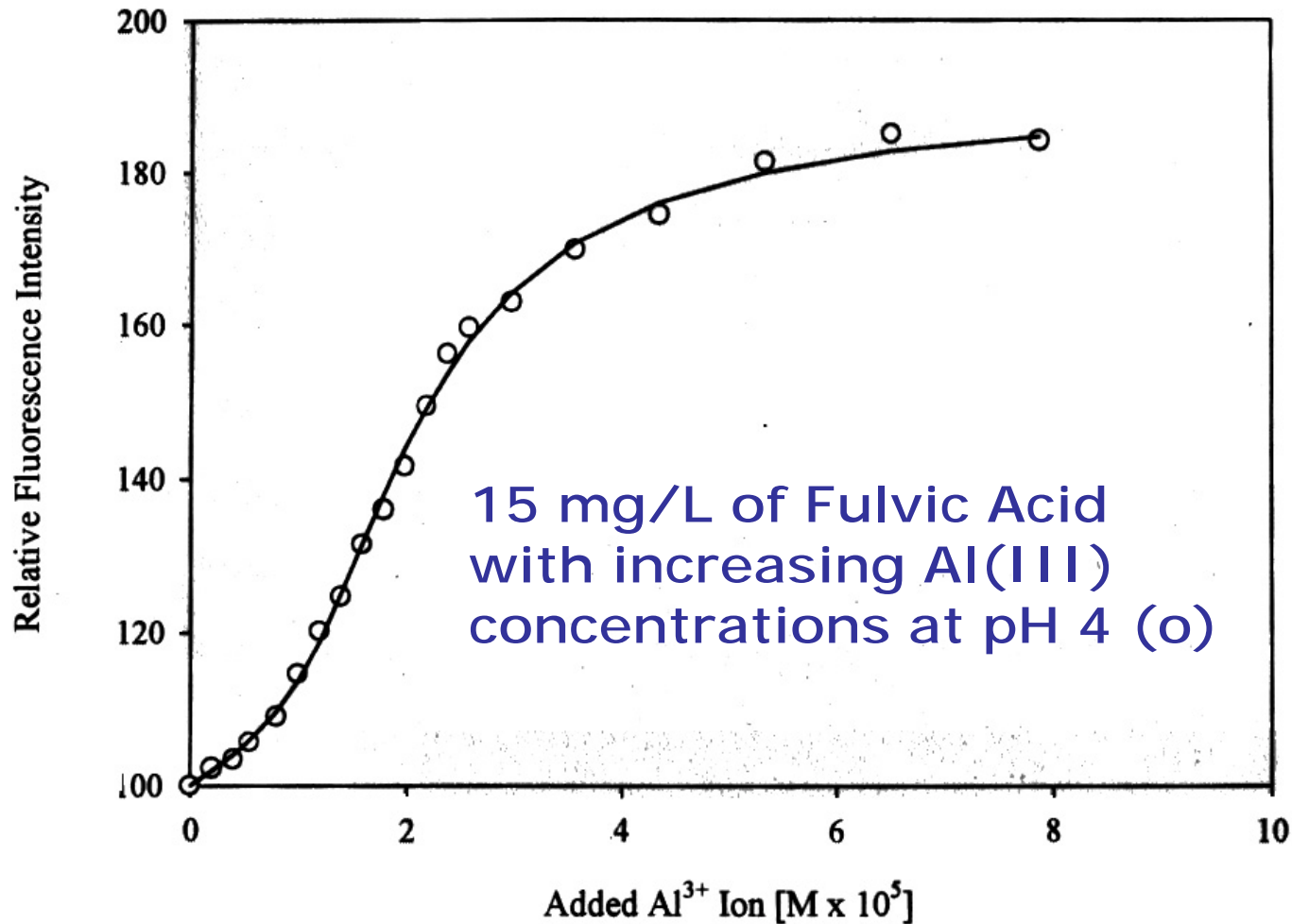
Excitation Emission Matrix

15 mg/L Fulvic
Acid at pH 4
with Al(III)

# Fluorescence Enhancement Curve with One-Site Model

15 mg/L of Fulvic Acid with increasing Al(III) concentrations at pH 4 (o)

Fluorescence Enhancement Curve with Two-Site Model

15 mg/L of Fulvic Acid with increasing Al(III) concentrations at pH 4 (o)

Individual Fluorescence Intensities Making Up the Overall "I"

$$I = I_{L1} + I_{ML1} + I_{L2} + I_{ML2}$$

Hypothetical Fluorescence Intensity Curves Showing Component Intensities
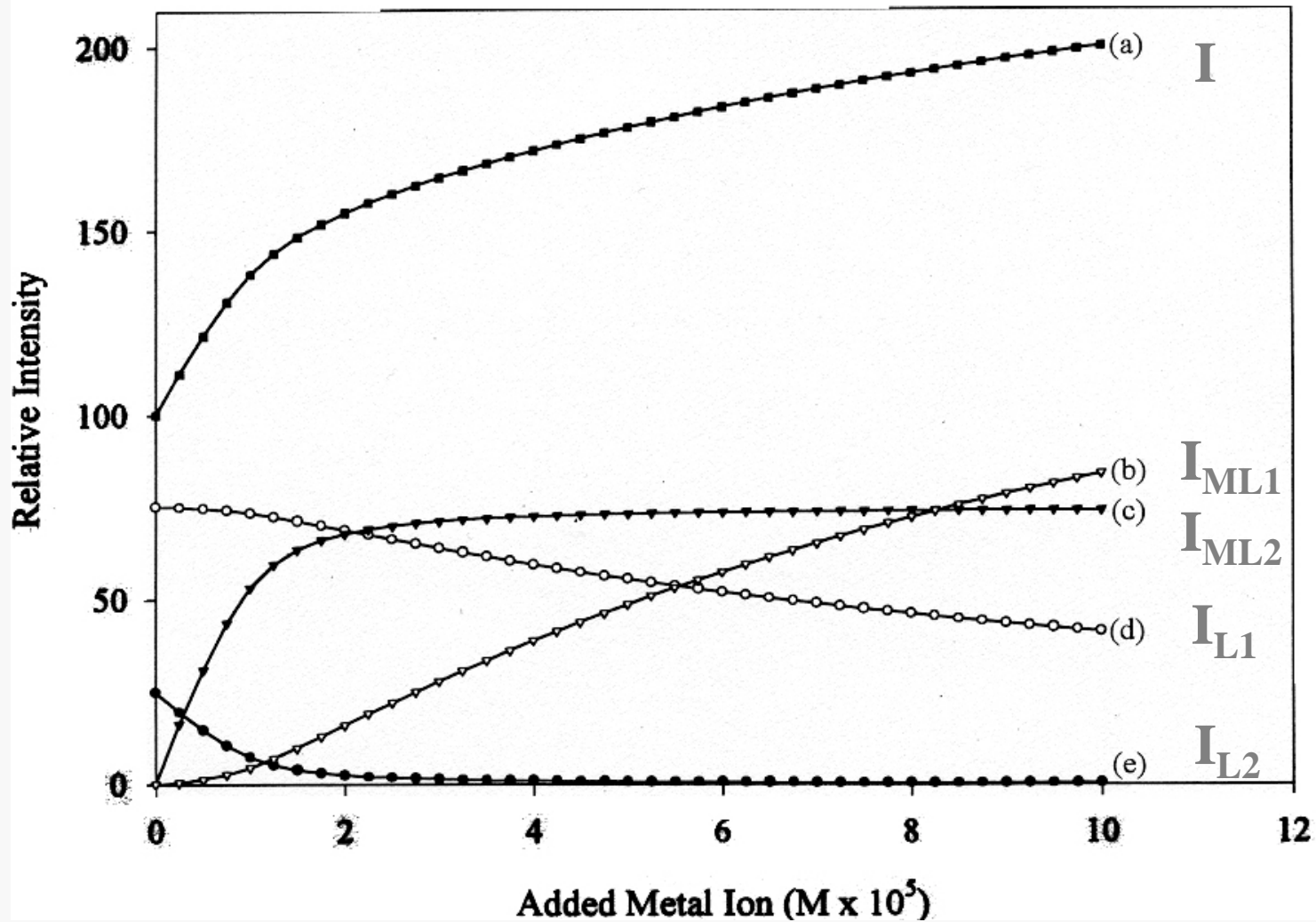
c) Long range radiative transfer

$$^1A^* + Q \rightarrow A + {}^1Q^*$$

Absorption spectrum of acceptor must overlap the fluorescence spectrum of donor

Occurs over distances of 50 Å or more
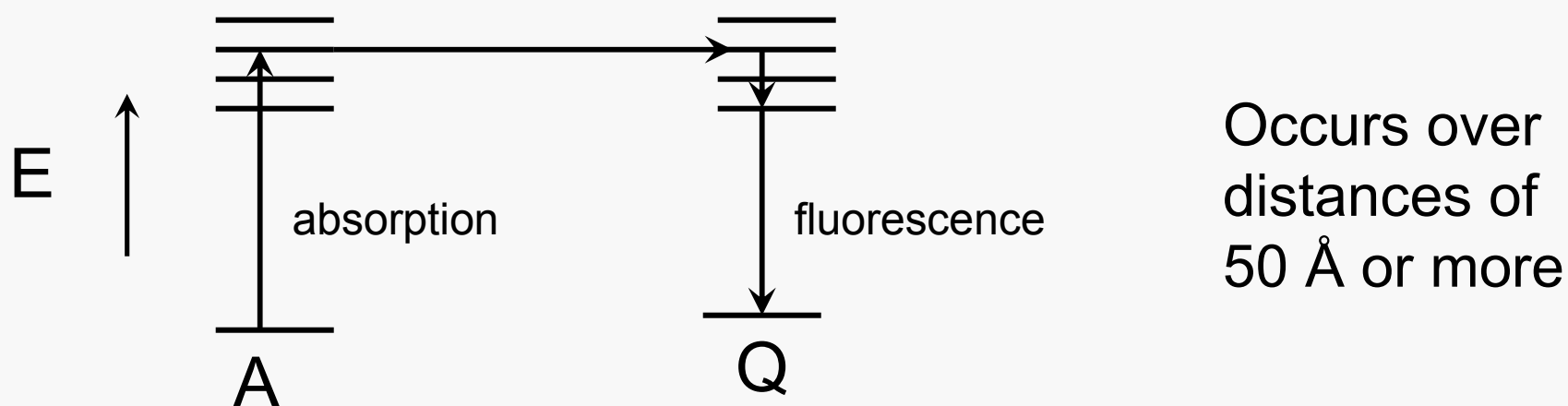
The solution to this problem (i.e. loss of energy) is to reduce the level of Q → usually $< 10^{-4}$ M is satisfactory

2) Oxygen quenching is also a problem (sometimes degas a solution to see if F goes up)

Another excited state process is **<u>eximer</u>** formation → an excited state dimer

$$^1A^* + A \; \rightarrow \; AA^* \; \rightarrow \; AA \; + \; h\nu \; \leftarrow \text{emission shifted to longer } \lambda$$

Heavy Atom Effect – atoms with high atomic number cause spin selection rules to be less rigidly obeyed → promotes IS at the expense of fluorescence

Observed if heavy atom is internal (in molecule) or external (in solvent)

Solvent Effects – essentially the same as for absorption → for π-π* transitions → increase solvent polarity → excited state stabilized relative to ground state → smaller ΔE → λ_{max} shifts to longer λ

Relationship between structure & fluorescence

Remembering that

$$\Phi_F = \frac{K_F}{K_F + K_{IC} + K_{IS}}$$

Also that larger ε → larger K_F (rate of fluorescence)

1)   Nature of Transition - π-π^* vs. n-π^*

Previously stated π-π^* $\rightarrow$ large ε (i.e. probable)

Therefore large K_F $\rightarrow$ large Φ_F generally

e.g. PAHs are efficient fluorophores

n-π^* $\rightarrow$ small ε $\rightarrow$ small K_F,small Φ_F (longer τ_F)

e.g. carbonyls rarely fluoresce efficiently

Fluorescence only observed for compounds
   with an extended π-electron system.
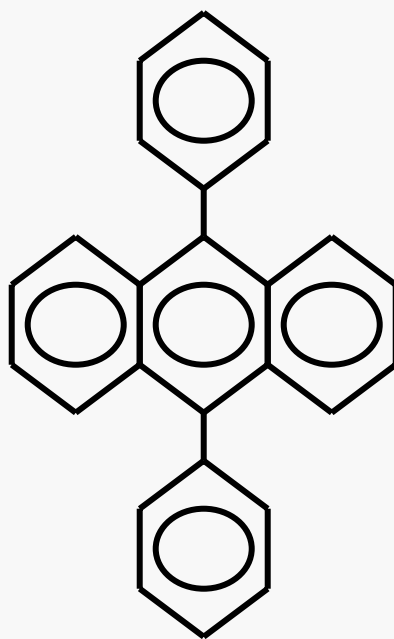
Saturated aliphatics don't fluoresce

Mainly aromatics

1) Effect of Conjugation – similar to UV-vis
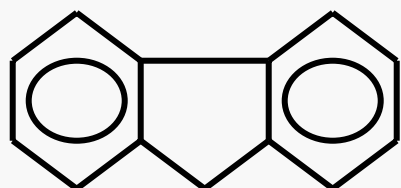
More conjugation → λ_{max} shifts to longer λ & ε

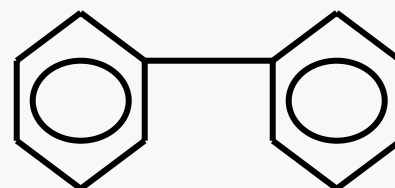increases → λ_F shifts to longer λ & Φ_F
   increases

has Φ_F = 1.0

3) Effect of structural rigidity - more rigid → less interaction with solvent → smaller K_{IC} → higher Φ_F

Fluorene
$\Phi_F = 1.0$

Biphenyl
$\Phi_F = 0.2$

4) Heavy atom effect → higher K_{IS} → smaller Φ_F
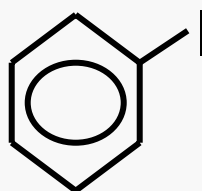
Internal →

External – no fluorescence in ethyl iodide

5) Effect of Substitution

Effect of Substitution on the Fluorescence of Benzene

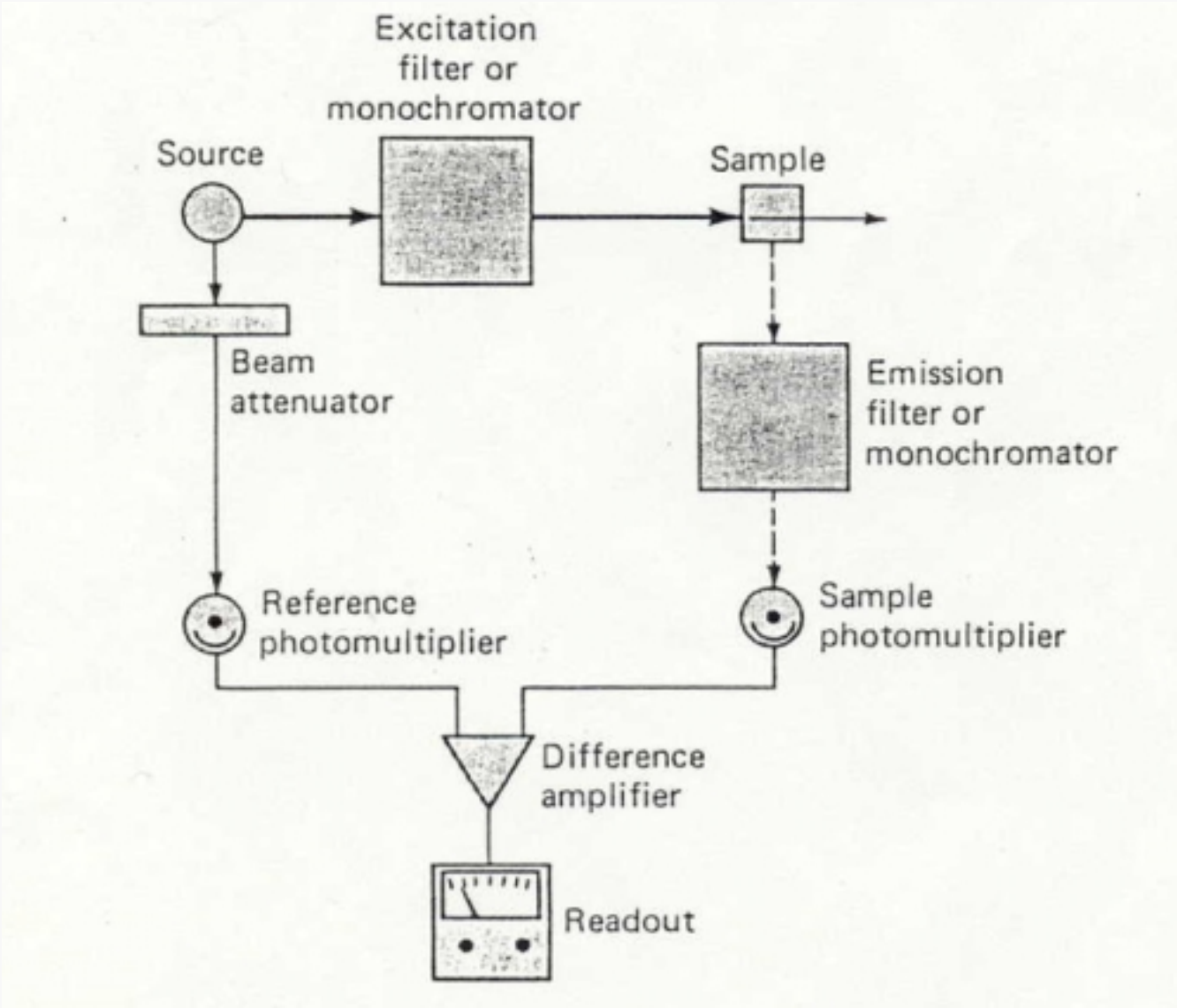| Compound	Formula	Wavelength of Fluorescence, nm	Relative Intensity of Fluorescence
Benzene	C_6H_6	270–310	10
Toluene	$C_6H_5CH_3$	270–320	17
Propylbenzene	$C_6H_5C_3H_7$	270–320	17
Fluorobenzene	C_6H_5F	270–320	10
Chlorobenzene	C_6H_5Cl	275–345	7
Bromobenzene	C_6H_5Br	290–380	5
Iodobenzene	C_6H_5I	—	0
Phenol	C_6H_5OH	285–365	18
Phenolate ion	$C_6H_5O^-$	310–400	10
Anisole	$C_6H_5OCH_3$	285–345	20
Aniline	$C_6H_5NH_2$	310–405	20
Anilinium ion	$C_6H_5NH_3^+$	—	0
Benzoic acid	C_6H_5COOH	310–390	3
Benzonitrile	C_6H_5CN	280–360	20
Nitrobenzene	$C_6H_5NO_2$	—	0

5) Effect of Substitution (conclusions)

   - no effect of alkyl groups

   - heavy atom effect

   - electron donating substituents - increase

   - electron withdrawing – kills fluorescence
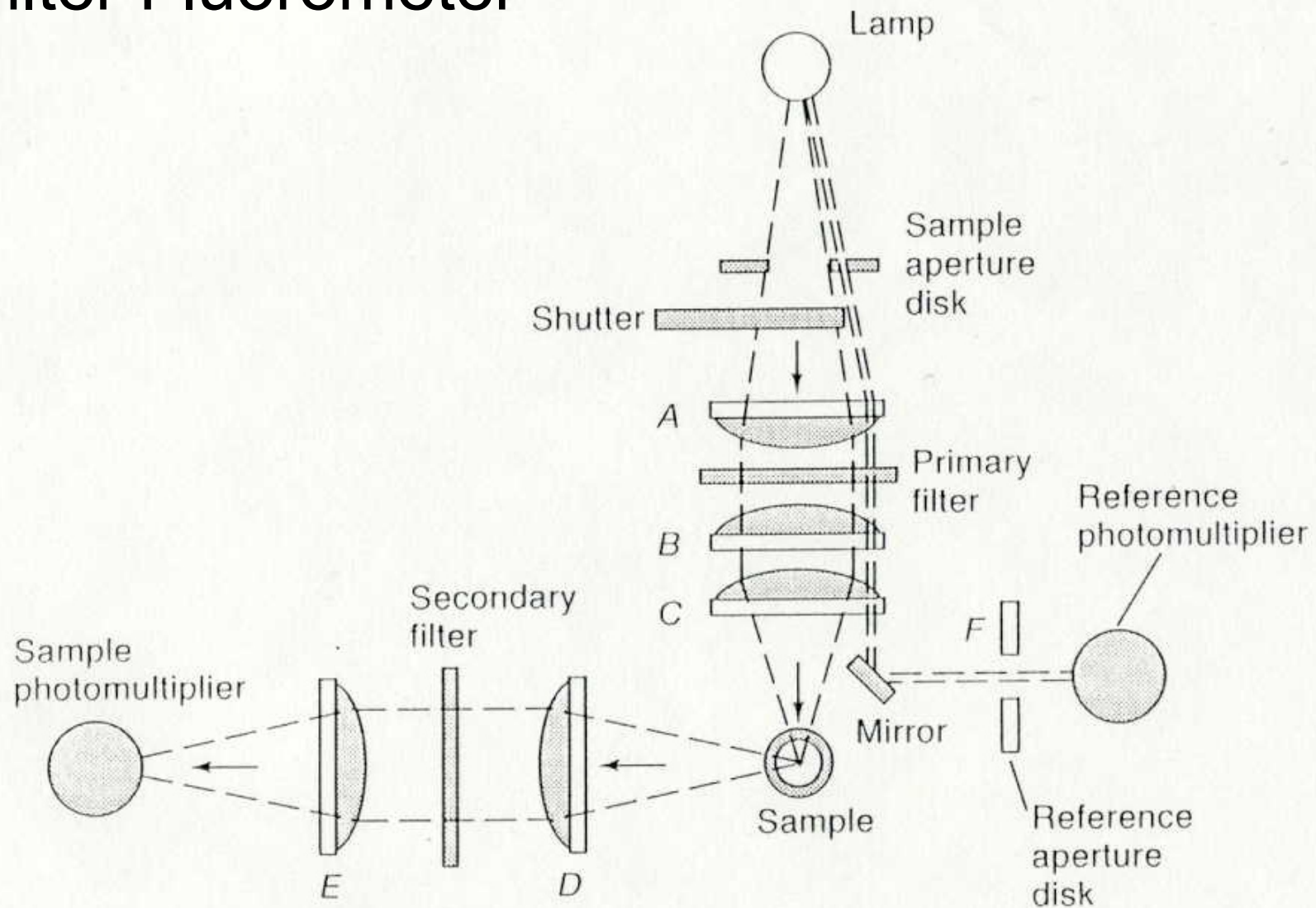
   - n-π^* generally no good

Applications of Fluorescence (general)

1) Quantitative analysis – usually trace

2) Derivatization – to convert non-fluorescent compounds to fluorescent

3) HPLC detection – with & without derivatization (pre or post column)

4) Study chemistry of process involving a change in fluorescence (I or λ)

5) Study excited state chemistry
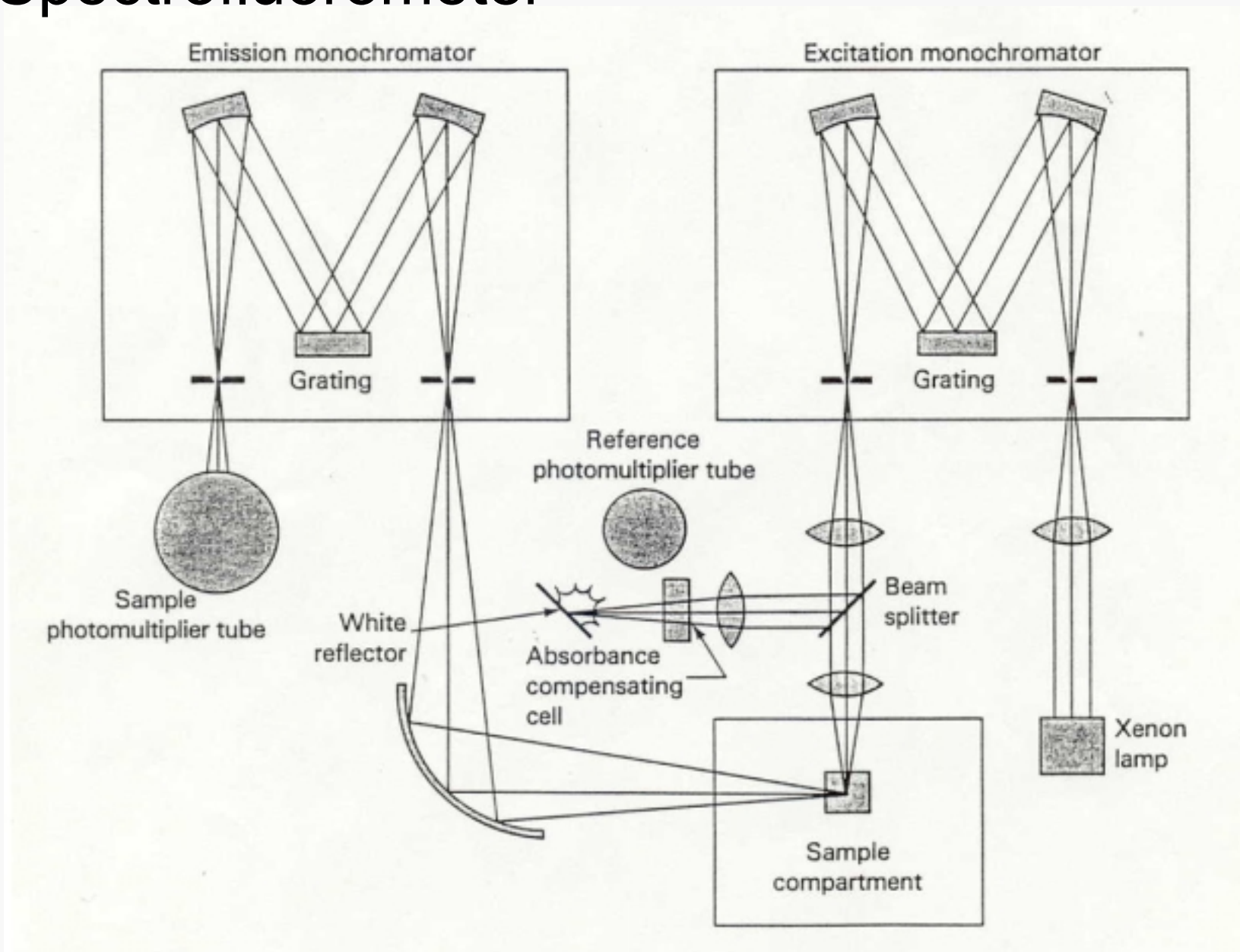
6) Qualitative identification – limited use

Fluorescence Instrumentation (basic diagram)

Filter Fluorometer

Spectrofluorometer

Phosphoroscope

Sample cell

Dewar flask with liquid nitrogen

Excitation shutter aperture

Emission shutter aperture

From excitation monochromator
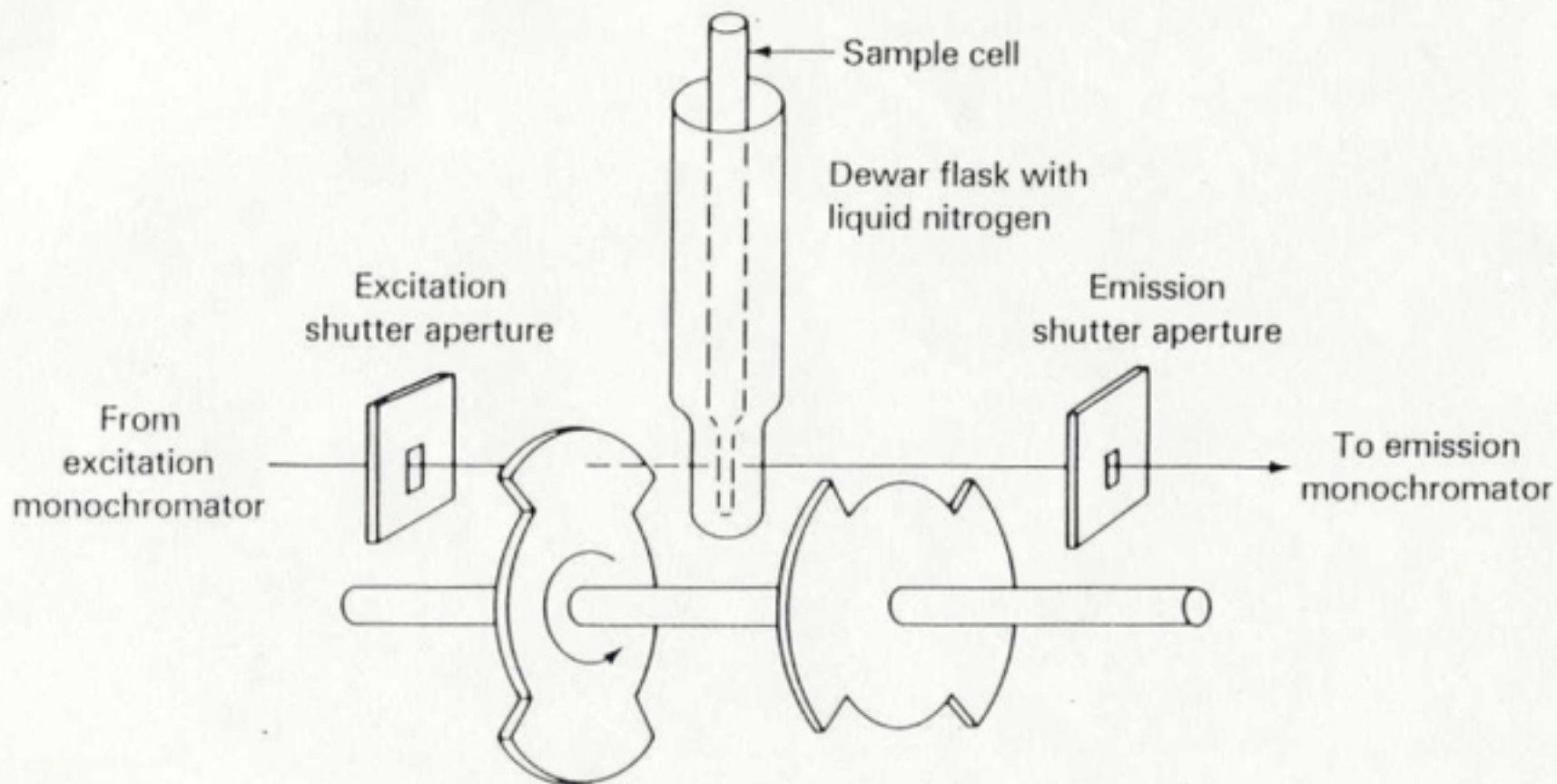
To emission monochromator

FIGURE 9–7   Schematic of a device for alternately exciting and observing phosphorescence. (Reprinted with permission from: T. C. O'Haver and J. D. Winefordner, *Anal. Chem.*, **1966,** *38*, 603. Copyright 1966 American Chemical Society.)