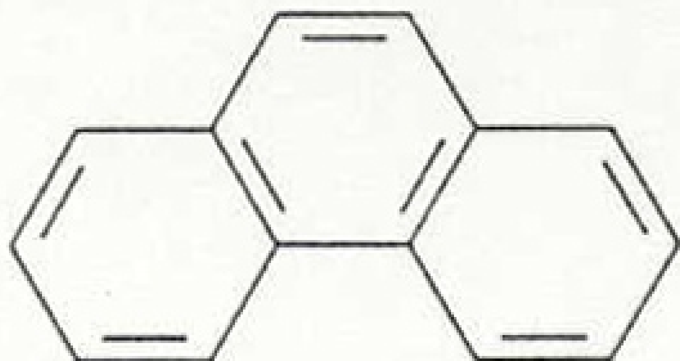
Spectra

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



Relative intensity

Excitation

Fluorescence

Phosphorescence

200 300 400 500 600

Wavelength, nm

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

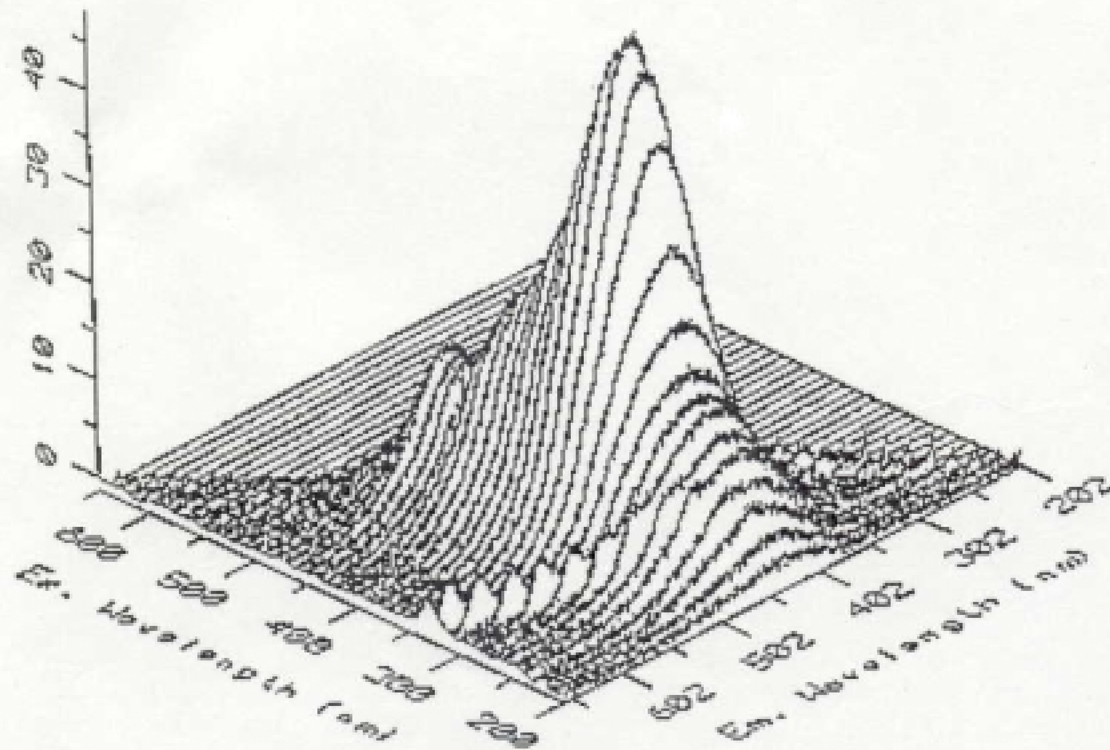
Degree of structure in fluorescence spectrum is due to the degree of solvation as discussed for absorption

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

Spectra

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

Fluorescence Emission Intensity



EEMs represented as contour plots

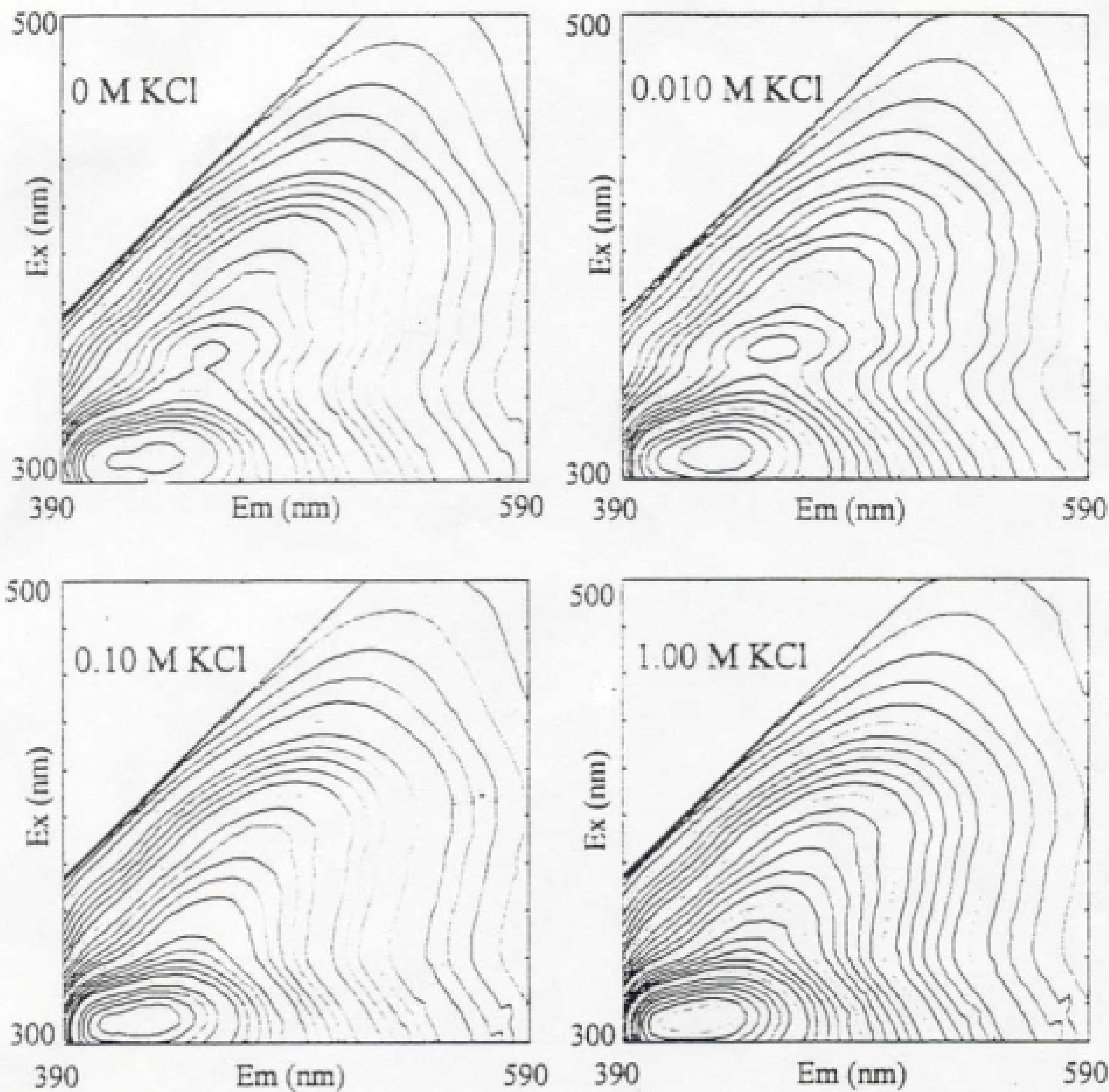
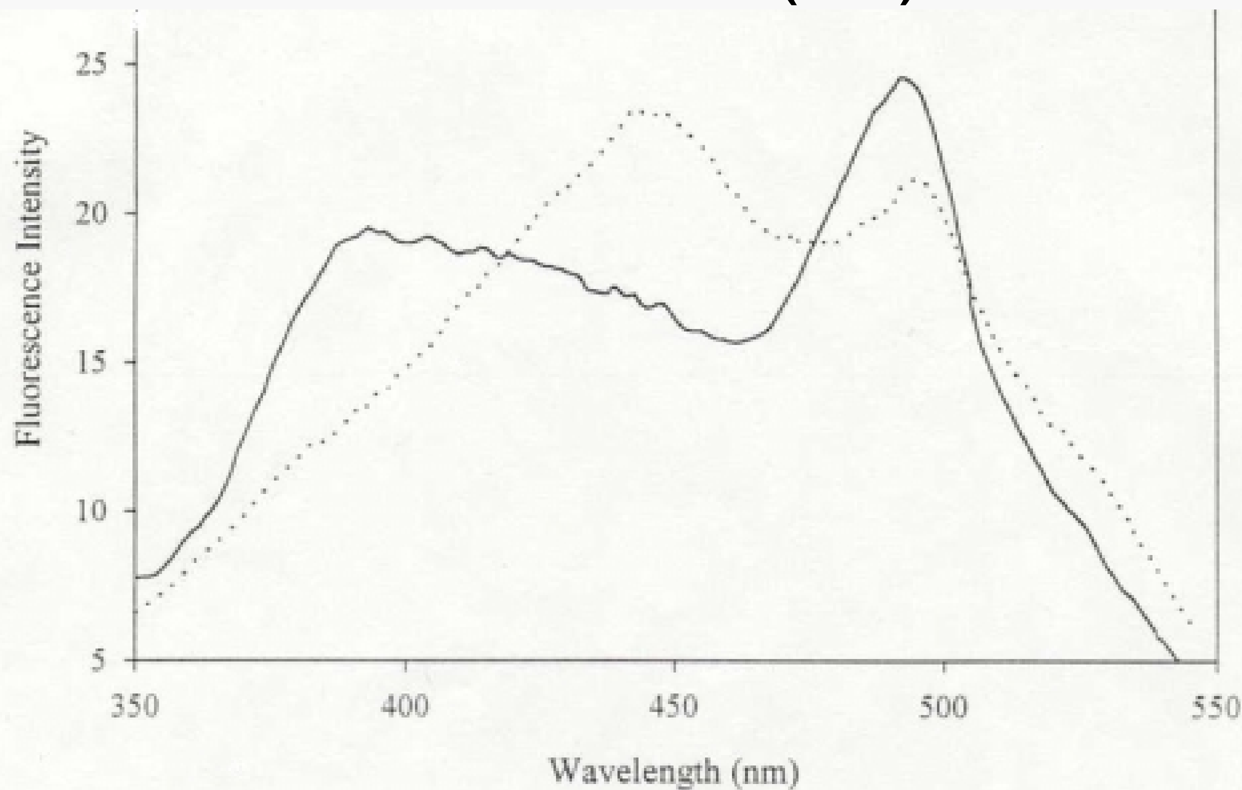


FIGURE 3. Absorbance-corrected EEMs of PFAR (50 mg/L, pH 6.0) at different ionic strengths.

Spectra

5) <u>Synchronous Scan Spectrum</u> – record fluorescence intensity while scanning both excitation and emission λ, usually at a constant offset (Δλ)



Synchronous scans of natural organic matter with Δλ = 25 nm at pH 4 & 9 (solid line)

Fluorescence Efficiency or Quantum Yield (Φ_F)

$$\Phi_F = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$

Fluorescence efficiency depends on the rate of fluorescence relative to the rates of other processes involving excited state deactivation

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

K_F: rate of fluorescence

K_{IC}: rate of radiationless deactivation or internal conversion

K_{IS}: rate of intersystem crossing

$$K_F = \frac{1}{\tau_F}$$

τ_F: fluorescence lifetime

Clearly anything that affects any of the rates involved in photophysical processes will affect Φ_F

e.g. temperature increases cause a decrease in Φ_F → higher temp increases K_{IC} which reduces Φ_F, K_F doesn't vary significantly with temp

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

Heating of solutions can be a problem with intense sources

Relationship between fluorescence intensity and concentration

$$I_F = K\Phi_F (I_o - I)$$

Instrumental factor (K)

Fluorescence quantum efficiency (Φ_F)

Fluorescence intensity (I_F)

Incident light intensity (I_o)

Transmitted light intensity emerging from sample unabsorbed (I)

Substitute Beer's Law

$$\log I_o/I = \varepsilon bC \quad \text{or} \quad I = I_o e^{-\varepsilon bC}$$

Result

$$I_F = K\,\Phi_F\,I_o\,(1 - e^{-\varepsilon bC})$$

This term in parentheses results in a series expansion of the form

$$\frac{(2.3\,\varepsilon\,b\,C)^n}{n!} + \ldots$$

where n = an integer increasing from 1

For εbC (i.e. A) < 0.05 terms past the first Are dropped giving

$$\boxed{I_F = K\,\Phi_F\,I_o\,\varepsilon\,b\,C}$$

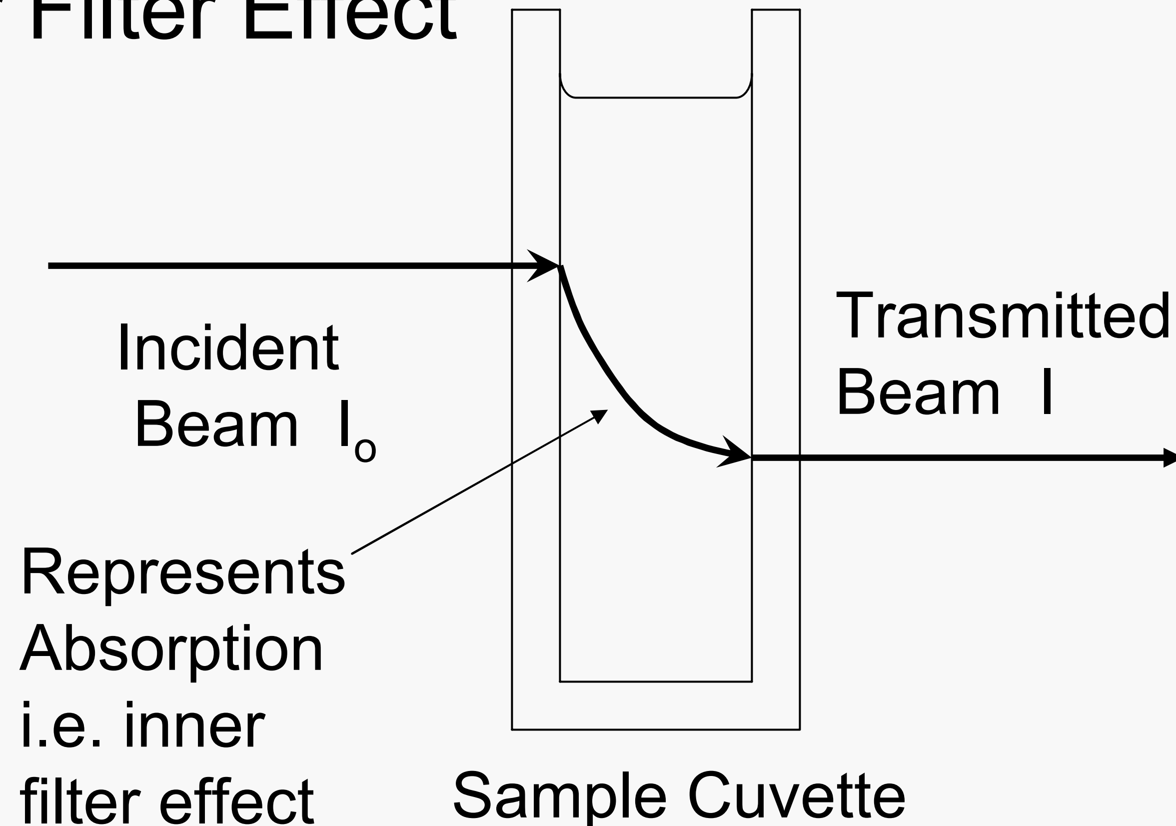
Note dependence on Φ_F, I_o, ε, b

Inner Filter Effects – if the absorbance of the sample is above 0.05, fluorescence intensity is decreased in two ways:

1) Primary Inner Filter Effect – excitation energy is decreased by absorption at the λ of excitation which decreases fluorescence
2) Secondary Inner Filter Effects – emitted fluorescence is absorbed by the sample at the emission λ which also reduces the amount of fluorescence measured

Primary Inner Filter Effect

At high ε b C



The front part of the cell is illuminated more efficiently than the back part → get less fluorescence from the back part of the cell

Inner Filter Effects – conclusions

- Inner filtering results in curved calibration curves
- Must know or measure absorbance at both the excitation and emission wavelengths
- Fluorescence is basically a trace technique having 1 - 3 orders of magnitude lower detection limits than absorbance
- Upper limit of fluorescence occurs around lower limit of absorbance (complimentary)
- Inner filtering is also called quenching

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

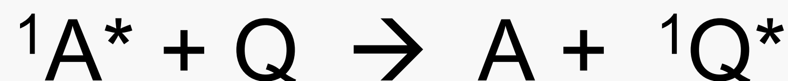
e.g. Phenol ground state $pK_a = 10.0$

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

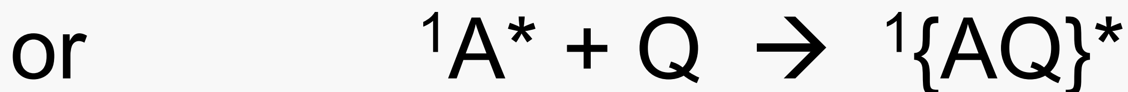
Effects of other solutes on excited state emission → can promote radiationless return to ground state = quenching

1) Energy Transfer



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

or



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

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

Occurs by

a) Q absorbs the emission of A

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

Stern-Volmer Equation

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

Fluorescence Quantum Yield in the absence of quencher (Φ_F^o)

With quencher (Φ_F)

Quenching constant (K_Q)

Fluorescence Lifetime of A (no Q) (τ_F^o)

Concentration of Quencher ($[Q]$)

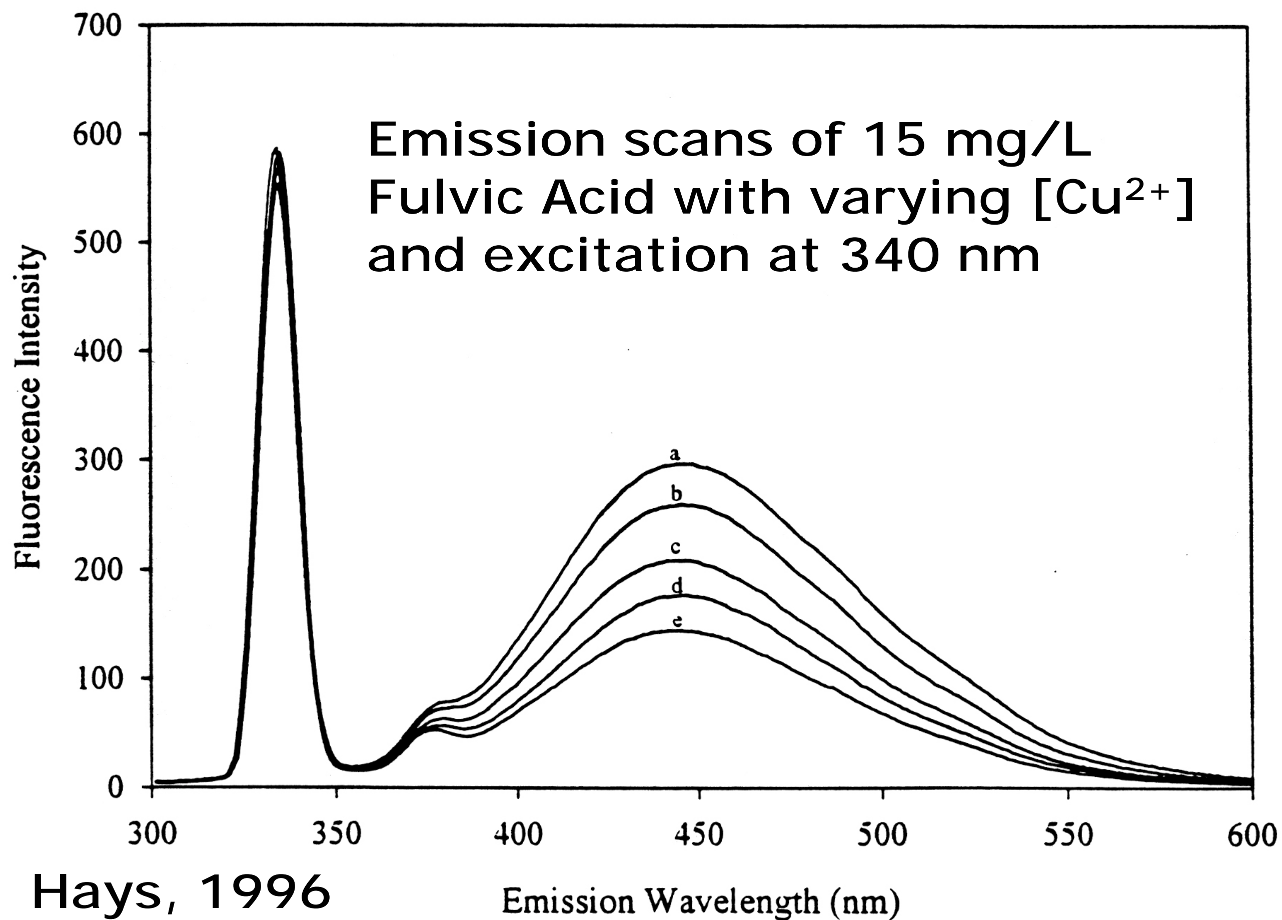
Two types of quenching are covered by the Stern-Volmer Equation:

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+}



Stern Volmer Equation

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

The Ryan Equation

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

1:1 Complex Formation

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

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

Equations for Fitting Data

Equation for One Site Binding

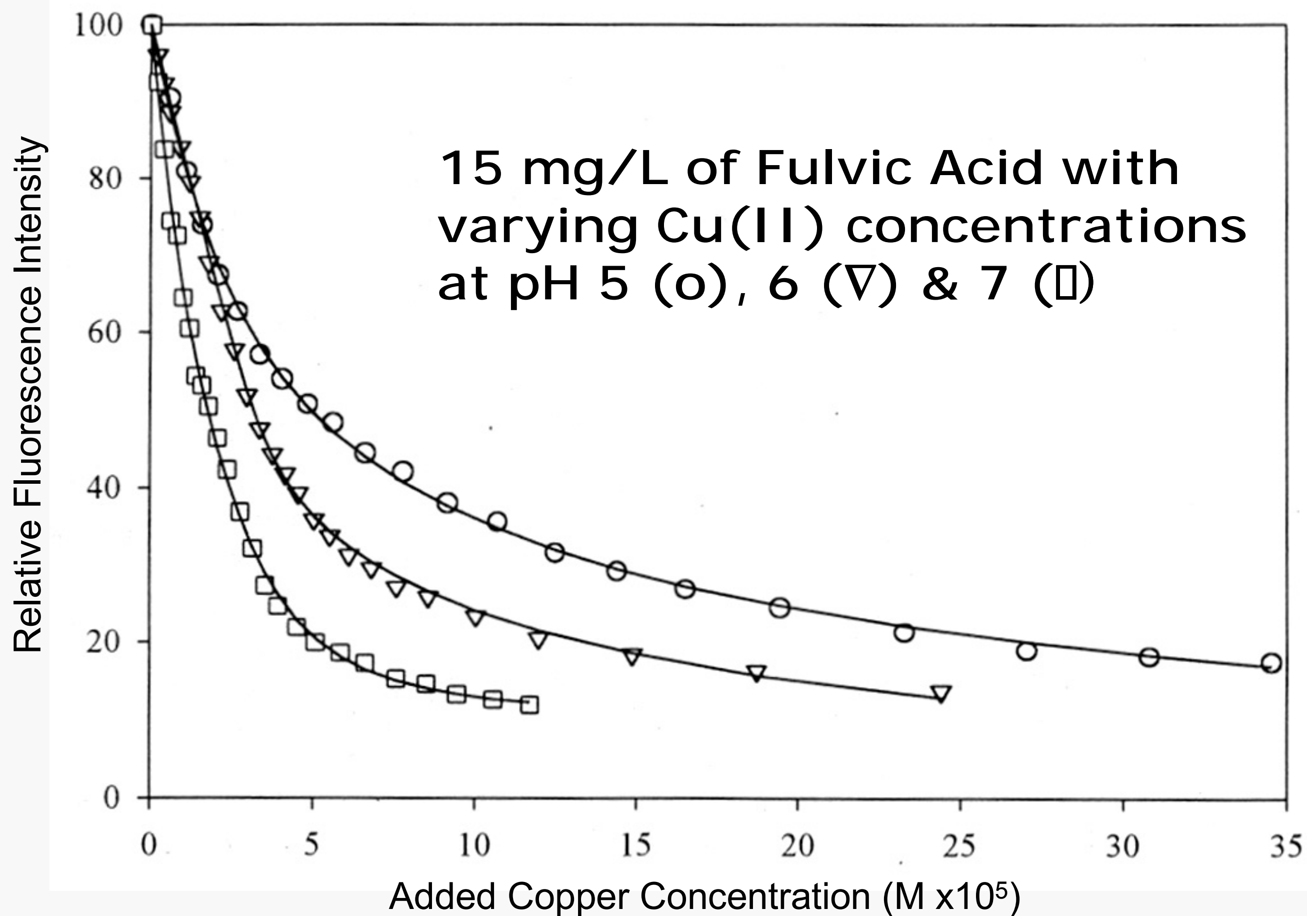
$$I=[200+2KI_{RES}C_M-I_{RES}[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_MC_L)^{0.5}]] / [2+2KC_M-[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_LC_M)^{0.5}]]$$

Equations for Two Site Binding

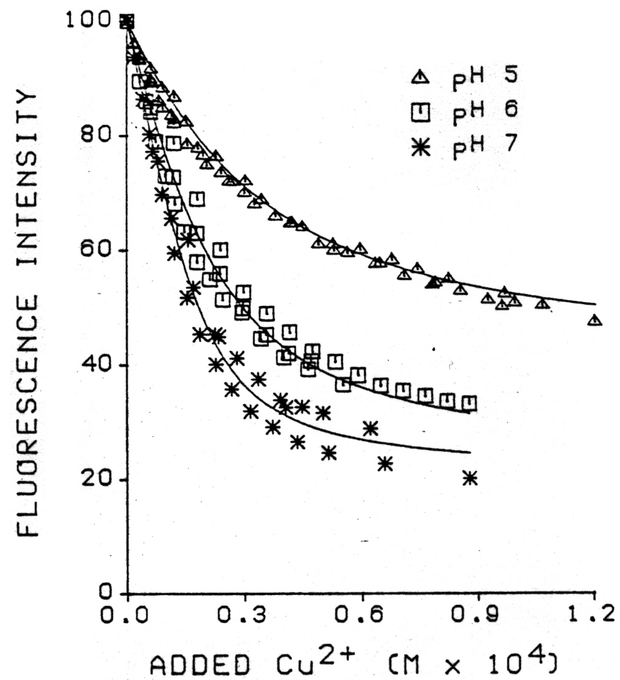
$$C_M = [M] + (K_1C_{L1}[M]/K_1[M] + 1)+(K_2C_{L2}[M]/K_2[M] + 1)+.... +K_nC_{Ln}[M]/K_n[M] + 1$$

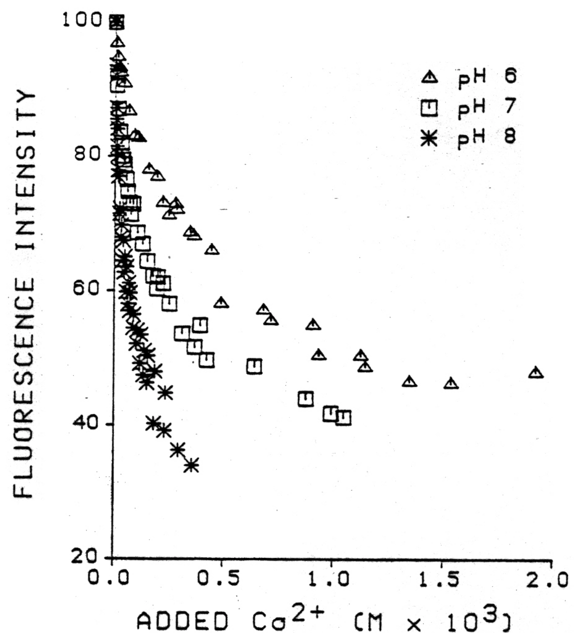
$$K_1K_2[M]^3 + \{K_1K_2(C_{L1}+C_{L2}-C_M) + K_1 + K_2\}[M]^2 + \{C_{L1}K_1+K_2C_{L2}-C_M(K_1 + K_2+1\}[M] - C_M = 0$$

Fluorescence Quenching Curves

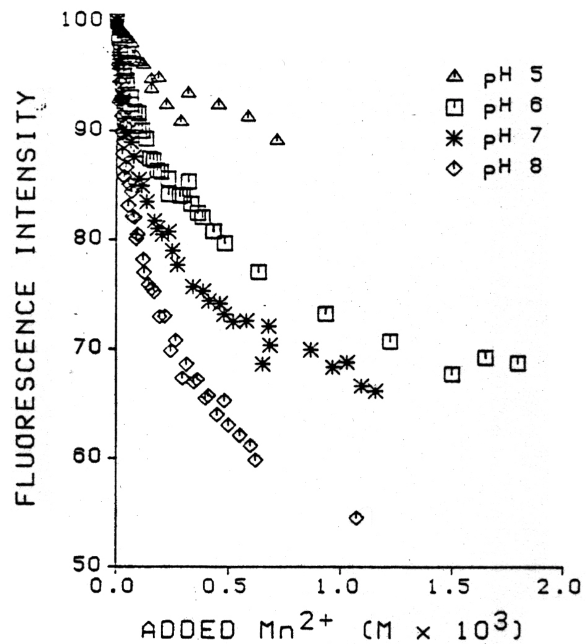


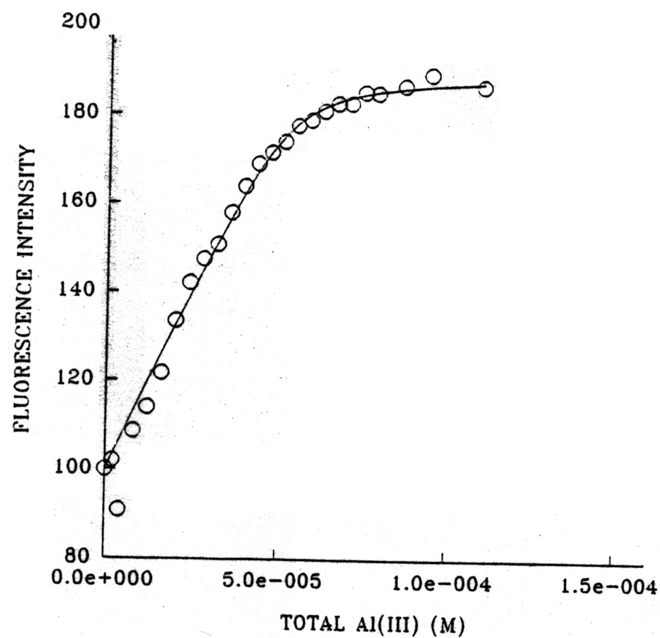
Hays, 1996



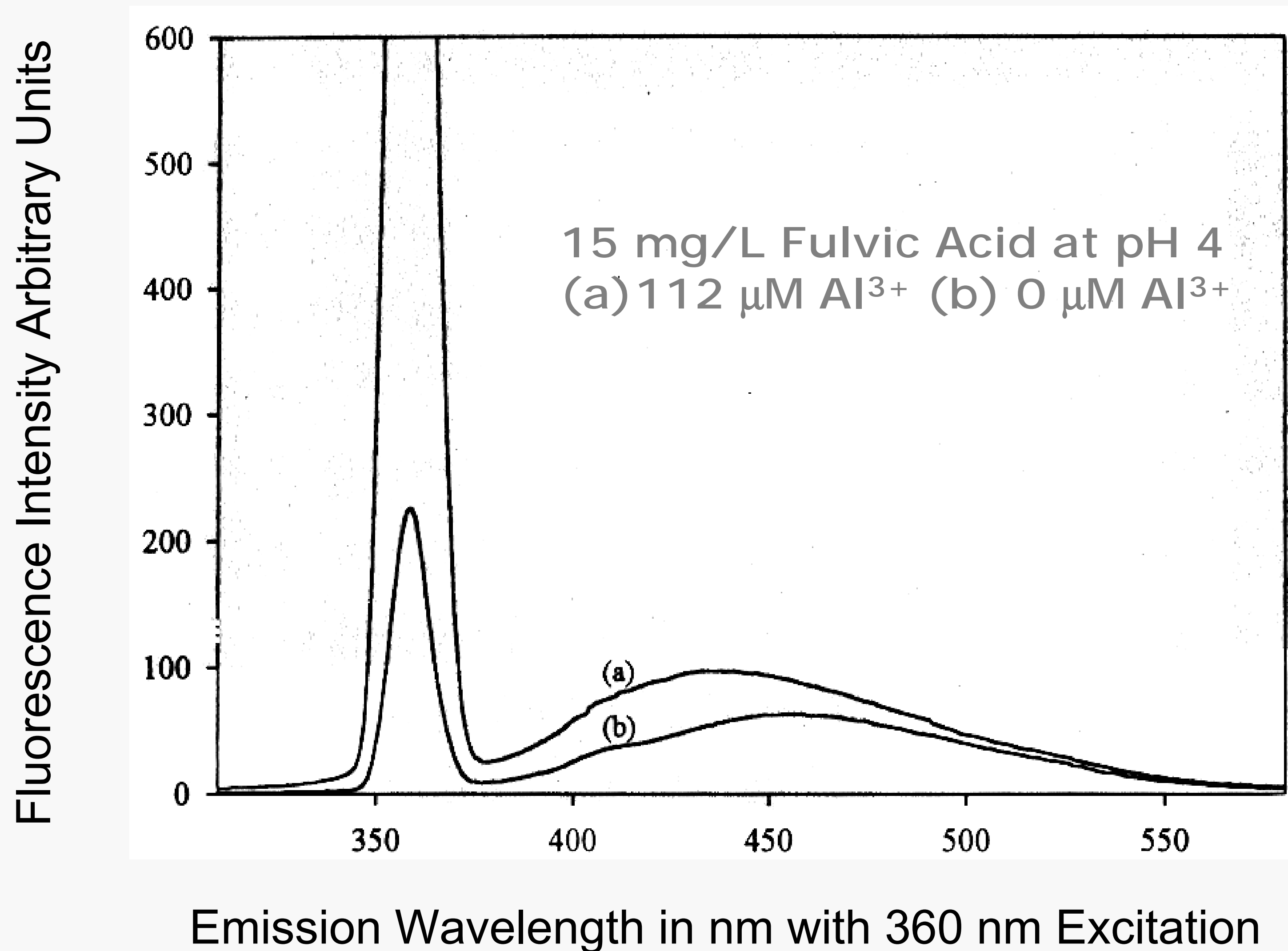


Fluorescence binding curves for Cu, Co, Mn & Al at pH values of 5-8

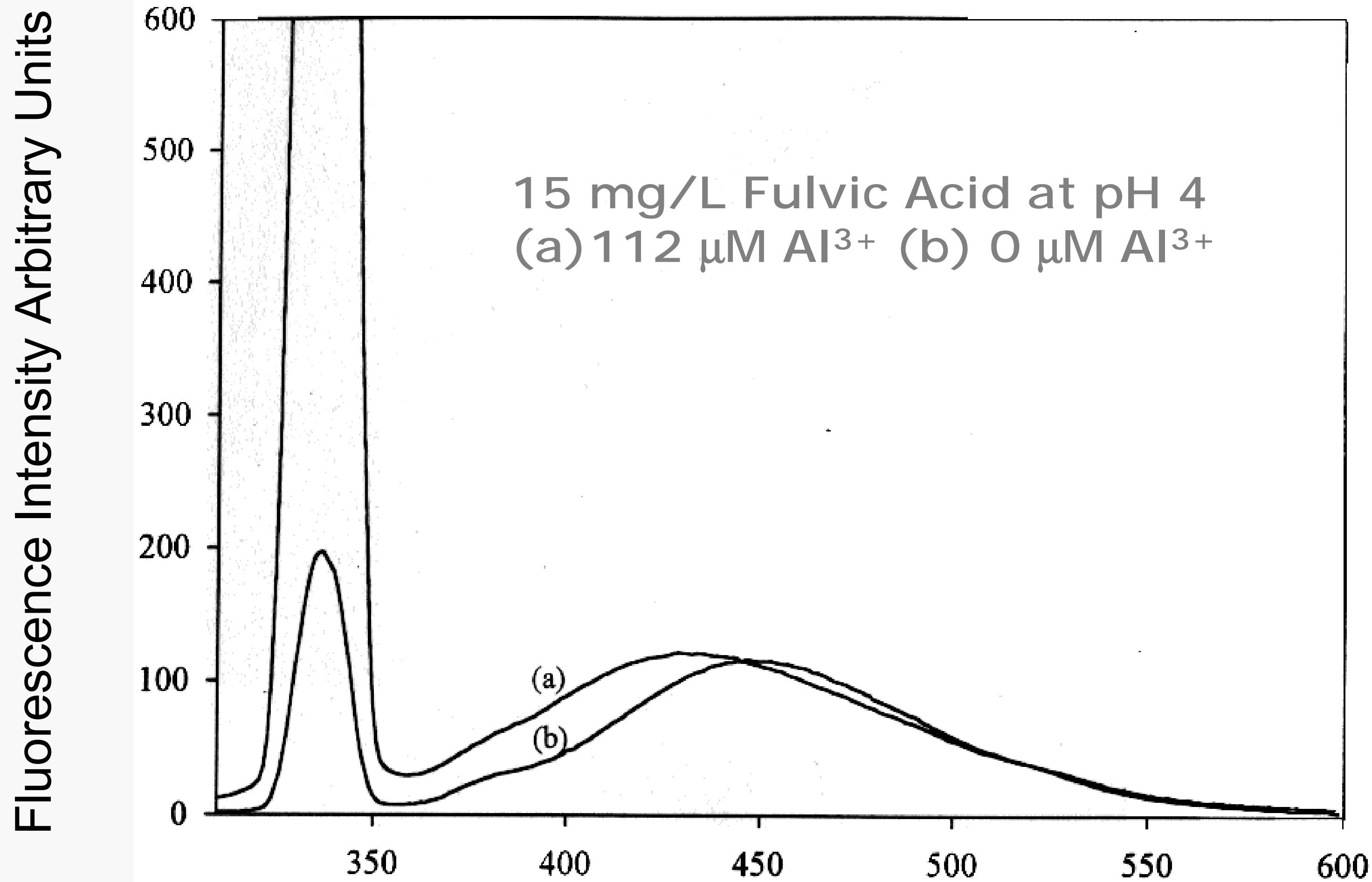




Fluorescence Enhancement

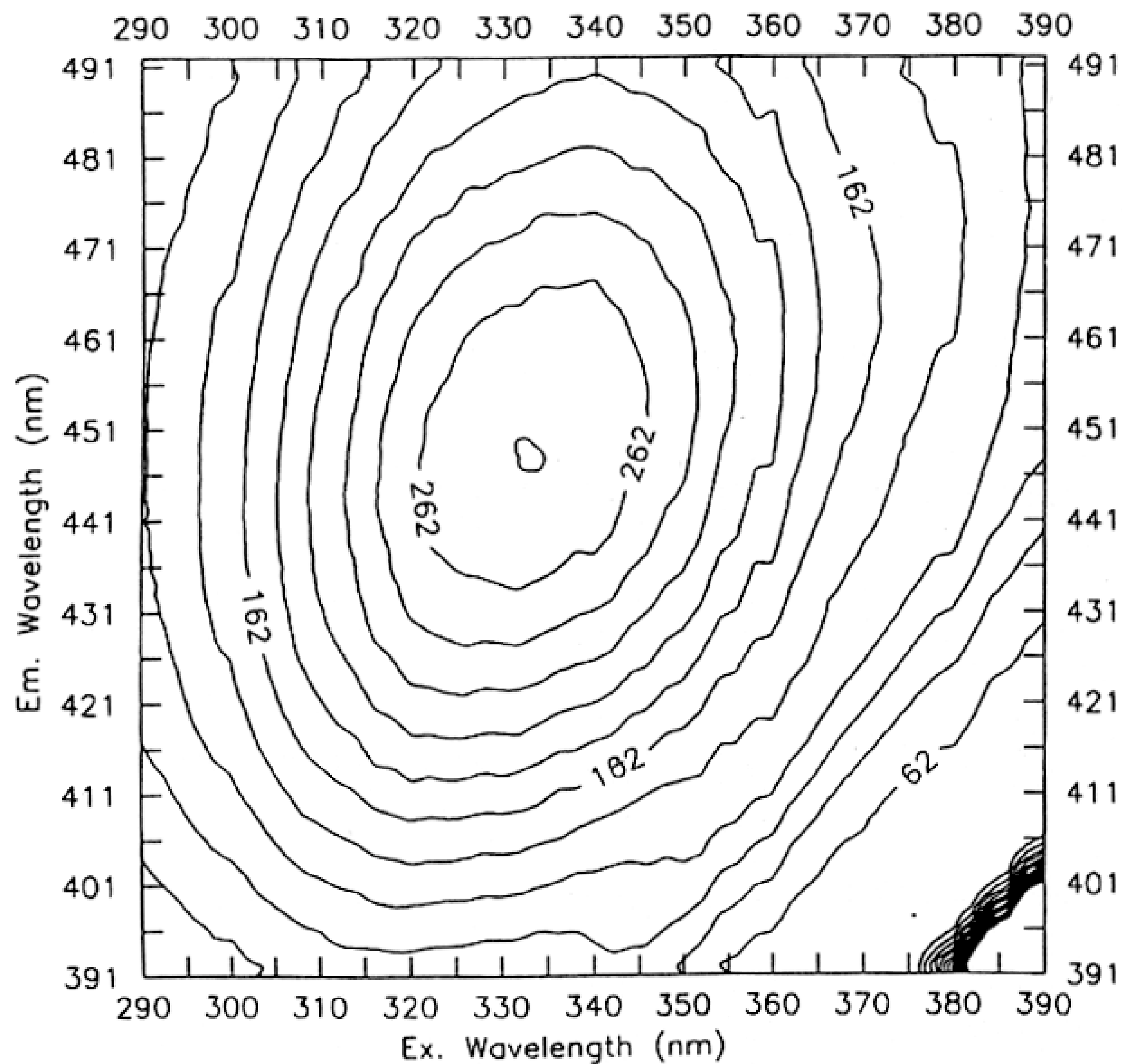


Fluorescence Enhancement



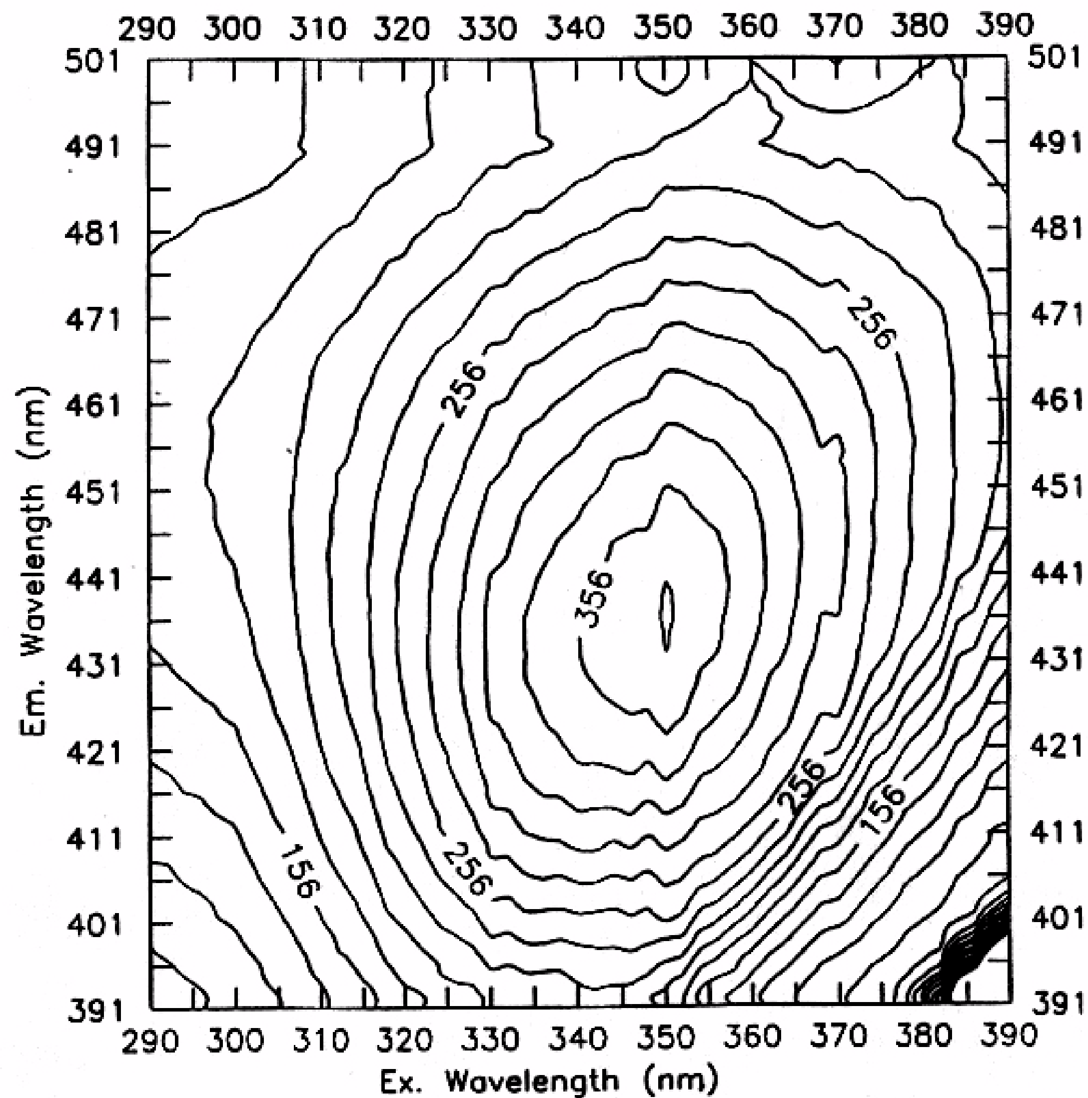
Emission Wavelength in nm with 340 nm Excitation

Excitation Emission Matrix



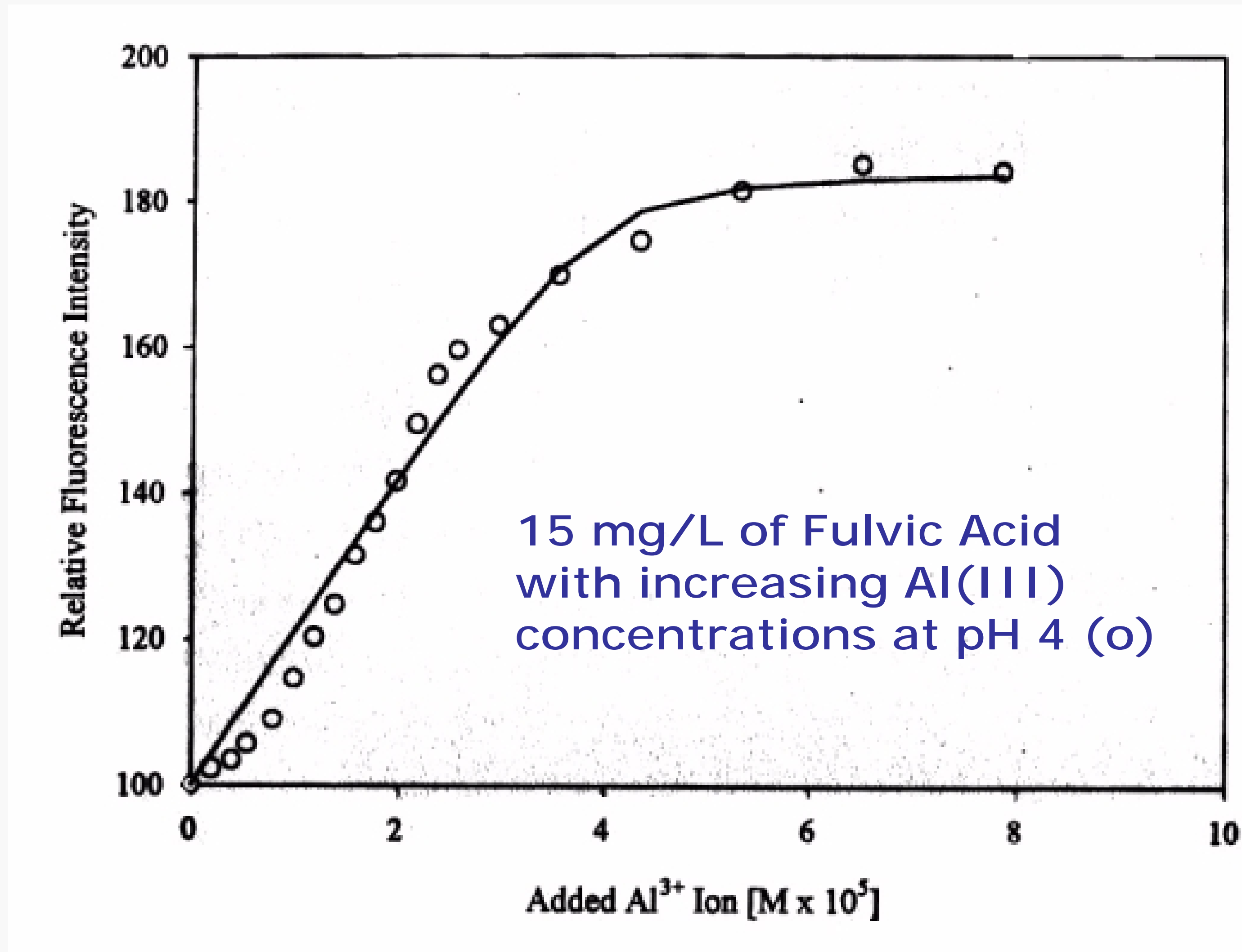
15 mg/L Fulvic Acid at pH 4 with no Al(III)

Excitation Emission Matrix

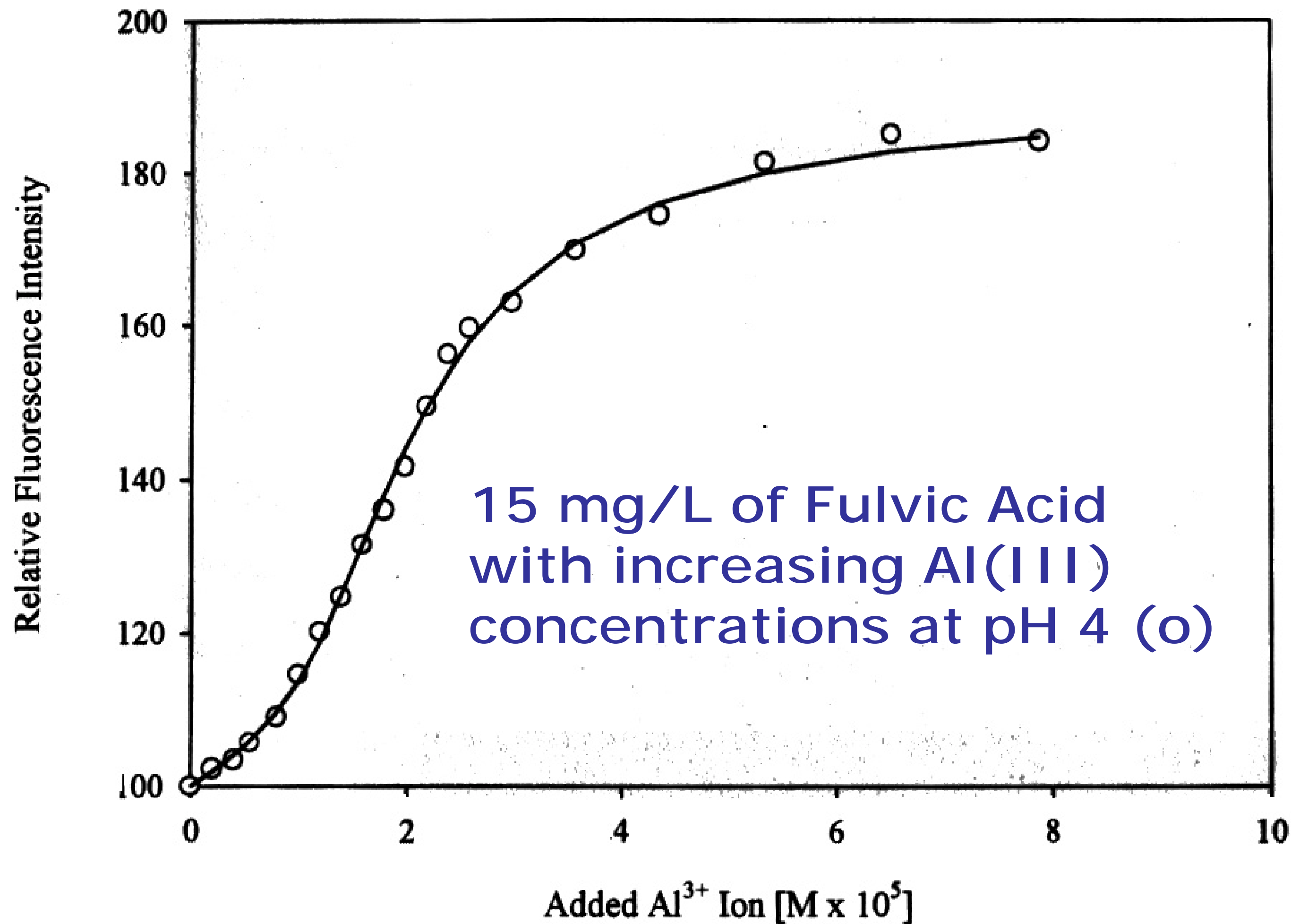


15 mg/L Fulvic Acid at pH 4 with Al(III)

Fluorescence Enhancement Curve with One-Site Model



Fluorescence Enhancement Curve with Two-Site Model



Individual Fluorescence Intensities Making Up the Overall "I"

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