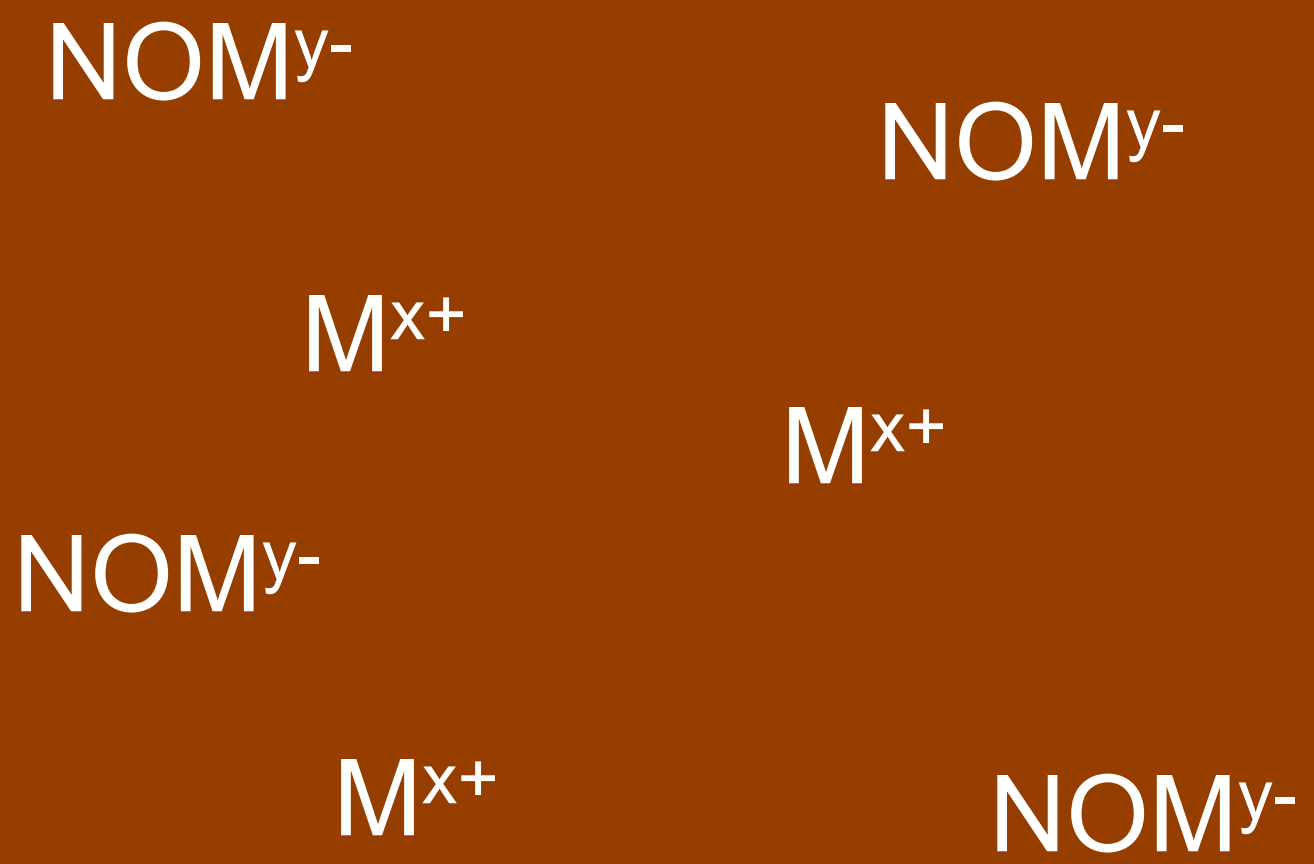


M^{x+} = metal ion, toxic or non, of charge x+ (e.g., Cu^{2+}, Al^{3+}, etc.)

NOM^{y-} = natural organic matter of varying negative charge y-

$$M^{x+} + NOM^{y-} \leftrightarrow M\text{-}NOM^{(x-y)-}$$

$$K = \frac{[M\text{-}NOM^{(x-y)-}]}{[M^{x+}][NOM^{y-}]}$$

K = equilibrium constant describing complexation reaction

$M\text{-}NOM^{(y-x)-}$ = metal complex of natural organic matter

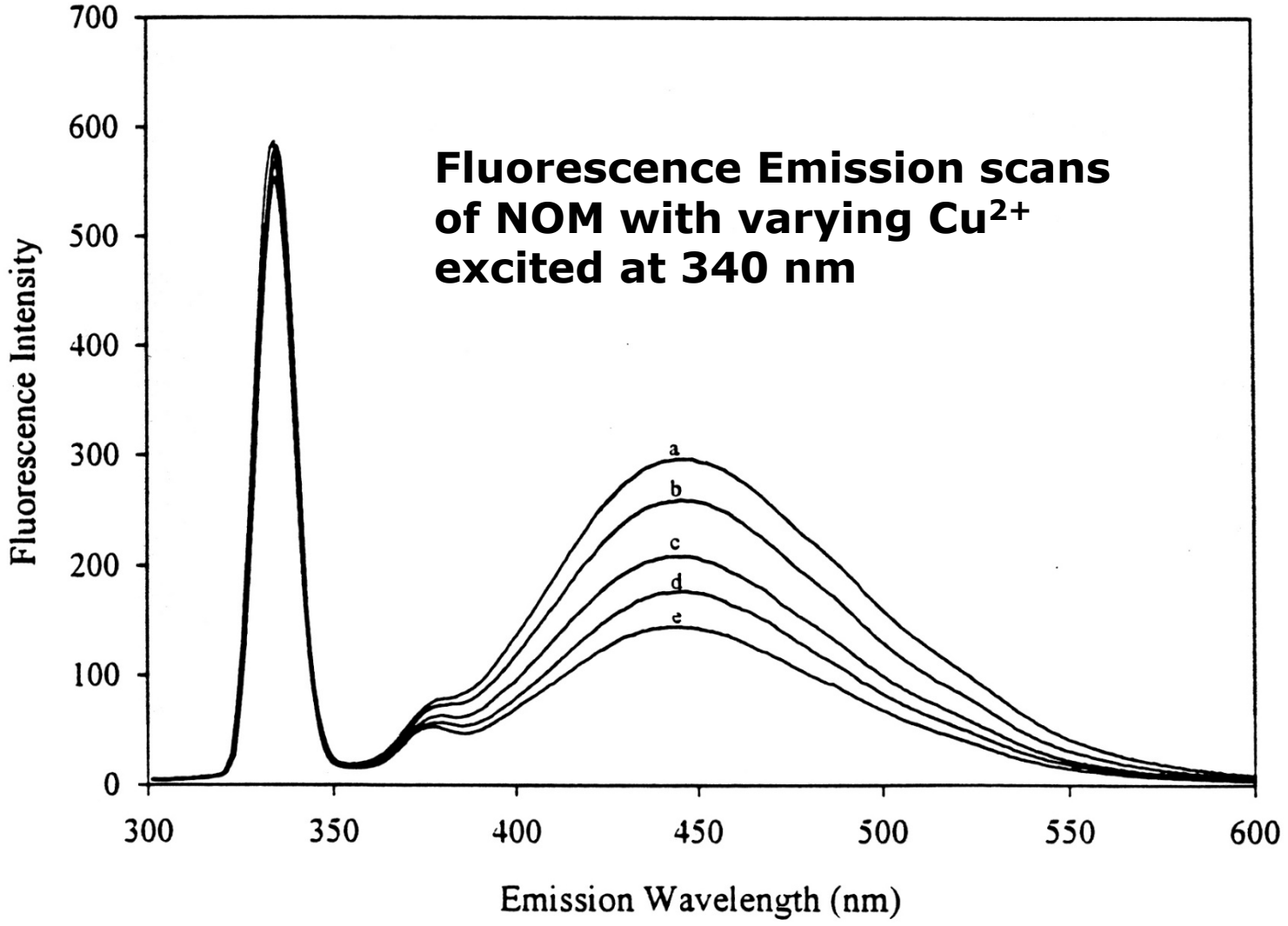
$M^{x+} + NOM^{y-} \leftrightarrow M\text{-}NOM^{(y-x)-}$

↑	↑	↑
measure	or maybe measure	or measure

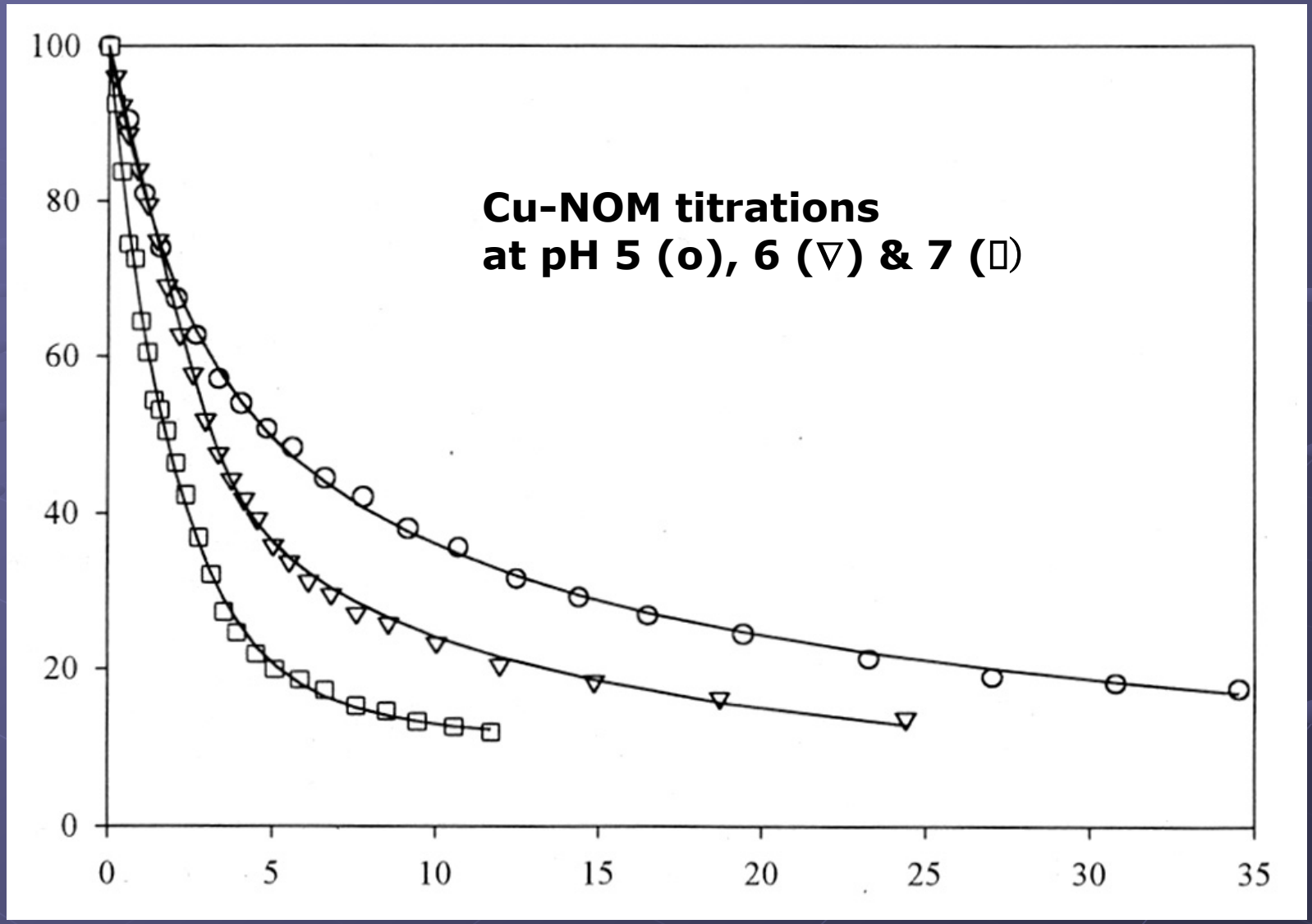
<u>Metal Speciation</u> = determination of the forms of metal in equilibrium with NOM

Measurement must not disturb equilibrium

Fluorescence Quenching



Fluorescence Quenching Curves



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

$$M + L = ML$$

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

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

Equations for Fitting Data

Equation for One Site Binding

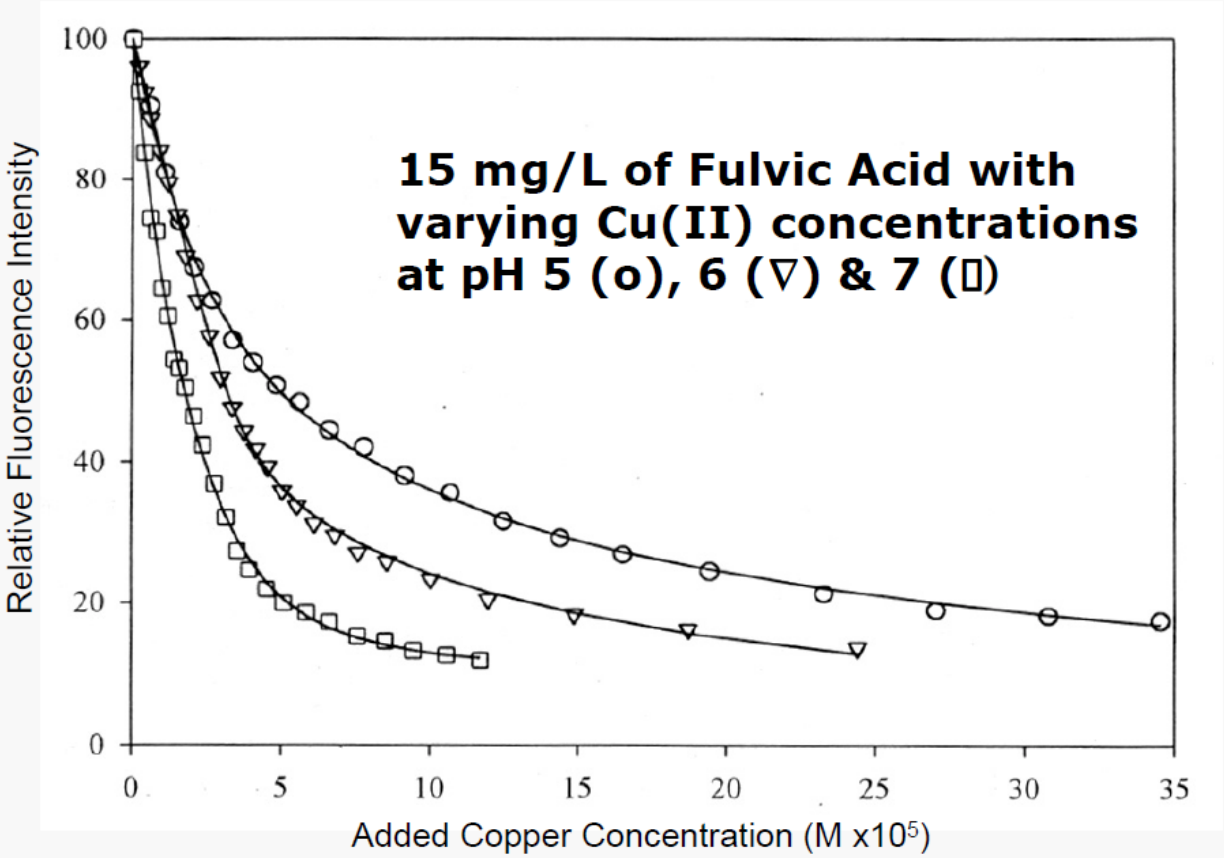
$$I=[200+2KI_{RES}C_M-I_{RES}[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_MC_L)^{0.5}]] /[2+2KC_M-[(KC_L+KC_M+1)-((KC_L+KC_M+1)^2-4K^2C_LC_M)^{0.5}]]$$

Equations for Two Site Binding

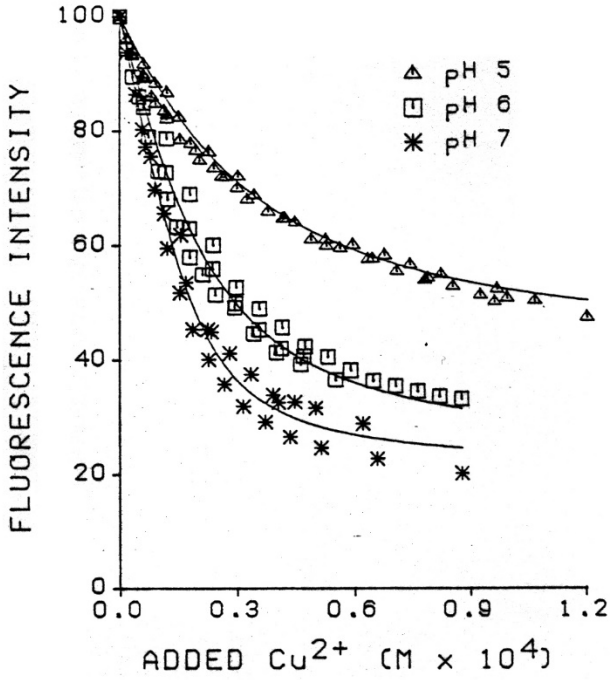
$$C_M = [M] + (K_1C_{L1}[M]/K_1[M] + 1)+(K_2C_{L2}[M]/K_2[M] + 1)+.... +K_nC_{Ln}[M]/K_n[M] + 1$$

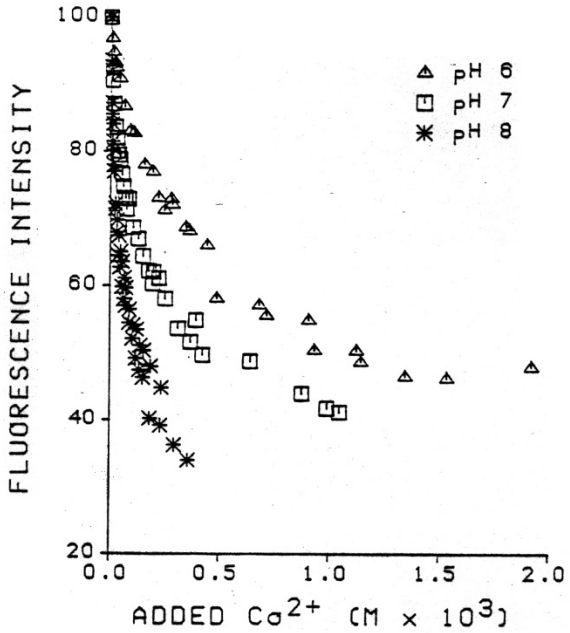
$$K_1K_2[M]^3 + \{K_1K_2(C_{L1}+C_{L2}-C_M) + K_1 + K_2\}[M]^2 + \{C_{L1}K_1+K_2C_{L2}-C_M(K_1 + K_2+1\}[M] - C_M = 0$$

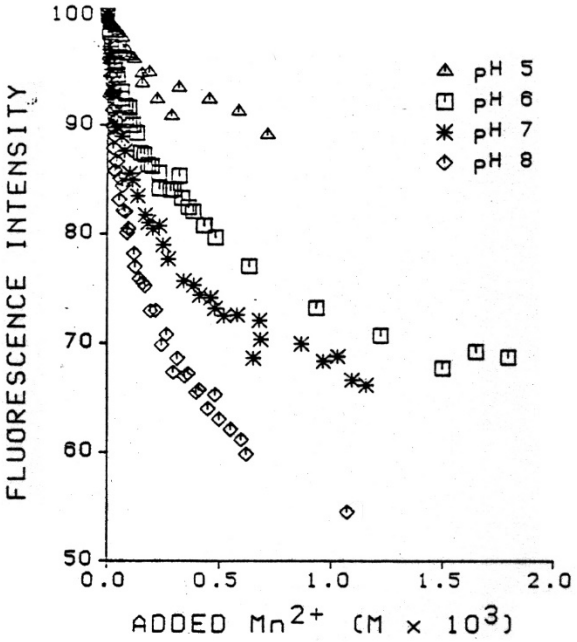
Fluorescence Quenching Curves

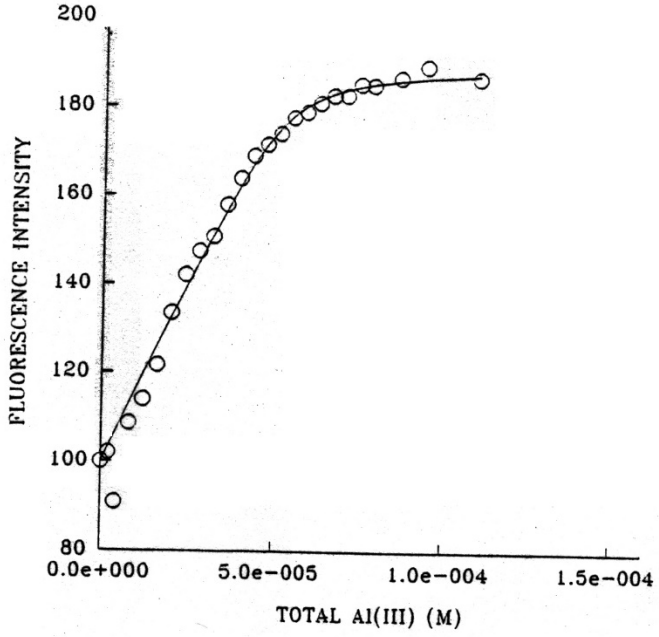


Hays, 1996





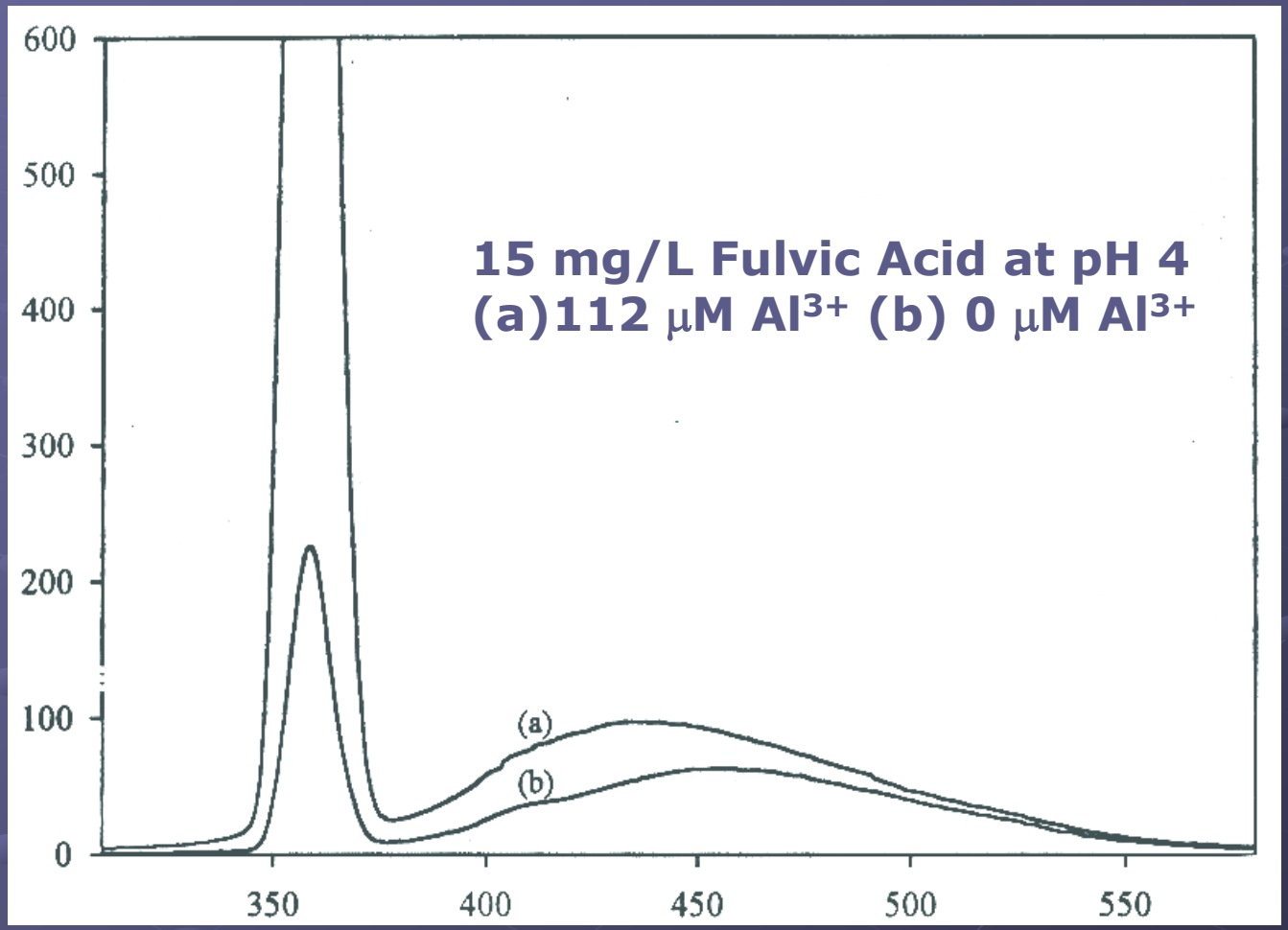




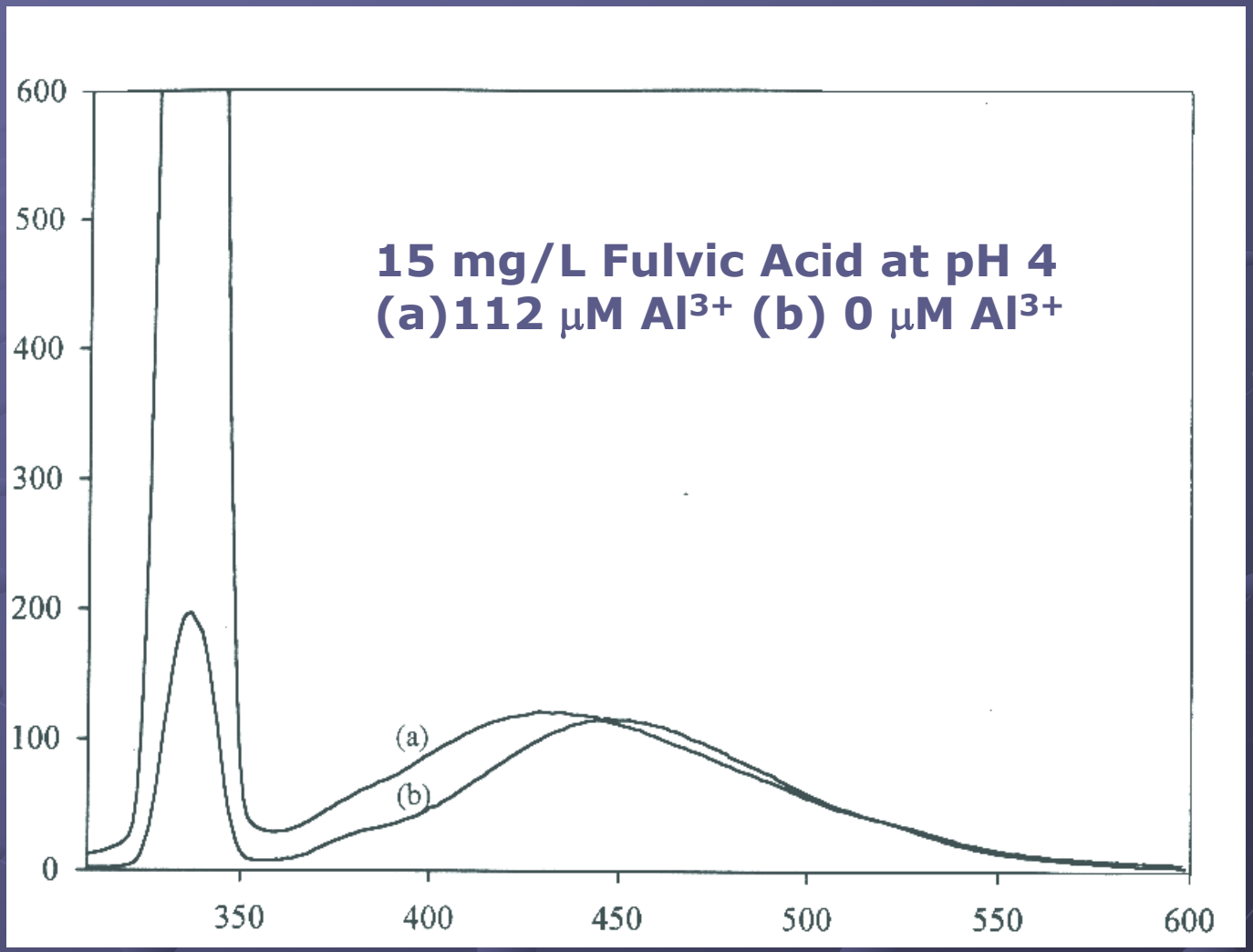
Fluorescence binding curves for Cu, Co, Mn & Al at pH values of 4-8

Fluorescence
- very sensitive
- does not disturb equilibrium
- few metals

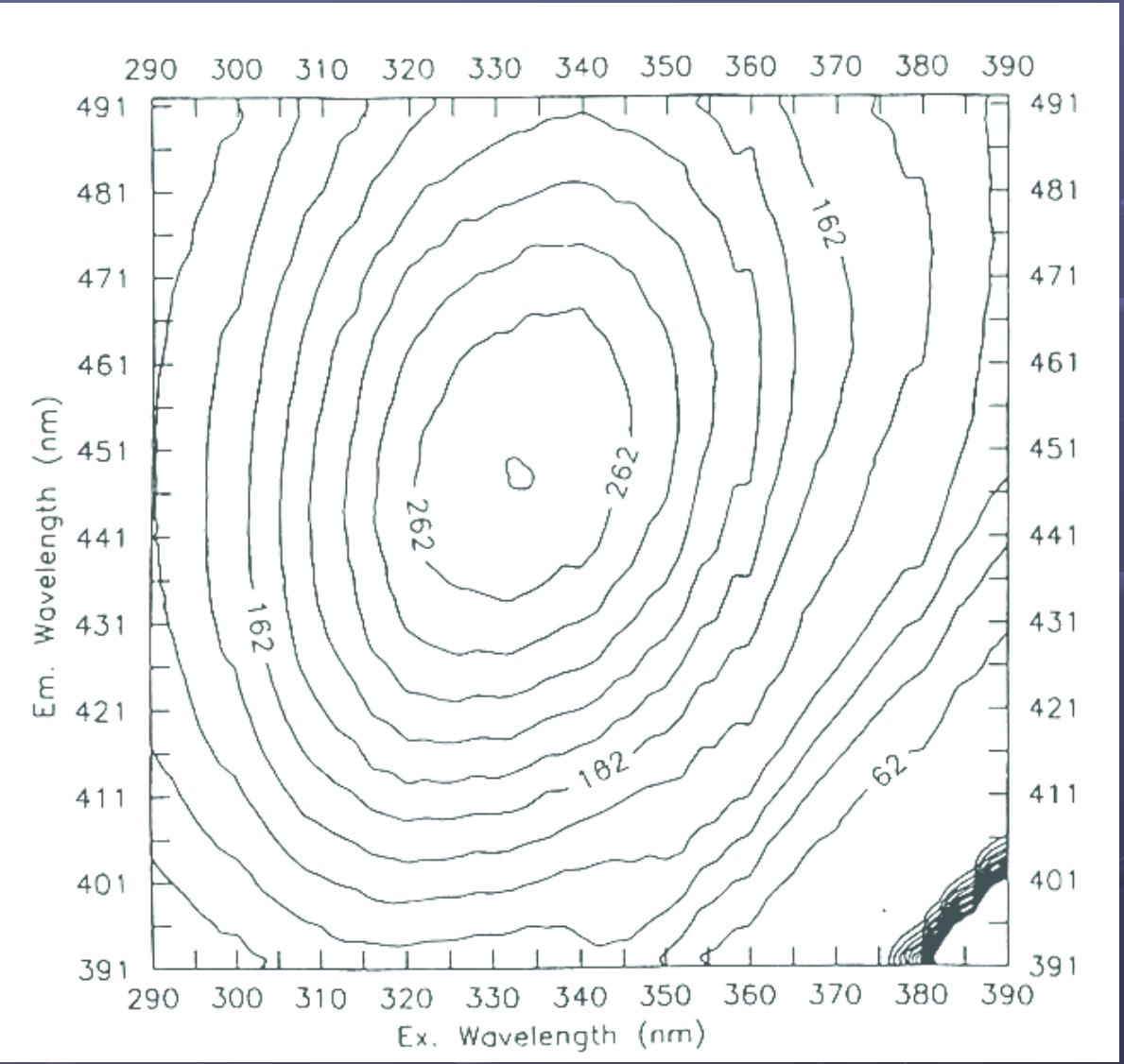
Fluorescence Enhancement



Fluorescence Enhancement

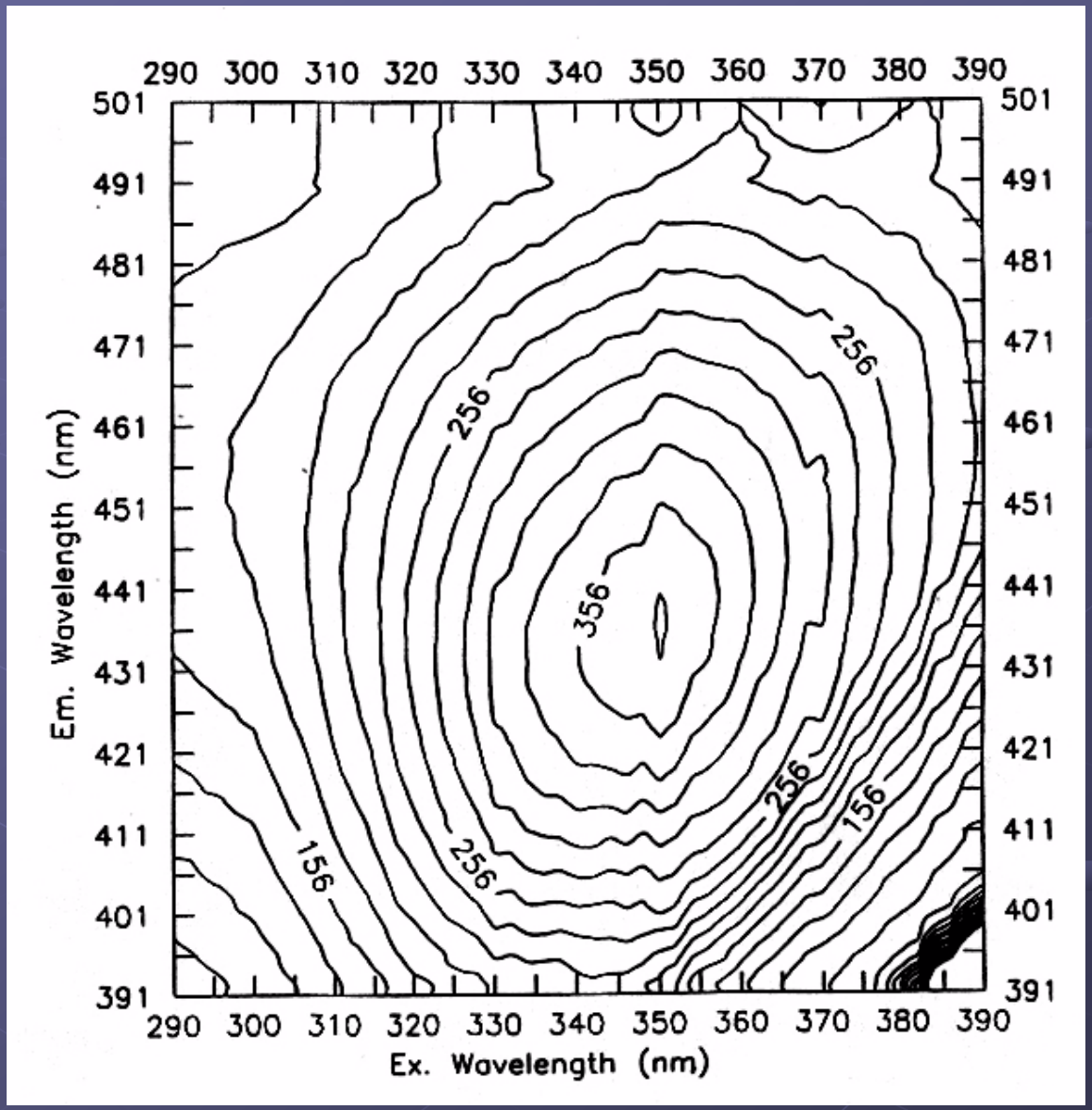


Excitation Emission Matrix



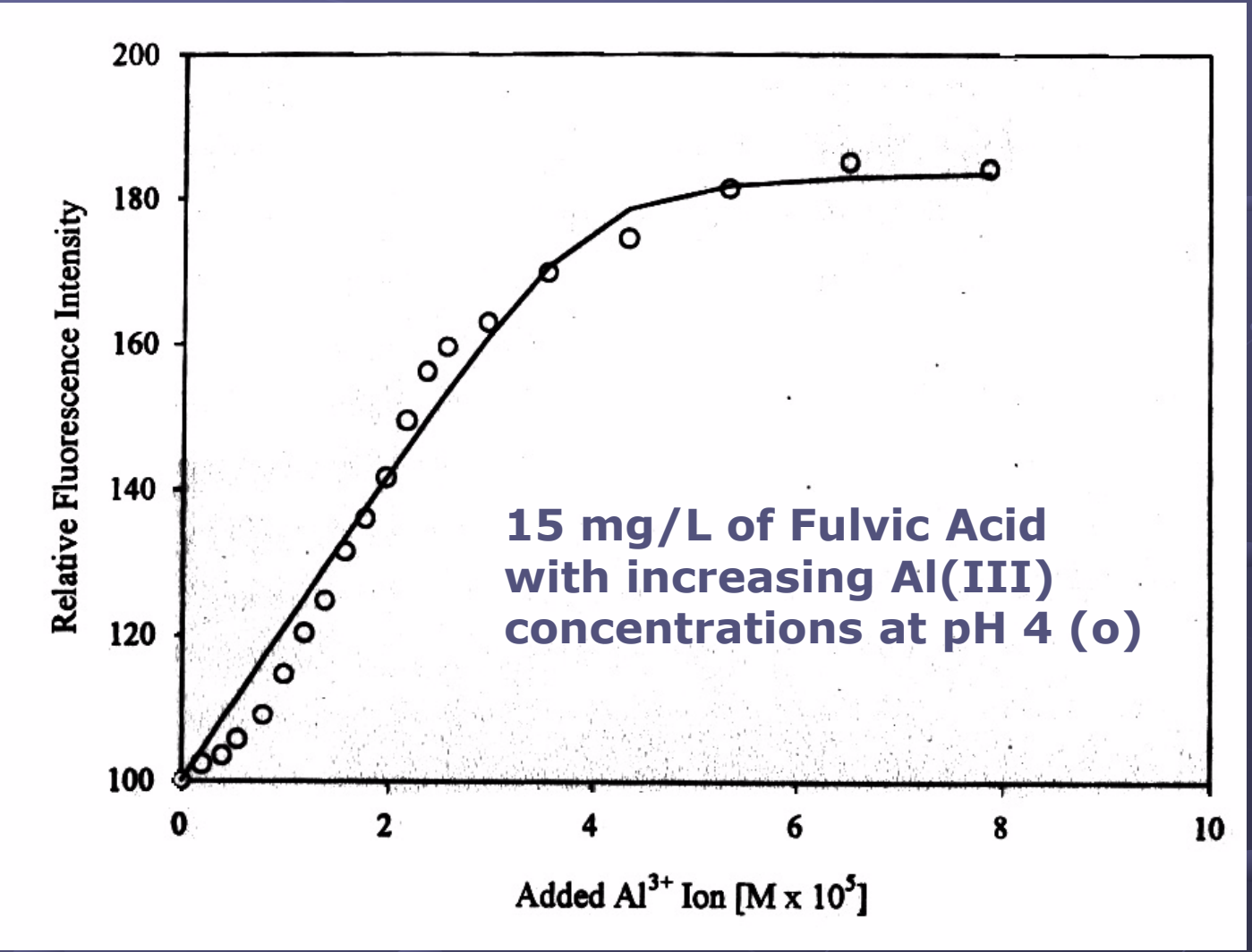
15 mg/L Fulvic Acid at pH 4 with no Al(III)

Excitation Emission Matrix

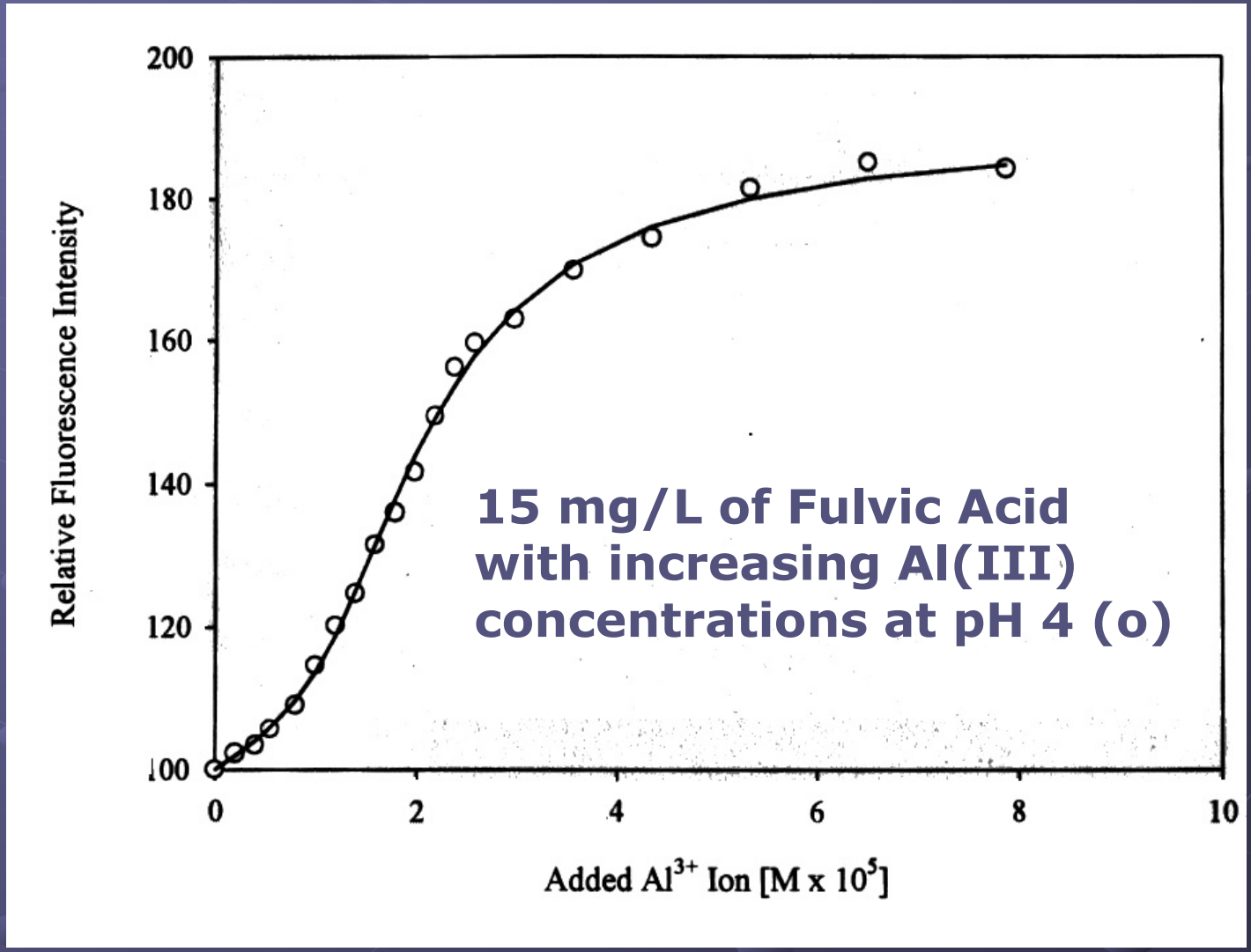


15 mg/L Fulvic Acid at pH 4 with Al(III)

Fluorescence Enhancement Curve with One-Site Model



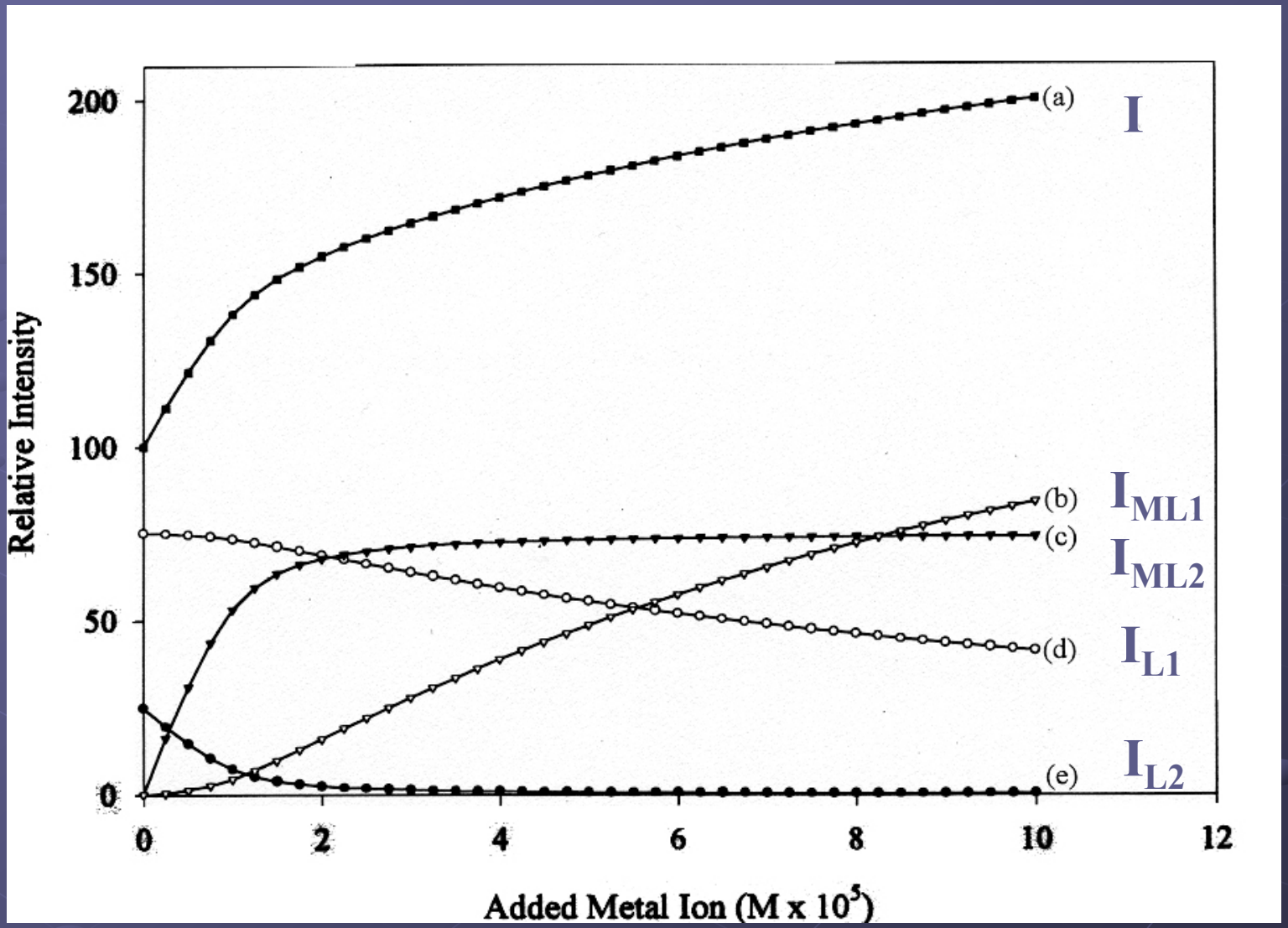
Fluorescence Enhancement Curve with Two-Site Model



Individual Fluorescence Intensities Making Up the Overall "I"

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

Hypothetical Fluorescence Intensity Curves Showing Component Intensities



Binding Data for Al^{3+} & FA
(15 mg/L)

pH	**4.00**
log K_1	**6.56 ± 0.30**
log K_2	**5.16 ± 0.12**
C_{L1}	**13.1 ± 1.5 μM**
C_{L2}	**6.0 ± 0.9 μM**
f_1	**0.07 ± 0.02**
I_{RES}	**186.2 ± 9.7**