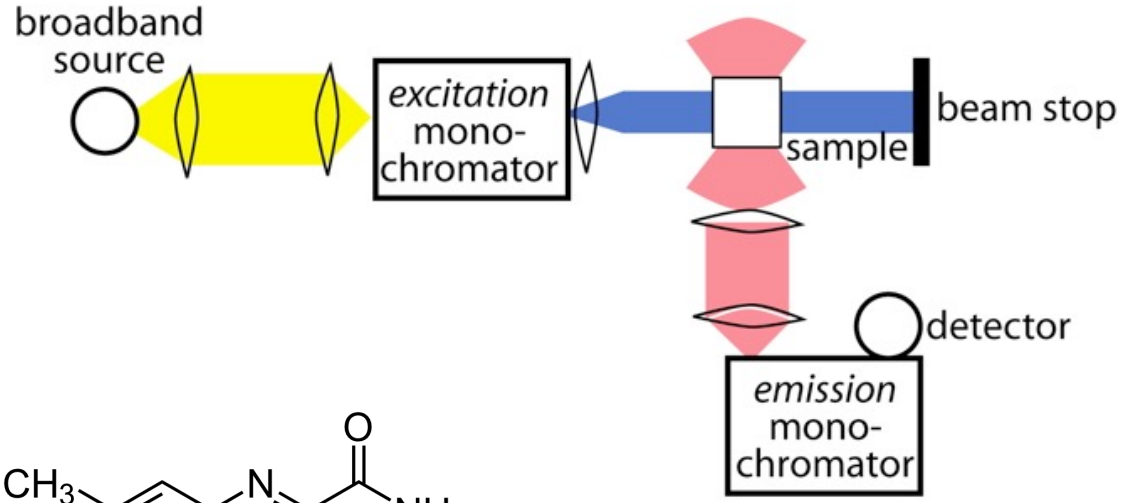
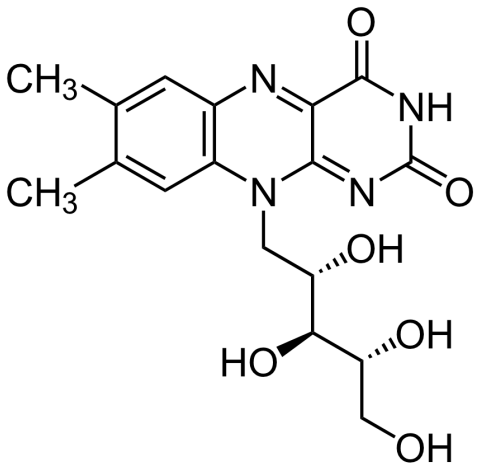
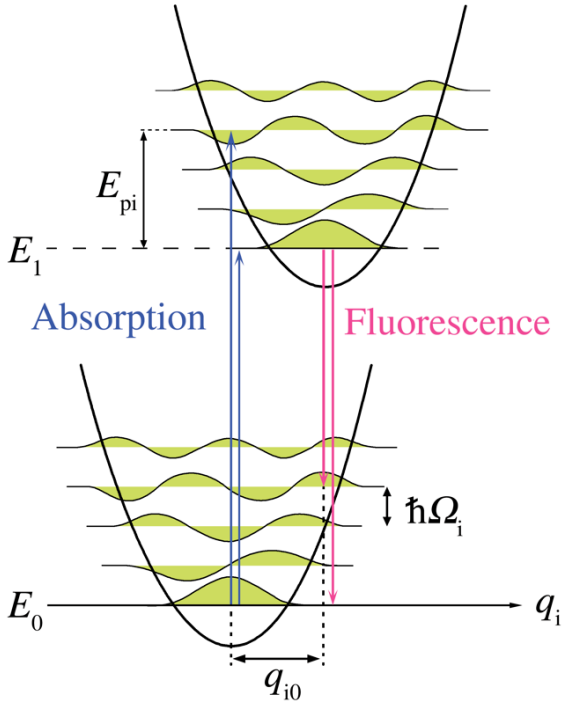
Absorption, Emission and Fluorescence Spectroscopies

Fluorescence Spectrometer





Riboflavin



R. Corn - Chem M3LC

Absorption versus Emission

Absorption is the process that consumes a photon and puts the atom or molecule in an excited state.

$$M + h\nu \rightarrow M^*$$

Emission is the process that creates a photon and takes the the atom or molecule in an excited state back to the ground state.

$$M^* \rightarrow M + h\nu$$

Fluorescence Spectroscopy

Fluorescence is the process that first consumes a photon and puts the atom or molecule in an excited state...

$$M + h\nu \rightarrow M^*$$

And then emits a photon of lower energy which takes the the atom or molecule back to the ground state.

$$M^* \rightarrow M + h\nu'$$

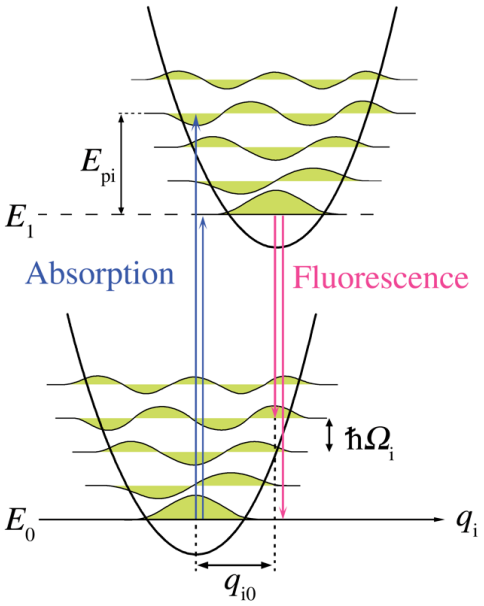
$$h\nu > h\nu'$$

Fluorescence Spectroscopy

Net reaction: $M + h\nu \rightarrow M^* \rightarrow M + h\nu'$

$h\nu > h\nu'$

The emitted photon has less energy than the absorbed photon because the molecule loses some energy (by vibrating and rotating) in the excited state:



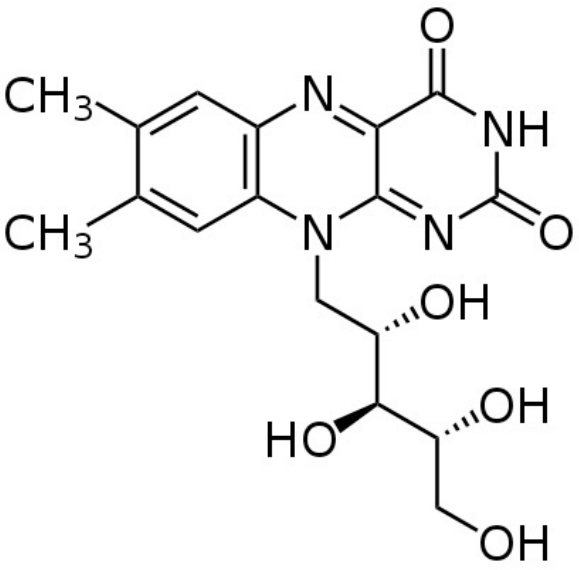
$M^* \dashrightarrow h\nu'$

$M^* \rightarrow M$

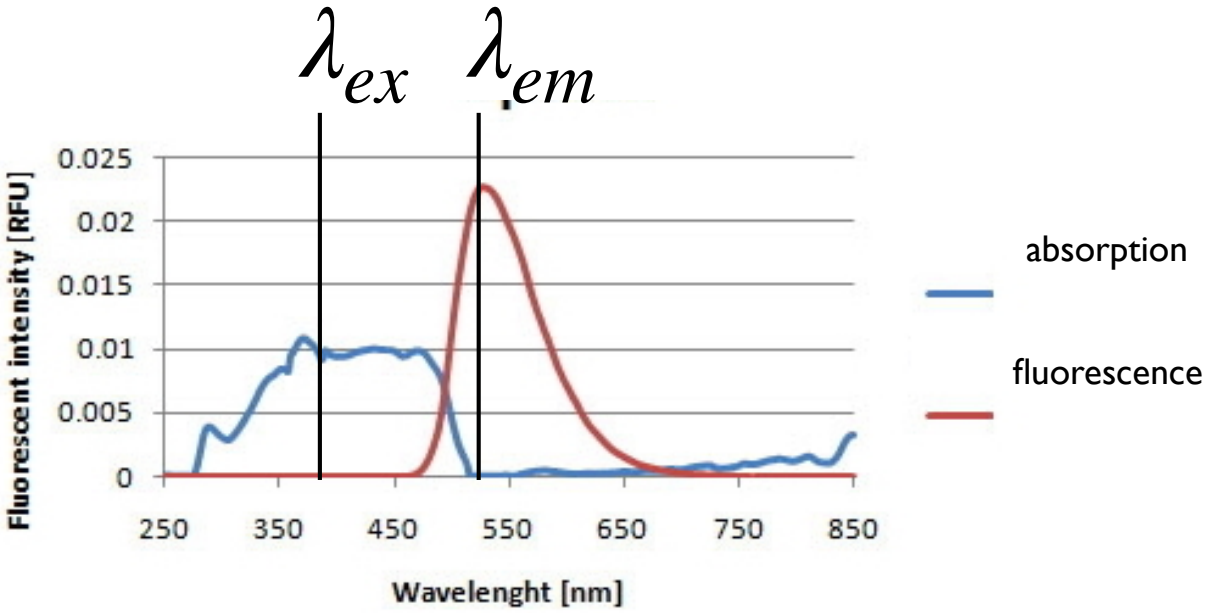
$h\nu > h\nu'$

Fluorescence Spectroscopy

The absorption and fluorescence spectra of riboflavin.



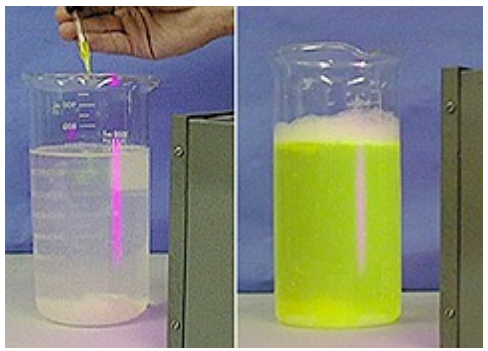
riboflavin



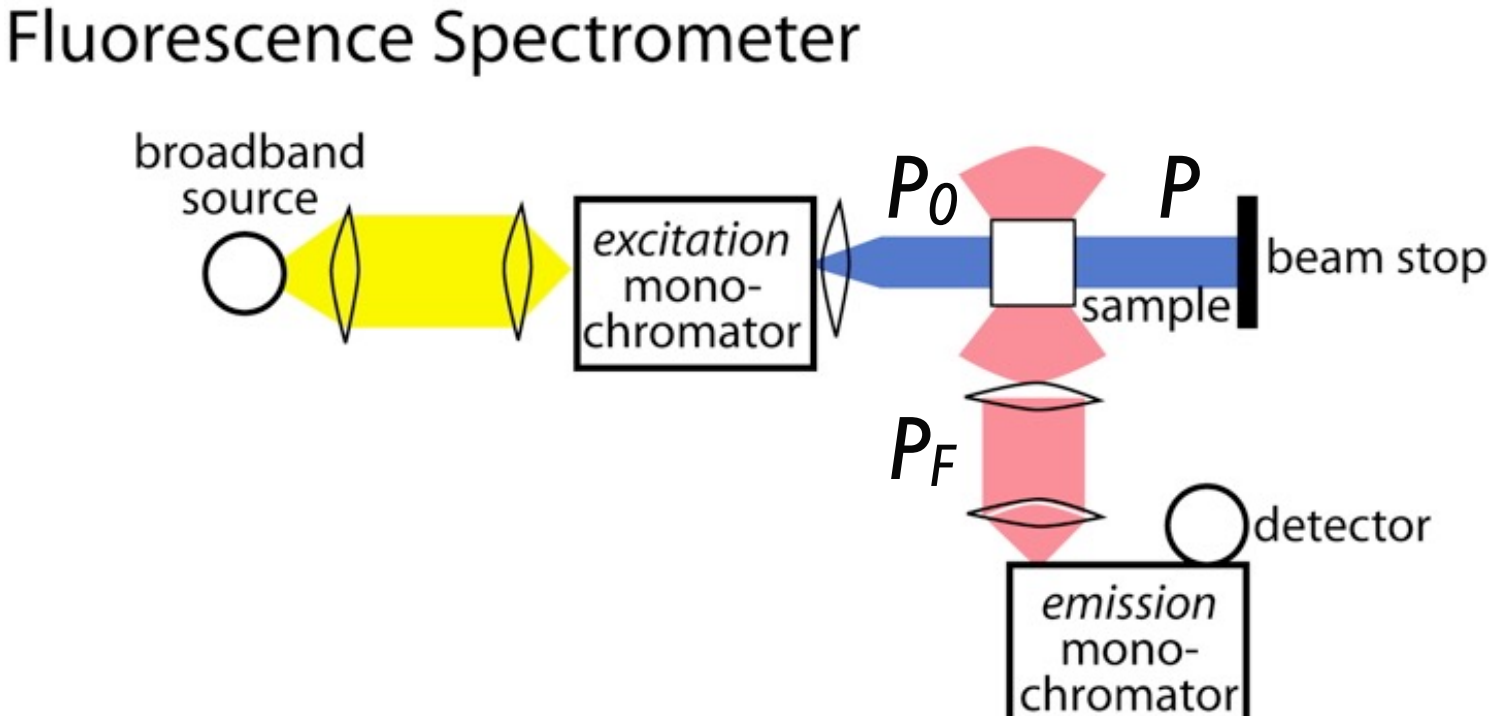
Fluorescence is always RED-shifted from the excitation.

$$\lambda_{em} > \lambda_{ex}$$

"Stoke's shift."



The Fluorescence Spectrum plots the amount of light emitted by a sample as a function of photon wavelength.



$\boldsymbol{P_0}$ = power of incident light beam (units: W)
$\boldsymbol{P}$ = power of transmitted light beam.
$\boldsymbol{P_F}$ = power of emitted fluorescence.

Quantitative Fluorescence Spectroscopy

The power of the emitted fluorescence is proportional to the absorbed power, P_0-P:

$$P_F = K'(P_0 - P)$$

substituting from Beer's law:

$$P = P_0 10^{-\epsilon dC} = P_0 e^{-2.303\epsilon dC}$$

$$P_F = K' P_0 (1 - e^{-2.303\epsilon dC})$$

$$P_F = K' P_0 (1 - (1 - 2.303\epsilon dC + \ldots))$$

$$\boxed{P_F \approx 2.303 K' P_0 \epsilon dC}$$

P_F is proportional to concentration at small εdC.

Method of Standard Addition

You can determine the concentration of an unknown concentration Cx by fluorescence using the method of standard addition. To a volumetric flask of volume Vt you add (i) a volume Vx of the unknown concentration Cx and (ii) volume Vs of a solution with a known concentration Cs.

For example:
Make five solutions by addition of Vs where Vs = nΔ n= 0 to 4; Δ = 5 mL

For each solution, the # of moles is CxVx + CsVs and volume is always Vt.

The concentration is: $$\frac{C_x V_x + C_s V_s}{V_t} = \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t}$$

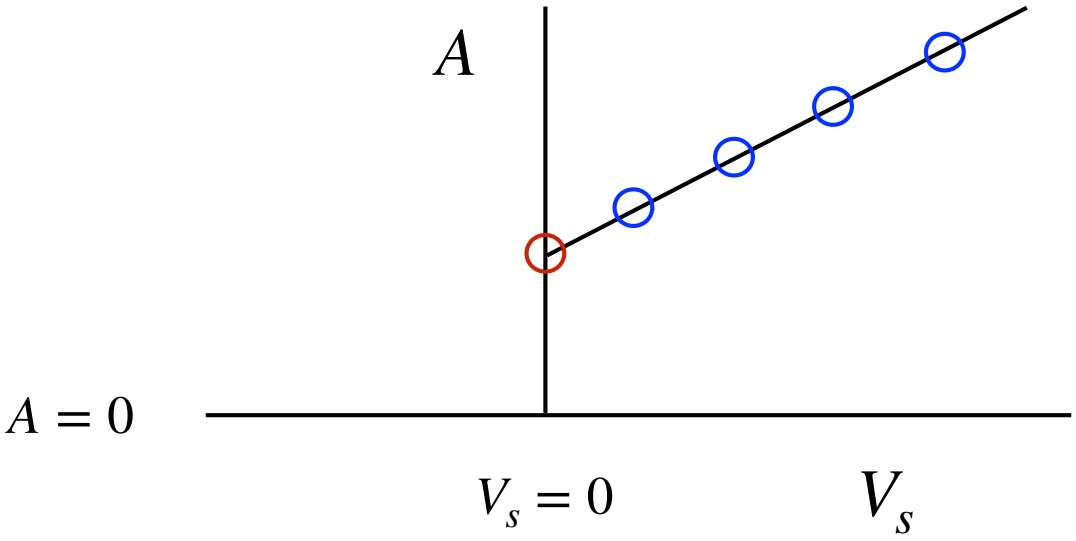
The Absorbance is: $$A = \epsilon d \left(\frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t} \right) = b + m V_s$$

Plot Absorbance vs Vs and fit with a straight line to get m & b.

Method of Standard Addition

$$A = \epsilon d\left(\frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t}\right) = b + mV_s$$

$$\frac{b}{m} = \frac{C_x V_x}{C_s} \longrightarrow \boxed{C_x = \frac{bC_s}{mVx}}$$



We can calculate Cx using b/m!

Method of Standard Addition

$$A = \epsilon d\left(\frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t}\right) = b + mV_s$$

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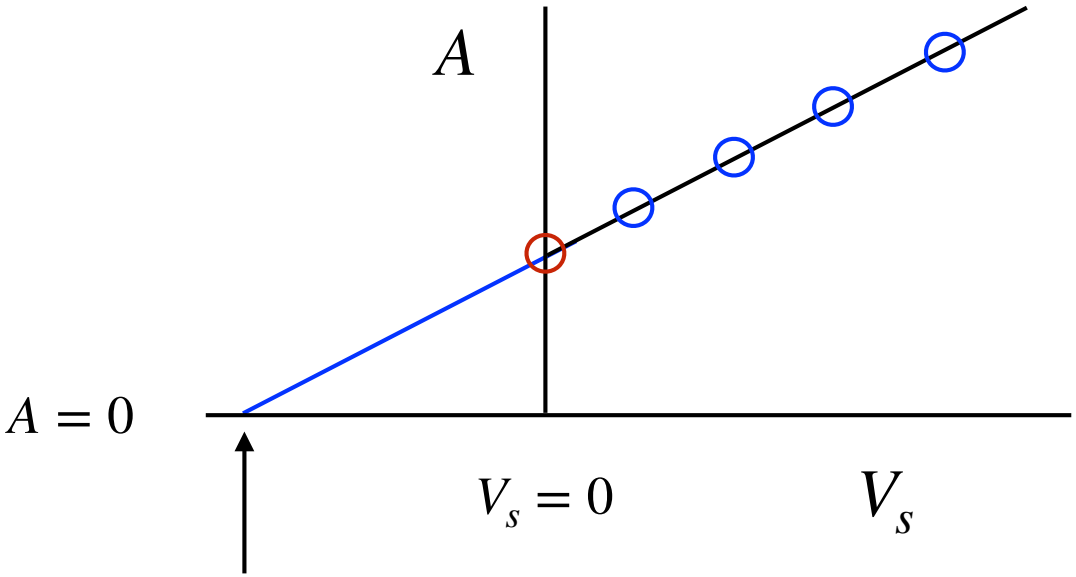


A=0 intercept gives you b/m!

Method of Standard Addition

$$A = \epsilon d\left(\frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t}\right) = b + mV_s$$

$$\frac{b}{m} = \frac{C_x V_x}{C_s} \longrightarrow \boxed{C_x = \frac{bC_s}{mVx}}$$

Standard deviation for Cx is sc:

$$s_c = \frac{s_r}{m}\sqrt{\frac{1}{N} + \frac{(\bar{y})^2}{m^2 S_{xx}}}$$

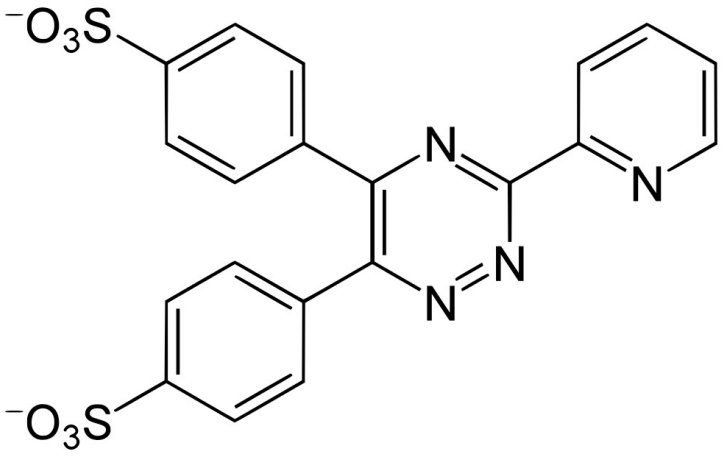
Book has different incorrect equation. See handout & paper.

95% confidence interval: $C_x \pm t_{N-2} s_c$

Metal-Ligand Complexation Equilibria

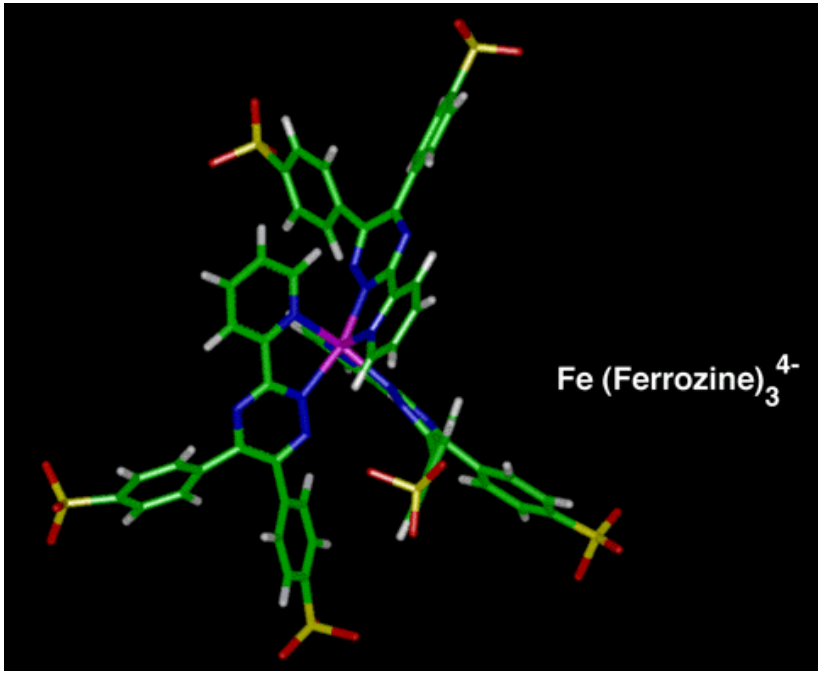
$$Fe^{2+} + 3Fz^{2-} \rightleftharpoons Fe(Fz)_3^{4-} \qquad K_f = 10^{15}$$

K_f = formation constant

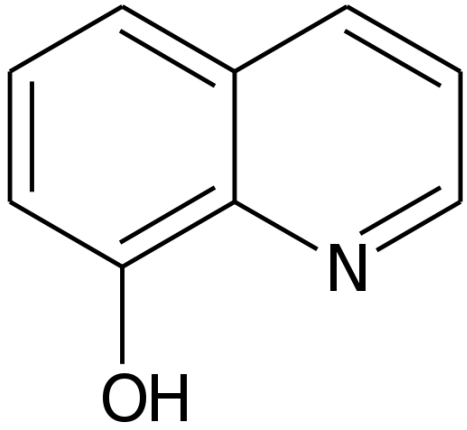


Ferrozine (Fz^{2-})
is a metal ligand

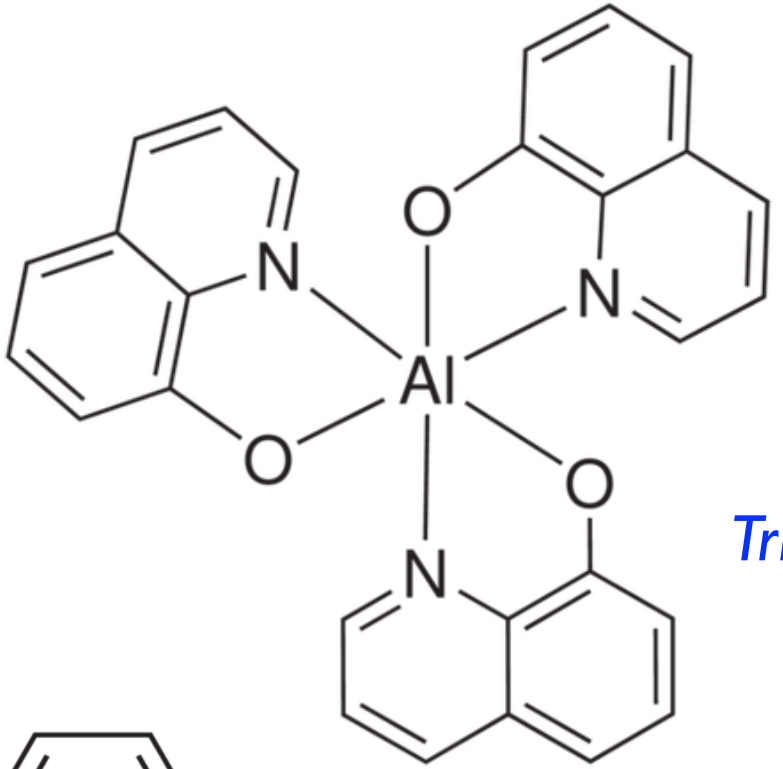
Three Ferrozine will form a metal-ligand complex with Fe^{2+}



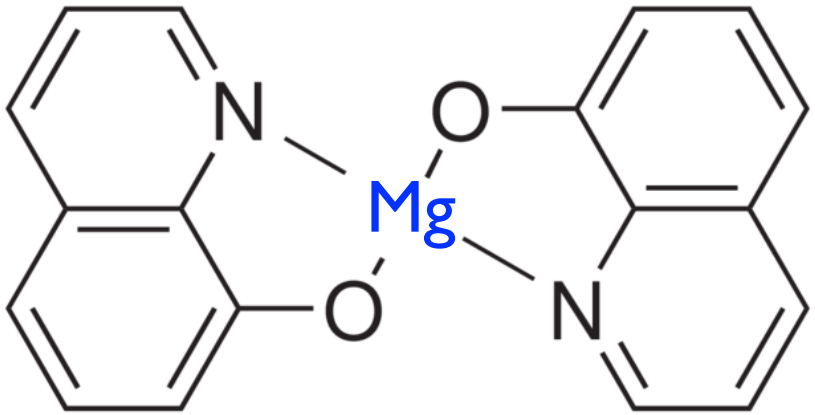
Hydroxyquinoline: a metal chelator that fluoresces upon binding!



8-hydroxyquinoline



Trivalent Cations



Divalent Cations

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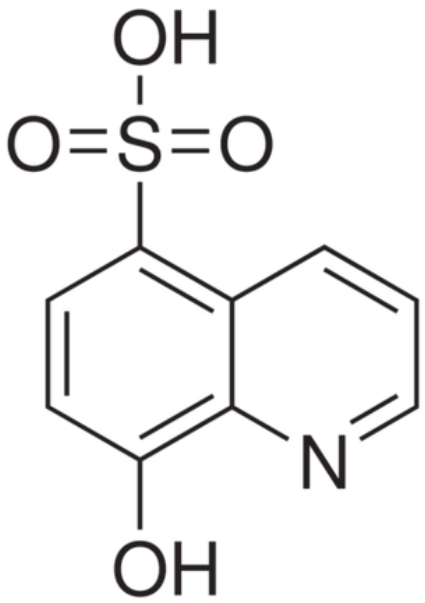


8-hydroxyquinoline-5-sulfonic Acid

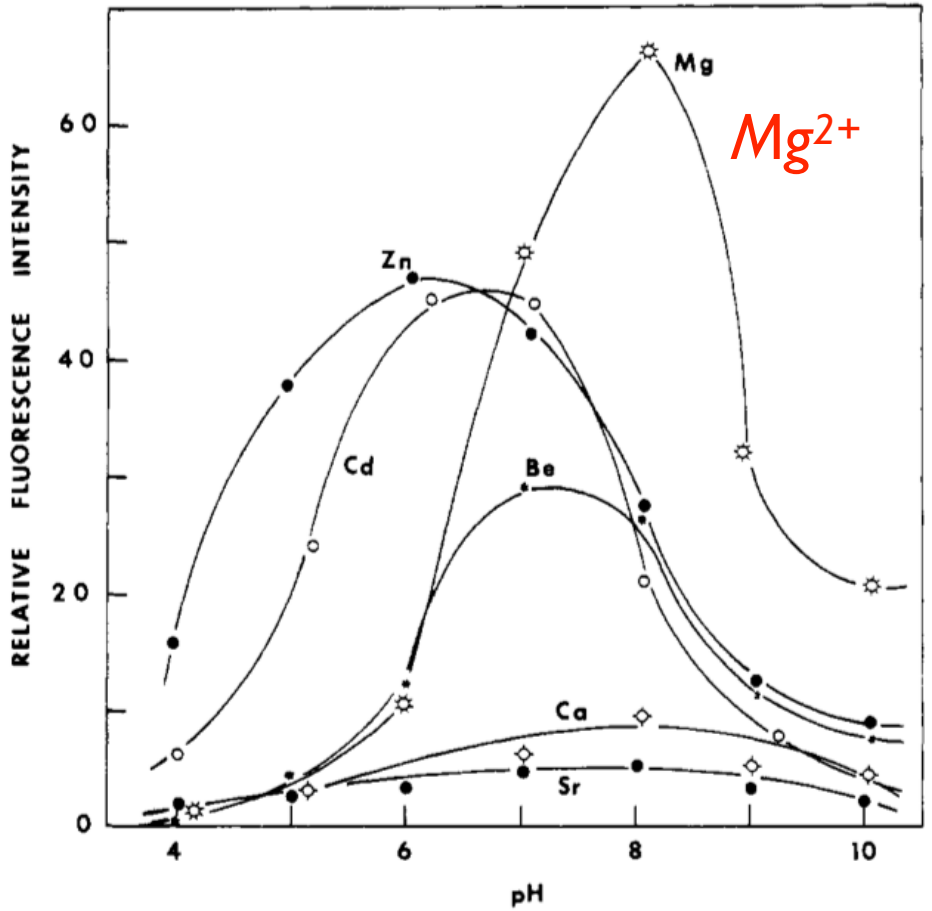


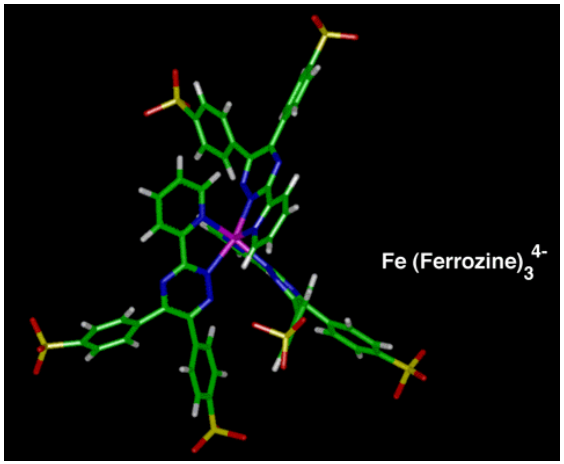
Figure 1. pH dependence of the fluorescnece intensities of group II metal–HQS chelates: Cd, 2 μM; all other metals in this and following figures, 20 μM; HQS, 1 mM.

Fluorometric Detection of Mg^{2+} in Seawater

Let's calculate the alpha fraction for Fe^{2+}:

$$Fe^{2+} + 3Fz^{2-} \rightleftharpoons Fe(Fz)_3^{4-} \qquad K_f = 10^{15}$$

$$K_f = \frac{[Fe(Fz)_3^{4-}]}{[Fe^{2+}][Fz^{2-}]^3}$$



$$C_{Fe(II)}^{tot} = [Fe^{2+}] + [Fe(Fz)_3^{4-}]$$

$$\alpha_{Fe^{2+}} = \frac{[Fe^{2+}]}{C_{Fe(II)}^{tot}} = \frac{1}{1 + K_f[Fz^{2-}]^3}$$

$$\alpha_{Fe(Fz)_3^{4-}} = 1 - \alpha_{Fe^{2+}}$$

only depends on K_f and $[Fz^{2-}]$!

Metal-Ligand Complexation Equilibria

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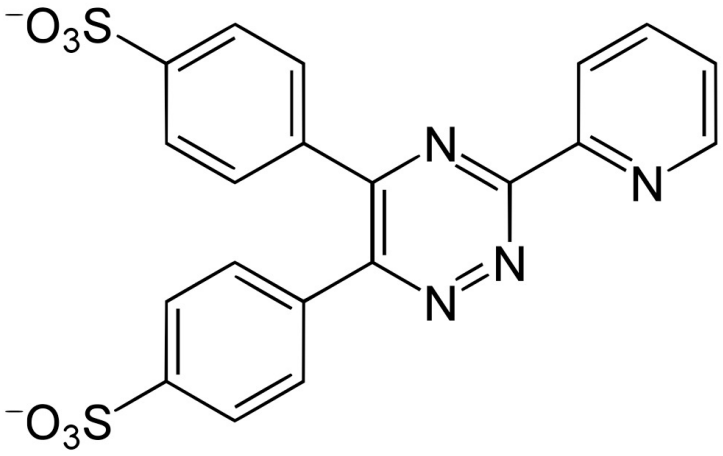

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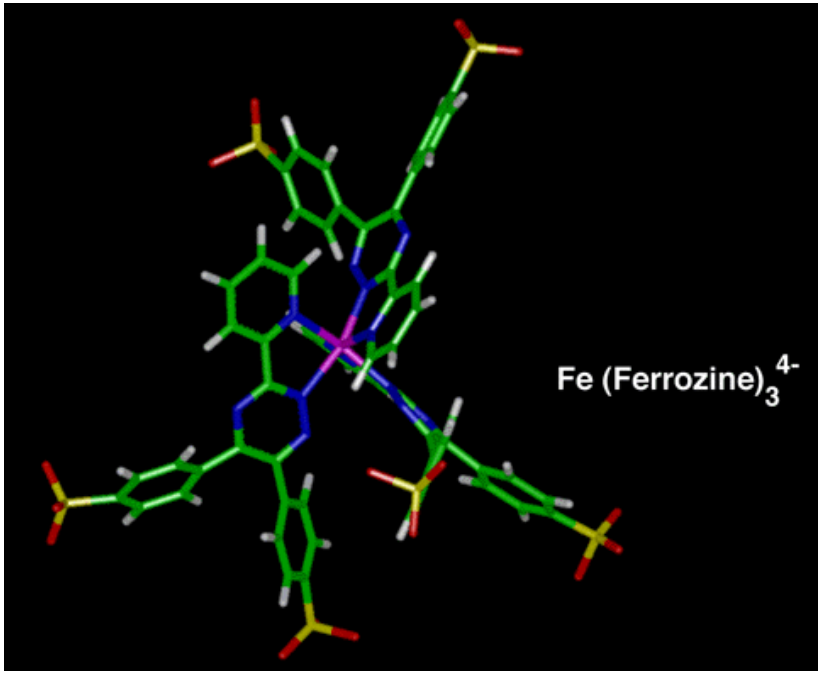

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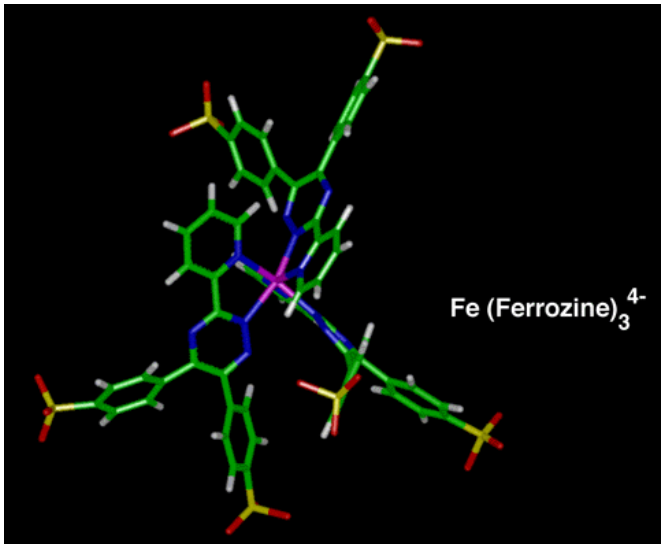

For example: $[Fz^{2-}] = 10^{-5}M$

$$\alpha_{Fe^{2+}} = 0.5$$

$$\alpha_{Fe(Fz)_3^{4-}} = 0.5$$

only depends on K_f and $[Fz^{2-}]$!

Alpha plot for Iron-Ferrozine complex