

Problem Session

2023. 7.15.

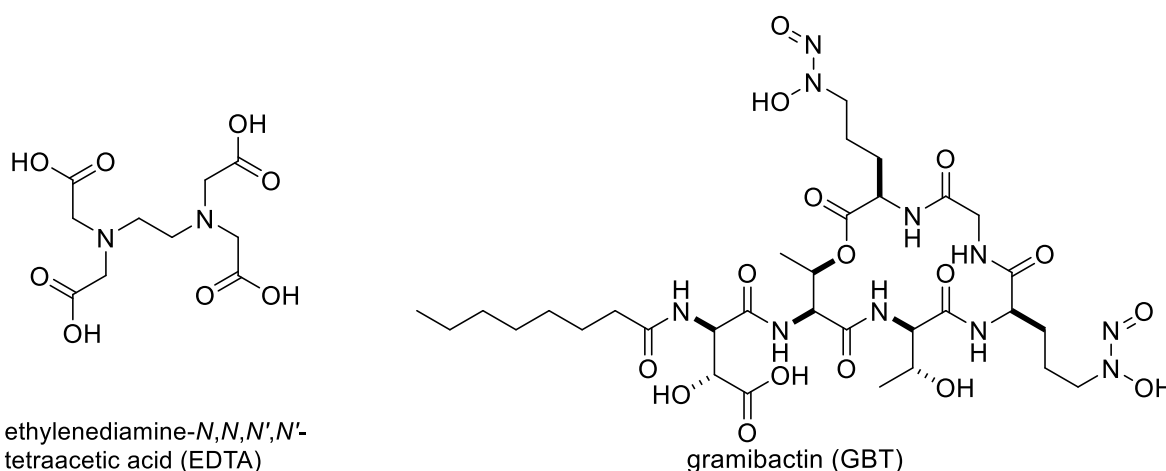
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Preface

Two metric parameters pM and $pL_{0.5}$ are commonly used to evaluate metal sequestering ability of ligands. The pM value is defined as $pM = -\log[M]$, where $[M]$ represents the concentration of the free ion $[M^{n+}]$ in the particular systems ($c_L/c_M = 10$, $c_M = 10^{-6}$ M, $pH = 7.4$, where c_L and c_M are total concentrations of a ligand and a metal ion, respectively), while the parameter $pL_{0.5}$ is defined as $pL_{0.5} = -\log c_L$ when 50% of the metal cation present in traces ($c_M \leq 10^{-12}$ M) bind to a ligand.

Problem

Ethylenediamine- N,N,N',N' -tetraacetic acid (EDTA, H_4Y) and gramibactin (GBT, H_4G) are both tetraprotic acids whose conjugate bases form 1:1 complexes with a ferric ion. Considerable equilibria and their equilibrium constants are listed in Table 1. All the other chemical species that appear in Table 1 are neglected in this problem.



- (1) Calculate pFe values of EDTA and GBT in $T = 298.15$ K.
- (2) Calculate $pL_{0.5}$ values of EDTA and GBT in $T = 298.15$ K when complexed with Fe^{3+} in the given three cases; (i) pH 2.5, (ii) pH 7.4, (iii) pH 10.5.

Table 1. Protonation constants for EDTA and GBT, Fe^{3+} hydrolysis, and $Fe^{3+}/EDTA$, Fe^{3+}/GBT complex formation constants ($\log \beta_{pqr}$) in $T = 298.15$ K.

$$pFe^{3+} + qL^{4-} + rH^+ \rightleftharpoons Fe_pL_qH_r^{(3p-4q+r)} \quad (L = Y \text{ or } G) \quad \beta_{pqr} = [Fe_pL_qH_r^{(3p-4q+r)}]/[Fe^{3+}]^p[L^{4-}]^q[H^+]^r$$

	species	$p:q:r$	$\log \beta_{pqr}$		species	$p:q:r$	$\log \beta_{pqr}$
EDTA	HY^{3-}	0:1:1	10.22	Fe^{3+}	$Fe(OH)^{2+}$	1:0:-1	-2.17
	H_2Y^{2-}	0:1:2	16.38		$Fe(OH)_2^+$	1:0:-2	-6.35
	H_3Y^-	0:1:3	19.09		$Fe(OH)_3$	1:0:-3	-14.25
	H_4Y	0:1:4	21.09		$Fe(OH)_4^-$	1:0:-4	-22.59
GBT	HG^{3-}	0:1:1	10.94	$Fe^{3+}/EDTA$	FeY^-	1:1:0	25.10
	H_2G^{2-}	0:1:2	16.65		$FeHY$	1:1:1	26.98
	H_3G^-	0:1:3	21.52		$FeY(OH)^{2-}$	1:1:-1	17.57
	H_4G	0:1:4	23.79	Fe^{3+}/GBT	FeG^-	1:1:0	27.61
					$FeG(OH)_2^{3-}$	1:1:-2	6.42

Topic: Quantitative evaluation of metal sequestering ability of ligands

1. Evaluation of metal sequestering ability

Since the sequestering ability of a ligand is strictly dependent on the stability of side species as well as that of the complex, it is necessary to consider competitive reactions involving simultaneous equilibria in different conditions. Therefore, the selectivity and the whole sequestering ability of a chelator toward a cation, as well as the comparison between two or more chelators, cannot be easily assessed by the simple analysis of single sets of stability constants of metal/ligand complexes in real conditions^{1,2}.

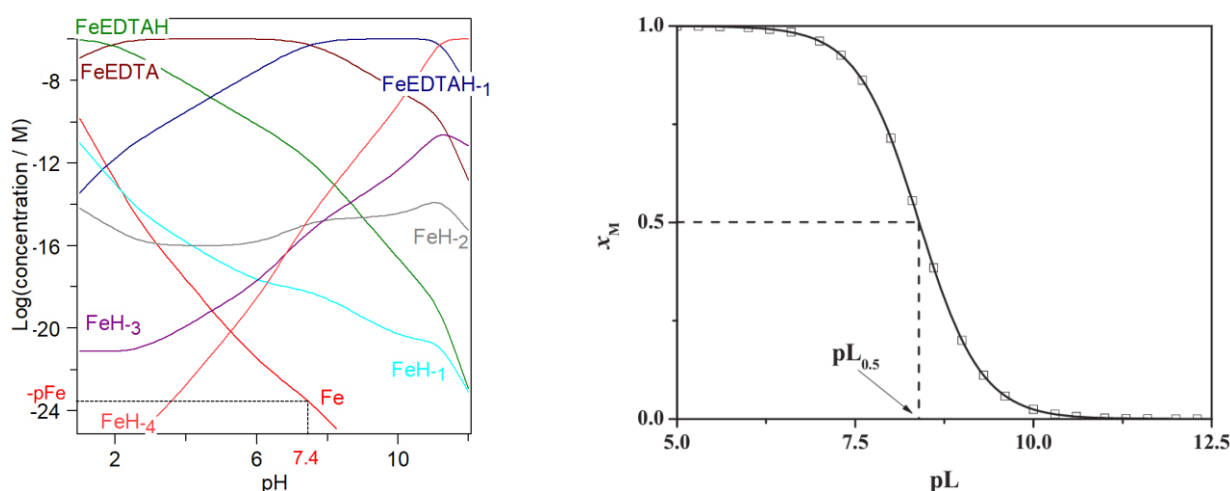
For this reason, the concentration of free metal ion in solution at equilibrium (pM) was introduced to compare the relative strength of different metal chelating agents³. In the view of fair comparison, pM is defined in $c_L/c_M = 10$, $c_M = 10^{-6}$ M, pH = 7.4 as the values are strictly conditional depending on total concentration of reagents and pH (Figure 1 left). Notably, this parameter should be used with care when performed on different cations because they undergo different hydrolysis.

To avoid the drawback of pM, the parameter $pL_{0.5}$ has been employed since it does not refer the free metal concentration as long as metal species are present as trace⁴. In the conditions, the fraction of metal complexed by the ligand x_M is expressed as sigmoidal dose-response curve of $pL (= -\log c_L)$ (Figure 1 right)

$$x_M = \frac{1}{1 + 10^{pL - pL_{0.5}}} \quad (1-1)$$

In principle, the $pL_{0.5}$ values can be advantageously used for the comparison of systems with the presence of other cations and ligands undergoing many different competing equilibria, even under different conditions of pH and temperature.

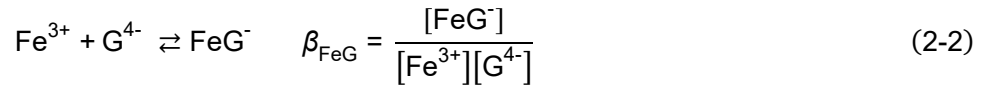
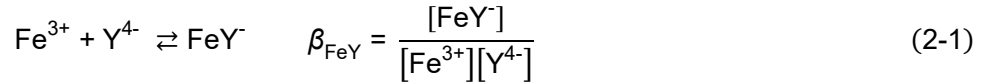
Figure 1. Speciation diagram for complex formation of EDTA with Fe^{3+} ($c_L = 10^{-5}$ M, $c_{Fe} = 10^{-6}$ M, pH = 7.4) (left)⁵ and generic sequestration diagram for the calculation of $pL_{0.5}$ (right).



2. Description of the total equilibrium system

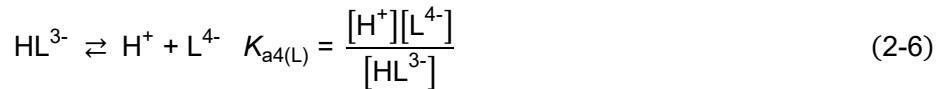
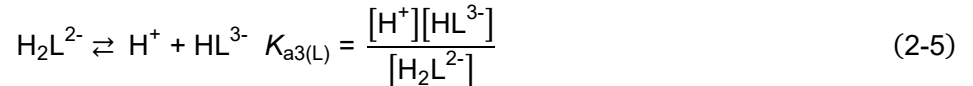
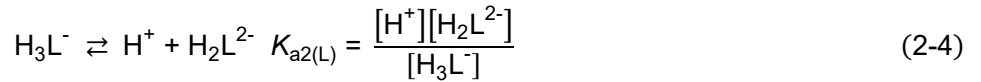
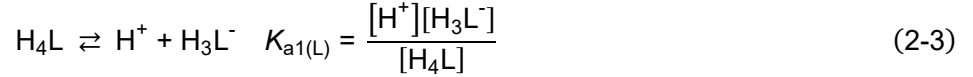
To demonstrate these parameters, the simplest one metal one ligand system in aqueous solution is considered in this problem. The sequestration ability of gramibactin^{1,6,7} was evaluated in comparison with EDTA. Complex formation reactions and competing side reactions are described in equilibria (2-1)–(2-13) with corresponding equilibrium constants.

- Complex formation constants

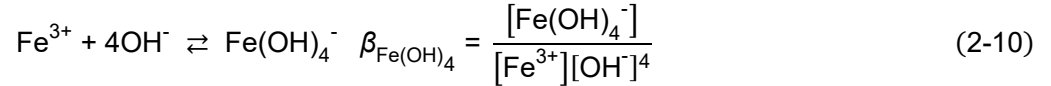
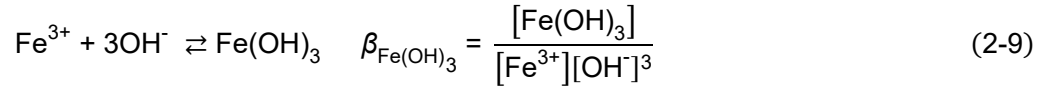
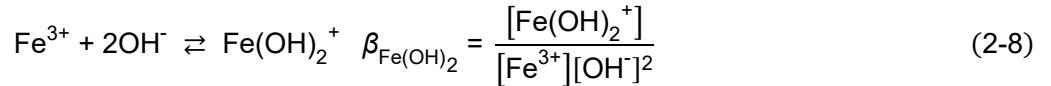
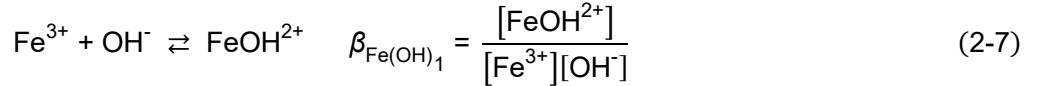


- Considerable competitive reactions

- Acid-base equilibrium of a ligand (L = Y or G)

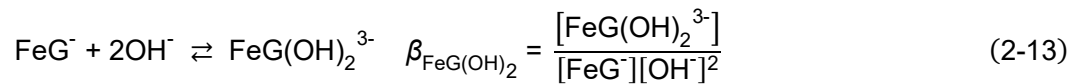
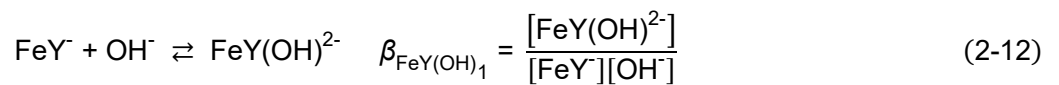
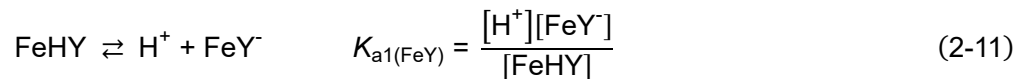


- Hydrolysis of Fe^{3+}



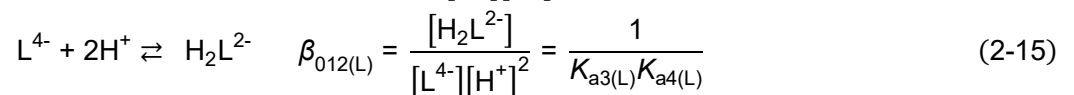
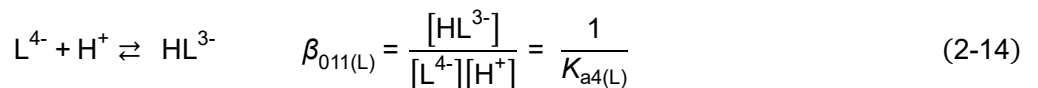
Polynuclear species $[\text{Fe}_2(\text{OH})_2]^{4+}$, $[\text{Fe}_3(\text{OH})_4]^{5+}$, and $[\text{Fe}_{12}(\text{OH})_{34}]^{2+}$ are considered as well as mononuclear ones for more precise iron(III) speciation though they are neglected in this problem to avoid troublesome calculation^{8,9}.

- Protonation and hydroxido species formation of a complex



Of note, equilibria (2-1)–(2-13) are transformed into $p\text{Fe}^{3+} + q\text{L}^{4-} + r\text{H}^+ \rightleftharpoons \text{Fe}_p\text{L}_q\text{H}_r^{(3p-4q+r)}$ for facile calculation as shown in Table 1. The resulting equilibria and corresponding equilibrium constants β_{pqr} are listed in equilibria (2-14)–(2-26). The overall equilibrium systems are described in Figure 2.

- Acid-base equilibrium of a ligand



$$L^{4-} + 3H^+ \rightleftharpoons H_3L^- \quad \beta_{013(L)} = \frac{[H_3L^-]}{[L^{4-}][H^+]^3} = \frac{1}{K_{a2(L)}K_{a3(L)}K_{a4(L)}} \quad (2-16)$$

$$L^{4-} + 4H^+ \rightleftharpoons H_4L \quad \beta_{014(L)} = \frac{[H_4L]}{[L^{4-}][H^+]^4} = \frac{1}{K_{a1(L)}K_{a2(L)}K_{a3(L)}K_{a4(L)}} \quad (2-17)$$

● Hydrolysis of Fe^{3+}

$$Fe^{3+} - H^+ + H_2O \rightleftharpoons FeOH^{2+} \quad \beta_{10-1(Fe)} = \frac{[FeOH^{2+}]}{[Fe^{3+}][H^+]^{-1}} = K_w \beta_{Fe(OH)_1} \quad (2-18)$$

$$Fe^{3+} - 2H^+ + 2H_2O \rightleftharpoons Fe(OH)_2^+ \quad \beta_{10-2(Fe)} = \frac{[Fe(OH)_2^+]}{[Fe^{3+}][H^+]^{-2}} = K_w^2 \beta_{Fe(OH)_2} \quad (2-19)$$

$$Fe^{3+} - 3H^+ + 3H_2O \rightleftharpoons Fe(OH)_3 \quad \beta_{10-3(Fe)} = \frac{[Fe(OH)_3]}{[Fe^{3+}][H^+]^{-3}} = K_w^3 \beta_{Fe(OH)_3} \quad (2-20)$$

$$Fe^{3+} - 4H^+ + 4H_2O \rightleftharpoons Fe(OH)_4^- \quad \beta_{10-4(Fe)} = \frac{[Fe(OH)_4^-]}{[Fe^{3+}][H^+]^{-4}} = K_w^4 \beta_{Fe(OH)_4} \quad (2-21)$$

where K_w represents ionic product of water ($K_w = [H^+][OH^-]$).

● Complex formation, protonation, and hydroxido species formation of a complex

$$Fe^{3+} + Y^{4-} \rightleftharpoons FeY^- \quad \beta_{110(FeY)} = \frac{[FeY^-]}{[Fe^{3+}][Y^{4-}]} = \beta_{FeY} \quad (2-22)$$

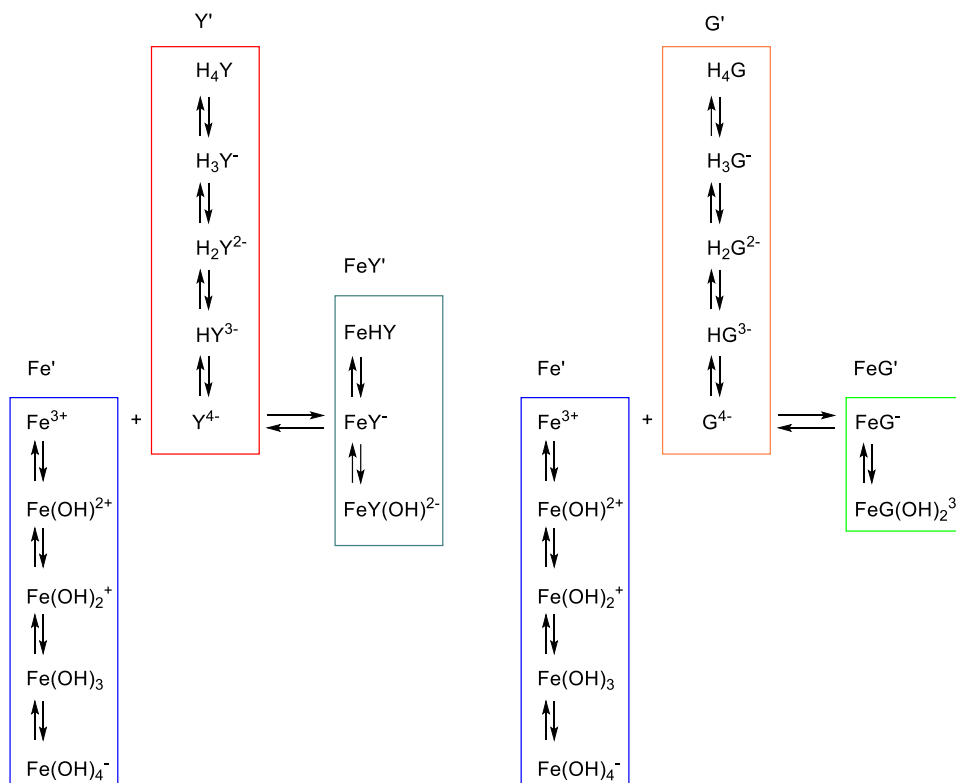
$$Fe^{3+} + Y^{4-} + H^+ \rightleftharpoons FeHY \quad \beta_{111(FeY)} = \frac{[FeHY]}{[Fe^{3+}][Y^{4-}][H^+]} = \frac{\beta_{FeY}}{K_{a1(FeY)}} \quad (2-23)$$

$$Fe^{3+} + Y^{4-} - H^+ + H_2O \rightleftharpoons FeY(OH)^{2-} \quad \beta_{11-1(FeY)} = \frac{[FeY(OH)^{2-}]}{[Fe^{3+}][Y^{4-}][H^+]^{-1}} = K_w \beta_{FeY} \beta_{FeY(OH)_1} \quad (2-24)$$

$$Fe^{3+} + G^{4-} \rightleftharpoons FeG^- \quad \beta_{110(FeG)} = \frac{[FeG^-]}{[Fe^{3+}][G^{4-}]} = \beta_{FeG} \quad (2-25)$$

$$Fe^{3+} + G^{4-} - 2H^+ + 2H_2O \rightleftharpoons FeG(OH)_2^{3-} \quad \beta_{11-2(FeG)} = \frac{[FeG(OH)_2^{3-}]}{[Fe^{3+}][G^{4-}][H^+]^{-2}} = K_w^2 \beta_{FeG} \beta_{FeG(OH)_2} \quad (2-26)$$

Figure 2. Expected chemical species in the complex formation equilibrium of Fe^{3+} -EDTA and Fe^{3+} -GBT.



3. Solution for the problem

The total equilibrium system was integrated into a single equilibrium using conditional formation constant β_{FeL}' .

$$Fe' + L' \rightleftharpoons FeL' \quad \beta_{FeL}' = \frac{[FeL']}{[Fe'][L']} \quad (3-1)$$

where

$$[L'] = [L^{4-}] + [HL^{3-}] + [H_2L^{2-}] + [H_3L^-] + [H_4L] \quad (3-2)$$

$$[Fe'] = [Fe^{3+}] + [FeOH^{2+}] + [Fe(OH)_2^+] + [Fe(OH)_3] + [Fe(OH)_4^-] \quad (3-3)$$

$$[FeY'] = [FeY^-] + [FeHY] + [FeY(OH)^{2-}] \quad (3-4)$$

$$[FeG'] = [FeG^-] + [FeG(OH)_2^{3-}] \quad (3-5)$$

Total concentrations of a ligand and a ferric ion c_L and c_{Fe} are described as

$$c_L = [L'] + [FeL'] \quad (3-6)$$

$$c_{Fe} = [Fe'] + [FeL'] \quad (3-7)$$

Here, side reaction coefficients α_L , α_{Fe} , and α_{FeL} are defined as

$$\alpha_L = \frac{[L']}{[L^{4-}]} \quad (3-8)$$

$$\alpha_{Fe} = \frac{[Fe']}{[Fe^{3+}]} \quad (3-9)$$

$$\alpha_{FeL} = \frac{[FeL']}{[FeL^-]} \quad (3-10)$$

The conditional formation constant β_{FeL}' is described by multiplying the original complex formation constant β_{FeL} by a coefficient including side reaction coefficients.

$$\beta_{FeL}' = \frac{[FeL']}{[Fe'][L']} = \frac{\alpha_{FeL}[FeL^-]}{\alpha_{Fe}\alpha_L[Fe^{3+}][L^{4-}]} = \frac{\alpha_{FeL}}{\alpha_{Fe}\alpha_L}\beta_{FeL} \quad (3-11)$$

In this problem, free metal ion concentration $[Fe^{3+}]$ is required to figure out pFe.

$$pFe = -\log[Fe^{3+}] \quad (3-12)$$

On the other hand, $pL_{0.5}$ becomes

$$\begin{aligned} pL_{0.5} &= -\log c_L = -\log([L'] + [FeL']) \\ &= -\log\{[L'](1 + [Fe']\beta_{FeL}')\} \end{aligned} \quad (3-13)$$

Since 50% of the metal cation bind to a ligand,

$$[Fe'] = [FeL'] = 0.5c_M \quad (3-14)$$

$$\beta_{FeL}' = \frac{1}{[L']} \quad (3-15)$$

Also, total concentration of a ferric iron is trace relative to that of a ligand.

$$c_{Fe} \ll c_L \Leftrightarrow c_{Fe} - [FeL'] \ll c_L - [FeL'] \Leftrightarrow [Fe'] \ll [L']$$

Therefore, eq. (3-13) finally becomes

$$\begin{aligned} pL_{0.5} &= -\log([L'] + [Fe']) \\ &= -\log[L'] \\ &= \log \beta_{FeL}' \end{aligned} \quad (3-16)$$

Accordingly, we need to calculate $[Fe^{3+}]$ and β_{FeL}' to solve the problem. These parameters are found in order of $\alpha \rightarrow \beta_{FeL}' \rightarrow [Fe'] \rightarrow [Fe^{3+}]$.

(1) pFe

• EDTA

α_Y is expressed as a function of $[H^+]$ from the combination of eqs. (2-14)–(2-17), and (3-2).

$$\begin{aligned} [Y'] &= [Y^{4-}] + [HY^{3-}] + [H_2Y^{2-}] + [H_3Y^-] + [H_4Y] \\ &= [Y^{4-}] \left(1 + \beta_{011(Y)}[H^+] + \beta_{012(Y)}[H^+]^2 + \beta_{013(Y)}[H^+]^3 + \beta_{014(Y)}[H^+]^4 \right) \end{aligned} \quad (3-17)$$

$$\alpha_Y = \frac{[Y']}{[Y^{4-}]} = 1 + \beta_{011(Y)}[H^+] + \beta_{012(Y)}[H^+]^2 + \beta_{013(Y)}[H^+]^3 + \beta_{014(Y)}[H^+]^4 \quad (3-18)$$

Similar transformations are carried out for α_{Fe} , and α_{FeY} .

$$\begin{aligned} [Fe'] &= [Fe^{3+}] + [FeOH^{2+}] + [Fe(OH)_2^+] + [Fe(OH)_3] + [Fe(OH)_4^-] \\ &= [Fe^{3+}] \left(1 + \beta_{10-1(Fe)}[H^+]^{-1} + \beta_{10-2(Fe)}[H^+]^{-2} + \beta_{10-3(Fe)}[H^+]^{-3} + \beta_{10-4(Fe)}[H^+]^{-4} \right) \end{aligned} \quad (3-19)$$

$$\alpha_{Fe} = \frac{[Fe']}{[Fe^{3+}]} = 1 + \beta_{10-1(Fe)}[H^+]^{-1} + \beta_{10-2(Fe)}[H^+]^{-2} + \beta_{10-3(Fe)}[H^+]^{-3} + \beta_{10-4(Fe)}[H^+]^{-4} \quad (3-20)$$

$$\begin{aligned} [FeY'] &= [FeY^-] + [FeHY] + [FeY(OH)^{2-}] \\ &= [FeY^-] \left(1 + \frac{\beta_{111(FeY)}}{\beta_{110(FeY)}}[H^+] + \frac{\beta_{11-1(FeY)}}{\beta_{110(FeY)}}[H^+]^{-1} \right) \end{aligned} \quad (3-21)$$

$$\alpha_{FeY} = \frac{[FeY']}{[FeY^-]} = 1 + \frac{\beta_{111(FeY)}}{\beta_{110(FeY)}}[H^+] + \frac{\beta_{11-1(FeY)}}{\beta_{110(FeY)}}[H^+]^{-1} \quad (3-22)$$

$[H^+] = 10^{-7.4}$ is assigned to eqs. (3-18), (3-20), and (3-22).

$$\alpha_Y = 1 + 661 + 38.0 + 7.76 \times 10^{-4} + 3.09 \times 10^{-9} = 700 \quad (3-23)$$

$$\alpha_{Fe} = 1 + 1.70 \times 10^5 + 2.82 \times 10^8 + 8.91 \times 10^7 + 1.02 \times 10^7 = 3.81 \times 10^8 \quad (3-24)$$

$$\alpha_{FeY} = 1 + 3.02 \times 10^{-6} + 0.741 = 1.74 \quad (3-25)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{FeY}' &= \frac{\alpha_{FeY}}{\alpha_{Fe}\alpha_Y} \beta_{FeY} \\ &= \frac{1.74}{3.81 \times 10^8 \times 700} 10^{25.10} \\ &= 8.21 \times 10^{13} \end{aligned} \quad (3-26)$$

From the combination of eqs. (3-6), (3-7), and (3-11), it is

$$\begin{aligned} \beta_{FeY}' &= \frac{[FeY']}{[Fe'][Y']} \\ &= \frac{[FeY']}{(c_{Fe} - [FeY'])(c_Y - [FeY'])} \end{aligned} \quad (3-27)$$

Assign values of $c_L = 10^{-5}$ M, $c_{Fe} = 10^{-6}$ M to eq. (3-27) and solve the quadratic equation of $[FeY']$ to find

$$[FeY'] = 1.00 \times 10^{-6} - 1.40 \times 10^{-15} \text{ M} \quad (3-28)$$

From eq. (3-7),

$$[Fe'] = 1.40 \times 10^{-15} \text{ M} \quad (3-29)$$

Isolating $[\text{Fe}^{3+}]$, eq. (3-20) finally becomes

$$[\text{Fe}^{3+}] = \frac{[\text{Fe}']}{\alpha_{\text{Fe}}} = \frac{1.40 \times 10^{-15}}{3.81 \times 10^8} = 3.67 \times 10^{-24} \text{ M} \quad (3-30)$$

$$\text{pFe} = -\log[\text{Fe}^{3+}] = 23.4 \quad (3-31)$$

- GBT

α_{G} is expressed as a function of $[\text{H}^+]$ from the combination of eqs. (2-14)–(2-17), and (3-2).

$$\begin{aligned} [\text{G}'] &= [\text{G}^{4-}] + [\text{HG}^{3-}] + [\text{H}_2\text{G}^{2-}] + [\text{H}_3\text{G}^-] + [\text{H}_4\text{G}] \\ &= [\text{G}^{4-}] \left(1 + \beta_{011(\text{G})}[\text{H}^+] + \beta_{012(\text{G})}[\text{H}^+]^2 + \beta_{013(\text{G})}[\text{H}^+]^3 + \beta_{014(\text{G})}[\text{H}^+]^4 \right) \end{aligned} \quad (3-32)$$

$$\alpha_{\text{G}} = \frac{[\text{G}']}{[\text{G}^{4-}]} = 1 + \beta_{011(\text{G})}[\text{H}^+] + \beta_{012(\text{G})}[\text{H}^+]^2 + \beta_{013(\text{G})}[\text{H}^+]^3 + \beta_{014(\text{G})}[\text{H}^+]^4 \quad (3-33)$$

Similar transformation is carried out for α_{FeG} .

$$\begin{aligned} [\text{FeG}'] &= [\text{FeG}^-] + [\text{FeG}(\text{OH})_2^{3-}] \\ &= [\text{FeG}^-] \left(1 + \frac{\beta_{11-2(\text{FeG})}}{\beta_{110(\text{FeG})}}[\text{H}^+]^2 \right) \end{aligned} \quad (3-34)$$

$$\alpha_{\text{FeG}} = \frac{[\text{FeG}']}{[\text{FeG}^-]} = 1 + \frac{\beta_{11-2(\text{FeG})}}{\beta_{110(\text{FeG})}}[\text{H}^+]^2 \quad (3-35)$$

$[\text{H}^+] = 10^{-7.4}$ is assigned to eqs. (3-33) and (3-35).

$$\alpha_{\text{G}} = 1 + 3.47 \times 10^3 + 70.8 + 0.209 + 1.55 = 3.54 \times 10^3 \quad (3-36)$$

$$\alpha_{\text{FeG}} = 1 + 4.07 \times 10^{-7} = 1.00 \quad (3-37)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{\text{FeG}}' &= \frac{\alpha_{\text{FeG}}}{\alpha_{\text{Fe}}\alpha_{\text{G}}} \beta_{\text{FeG}} \\ &= \frac{1.00}{3.81 \times 10^8 \times 3.54 \times 10^3} 10^{27.61} \\ &= 3.02 \times 10^{15} \end{aligned} \quad (3-38)$$

From the combination of eqs. (3-6), (3-7), and (3-11), it is

$$\begin{aligned} \beta_{\text{FeG}}' &= \frac{[\text{FeG}']}{[\text{Fe}'][\text{G}']} \\ &= \frac{[\text{FeG}']}{(c_{\text{Fe}} - [\text{FeG}'])(c_{\text{G}} - [\text{FeG}'])} \end{aligned} \quad (3-39)$$

Assign values of $c_{\text{L}} = 10^{-5} \text{ M}$, $c_{\text{Fe}} = 10^{-6} \text{ M}$ to eq. (3-39) and solve the quadratic equation of $[\text{FeY}']$ to find

$$[\text{FeG}'] = 1.00 \times 10^{-6} - 3.51 \times 10^{-17} \text{ M} \quad (3-40)$$

From eq. (3-7),

$$[\text{Fe}'] = 3.51 \times 10^{-17} \text{ M} \quad (3-41)$$

Isolating $[\text{Fe}^{3+}]$, eq. (3-20) finally becomes

$$[\text{Fe}^{3+}] = \frac{[\text{Fe}']}{\alpha_{\text{Fe}}} = \frac{3.51 \times 10^{-17}}{3.81 \times 10^8} = 9.21 \times 10^{-26} \text{ M} \quad (3-42)$$

$$\text{pFe} = -\log[\text{Fe}^{3+}] = 25.0 \quad (3-43)$$

Calculation of pFe values indicated that the sequestering ability of GBT is 40 times higher than that of EDTA.

(2) $pL_{0.5}$

(ii) $pH = 7.4$

$pL_{0.5}$ values are found by integrating eqs. (3-16), (3-26), and (3-38).

- EDTA

$$\begin{aligned} pL_{0.5} &= \log \beta_{FeY}' \\ &= \log(8.21 \times 10^{13}) = 13.9 \end{aligned} \quad (3-44)$$

- GBT

$$\begin{aligned} pL_{0.5} &= \log \beta_{FeG}' \\ &= \log(3.02 \times 10^{15}) = 15.5 \end{aligned} \quad (3-45)$$

(i) $pH = 2.5$

$[H^+] = 10^{-2.5}$ is assigned to eqs. (3-18), (3-20), (3-22), (3-33) and (3-35).

- EDTA

$$\alpha_Y = 1 + 3.16 \times 10^{-2} + 2.40 \times 10^{11} + 3.89 \times 10^{11} + 1.23 \times 10^{11} = 7.52 \times 10^{11} \quad (3-46)$$

$$\alpha_{Fe} = 1 + 2.14 + 4.47 \times 10^{-2} + 1.78 \times 10^{-7} + 2.57 \times 10^{-13} = 3.18 \quad (3-47)$$

$$\alpha_{FeY} = 1 + 0.240 + 9.33 \times 10^{-6} = 1.24 \quad (3-48)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{FeY}' &= \frac{\alpha_{FeY}}{\alpha_{Fe}\alpha_Y} \beta_{FeY} \\ &= \frac{1.24}{3.18 \times 7.52 \times 10^{11}} 10^{25.10} \\ &= 6.52 \times 10^{12} \end{aligned} \quad (3-49)$$

$$pL_{0.5} = \log \beta_{FeY}' = 12.8 \quad (3-50)$$

- GBT

$$\alpha_G = 1 + 2.75 \times 10^8 + 4.47 \times 10^{11} + 1.05 \times 10^{14} + 6.17 \times 10^{13} = 1.67 \times 10^{14} \quad (3-51)$$

$$\alpha_{FeG} = 1 + 6.46 \times 10^{-17} = 1.00 \quad (3-52)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{FeG}' &= \frac{\alpha_{FeG}}{\alpha_{Fe}\alpha_G} \beta_{FeG} \\ &= \frac{1.00}{3.18 \times 1.67 \times 10^{14}} 10^{27.61} \\ &= 7.67 \times 10^{12} \end{aligned} \quad (3-53)$$

$$pL_{0.5} = \log \beta_{FeY}' = 12.9 \quad (3-54)$$

(iii) $pH = 10.5$

$[H^+] = 10^{-2.5}$ is assigned to eqs. (3-18), (3-20), (3-22), (3-33) and (3-35).

- EDTA

$$\alpha_Y = 1 + 0.524 + 2.40 \times 10^{-5} + 3.89 \times 10^{-13} + 1.23 \times 10^{-21} = 1.52 \quad (3-55)$$

$$\alpha_{Fe} = 1 + 2.14 \times 10^8 + 4.47 \times 10^{14} + 1.78 \times 10^{17} + 2.57 \times 10^{19} = 2.59 \times 10^{19} \quad (3-56)$$

$$\alpha_{FeY} = 1 + 2.40 \times 10^{-9} + 933 = 934 \quad (3-57)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{FeY}' &= \frac{\alpha_{FeY}}{\alpha_{Fe}\alpha_Y} \beta_{FeY} \\ &= \frac{1.52}{2.59 \times 10^{19} \times 934} 10^{25.10} \end{aligned}$$

$$= 2.98 \times 10^8 \quad (3-58)$$

$$pL_{0.5} = \log \beta_{FeY}' = 8.47 \quad (3-59)$$

● GBT

$$\alpha_G = 1 + 2.75 + 4.47 \times 10^{-5} + 1.05 \times 10^{-10} + 6.17 \times 10^{-19} = 3.75 \quad (3-60)$$

$$\alpha_{FeG} = 1 + 0.646 = 1.65 \quad (3-61)$$

Therefore, eq. (3-11) becomes

$$\begin{aligned} \beta_{FeG}' &= \frac{\alpha_{FeG}}{\alpha_{Fe}\alpha_G} \beta_{FeG} \\ &= \frac{1.65}{2.59 \times 10^{19} \times 3.75} 10^{27.61} \\ &= 6.90 \times 10^7 \end{aligned} \quad (3-62)$$

$$pL_{0.5} = \log \beta_{FeY}' = 7.84 \quad (3-63)$$

Overall, Calculation of $pL_{0.5}$ values indicated that the sequestering ability of GBT is 37 times higher than that of EDTA at pH 7.4, but at the same level at pH 2.5, and 4 times weaker at pH 10.5. The relationship between pH and $pL_{0.5}$ values of GBT and EDTA are described in Figure 3.

Example of application of $pL_{0.5}$ value calculation¹⁰

Helicobacter pylori produces Hpn protein which help the intracellular modulation of the amount of Ni^{2+} . Since histidine potentially participates in the complex formation with neighboring residues, the $pL_{0.5}$ values of N-terminal domain of Hpn proteins (MAHHEEQHG-Am) was investigated with that of its mutants obtained by substitution of one His residue with Ala (Ala-scan) to understand the role of each His in the coordination of metal ions. The binding affinities was compared for Cu^{2+} , Ni^{2+} and Zn^{2+} ions, which are potentially competing metals *in vivo* (Table 2).

The $pL_{0.5}$ values confirmed that His-3 plays an important role in binding Cu^{2+} and Ni^{2+} . Also, it was revealed that the metal binding affinities follow the order $Cu^{2+} > Ni^{2+} > Zn^{2+}$. In fact, the 3-residues amino terminal metal-binding motif (MAH) works as the most efficient binding site for Cu^{2+} and Ni^{2+} , while macrochelate Zn^{2+} complexes are formed thanks to the presence of several suitable anchoring sites (His and Glu).

The order of values suggested that the function of Hpn to capture Ni^{2+} is perturbed by the presence of Cu^{2+} even if in low amount. Actually, the interaction of Ni^{2+} -WT complex with Cu^{2+} resulted in substitution of chelated metal species. The removed Ni^{2+} ion is proposed to be just released or moved to the secondary binding site to form a hetero-binuclear complex (Figure 4).

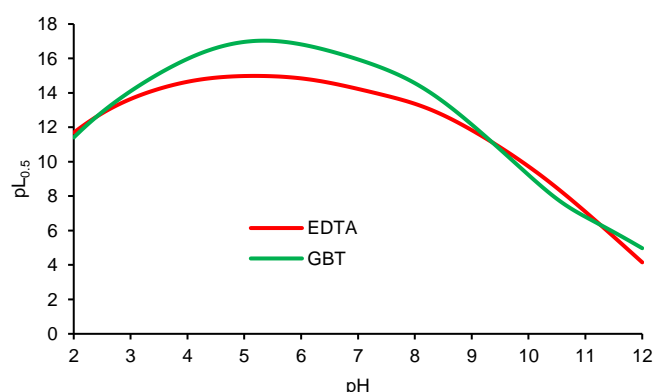
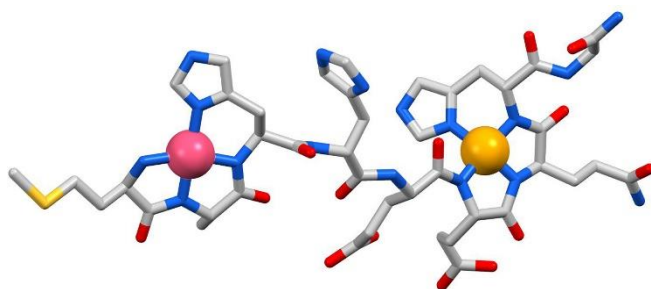


Figure 3. The pH profile of the $pL_{0.5}$ values for EDTA and gramibactin.

Table 2. $pL_{0.5}$ values for different metal complexes with N-terminal domain of WT and that of its mutants at pH = 7.4.

	Cu^{2+}	Ni^{2+}	Zn^{2+}
WT	12.27	8.21	5.49
H3A	9.23	5.28	4.34
H4A	12.25	8.23	4.14
H8A	12.24	7.95	3.76

Figure 4. Proposed molecular structure for the hetero-binuclear complex of N-terminal domain of Hpn protein.



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