

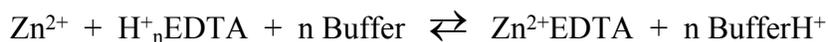
Supplemental Information

“Thermodynamics of Pb(II) and Zn(II) binding to MT-3, a neurologically important metallothionein”

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Description of the determination of the formation enthalpy (ΔH) and stability constant (K) for the formation of metal-EDTA complexes at a specific pH.

To provide more detail about how data from the NIST database was used in the thermodynamic analyses, the treatment of Zn^{2+} EDTA formation at pH 6.0 in Bis-Tris buffer is outlined in detail. An analogous approach was used for Pb^{2+} EDTA formation at pH 6.0 in MES buffer.



Enthalpy of Formation: ΔH

$$\Delta H_{Zn-EDTA}(pH\ 6.0) = -n\Delta H_{EDTA-H} + \Delta H_{Zn-EDTA} + n\Delta H_{Buffer-H}$$

Here, n is the number of protons that are displaced from the EDTA by the metal ion and subsequently protonate the buffer.

To determine n , the protonation state of the EDTA at the desired pH is found with the Henderson-Hasselbalch relationship.

$$pH = pK_a + \log([A^-]/[HA])$$

Since two of the pK_a values of EDTA are well below pH 6.0, they are not included in the analysis. The percentage of protonation associated with pK_a 6.13 is determined by the following analysis.

$$\begin{aligned} 6.0 &= 6.13 + \log([A^-]/[HA]) \\ -0.13 &= \log([A^-]/[HA]) \\ 10^{-0.13} &= [A^-]/[HA] \\ 0.741 &= [A^-]/[HA] = X \end{aligned}$$

EDTA (NIST database)

	pKa	ΔH
4H+	2	0.3
3H+	2.69	1.5
2H+	6.13	-4.2
1H+	9.52	-5.6

$$\% \text{ protonated} = [A^-] + [HA] = [HA]X + [HA] = [HA](X + 1)$$

$$\% \text{ protonated} = \frac{[HA]}{(X + 1)}$$

$$\% \text{ protonated} = \frac{100\%}{(0.741 + 1)} = 57.4 \%$$

Repeating this calculation for pK_a 9.52 results in 99.7% protonation. This gives an overall EDTA protonation of $0.574 + 0.997 = 1.57$ protons at pH 6.0.

Now, using values for $\Delta H_{\text{Buffer-H}}$ and $\Delta H_{\text{Zn-EDTA}}$ from NIST, the above equation is solved.

$$\Delta H_{\text{Zn-EDTA}}(\text{pH } 6.0)$$

$$= -n\Delta H_{\text{EDTA-H}} + \Delta H_{\text{Zn-EDTA}} + n\Delta H_{\text{Buffer-H}}$$

$$= -0.574(-4.2 \text{ kcal/mol}) - 0.997(-5.6 \text{ kcal/mol}) + (-4.7 \text{ kcal/mol}) + 1.57(-6.86 \text{ kcal/mol})$$

$$= -7.5 \text{ kcal/mol}$$

Stability Constant: K

The NIST value for the $\text{Zn}^{2+}\text{EDTA}$ stability constant is independent of pH, so this value needs to be adjusted to the experimental pH to account for proton competition with Zn^{2+} for EDTA.

$$\begin{aligned} K_{\text{Zn-EDTA}}(\text{pH } 6.0) &= \frac{K_{\text{Zn-EDTA}}}{[1 + K_{\text{EDTA-H1}}[\text{H}^+] + K_{\text{EDTA-H1}}K_{\text{EDTA-H2}}[\text{H}^+]^2]} \\ &= \frac{3.16 \times 10^{16}}{[1 + (10^{9.52})(10^{-6.0}) + (10^{9.52})(10^{6.13})(10^{-6.0})^2]} \\ &= \frac{3.16 \times 10^{16}}{[1 + (3.3 \times 10^9)(1 \times 10^{-6}) + (3.3 \times 10^9)(1.3 \times 10^6)(1 \times 10^{-12})]} \\ &= \frac{3.16 \times 10^{16}}{1 + 3300 + 4290} \\ &= 4.1 \times 10^{12} \end{aligned}$$