

SECTION-II

BINDING OF Ca^{2+} , Mg^{2+} AND H^+ TO EGTA: FUNDAMENTAL EQUATIONS; H^+ -IONS RELEASED ON Ca^{2+} BINDING AND CORRECTION OF TABULATED STOICHIOMETRIC CONSTANTS FOR IONIC STRENGTH AND TEMPERATURE

The equations for the correction of the tabulated constants for ionic strength and temperature (from page 49 on) were derived by Professor (now emeritus) Paul Schindler, Department of Chemistry, University of Bern, Switzerland.

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DEFINITIONS

$$\alpha = \left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}}\right)$$

OR

$$\alpha = (1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)})$$

$$\beta = \left(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * \frac{[H^+]}{K_{H-1}}\right)$$

OR

$$\beta = (1 + 10^{(pK_{Ca-2} - pK_{Ca-1})} * 10^{(pK_{H-1} - pH)})$$

$$\gamma = \left(1 + \frac{K_{Ca-2}}{K_{Ca-1}} * \frac{K_{H-1}}{[H^+]}\right)$$

OR

$$\gamma = (1 + 10^{(pK_{Ca-1} - pK_{Ca-2})} * 10^{(pH - pK_{H-1})})$$

$$\partial = \left\{ \frac{[H^+]}{K_{H-1}} \left(\frac{[Ca^{2+}]}{K_{Ca-2}} + 1 \right) + 2 * \frac{[H^+]^2}{K_{H-1}K_{H-2}} + 3 * \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + 4 * \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right\}$$

OR

$$\partial = \{ 10^{(pK_{H-1} - pH)} (10^{(pK_{Ca-2} - pCa)} + 1) + 2 * 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + \dots + 4 * 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)} \}$$

$$\varepsilon = \left(\frac{[Ca^{2+}]}{K_{Ca-2}} \frac{[H^+]}{K_{H-1}} + \frac{[Mg^{2+}]}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}} + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + 2 \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + 3 \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + 4 \frac{[H_4-EGTA]}{[EGTA^{4-}]} \right)$$

OR

$$\varepsilon = \{ 10^{(pK_{Ca-2} - pCa)} 10^{(pK_{H-1} - pH)} + 10^{(pK_{Mg-2} - pMg)} 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} - pH)} + \dots + 4 * 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)} \}$$

Important: All units are in mol/l. Failure to comply with this will lead to errors.

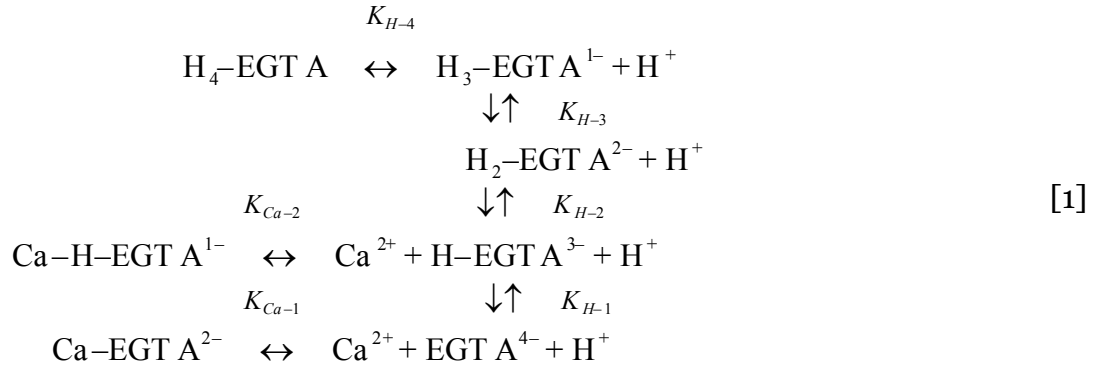
INTRODUCTION

In Part 1 of this Section the equations for the binding of Ca^{2+} and H^+ to EGTA are developed. The H^+ release on Ca^{2+} binding is also considered. In Part II, the simultaneous binding of both Ca^{2+} and Mg^{2+} to EGTA will be considered and Part III considers methods for correcting the tabulated constants to different ionic strengths and temperatures.

PART I: BINDING OF Ca^{2+} AND H^+ TO EGTA

EQUILIBRIUM CONSTANTS

The binding of Ca^{2+} and H^+ to EGTA is illustrated below.



The protonation equilibrium constants are:

$$\frac{[\text{H}^+][\text{EGT A}^{4-}]}{[\text{H-EGT A}^{3-}]} = K_{H-1} \quad \text{and} \quad \frac{[\text{H}^+][\text{H-EGT A}^{3-}]}{[\text{H}_2\text{-EGT A}^{2-}]} = K_{H-2} \quad [2][3]$$

$$\frac{[\text{H}^+][\text{H}_2\text{-EGT A}^{2-}]}{[\text{H}_3\text{-EGT A}^{1-}]} = K_{H-3} \quad \text{and} \quad \frac{[\text{H}^+][\text{H}_3\text{-EGT A}^{1-}]}{[\text{H}_4\text{-EGT A}]} = K_{H-4} \quad [4][5]$$

The Ca^{2+} equilibrium constants are:

$$\frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]}{[\text{Ca-EGT A}^{2-}]} = K_{Ca-1} \quad \text{and} \quad \frac{[\text{Ca}^{2+}][\text{H-EGT A}^{3-}]}{[\text{Ca-H-EGT A}^{1-}]} = K_{Ca-2} \quad [6][7]$$

K_{app} IN TERMS OF [H⁺] AND H⁺/Ca²⁺-EQUILIBRIUM CONSTANTS

The apparent equilibrium constant (K_{app}) is defined as follows (see also Section-I, page 18):

$$\frac{[\text{Ca}^{2+}](\text{[EGT A}^{4-}] + [\text{H-EGT A}^{3-}] + [\text{H}_2\text{-EGT A}^{2-}] + [\text{H}_3\text{-EGT A}^{1-}] + [\text{H}_4\text{-EGT A}])}{([\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}])} = K_{\text{app}} \quad [8]$$

or

$$\frac{[\text{Ca}^{2+}]}{[\text{Ca-EGT A}^{2-}]} * \frac{([\text{EGT A}^{4-}] + [\text{H-EGT A}^{3-}] + [\text{H}_2\text{-EGT A}^{2-}] + [\text{H}_3\text{-EGT A}^{1-}] + [\text{H}_4\text{-EGT A}])}{(1 + \frac{[\text{Ca-H-EGT A}^{1-}]}{[\text{Ca-EGT A}^{2-}]})} = K_{\text{app}} \quad [9]$$

but from equation [6]

$$\frac{[\text{Ca}^{2+}]}{[\text{Ca-EGT A}^{2-}]} = \frac{K_{\text{Ca-1}}}{[\text{EGT A}^{4-}]} \quad [10]$$

which on substitution in [9] gives:

$$\frac{K_{\text{Ca-1}}(1 + \frac{[\text{H-EGT A}^{3-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_2\text{-EGT A}^{2-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_3\text{-EGT A}^{1-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_4\text{-EGT A}]}{[\text{EGT A}^{4-}]})}{(1 + \frac{[\text{Ca-H-EGT A}^{1-}]}{[\text{Ca-EGT A}^{2-}]})} = K_{\text{app}} \quad [11]$$

combining equations [6] and [7] gives:

$$\frac{[\text{Ca-H-EGT A}^{1-}]}{[\text{Ca-EGT A}^{2-}]} = \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} * \frac{[\text{H-EGT A}^{3-}]}{[\text{EGT A}^{4-}]} \quad [12]$$

from equation [2]

$$\frac{[\text{H-EGT A}^{3-}]}{[\text{EGT A}^{4-}]} = \frac{[\text{H}^+]}{K_{\text{H-1}}}$$

it follows that:

$$\frac{[\text{Ca-H-EGT A}^{1-}]}{[\text{Ca-EGT A}^{2-}]} = \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} \cdot \frac{[\text{H}^+]}{K_{\text{H-1}}} \quad [13]$$

$[H\text{-EGT A}^{2-}]/[EGT A^{4-}]$ etc. in terms of $[H^+]$ and equilibrium constants from equation [2]:

$$[\text{H-EGT A}^{3-}] = \frac{[\text{EGT A}^{4-}][\text{H}^+]}{K_{\text{H-1}}} \quad [14]$$

substituting in equation [3]

$$\frac{[\text{H}^+]}{[\text{H}_2\text{-EGT A}^{2-}]} \cdot \frac{[\text{EGT A}^{4-}][\text{H}^+]}{K_{\text{H-1}}} = K_{\text{H-2}} \quad [15]$$

on simplification this gives:

$$\frac{[\text{H}_2\text{-EGT A}^{2-}]}{[\text{EGT A}^{4-}]} = \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} \quad [16]$$

similarly we get

$$\frac{[\text{H}_3\text{-EGT A}^{1-}]}{[\text{EGT A}^{4-}]} = \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} \quad \text{and} \quad \frac{[\text{H}_4\text{-EGT A}]}{[\text{EGT A}^{4-}]} = \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \quad [17][18]$$

substitution in [11] gives:

$$\frac{K_{\text{Ca-1}} \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right)}{\left(1 + \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} \cdot \frac{[\text{H}^+]}{K_{\text{H-1}}} \right)} = K_{\text{app}} \quad [19]$$

or in pK and pH notation :

$$\frac{K_{\text{Ca-1}} (1 + 10^{(\text{pK}_{\text{H-1}} - \text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} - 2\text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} - 3\text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} + \text{pK}_{\text{H-4}} - 4\text{pH})})}{\left(1 + \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} \cdot 10^{(\text{pK}_{\text{H-1}} - \text{pH})} \right)} = K_{\text{app}} \quad [20]$$

to simplify let:

$$\alpha = (1 + 10^{(\text{pK}_{\text{H-1}} - \text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} - 2\text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} - 3\text{pH})} + 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} + \text{pK}_{\text{H-4}} - 4\text{pH})}) \quad [21]$$

$$\text{If } \beta = \left(1 + \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} \cdot 10^{(\text{pK}_{\text{H-1}} - \text{pH})} \right) \quad [22]$$

It follows that:

$$\frac{K_{Ca-1} \alpha}{\beta} = K_{app} \quad [23]$$

1) Alternate method of expressing K_{app}

Harrison & Bers (1989) use binding constants instead of equilibrium constants and define the apparent constant as follows:

$$K_{app}^B = \frac{K_{Ca-1}^B}{(1 + [H^+]K_{H-1}^B + [H^+]^2K_{H-1}^BK_{H-2}^B + [H^+]^3K_{H-1}^BK_{H-2}^BK_{H-3}^B + [H^+]^4K_{H-1}^BK_{H-2}^BK_{H-3}^BK_{H-4}^B)} + \frac{K_{Ca-2}^B}{(\frac{1}{[H^+]K_{H-1}^B} + 1 + [H^+]K_{H-2}^B + [H^+]^2K_{H-2}^BK_{H-3}^B + [H^+]^3K_{H-2}^BK_{H-3}^BK_{H-4}^B)} \quad [24]$$

where K^B defines a binding constant.

Converting to equilibrium constants gives:

$$\frac{1}{K_{app}} = \frac{1}{K_{Ca-1}(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}})} + \frac{1}{K_{Ca-2}(\frac{K_{H-1}}{[H^+]} + 1 + \frac{[H^+]}{K_{H-2}} + \frac{[H^+]^2}{K_{H-2}K_{H-3}} + \frac{[H^+]^3}{K_{H-2}K_{H-3}K_{H-4}})} \quad [25]$$

It follows that:

$$\frac{1}{K_{app}} = \frac{1}{K_{Ca-1}\alpha} + \frac{1}{K_{Ca-2}\frac{K_{H-1}}{[H^+]}(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}})} \quad [26]$$

and

$$\frac{1}{K_{app}} = \frac{1}{K_{Ca-1}\alpha} + \frac{1}{K_{Ca-2}\frac{K_{H-1}}{[H^+]}\alpha} \quad [27]$$

this simplifies to:

$$\frac{1}{K_{app}} = \frac{K_{Ca-2}K_{H-1} + K_{Ca-1}[H^+]}{K_{Ca-1}K_{Ca-2}K_{H-1}\alpha} \quad [28]$$

re-arrangement gives:

$$\frac{1}{K_{app}} = \frac{K_{Ca-2}K_{H-1}(1 + \frac{K_{Ca-1}[H^+]}{K_{Ca-2}K_{H-1}})}{K_{Ca-1}K_{Ca-2}K_{H-1}\alpha} \quad [29]$$

which simplifies to:

$$K_{app} = \frac{K_{Ca-1}\alpha}{(1 + \frac{K_{Ca-1}[H^+]}{K_{Ca-2}K_{H-1}})} \text{ or } K_{app} = \frac{K_{Ca-1}\alpha}{\beta} \quad [30][31]$$

2) Conversion of K_{app} from pH_1 to K_{app} at pH_2 within the pH range of 5 to 8

Smith & Miller (1985) use the following equation to convert K_{app} from pH_1 to pH_2 .

$$-\log K_{app-2} = -\log K_{app-1} + 2(pH_2 - pH_1) \quad [32]$$

This can be derived as follows;

At pH values between 6 and 8 the third and fourth protonation constants can be neglected.

Under these circumstances K_{app} becomes:

$$\frac{K_{Ca-1}(1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)})}{(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH)})} = K_{app} \quad [33]$$

since $10^{(pK_1 + pK_2 - 2 * pH)} \gg (1 + 10^{(pK_1 - pH)})$ as a first approximation equation [33] can be written:

$$\frac{K_{Ca-1}(10^{(pK_{H-1} + pK_{H-2} - 2pH)})}{(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH)})} = K_{app} \quad [34]$$

It then follows at pH_1 and pH_2 :

$$\frac{K_{Ca-1}(10^{(pK_{H-1} + pK_{H-2} - 2pH_1)})}{(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH_1)})} * \frac{(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH_2)})}{K_{Ca-1}(10^{(pK_{H-1} + pK_{H-2} - 2pH_2)})} = \frac{K_{app-1}}{K_{app-2}} \quad [35]$$

since $(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH_1)})$ and $(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH_2)})$ are approximately equal, equation [35] simplifies to:

$$\frac{(10^{(pK_{H-1} + pK_{H-2} - 2pH_1)})}{(10^{(pK_{H-1} + pK_{H-2} - 2pH_2)})} = \frac{K_{app-1}}{K_{app-2}} \quad [36]$$

or

$$\frac{(10^{(pK_{H-1} + pK_{H-2})} * 10^{-2pH_1})}{(10^{(pK_{H-1} + pK_{H-2})} * 10^{-2pH_2})} = \frac{K_{app-1}}{K_{app-2}} \quad [37]$$

and

$$\frac{10^{-2pH_1}}{10^{-2pH_2}} = \frac{K_{app-1}}{K_{app-2}} \quad [38]$$

It follows that:

$$\log\left(\frac{10^{-2pH_1}}{10^{-2pH_2}}\right) = \log\left(\frac{K_{app-1}}{K_{app-2}}\right) \quad [39]$$

and

$$\log(10^{-2pH_1}) - \log(10^{-2pH_2}) = \log(K_{app-1}) - \log(K_{app-2}) \quad [40]$$

which simplifies to:

$$-\log(K_{app-1}) + 2(pH_2 - pH_1) = -\log(K_{app-2}) \quad [41]$$

IONIZED AND BOUND CALCIUM IN EGTA SOLUTIONS

1) Ionized calcium $[Ca^{2+}]$

The derivation of the $[Ca^{2+}]$ is identical to the binding of Mg^{2+} to ATP, (Section-I, equation [84], page 14). For Ca^{2+} and EGTA it is:

$$[Ca^{2+}] = \frac{-A + \sqrt{A^2 + 4[Ca]_T K_{app}}}{2} \quad [42]$$

where

$$A = (K_{app} + [EGTA]_T - [Ca]_T) \quad [43]$$

2) Bound calcium: $[Ca-H-EGTA^{1-}]$

$$[EGTA]_T = [EGTA^{4-}] + [H-EGTA^{3-}] + [H_2-EGTA^{2-}] + [H_3-EGTA^{1-}] + [H_4-EGTA] + [Ca-H-EGTA^{1-}] + [Ca-EGTA^{2-}] \quad [44]$$

and

$$[Ca]_T = [Ca^{2+}] + [Ca-EGTA^{2-}] + [Ca-H-EGTA^{2-}] \quad [45]$$

it follows that:

$$[Ca]_T = [Ca^{2+}] + [Ca-H-EGTA^{1-}](1 + \frac{[Ca-EGTA^{2-}]}{[Ca-H-EGTA^{1-}]}) \quad [46]$$

and from equations [7] and [13]

$$[Ca]_T = \frac{K_{Ca-2}[Ca-H-EGTA^{1-}]}{[H-EGTA^{3-}]} + [Ca-H-EGTA^{1-}](1 + \frac{K_{Ca-2}K_{H-1}}{K_{Ca-1}[H^+]}) \quad [47]$$

and substituting for $[H-EGTA^{3-}]$ from equation [2] gives:

$$[Ca]_T = \frac{K_{H-1}K_{Ca-2}[Ca-H-EGTA^{1-}]}{[H^+][EGTA^{4-}]} + [Ca-H-EGTA^{1-}](1 + \frac{K_{Ca-2}K_{H-1}}{K_{Ca-1}[H^+]}) \quad [48]$$

$[EGTA^{4-}]$ in terms of equilibrium constants and $[Ca-H-EGTA^{1-}]$

from equations [44] it follows that:

$$[EGTA]_T = [EGTA^{4-}](1 + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + \frac{[H_4-EGTA]}{[EGTA^{4-}]} + [Ca-H-EGTA^{1-}](1 + \frac{[Ca-EGTA^{2-}]}{[Ca-H-EGTA^{1-}]}) \quad [49]$$

from equations [13] and equations [14] to [18] we get:

$$[EGTA]_T = [EGTA^{4-}](1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}}) + [Ca-H-EGTA^{1-}](1 + \frac{K_{Ca-2}}{K_{Ca-1}} * \frac{K_{H-1}}{[H^+]}) \quad [50]$$

it follows that:

$$[\text{EGT A}^{4-}] = \frac{[\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}](1 + \frac{K_{\text{Ca-2}} * K_{\text{H-1}}}{K_{\text{Ca-1}} [\text{H}^+]})}{(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}})} \quad [51]$$

to simplify:

$$\alpha = (1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}})$$

and let

$$\gamma = (1 + \frac{K_{\text{Ca-2}} * K_{\text{H-1}}}{K_{\text{Ca-1}} [\text{H}^+]}) \quad [52]$$

thus equation [51] becomes:

$$[\text{EGT A}^{4-}] = \frac{([\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}]\gamma)}{\alpha} \quad [53]$$

substitution of equation [53] in equation [48] gives:

$$[\text{Ca}]_T = \frac{K_{\text{H-1}}K_{\text{Ca-2}}[\text{Ca-H-EGT A}^{1-}]}{[\text{H}^+]\frac{([\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}]\gamma)}{\alpha}} + [\text{Ca-H-EGT A}^{1-}]\gamma \quad [54]$$

or

$$[\text{Ca}]_T = \frac{K_{\text{H-1}}K_{\text{Ca-2}}[\text{Ca-H-EGT A}^{1-}]\alpha}{[\text{H}^+]([\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}]\gamma)} + [\text{Ca-H-EGT A}^{1-}]\gamma \quad [55]$$

which on simplification gives:

$$[\text{Ca}]_T[\text{H}^+]([\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}]\gamma) = K_{\text{H-1}}K_{\text{Ca-2}}[\text{Ca-H-EGT A}^{1-}]\alpha + [\text{Ca-H-EGT A}^{1-}]\gamma\{[\text{H}^+]([\text{EGTA}]_T - [\text{Ca-H-EGT A}^{1-}]\gamma)\} \quad [56]$$

further simplification and collecting terms gives:

$$[\text{Ca-H-EGT A}^{1-}]^2 - (\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{K_{\text{H-1}}K_{\text{Ca-2}}\alpha}{[\text{H}^+]\gamma^2})[\text{Ca-H-EGT A}^{1-}] + \frac{[\text{Ca}]_T[\text{EGTA}]_T}{\gamma^2} = 0 \quad [57]$$

the solution of the quadratic is:

$$[\text{Ca-H-EGTA}] = \frac{B - \sqrt{B^2 - \frac{4[\text{Ca}]_T[\text{EGTA}]_T}{\gamma^2}}}{2} \quad [58]$$

where

$$B = \left(\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{K_{H-1}K_{Ca-2}\alpha}{[\text{H}^+]^2} \right) \quad [59]$$

and

$$\alpha = \left(1 + \frac{[\text{H}^+]}{K_{H-1}} + \frac{[\text{H}^+]^2}{K_{H-1}K_{H-2}} + \frac{[\text{H}^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[\text{H}^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right)$$

$$\gamma = \left(1 + \frac{K_{Ca-2} * K_{H-1}}{K_{Ca-1} [\text{H}^+]} \right)$$

or

$$\gamma = \frac{K_{Ca-1}[\text{H}^+] + K_{Ca-2}K_{H-1}}{K_{Ca-1}[\text{H}^+]}$$

substituting in equation [59] gives:

$$B = \left(\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{1}{\gamma} \frac{K_{H-1}K_{Ca-2}\alpha}{[\text{H}^+]} * \frac{K_{Ca-1}[\text{H}^+]}{K_{Ca-1}[\text{H}^+] + K_{Ca-2}K_{H-1}} \right) \quad [60]$$

or

$$B = \left(\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{1}{\gamma} \frac{K_{H-1}K_{Ca-1}K_{Ca-2}\alpha}{(K_{Ca-1}[\text{H}^+] + K_{Ca-2}K_{H-1})} \right) \quad [61]$$

dividing term 3 by $K_{Ca-2}K_{H-1}$ gives:

$$B = \left(\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{1}{\gamma} \frac{K_{Ca-1}\alpha}{\left(1 + \frac{K_{Ca-1}[\text{H}^+]}{K_{Ca-2}K_{H-1}} \right)} \right) \quad [62]$$

since

$$K_{\text{app}} = \frac{K_{Ca-1}\alpha}{\left(1 + \frac{K_{Ca-1}[\text{H}^+]}{K_{Ca-2}K_{H-1}} \right)}$$

it follows that

$$B = \left(\frac{[\text{EGTA}]_T}{\gamma} + \frac{[\text{Ca}]_T}{\gamma} + \frac{K_{\text{app}}}{\gamma} \right) \quad [63]$$

3) Bound calcium: [Ca-EGTA²⁻]

From equations [6], [13] and [45]

$$[\text{Ca}]_T = \frac{K_{\text{Ca-1}}[\text{Ca-EGT A}^{2-}]}{[\text{EGT A}^{4-}]} + [\text{Ca-EGT A}^{2-}]\left(1 + \frac{K_{\text{Ca-1}}[\text{H}^+]}{K_{\text{Ca-2}}K_{\text{H-1}}}\right) \quad [64]$$

From equation [44] in terms of equilibrium constants and [Ca-EGTA²⁻]

$$[\text{EGTA}]_T = [\text{EGT A}^{4-}]\left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}}\right) + [\text{Ca-EGT A}^{2-}]\left(1 + \frac{K_{\text{Ca-1}} * [\text{H}^+]}{K_{\text{Ca-2}} K_{\text{H-1}}}\right) \quad [65]$$

but:

$$\alpha = \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}}\right)$$

and

$$\beta = \left(1 + \frac{K_{\text{Ca-1}} * [\text{H}^+]}{K_{\text{Ca-2}} K_{\text{H-1}}}\right)$$

so

$$[\text{EGT A}^{4-}] = \frac{([\text{EGTA}]_T - [\text{Ca-EGT A}^{2-}]\beta)}{\alpha} \quad [66]$$

substitution in equation [64] gives:

$$[\text{Ca}]_T = \frac{K_{\text{Ca-2}}[\text{Ca-EGTA}^{2-}]\alpha}{([\text{EGTA}]_T - [\text{Ca-EGT A}^{2-}]\beta)} + [\text{Ca-EGTA}^{2-}]\beta \quad [67]$$

Simplification gives:

$$[\text{Ca-EGTA}^{2-}]^2 - \left(\frac{[\text{EGTA}]_T}{\beta} + \frac{[\text{Ca}]_T}{\beta} + \frac{K_{\text{Ca-1}}\alpha}{\beta^2}\right)[\text{Ca-EGT A}^{2-}] + \frac{[\text{Ca}]_T[\text{EGTA}]_T}{\beta^2} = 0 \quad [68]$$

The solution of the quadratic is:

$$[\text{Ca-EGT A}^{2-}] = \frac{C - \sqrt{C^2 - \frac{4[\text{Ca}]_T[\text{EGTA}]_T}{\beta^2}}}{2} \quad [69]$$

where

$$C = \left(\frac{[\text{EGTA}]_T}{\beta} + \frac{[\text{Ca}]_T}{\beta} + \frac{K_{\text{Ca-1}}\alpha}{\beta^2} \right) \quad [70]$$

or

$$C = \left(\frac{[\text{EGTA}]_T}{\beta} + \frac{[\text{Ca}]_T}{\beta} + \frac{K_{\text{app}}}{\beta} \right) \quad [71]$$

$$\alpha = \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right)$$

$$\beta = \left(1 + \frac{K_{\text{Ca-1}} * [\text{H}^+]}{K_{\text{Ca-2}} K_{\text{H-1}}} \right)$$

CONCENTRATIONS OF EGTA NOT BOUND TO Ca^{2+}

1) $[\text{EGTA}^{4-}]$ (derivation 1)

Equation [44], namely

$$[\text{EGTA}]_T = [\text{EGTA}^{4-}] + [\text{H-EGTA}^{3-}] + [\text{H}_2\text{-EGTA}^{2-}] + [\text{H}_3\text{-EGTA}^{1-}] + [\text{H}_4\text{-EGTA}] + [\text{Ca-H-EGTA}^{1-}] + [\text{Ca-EGTA}^{2-}]$$

can also be written as:

$$[\text{EGTA}]_T = [\text{EGTA}^{4-}]\alpha + ([\text{Ca}]_T - [\text{Ca}^{2+}]) \quad [72]$$

and

$$[\text{Ca}^{2+}] = [\text{EGTA}^{4-}]\alpha + [\text{Ca}]_T - [\text{EGTA}]_T \quad [73]$$

substituting equation [42] for $[\text{Ca}^{2+}]$ gives:

$$\frac{-A + \sqrt{A^2 + 4[\text{Ca}]_T K_{\text{app}}}}{2} = [\text{EGTA}^{4-}]\alpha + [\text{Ca}]_T - [\text{EGTA}]_T \quad [74]$$

$$A^2 + 4[\text{Ca}]_T K_{\text{app}} = (2[\text{EGTA}^{4-}]\alpha + 2[\text{Ca}]_T - 2[\text{EGTA}]_T + A)^2 \quad [75]$$

but $A = (K_{\text{app}} + [\text{EGTA}]_T - [\text{Ca}]_T)$ and substitution in equation [75] gives:

$$(K_{app} + [EGTA]_T - [Ca]_T)^2 + 4[Ca]_T K_{app} = (2[EGTA]_T \alpha + 2[Ca]_T - 2[EGTA]_T + K_{app} + [EGTA]_T - [Ca]_T)^2 \quad [76]$$

or

$$\{K_{app} - ([Ca]_T - [EGTA]_T)\}^2 + 4[Ca]_T K_{app} = \{2[EGTA]_T \alpha + K_{app} + ([Ca]_T - [EGTA]_T)\}^2 \quad [77]$$

simplifying each side in turn.

$$LHS = K_{app}^2 + 2K_{app}[Ca]_T + 2K_{app}[EGTA]_T + ([Ca]_T - [EGTA]_T)^2 \quad [78]$$

and

RHS =

$$4\alpha^2[EGTA]_T^2 + 4\alpha K_{app}[EGTA]_T + 4\alpha[Ca]_T[EGTA]_T - 4\alpha[EGTA]_T[EGTA]_T + K_{app}^2 + 2K_{app}[Ca]_T - 2K_{app}[EGTA]_T + ([Ca]_T - [EGTA]_T)^2 \quad [79]$$

equating both sides and simplifying gives:

$$4\alpha^2[EGTA]_T^2 + (4\alpha K_{app} + 4\alpha[Ca]_T - 4\alpha[EGTA]_T)[EGTA]_T - 4K_{app}[EGTA]_T = 0 \quad [80]$$

$$[EGTA]_T^2 - \left(\frac{[EGTA]_T}{\alpha} - \frac{[Ca]_T}{\alpha} - \frac{K_{app}}{\alpha}\right)[EGTA]_T - \frac{K_{app}[EGTA]_T}{\alpha^2} = 0 \quad [81]$$

however, from equation [23]

$$K_{app} = \frac{K_{Ca-1}\alpha}{\beta}$$

where

$$\alpha = \left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}}\right)$$

and

$$\beta = \left(1 + \frac{K_{Ca-1} * [H^+]}{K_{Ca-2} K_{H-1}}\right)$$

substitution into equation [81] gives:

$$[EGTA]_T^2 - \left(\frac{[EGTA]_T}{\alpha} - \frac{[Ca]_T}{\alpha} - \frac{K_{Ca-1}}{\beta}\right)[EGTA]_T - \frac{K_{Ca-1}[EGTA]_T}{\alpha\beta} = 0 \quad [82]$$

the solution of quadratic is:

$$[\text{EGTA}^{4-}] = \frac{D + \sqrt{D^2 + \frac{4K_{\text{Ca-1}}[\text{EGTA}]_{\text{T}}}{\alpha\beta}}}{2} \quad [83]$$

where

$$D = \left(\frac{[\text{EGTA}]_{\text{T}}}{\alpha} - \frac{[\text{Ca}]_{\text{T}}}{\alpha} - \frac{K_{\text{Ca-1}}}{\beta} \right) \quad [84]$$

$$\alpha = \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right)$$

$$\beta = \left(1 + \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} * \frac{[\text{H}^+]}{K_{\text{H-1}}} \right)$$

Once $[\text{EGTA}^{4-}]$ has been determined the other concentrations can be determined as follows:

$$2) [\text{H-EGTA}^{3-}] = [\text{EGTA}^{4-}] \frac{[\text{H}^+]}{K_{\text{H-1}}} \quad [85]$$

$$3) [\text{H}_2\text{-EGTA}^{2-}] = [\text{EGTA}^{4-}] \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} \quad [86]$$

$$4) [\text{H}_3\text{-EGTA}^{1-}] = [\text{EGTA}^{4-}] \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} \quad [87]$$

$$5) [\text{H}_4\text{-EGTA}] = [\text{EGTA}^{4-}] \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \quad [88]$$

or in pK and pH terminology

$$2) [\text{H-EGTA}^{3-}] = [\text{EGTA}^{4-}] 10^{(\text{pK}_{\text{H-1}} - \text{pH})} \quad [89]$$

$$3) [\text{H}_2\text{-EGTA}^{2-}] = [\text{EGTA}^{4-}] 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} - 2\text{pH})} \quad [90]$$

$$4) [\text{H}_3\text{-EGTA}^{1-}] = [\text{EGTA}^{4-}] 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} - 3\text{pH})} \quad [91]$$

$$5) [\text{H}_4\text{-EGTA}] = [\text{EGTA}^{4-}] 10^{(\text{pK}_{\text{H-1}} + \text{pK}_{\text{H-2}} + \text{pK}_{\text{H-3}} + \text{pK}_{\text{H-4}} - 4\text{pH})} \quad [92]$$

6) $[\text{EGTA}^{4-}]$ (Derivation 2: Smith, 1983)

In his PhD Thesis G. Smith (1983) presents a different derivation for $[\text{EGTA}^{4-}]$. On page 14 of the thesis he starts with the following equations:

$$[\text{EGT A}]_{\text{T}} = [\text{EGT A}^{4-}]\alpha + [\text{Ca}^{2+}][\text{EGT A}^{4-}]\beta^{\text{I}} \quad [93]$$

and

$$[\text{Ca}]_{\text{T}} = [\text{Ca}^{2+}] + [\text{Ca}^{2+}][\text{EGT A}^{4-}]\beta^{\text{I}} \quad [94]$$

His equations 1,13a and 1,13b

“ α ” is defined as in equation [21], but “ β^{I} ” is defined in terms of equilibrium constants as follows:

$$\beta^{\text{I}} = \left(\frac{1}{K_{\text{Ca-1}}} + \frac{[\text{H}^+]}{K_{\text{Ca-2}}K_{\text{H-1}}} \right) \quad [95]$$

rearranging gives:

$$\beta^{\text{I}} = \frac{1}{K_{\text{Ca-1}}} \left(1 + \frac{K_{\text{Ca-1}}[\text{H}^+]}{K_{\text{Ca-2}}K_{\text{H-1}}} \right) \text{ or } \beta^{\text{I}} = \frac{1}{K_{\text{Ca-1}}} \beta \quad [96][97]$$

where β is defined as in equation [22]. Substituting equation [97] in equations [93] and [89] gives:

$$[\text{EGT A}]_{\text{T}} = [\text{EGT A}^{4-}]\alpha + \frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]}{K_{\text{Ca-1}}} \beta \quad [98]$$

$$[\text{Ca}]_{\text{T}} = [\text{Ca}^{2+}] + \frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]}{K_{\text{Ca-1}}} \beta \quad [99]$$

The derivation of equations [98] and [99] is as follows:

$$[\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}] = ([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) \quad [100]$$

$$[\text{Ca-EGT A}^{2-}]\left(1 + \frac{[\text{Ca-H-EGT A}^{1-}]}{[\text{Ca-EGT A}^{2-}]}\right) = ([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) \quad [101]$$

and

$$[\text{Ca-EGT A}^{2-}]\left(1 + \frac{K_{\text{Ca-1}}[\text{H}^+]}{K_{\text{Ca-2}}K_{\text{H-1}}}\right) = ([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) \quad [102]$$

$$[\text{Ca-EGT A}^{2-}] = \frac{([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}])}{(1 + \frac{K_{\text{Ca-1}}[\text{H}^+]}{K_{\text{Ca-2}}K_{\text{H-1}}})} \quad [103]$$

$$[\text{Ca-EGT A}^{2-}] = \frac{([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}])}{\beta} \quad [104]$$

substitution in [6] gives:

$$\frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]\beta}{([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}])} = K_{\text{Ca-1}} \quad [105]$$

and

$$([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) = \frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]\beta}{K_{\text{Ca-1}}} \quad [106]$$

since

$$[\text{EGT A}]_{\text{T}} = [\text{EGT A}^{4-}]\alpha + ([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) \quad \text{and} \quad [\text{Ca}]_{\text{T}} = [\text{Ca}^{2+}] + ([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}])$$

equations [98] and [99] follow. Equation [99] can be written

$$[\text{Ca}]_{\text{T}} = [\text{Ca}^{2+}](1 + \frac{[\text{EGT A}^{4-}]\beta}{K_{\text{Ca-1}}}) \quad [107]$$

$$[\text{Ca}^{2+}] = \frac{[\text{Ca}]_{\text{T}}}{(1 + \frac{[\text{EGT A}^{4-}]\beta}{K_{\text{Ca-1}}})} \quad [108]$$

$$[\text{Ca}^{2+}] = \frac{K_{\text{Ca-1}}[\text{Ca}]_{\text{T}}}{(K_{\text{Ca-1}} + [\text{EGT A}^{4-}]\beta)} \quad [109]$$

substituting in equation [98] gives

$$[\text{EGT A}]_{\text{T}} = [\text{EGT A}^{4-}]\alpha + \frac{K_{\text{Ca-1}}[\text{Ca}]_{\text{T}}}{(K_{\text{Ca-1}} + [\text{EGT A}^{4-}]\beta)} * \frac{[\text{EGT A}^{4-}]\beta}{K_{\text{Ca-1}}} \quad [110]$$

$$[\text{EGT A}]_{\text{T}} = [\text{EGT A}^{4-}]\alpha + \frac{[\text{Ca}]_{\text{T}}[\text{EGT A}^{4-}]\beta}{(K_{\text{Ca-1}} + [\text{EGT A}^{4-}]\beta)} \quad [110]$$

simplification and collecting terms gives:

$$[\text{EGT A}^{4-}]^2 - \left(\frac{[\text{EGTA}]_T}{\alpha} - \frac{[\text{Ca}]_T}{\alpha} - \frac{K_{\text{Ca-1}}}{\beta} \right) [\text{EGT A}^{4-}] - \frac{K_{\text{Ca-1}} [\text{EGTA}]_T}{\alpha\beta} = 0 \quad [112]$$

which is identical to equation [82].

EGTA⁴⁻ AND ITS PROTONATED FORMS: ALTERNATIVE METHOD

[EGTA⁴⁻] can be calculated from equation [82] and the protonated forms using this from equations [85] to [87]. However, this has the disadvantage that the [EGTA⁴⁻] can be at certain pH values very low, which can introduce errors into the calculation of the protonated forms. A way round this, is as shown below to calculate the various forms directly using the calculated [Ca²⁺].

Equation [44] can be written:

$$[\text{EGTA}]_T - ([\text{Ca-H-EGT A}^{1-}] + [\text{Ca-EGT A}^{2-}]) = [\text{EGT A}^{4-}] + [\text{H-EGT A}^{3-}] + [\text{H}_2\text{-EGT A}^{2-}] + [\text{H}_3\text{-EGT A}^{1-}] + [\text{H}_4\text{-EGT A}] \quad [113]$$

From equation [45]:

$$([\text{Ca-H-EGT A}^{1-}] + [\text{Ca-EGT A}^{2-}]) = ([\text{Ca}]_T - [\text{Ca}^{2+}]) \quad [114]$$

Substituting equation [114] in equation [113] gives:

$$[\text{EGTA}]_T - ([\text{Ca}]_T - [\text{Ca}^{2+}]) = [\text{EGT A}^{4-}] + [\text{H-EGT A}^{3-}] + [\text{H}_2\text{-EGT A}^{2-}] + [\text{H}_3\text{-EGT A}^{1-}] + [\text{H}_4\text{-EGT A}] \quad [115]$$

If the [Ca²⁺] is calculated at the given pH and [Ca]_T, then as shown below [EGTA⁴⁻] and its protonated forms can be calculated directly. To simplify the equations let:

$$[\text{EGTA}]_{\text{Free}} = [\text{EGTA}]_T - ([\text{Ca}]_T - [\text{Ca}^{2+}]) \quad [116]$$

1) [EGTA⁴⁻]

From equation [108]:

$$[\text{EGTA}]_{\text{Free}} = [\text{EGTA}^{4-}] \left(1 + \frac{[\text{H-EGTA}^{3-}]}{[\text{EGTA}^{4-}]} + \frac{[\text{H}_2\text{-EGTA}^{2-}]}{[\text{EGTA}^{4-}]} + \frac{[\text{H}_3\text{-EGTA}^{1-}]}{[\text{EGTA}^{4-}]} + \frac{[\text{H}_4\text{-EGTA}]}{[\text{EGTA}^{4-}]} \right) \quad [117]$$

rearranging gives:

$$[\text{EGTA}^{4-}] = \frac{[\text{EGTA}]_{\text{Free}}}{\alpha} \quad [118]$$

2) [H-EGTA³⁻]

Express both [EGTA⁴⁻] and protonated forms in terms of [H-EGTA³⁻] using equations [2] to [5]:

$$[\text{EGTA}^{4-}] = \frac{K_{\text{H-1}}}{[\text{H}^+]} * [\text{H-EGTA}^{3-}] \quad [119]$$

$$[\text{H}_2\text{-EGTA}^{2-}] = \frac{[\text{H}^+]}{K_{\text{H-2}}} * [\text{H-EGTA}^{3-}] \quad [120]$$

$$[\text{H}_3\text{-EGTA}^{1-}] = \frac{[\text{H}^+]}{K_{\text{H-3}}} * [\text{H}_2\text{-EGTA}^{2-}] \quad [121]$$

or

$$[\text{H}_3\text{-EGTA}^{1-}] = \frac{[\text{H}^+]^2}{K_{\text{H-2}} K_{\text{H-3}}} * [\text{H-EGTA}^{3-}] \quad [122]$$

$$[\text{H}_4\text{-EGTA}] = \frac{[\text{H}^+]}{K_{\text{H-4}}} * [\text{H}_3\text{-EGTA}^{1-}] \quad [123]$$

or

$$[\text{H}_3\text{-EGTA}^{1-}] = \frac{[\text{H}^+]^3}{K_{\text{H-2}} K_{\text{H-3}} K_{\text{H-4}}} * [\text{H-EGTA}^{3-}] \quad [124]$$

which gives:

$$[\text{H-EGTA}^{3-}] = \frac{[\text{EGTA}]_{\text{Free}}}{\left(\frac{K_{\text{H-1}}}{[\text{H}^+]} + 1 + \frac{[\text{H}^+]}{K_{\text{H-2}}} + \frac{[\text{H}^+]^2}{K_{\text{H-2}} K_{\text{H-3}}} + \frac{[\text{H}^+]^3}{K_{\text{H-2}} K_{\text{H-3}} K_{\text{H-4}}} \right)} \quad [125]$$

3) [H₂-EGTA²⁻]

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}}}{[\text{H}^+]} * [\text{H-EGT A}^{3-}] \quad [126]$$

or

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}} K_{\text{H-2}}}{[\text{H}^+]^2} * [\text{H}_2\text{-EGT A}^{2-}] \quad [127]$$

$$[\text{H-EGT A}^{1-}] = \frac{K_{\text{H-2}}}{[\text{H}^+]} * [\text{H}_2\text{-EGT A}^{2-}] \quad [128]$$

$$[\text{H}_3\text{-EGT A}^{1-}] = \frac{[\text{H}^+]}{K_{\text{H-3}}} * [\text{H}_2\text{-EGT A}^{2-}] \quad [129]$$

$$[\text{H}_4\text{-EGTA}] = \frac{[\text{H}^+]}{K_{\text{H-3}}} * [\text{H}_3\text{-EGT A}^{1-}] \quad [130]$$

or

$$[\text{H}_4\text{-EGTA}] = \frac{[\text{H}^+]^2}{K_{\text{H-3}} K_{\text{H-4}}} * [\text{H}_2\text{-EGT A}^{2-}] \quad [131]$$

which gives:

$$[\text{H}_2\text{-EGT A}^{2-}] = \frac{[\text{EGTA}]_{\text{Free}}}{\left(\frac{K_{\text{H-1}} K_{\text{H-2}}}{[\text{H}^+]^2} + \frac{K_{\text{H-2}}}{[\text{H}^+]} + 1 + \frac{[\text{H}^+]}{K_{\text{H-3}}} + \frac{[\text{H}^+]^2}{K_{\text{H-3}} K_{\text{H-4}}} \right)} \quad [132]$$

4) $[\text{H}_3\text{-EGTA}^{1-}]$

From equation [127]

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}} K_{\text{H-2}}}{[\text{H}^+]^2} * [\text{H}_2\text{-EGT A}^{2-}]$$

or

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}} K_{\text{H-2}} K_{\text{H-3}}}{[\text{H}^+]^3} * [\text{H}_3\text{-EGT A}^{1-}] \quad [133]$$

From equation [128]

$$[\text{H-EGT A}^{3-}] = \frac{K_{\text{H-2}}}{[\text{H}^+]} * [\text{H}_2\text{-EGT A}^{2-}]$$

or

$$[\text{H-EGT A}^{3-}] = \frac{K_{\text{H-2}}K_{\text{H-3}}}{[\text{H}^+]^2} * [\text{H}_3\text{-EGT A}^{1-}] \quad [134]$$

$$[\text{H}_2\text{-EGT A}^{2-}] = \frac{K_{\text{H-3}}}{[\text{H}^+]} * [\text{H}_3\text{-EGT A}^{1-}] \quad [135]$$

$$[\text{H}_4\text{-EGTA}] = \frac{[\text{H}^+]}{K_{\text{H-4}}} * [\text{H}_3\text{-EGT A}^{1-}] \quad [136]$$

which gives:

$$[\text{H}_3\text{-EGT A}^{1-}] = \frac{[\text{EGTA}]_{\text{Free}}}{\left(\frac{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}}{[\text{H}^+]^3} + \frac{K_{\text{H-2}}K_{\text{H-3}}}{[\text{H}^+]^2} + \frac{K_{\text{H-3}}}{[\text{H}^+]} + 1 + \frac{[\text{H}^+]}{K_{\text{H-4}}} \right)} \quad [137]$$

5) [\text{H}_4\text{-EGTA}]

From equation [133]

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}}{[\text{H}^+]^3} * [\text{H}_3\text{-EGT A}^{1-}] \quad [138]$$

or

$$[\text{EGT A}^{4-}] = \frac{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}}{[\text{H}^+]^4} * [\text{H}_4\text{-EGTA}] \quad [139]$$

From equation [134]

$$[\text{H-EGT A}^{3-}] = \frac{K_{\text{H-2}}K_{\text{H-3}}}{[\text{H}^+]^2} * [\text{H}_3\text{-EGT A}^{1-}]$$

or

$$[\text{H-EGT A}^{3-}] = \frac{K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}}{[\text{H}^+]^2} * [\text{H}_4\text{-EGTA}] \quad [140]$$

From equation [135]

$$[\text{H}_2\text{-EGT A}^{2-}] = \frac{K_{\text{H-3}}}{[\text{H}^+]} * [\text{H}_3\text{-EGT A}^{1-}]$$

or

$$[H_2-EGT A^{2-}] = \frac{K_{H-3}K_{H-4}}{[H^+]^2} * [H_4-EGTA] \quad [141]$$

$$[H_3-EGT A^{1-}] = \frac{K_{H-4}}{[H^+]} * [H_4-EGTA]$$

which gives:

$$[H_4-EGTA] = \frac{[EGTA]_{Free}}{\left(\frac{K_{H-1}K_{H-2}K_{H-3}K_{H-4}}{[H^+]^4} + \frac{K_{H-2}K_{H-3}K_{H-4}}{[H^+]^3} + \frac{K_{H-3}K_{H-4}}{[H^+]^2} + \frac{K_{H-4}}{[H^+]} + 1 \right)} \quad [142]$$

TITRATION OF EGTA WITH KOH

This is based on Section-I, page 32. The derivation is for a solution of EGTA, in a background of KCl, with or without added $CaCl_2$.

1). Initial solution:

Cations:

$$[H^+] + [K^+]_{Back} + 2[Ca^{2+}] \quad [143]$$

Anions

$$[Cl^-] + [OH^-] + [EGTA]_T \quad [144]$$

where

$$[EGTA]_T = 4[EGT A^{4-}] + 3[H-EGT A^{3-}] + 2[H_2-EGT A^{2-}] + [H_3-EGT A^{1-}] + 2[Ca-EGT A^{2-}] + [Ca-H-EGT A^{1-}] \quad [145]$$

[EGTA]_T in terms of [EGTA⁴⁻]

$$[EGTA]_T = [EGT A^{4-}] \left(4 + \frac{3[H-EGT A^{3-}]}{[EGT A^{4-}]} + \frac{2[H_2-EGT A^{2-}]}{[EGT A^{4-}]} + \frac{[H_3-EGT A^{1-}]}{[EGT A^{4-}]} + \frac{2[Ca-EGT A^{2-}]}{[EGT A^{4-}]} + \frac{[Ca-H-EGT A^{1-}]}{[EGT A^{4-}]} \right) \quad [146]$$

Using equations [2] to [7] gives:

$$[EGTA]_T = [EGT A^{4-}] \left(4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H-EGT A^{3-}]}{[EGT A^{4-}]} * \frac{[Ca-H-EGT A^{1-}]}{[H-EGT A^{3-}]} \right) \quad [147]$$

$$[EGTA]_T = [EGT A^{4-}] \left(4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H^+][Ca^{2+}]}{K_{H-1}K_{Ca-2}} \right) \quad [148]$$

Because of electrical neutrality equations [143] and [144] can be equated

$$[H^+] + [K^+]_{Back} + 2[Ca^{2+}] = [Cl^-] + [OH^-] + [EGTA]_T \quad [149]$$

The ionic product of water (K_w) means that:

$$[H^+][OH^-] = K_w \text{ or } [OH^-] = \frac{K_w}{[H^+]} \quad [150][151]$$

substituting [151] in [149] gives:

$$[H^+] + [K^+]_{Back} + 2[Ca^{2+}] = [Cl^-] + \frac{K_w}{[H^+]} + [EGTA]_T \quad [152]$$

2) Titrating initial solution

Titration of the initial background solution with KOH modifies equation [152] as follows:

$$[H^+] + [K^+]_{Back} + [K^+]_{Alkali} + 2[Ca^{2+}] = [Cl^-] + \frac{K_w}{[H^+]} + [EGTA]_T \quad [153]$$

and the [KOH] added is given by:

$$[K^+]_{Alkali} = [EGTA]_T + ([Cl^-] + \frac{K_w}{[H^+]} - [H^+] + [K^+]_{Back} - 2[Ca^{2+}]) \quad [154]$$

substituting equation [148] in equation [154] gives

$$\begin{aligned} [K^+]_{Alkali} = & [EGTA]_T \left(4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right) \\ & + [EGTA]_T \left(\frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H^+][Ca^{2+}]}{K_{H-1}K_{Ca-2}} \right) \\ & + ([Cl^-] + \frac{K_w}{[H^+]} - [H^+] - [K^+]_{Back} - 2[Ca^{2+}]) \end{aligned} \quad [155]$$

In the background solution:

$$[Cl^-] = [K^+]_{Back} + 2[Ca^{2+}]_T \quad [156]$$

Substituting in equation [155] gives:

$$\begin{aligned}
[K^+]_{\text{Alkali}} = & [EGT A^{4-}](4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}}) \\
& + [EGT A^{4-}](\frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H^+][Ca^{2+}]}{K_{H-1}K_{Ca-2}}) \\
& + ([K^+]_{\text{Back}} + 2[Ca^{2+}]_T + \frac{K_w}{[H^+]} - [H^+] - [K^+]_{\text{Back}} - 2[Ca^{2+}])
\end{aligned} \tag{157}$$

which simplifies to:

$$\begin{aligned}
[K^+]_{\text{Alkali}} = & [EGT A^{4-}](4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}}) \\
& + [EGT A^{4-}](\frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H^+][Ca^{2+}]}{K_{H-1}K_{Ca-2}}) \\
& + (2[Ca^{2+}]_T - 2[Ca^{2+}] + \frac{K_w}{[H^+]} - [H^+])
\end{aligned} \tag{158}$$

and KOH per mol [EGTA]_T is given by:

$$\begin{aligned}
\frac{[K^+]_{\text{Alkali}}}{[EGTA]_T} = & \frac{[EGT A^{4-}]}{[EGTA]_T}(4 + \frac{3[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}}) \\
& + \frac{[EGT A^{4-}]}{[EGTA]_T}(\frac{2[Ca^{2+}]}{K_{Ca-1}} + \frac{[H^+][Ca^{2+}]}{K_{H-1}K_{Ca-2}}) \\
& + \frac{1}{[EGTA]_T}(2[Ca^{2+}]_T - 2[Ca^{2+}] + \frac{K_w}{[H^+]} - [H^+])
\end{aligned} \tag{159}$$

3) Comments

1) This does not account for the volume change on titration, but this can easily be done by dividing through by V, the total volume after each step in the titration.

2) In the third term in equation [159]:

$$\frac{1}{[EGTA]_T}(2[Ca^{2+}]_T - 2[Ca^{2+}] + \frac{K_w}{[H^+]} - [H^+])$$

Smith & Miller (1985) have:

$$\frac{1}{[EGTA]_T}([Cl^-] - 2[Ca^{2+}] + \frac{K_w}{[H^+]} - [H^+])$$

This arises because I have included the background [K⁺] in the neutrality equation (equation [152]).

3) K_w in mol/l at 25°C is equal to $10^{-13.995}$ making pK_w 13.995 It is often convenient to use mmol/l instead of mol/l. Under these circumstances K_w becomes $10^{-7.995}$ and pK_w 7.995. If concentrations are in mmol/l then:

$$\frac{[H^+](\text{mmol/l}) * [OH^-](\text{mmol/l})}{1000} = 10^{-13.995} \quad [160]$$

or

$$[H^+](\text{mmol/l}) * [OH^-](\text{mmol/l}) = 10^{-7.995} \quad [161]$$

[H⁺] BOUND TO EGTA

The total concentration of H⁺ bound to EGTA is as follows:

$$[H^+]_B = [Ca-H-EGTA^{1-}] + [H-EGTA^{3-}] + 2*[H_2-EGTA^{2-}] + 3*[H_3-EGTA^{1-}] + 4*[H_4-EGTA] \quad [162]$$

from equation [7]

$$[Ca-H-EGTA^{1-}] = \frac{[Ca^{2+}][H-EGTA^{3-}]}{K_{Ca-2}} \quad [163]$$

and from equation [2]

$$[Ca-H-EGTA^{1-}] = \frac{[Ca^{2+}]}{K_{Ca-2}} * \frac{[H^+][EGTA^{4-}]}{K_{H-1}} \quad [164]$$

substitution in equation [162] gives

$$[H^+]_B = \frac{[Ca^{2+}]}{K_{Ca-2}} * \frac{[H^+][EGTA^{4-}]}{K_{H-1}} + [H-EGTA^{3-}] + 2*[H_2-EGTA^{2-}] + 3*[H_3-EGTA^{1-}] + 4*[H_4-EGTA] \quad [165]$$

$$[H^+]_B = [EGTA^{4-}] \left(\frac{[Ca^{2+}]}{K_{Ca-2}} * \frac{[H^+]}{K_{H-1}} + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + 2 * \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + 3 * \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + 4 * \frac{[H_4-EGTA]}{[EGTA^{4-}]} \right) \quad [166]$$

or

$$[H^+]_B = [EGTA^{4-}] \left(\frac{[Ca^{2+}]}{K_{Ca-2}} * \frac{[H^+]}{K_{H-1}} + \frac{[H^+]}{K_{H-1}} + 2 * \frac{[H^+]^2}{K_{H-1}K_{H-2}} + 3 * \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + 4 * \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right) \quad [167]$$

or

$$[H^+]_B = [EGTA^{4-}] \left\{ \frac{[H^+]}{K_{H-1}} \left(\frac{[Ca^{2+}]}{K_{Ca-2}} + 1 \right) + 2 * \frac{[H^+]^2}{K_{H-1}K_{H-2}} + 3 * \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + 4 * \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right\} \quad [168]$$

[EGTA⁴⁻] in terms of [Ca²⁺], [Ca]_T and [EGTA]_T

$$[\text{EGT A}]_T = [\text{EGT A}^{4-}] + [\text{H-EGT A}^{3-}] + [\text{H}_2\text{-EGT A}^{2-}] + [\text{H}_3\text{-EGT A}^{1-}] + [\text{H}_4\text{-EGT A}] + [\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}] \quad [169]$$

and

$$[\text{Ca}]_T = [\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}] + [\text{Ca}^{2+}] \quad [170]$$

or

$$[\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}] = [\text{Ca}]_T - [\text{Ca}^{2+}] \quad [171]$$

substitution of equation [171] in equation [169] and simplification gives:

$$[\text{EGT A}]_T = [\text{EGT A}^{4-}] \left(1 + \frac{[\text{H-EGT A}^{3-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_2\text{-EGT A}^{2-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_3\text{-EGT A}^{1-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_4\text{-EGT A}]}{[\text{EGT A}^{4-}]} \right) + ([\text{Ca}]_T - [\text{Ca}^{2+}]) \quad [172]$$

substitution similar to equation [19] gives:

$$[\text{EGT A}]_T = [\text{EGT A}^{4-}] \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right) + ([\text{Ca}]_T - [\text{Ca}^{2+}]) \quad [173]$$

or

$$[\text{EGT A}^{4-}] = \frac{\{[\text{EGT A}]_T - ([\text{Ca}]_T - [\text{Ca}^{2+}])\}}{\left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right)} \quad [174]$$

substitution in equation [168] gives:

$$[\text{H}^+]_B = \{[\text{EGT A}]_T - ([\text{Ca}]_T - [\text{Ca}^{2+}])\} \frac{\left\{ \frac{[\text{H}^+]}{K_{\text{H-1}}} \left(\frac{[\text{Ca}^{2+}]}{K_{\text{Ca-2}}} + 1 \right) + 2 * \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + 3 * \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + 4 * \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right\}}{\left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right)} \quad [175]$$

or

$$[\text{H}^+]_B = \{[\text{EGT A}]_T - ([\text{Ca}]_T - [\text{Ca}^{2+}])\} * \frac{\partial}{\alpha} \quad [176]$$

where;

$$\partial = \left\{ \frac{[\text{H}^+]}{K_{\text{H-1}}} \left(\frac{[\text{Ca}^{2+}]}{K_{\text{Ca-2}}} + 1 \right) + 2 * \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + 3 * \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + 4 * \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right\} \quad [177]$$

and α is as defined above.

In terms of pH, pK and pCa we get:

$$[H^+]_B = \{[EGTA]_T - ([Ca]_T - 10^{-pCa})\} \frac{\partial}{\alpha} \quad [178]$$

and

$$\frac{\partial}{\alpha} = \frac{\{10^{(pK_{H-1} - pH)}(10^{(pK_{Ca-2} - pCa)} + 1) + 2 \cdot 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 3 \cdot 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 4 \cdot 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)}\}}{(1 + 10^{(pK_{H-1} - pH)}) + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)}} \quad [179]$$

[H⁺] RELEASED WHEN EGTA BINDS Ca²⁺

The concentration of H⁺-ions released when EGTA binds Ca²⁺ can be calculated as follows:

$$\Delta[H^+] = [H^+]_B([Ca]_T = 0) - [H^+]_B([Ca]_T = [Ca]_T) \quad [180]$$

Since $([Ca]_T - [Ca^{2+}])$ tends to zero at $[Ca]_T$ of zero, it follows from equation [178],

$$[H^+]_B([Ca]_T = 0) = \frac{[EGTA]_T \{10^{(pK_{H-1} - pH)} + 2 \cdot 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 3 \cdot 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 4 \cdot 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)}\}}{\alpha} \quad [181]$$

Note: the term $(1 + 10^{(pK_{Ca-2} - pCa)})$ tends to 1 in the ∂ term

$$[H^+]_B([Ca]_T = [Ca]_T) = \{[EGTA]_T - ([Ca]_T - 10^{-pCa})\} \frac{\partial}{\alpha} \quad [182]$$

subtracting equation [182] from [183] and simplifying gives:

$$\Delta[H^+] = \frac{([Ca]_T - 10^{-pCa})\partial - [EGTA]_T 10^{\{(pK_{H-1} - pH) + (pK_{Ca-2} - pCa)\}}}{\alpha} \quad [183]$$

or

$$\Delta[H^+] = \frac{([Ca]_T - 10^{-pCa})\partial}{\alpha} - \frac{[EGTA]_T 10^{\{(pK_{H-1} - pH) + (pK_{Ca-2} - pCa)\}}}{\alpha} \quad [184]$$

To calculate N, (the number of H⁺ ions released per Ca²⁺-ions bound) $\Delta[H^+]$ has to be divided by the amount of Ca²⁺ bound, namely $([Ca]_T - [Ca^{2+}])$. Equation [184] becomes:

$$N = \frac{([Ca]_T - [Ca^{2+}])\partial}{\alpha([Ca]_T - [Ca^{2+}])} - \frac{[EGTA]_T 10^{\{(pK_{H-1} - pH) + (pK_{Ca-2} - pCa)\}}}{\alpha([Ca]_T - [Ca^{2+}])} \quad [185]$$

which becomes:

$$N = \frac{\partial}{\alpha} - \frac{[\text{EGTA}]_T 10^{\{(\text{pK}_{\text{H-1}} - \text{pH}) + (\text{pK}_{\text{Ca-2}} - \text{pCa})\}}}{\alpha([\text{Ca}]_T - [\text{Ca}^{2+}])} \quad [186]$$

N (H⁺ RELEASE/Ca²⁺ BOUND) AS A FUNCTION OF EQUILIBRIUM CONSTANTS

Equation [186] seemed an odd result as it appears that N depends on both the [EGTA]_T and the amount bound and N could thus vary according to concentrations of EGTA and Ca²⁺. However, as shown below this is not the case.

The term delta, namely:

$$\partial = \left\{ \frac{[\text{H}^+]}{\text{K}_{\text{H-1}}} \left(\frac{[\text{Ca}^{2+}]}{\text{K}_{\text{Ca-2}}} + 1 \right) + 2 * \frac{[\text{H}^+]^2}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}}} + 3 * \frac{[\text{H}^+]^3}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}} \text{K}_{\text{H-3}}} + 4 * \frac{[\text{H}^+]^4}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}} \text{K}_{\text{H-3}} \text{K}_{\text{H-4}}} \right\}$$

can be written for simplicity as:

$$\partial = \left\{ \frac{[\text{H}^+]}{\text{K}_{\text{H-1}}} \left(\frac{[\text{Ca}^{2+}]}{\text{K}_{\text{Ca-2}}} + 1 \right) + \Sigma \right\} \quad [187]$$

where

$$\Sigma = \left(2 * \frac{[\text{H}^+]^2}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}}} + 3 * \frac{[\text{H}^+]^3}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}} \text{K}_{\text{H-3}}} + 4 * \frac{[\text{H}^+]^4}{\text{K}_{\text{H-1}} \text{K}_{\text{H-2}} \text{K}_{\text{H-3}} \text{K}_{\text{H-4}}} \right) \quad [188]$$

and from equation [72]

$$[\text{EGTA}]_T = [\text{EGTA}^4] \alpha + ([\text{Ca}]_T - [\text{Ca}^{2+}])$$

substituting in equation [186] and changing to concentrations from p values:

$$N = \frac{1}{\alpha} \left\{ \frac{[\text{H}^+]}{\text{K}_{\text{H-1}}} * \frac{[\text{Ca}^{2+}]}{\text{K}_{\text{Ca-2}}} + \frac{[\text{H}^+]}{\text{K}_{\text{H-1}}} + \Sigma - \frac{[\text{EGTA}^4] \alpha + ([\text{Ca}]_T - [\text{Ca}^{2+}])}{([\text{Ca}]_T - [\text{Ca}^{2+}])} * \frac{[\text{H}^+]}{\text{K}_{\text{H-1}}} * \frac{[\text{Ca}^{2+}]}{\text{K}_{\text{Ca-2}}} \right\} \quad [189]$$

and

$$N = \frac{1}{\alpha} \left\{ \frac{[H^+]}{K_{H-1}} * \frac{[Ca^{2+}]}{K_{Ca-2}} + \frac{[H^+]}{K_{H-1}} + \Sigma - \left(\frac{[EGTA^{4-}]\alpha}{([Ca]_T - [Ca^{2+}])} + 1 \right) \frac{[H^+]}{K_{H-1}} * \frac{[Ca^{2+}]}{K_{Ca-2}} \right\} \quad [190]$$

which simplifies to:

$$N = \frac{1}{\alpha} \left(\frac{[H^+]}{K_{H-1}} + \Sigma - \frac{[EGTA^{4-}]\alpha [Ca^{2+}]}{([Ca]_T - [Ca^{2+}])} * \frac{[H^+]}{K_{H-1}K_{Ca-2}} \right) \quad [191]$$

But $\frac{[EGTA^{4-}]\alpha [Ca^{2+}]}{([Ca]_T - [Ca^{2+}])} = K_{app}$ and equation [191] becomes;

$$N = \frac{1}{\alpha} \left(\frac{[H^+]}{K_{H-1}} + \Sigma - \frac{K_{app} [H^+]}{K_{H-1}K_{Ca-2}} \right) \quad [192]$$

or

$$N = \frac{1}{\alpha} \left\{ \frac{[H^+]}{K_{H-1}} \left(1 - \frac{K_{app}}{K_{Ca-2}} \right) + \Sigma \right\} \quad [193]$$

Substituting for Σ gives:

$$N = \frac{1}{\alpha} \left\{ \frac{[H^+]}{K_{H-1}} \left(1 - \frac{K_{app}}{K_{Ca-2}} \right) + 2 * \frac{[H^+]^2}{K_{H-1}K_{H-2}} + 3 * \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + 4 * \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right\} \quad [194]$$

or

$$N = \frac{1}{\alpha} \left\{ \frac{[H^+]}{K_{H-1}} \left(1 - \frac{K_{Ca-1}}{K_{Ca-2}} \frac{\alpha}{\beta} \right) + 2 * \frac{[H^+]^2}{K_{H-1}K_{H-2}} + 3 * \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + 4 * \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right\} \quad [195]$$

which means that N is independent of the both $[EGTA]_T$ and $[Ca]_T$.

CALCULATION OF K_{app} FROM H^+ -RELEASE

The apparent equilibrium constant K_{app} can be calculated from the H^+ release if N is known. At a given N:

$$Ca-Bound = \frac{\Delta H^+}{N} \quad [196]$$

$$Ca-free = [Ca]_T - \frac{\Delta H^+}{N} \quad [197]$$

$$[\text{EGTA}]_F = [\text{EGTA}]_T - \frac{\Delta H^+}{N} \quad [198]$$

From these definitions:

$$\frac{([\text{Ca}]_T - \frac{\Delta H^+}{N})([\text{EGTA}]_T - \frac{\Delta H^+}{N})}{\frac{\Delta H^+}{N}} = K_{\text{app}} \quad [199]$$

simplification gives:

$$\frac{(N^*[\text{Ca}]_T - \Delta H^+)(N^*[\text{EGTA}]_T - \Delta H^+)}{N^* \Delta H^+} = K_{\text{app}} \quad [200]$$

PART II: BINDING OF Ca^{2+} Mg^{2+} AND H^+ TO EGTA

In the previous section the basic equations for Ca^{2+} binding to EGTA were developed. However, buffer solutions made to mimic intracellular conditions also contain Mg^{2+} and as EGTA binds both, the relevant equations when both cations are present will be developed in this section.

Mg^{2+} EQUILIBRIUM CONSTANTS

There are two binding sites for Mg^{2+} and the equilibrium constants are defined as follows:

$$\frac{[\text{Mg}^{2+}][\text{EGTA}^{4-}]}{[\text{Mg-EGTA}^{2-}]} = K_{\text{Mg-1}} \quad \text{and} \quad \frac{[\text{Mg}^{2+}][\text{H-EGTA}^{3-}]}{[\text{Mg-H-EGTA}^{1-}]} = K_{\text{Mg-2}} \quad [201][202]$$

DEFINITIONS OF K_{app} FOR Mg^{2+} AND Ca^{2+}

These are defined as follows:

$$\frac{[\text{Mg}^{2+}]([\text{EGTA}^{4-}] + [\text{H-EGTA}^{3-}] + [\text{H}_2\text{-EGTA}^{2-}] + [\text{H}_3\text{-EGTA}^{1-}] + [\text{H}_4\text{-EGTA}])}{([\text{Mg-EGTA}^{2-}] + [\text{Mg-H-EGTA}^{1-}])} = K_{\text{Mg-app}} \quad [203]$$

$$\frac{[\text{Ca}^{2+}]([\text{EGTA}^{4-}] + [\text{H-EGTA}^{3-}] + [\text{H}_2\text{-EGTA}^{2-}] + [\text{H}_3\text{-EGTA}^{1-}] + [\text{H}_4\text{-EGTA}])}{([\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}])} = K_{\text{Ca-app}} \quad [204]$$

similarly as for equation [20] these can be expressed as follows:

$$K_{Mg-app} = \frac{K_{Mg-1}(1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)})}{(1 + \frac{K_{Mg-1}}{K_{Mg-2}} * 10^{(pK_{H-1} - pH)})}$$

[205]

$$K_{Ca-app} = \frac{K_{Ca-1}(1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)})}{(1 + \frac{K_{Ca-1}}{K_{Ca-2}} * 10^{(pK_{H-1} - pH)})}$$

[206]

It is also possible to define a combined apparent constant for Ca^{2+} measurements in the solution.

$$K_{Ca/Mg-app} = \frac{[Ca^{2+}][EGTA^{4-}] + [H-EGTA^{3-}] + [H_2-EGTA^{2-}] + [H_3-EGTA^{1-}] + [H_4-EGTA] + [Mg-EGTA^{2-}] + [Mg-H-EGTA^{1-}]}{([Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}])}$$

[207]

This arises because this is what would be estimated in a buffer solution containing both Ca^{2+} and Mg^{2+} .

$$\text{as } \frac{[Ca^{2+}]\{[EGTA]_T - ([Ca]_T - [Ca^{2+}])\}}{([Ca]_T - [Ca^{2+}])} = K_{Ca/Mg-app}, \text{ by definition}$$

[208]

DEFINITIONS OF TOTAL CALCIUM, MAGNESIUM AND EGTA

$$[Ca]_T = [Ca^{2+}] + [Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}]$$

[209]

$$[Mg]_T = [Mg^{2+}] + [Mg-EGTA^{2-}] + [Mg-H-EGTA^{1-}]$$

[210]

$$[EGTA]_T = [EGTA^{4-}] + [H-EGTA^{3-}] + [H_2-EGTA^{2-}] + [H_3-EGTA^{1-}] + [H_4-EGTA] + [Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}] + [Mg-EGTA^{2-}] + [Mg-H-EGTA^{1-}]$$

[211]

To simplify let that fraction of EGTA bound neither to Mg^{2+} nor to Ca^{2+} be $[EGTA]_F$:

$$[EGTA]_F = [EGTA^{4-}] + [H-EGTA^{3-}] + [H_2-EGTA^{2-}] + [H_3-EGTA^{1-}] + [H_4-EGTA]$$

[212]

IONIZED AND BOUND Ca/Mg AND UNPROTONATED AND PROTONATED EGTA

1) Ionized calcium: $[Ca^{2+}]$

$$[EGTA]_T = [EGTA]_F + [Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}] + [Mg-EGTA^{2-}] + [Mg-H-EGTA^{1-}] \quad [213]$$

and

$$[EGTA]_T = [EGTA]_F + ([Ca]_T - [Ca^{2+}]) + ([Mg]_T - [Mg^{2+}]) \quad [214]$$

Deriving $[EGTA]_F/Mg^{2+}$ in terms of total concentrations and equilibrium constants

a) $[EGTA]_F$

$$\frac{[Ca^{2+}][EGTA]_F}{([Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}])} = K_{Ca-app} \quad [215]$$

$$\frac{[Ca^{2+}][EGTA]_F}{([Ca]_T - [Ca^{2+}])} = K_{Ca-app} \quad [216]$$

$$[EGTA]_F = \frac{K_{Ca-app}([Ca]_T - [Ca^{2+}])}{[Ca^{2+}]} \quad [217]$$

b) Mg^{2+}

$$\frac{[Mg^{2+}][EGTA]_F}{([Mg]_T - [Mg^{2+}])} = K_{Mg-app} \quad [218]$$

$$[Mg^{2+}] = \frac{K_{Mg-app}([Mg]_T - [Mg^{2+}])}{[EGTA]_F} \quad [219]$$

whence:

$$[Mg^{2+}] = \frac{K_{Mg-app}[Mg]_T}{[EGTA]_F + K_{Mg-app}} \quad [220]$$

substitution with equation [216] gives,

$$[Mg^{2+}] = \frac{K_{Mg-app}[Mg]_T [Ca^{2+}]}{K_{Ca-app}([Ca]_T - [Ca^{2+}]) + K_{Mg-app}[Ca^{2+}]} \quad [221]$$

and

$$([Mg]_T - [Mg^{2+}]) = \frac{K_{Ca-app}[Mg]_T ([Ca]_T - [Ca^{2+}])}{K_{Ca-app}([Ca]_T - [Ca^{2+}]) + K_{Mg-app}[Ca^{2+}]} \quad [222]$$

substitution of equations [215] and [222] in [214] gives:

$$[EGTA]_T = \frac{K_{Ca-app}([Ca]_T - [Ca^{2+}])}{[Ca^{2+}]} + ([Ca]_T - [Ca^{2+}]) + \frac{K_{Ca-app}[Mg]_T ([Ca]_T - [Ca^{2+}])}{K_{Ca-app}([Ca]_T - [Ca^{2+}]) + K_{Mg-app}[Ca^{2+}]} \quad [223]$$

on simplification this gives:

$$A[\text{Ca}^{2+}]^3 + B[\text{Ca}^{2+}]^2 + C[\text{Ca}^{2+}] + D = 0 \quad [224]$$

$$A = (K_{\text{Ca-app}} - K_{\text{Mg-app}})$$

$$B = [\text{EGTA}]_T(K_{\text{Ca-app}} - K_{\text{Mg-app}}) + [\text{Ca}]_T(K_{\text{Mg-app}} - 2K_{\text{Ca-app}}) - [\text{Mg}]_T K_{\text{Ca-app}} - K_{\text{Ca-app}} K_{\text{Mg-app}} + K_{\text{Ca-app}}^2$$

$$C = [\text{Ca}]_T K_{\text{Ca-app}} ([\text{Ca}]_T + [\text{Mg}]_T) + [\text{Ca}]_T K_{\text{Ca-app}} (K_{\text{Mg-app}} - 2K_{\text{Ca-app}}) - [\text{Ca}]_T [\text{EGTA}]_T K_{\text{Ca-app}}$$

$$D = [\text{Ca}]_T^2 K_{\text{Ca-app}}^2$$

2) Ionized magnesium : [Mg²⁺]

A similar procedure to that above can be used to derive the [Mg²⁺] i.e. equation [214]

$$[\text{EGTA}]_T = [\text{EGTA}]_F + ([\text{Ca}]_T - [\text{Ca}^{2+}]) + ([\text{Mg}]_T - [\text{Mg}^{2+}])$$

Deriving [EGTA]_F and Ca²⁺ in terms of total concentrations and equilibrium constants

a) [EGTA]_F

$$\frac{[\text{Mg}^{2+}][\text{EGT A}]_F}{([\text{Mg-EGT A}^{2-}] + [\text{Mg-H-EGT A}^{1-}])} = K_{\text{Mg-app}} \quad [225]$$

$$\frac{[\text{Mg}^{2+}][\text{EGT A}]_F}{([\text{Mg}]_T - [\text{Mg}^{2+}])} = K_{\text{Mg-app}} \quad [226]$$

$$[\text{EGT A}]_F = \frac{K_{\text{Mg-app}}([\text{Mg}]_T - [\text{Mg}^{2+}])}{[\text{Mg}^{2+}]} \quad [227]$$

b) Ca²⁺

$$\frac{[\text{Ca}^{2+}][\text{EGT A}]_F}{([\text{Ca}]_T - [\text{Ca}^{2+}])} = K_{\text{Ca-app}} \quad [228]$$

$$[\text{Ca}^{2+}] = \frac{K_{\text{Ca-app}}[\text{Ca}]_T}{[\text{EGT A}]_F + K_{\text{Ca-app}}} \quad [229]$$

substitution with equation [221] gives:

$$[\text{Ca}^{2+}] = \frac{K_{\text{Ca-app}} [\text{Ca}]_{\text{T}} [\text{Mg}^{2+}]}{K_{\text{Mg-app}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}]) + K_{\text{Ca-app}} [\text{Mg}^{2+}]} \quad [230]$$

and

$$([\text{Ca}]_{\text{T}} - [\text{Ca}^{2+}]) = \frac{K_{\text{Mg-app}} [\text{Ca}]_{\text{T}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}])}{K_{\text{Mg-app}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}]) + K_{\text{Ca-app}} [\text{Mg}^{2+}]} \quad [231]$$

Substituting in equation [214] gives:

$$[\text{EGTA}]_{\text{T}} = \frac{K_{\text{Mg-app}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}])}{[\text{Mg}^{2+}]} + \frac{K_{\text{Mg-app}} [\text{Ca}]_{\text{T}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}])}{K_{\text{Mg-app}} ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}]) + K_{\text{Ca-app}} [\text{Mg}^{2+}]} + ([\text{Mg}]_{\text{T}} - [\text{Mg}^{2+}]) \quad [232]$$

on simplification this gives:

$$A[\text{Mg}^{2+}]^3 + B[\text{Mg}^{2+}]^2 + C[\text{Mg}^{2+}] + D = 0 \quad [233]$$

where:

$$A = (K_{\text{Mg-app}} - K_{\text{Ca-app}})$$

$$B = [\text{EGTA}]_{\text{T}} (K_{\text{Mg-app}} - K_{\text{Ca-app}}) + [\text{Mg}]_{\text{T}} (K_{\text{Ca-app}} - 2K_{\text{Mg-app}}) - [\text{Ca}]_{\text{T}} K_{\text{Mg-app}} - K_{\text{Ca-app}} K_{\text{Mg-app}} + K_{\text{Mg-app}}^2$$

$$C = [\text{Mg}]_{\text{T}} K_{\text{Mg-app}} ([\text{Mg}]_{\text{T}} + [\text{Ca}]_{\text{T}}) + [\text{Mg}]_{\text{T}} K_{\text{Mg-app}} (K_{\text{Ca-app}} - 2K_{\text{Mg-app}}) - [\text{Mg}]_{\text{T}} [\text{EGTA}]_{\text{T}} K_{\text{Mg-app}}$$

$$D = K_{\text{Mg-app}}^2 [\text{Mg}]_{\text{T}}^2$$

3) [EGTA] not bound to either Ca^{2+} or Mg^{2+} : [EGTA]_F

equation [213] namely:

$$[\text{EGTA}]_{\text{T}} = [\text{EGTA}]_{\text{F}} + [\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}] + [\text{Mg-EGTA}^{2-}] + [\text{Mg-H-EGTA}^{1-}]$$

Derivation of bound Ca^{2+} and Mg^{2+} in terms of [EGTA]_F and equilibrium constants

$$[\text{Ca}^{2+}] = \frac{K_{\text{Ca-app}} ([\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}])}{[\text{EGTA}]_{\text{F}}} \quad [234]$$

and from equation [116]

$$[\text{Ca}]_{\text{T}} = \frac{K_{\text{Ca-app}} ([\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}])}{[\text{EGTA}]_{\text{F}}} + [\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}] \quad [235]$$

$$[\text{Ca}]_T = ([\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}]) \left(1 + \frac{K_{\text{Ca-app}}}{[\text{EGTA}]_F}\right) \quad [236]$$

whence

$$([\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}]) = \frac{[\text{Ca}]_T}{\left(1 + \frac{K_{\text{Ca-app}}}{[\text{EGTA}]_F}\right)} \quad [237]$$

and

$$([\text{Ca-EGT A}^{2-}] + [\text{Ca-H-EGT A}^{1-}]) = \frac{[\text{Ca}]_T [\text{EGTA}]_F}{([\text{EGTA}]_F + K_{\text{Ca-app}})} \quad [238]$$

similarly:

$$([\text{Mg-EGT A}^{2-}] + [\text{Mg-H-EGT A}^{1-}]) = \frac{[\text{Mg}]_T [\text{EGTA}]_F}{([\text{EGTA}]_F + K_{\text{Mg-app}})} \quad [239]$$

substitution in equation [213] gives:

$$[\text{EGTA}]_T = [\text{EGTA}]_F + \frac{[\text{Ca}]_T [\text{EGTA}]_F}{([\text{EGTA}]_F + K_{\text{Ca-app}})} + \frac{[\text{Mg}]_T [\text{EGTA}]_F}{([\text{EGTA}]_F + K_{\text{Mg-app}})} \quad [240]$$

simplification gives:

$$A[\text{EGTA}]_F^3 + B[\text{EGTA}]_F^2 + C[\text{EGTA}]_F + D = 0 \quad [241]$$

where

$$A = 1$$

$$B = [\text{Ca}]_T + [\text{Mg}]_T - [\text{EGTA}]_T + K_{\text{Ca-app}} + K_{\text{Mg-app}}$$

$$C = [\text{Ca}]_T K_{\text{Mg-app}} + [\text{Mg}]_T K_{\text{Ca-app}} - [\text{EGTA}]_T (K_{\text{Ca-app}} + K_{\text{Mg-app}}) + K_{\text{Ca-app}} K_{\text{Mg-app}}$$

$$D = -[\text{EGTA}]_T K_{\text{Ca-app}} K_{\text{Mg-app}}$$

4) Free [EGTA]: [EGTA⁴⁻]

Note: This is derived for completion. For accuracy [EGTA⁴⁻] should be calculated from [EGTA]_F.

From the definition of [EGTA]_F, equation [212] we get:

$$[\text{EGTA}]_F = [\text{EGT A}^{4-}] \left(1 + \frac{[\text{H-EGT A}^{3-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_2\text{-EGT A}^{2-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_3\text{-EGT A}^{1-}]}{[\text{EGT A}^{4-}]} + \frac{[\text{H}_4\text{-EGT A}]}{[\text{EGT A}^{4-}]}\right)$$

or

$$[\text{EGTA}]_F = [\text{EGTA}^{4-}] \alpha \text{ and } [\text{EGTA}^{4-}] = \frac{[\text{EGTA}]_F}{\alpha} \quad [242][243]$$

substituting in equation [241] gives:

$$A[\text{EGTA}^{4-}]^3 \alpha^3 + B[\text{EGTA}^{4-}]^2 \alpha^2 + C[\text{EGTA}^{4-}] \alpha + D = 0 \quad [244]$$

and

$$A[\text{EGTA}^{4-}]^3 + \frac{B}{\alpha}[\text{EGTA}^{4-}]^2 + \frac{C}{\alpha^2}[\text{EGTA}^{4-}] + \frac{D}{\alpha^3} = 0 \quad [245]$$

redefining $A = A$, $B = \frac{B}{\alpha}$, $C = \frac{C}{\alpha^2}$, and $D = \frac{D}{\alpha^3}$ gives:

$$A[\text{EGTA}^{4-}]^3 + B[\text{EGTA}^{4-}]^2 + C[\text{EGTA}^{4-}] + D = 0 \quad [246]$$

where

$$A = 1$$

$$B = \frac{([\text{Ca}]_T + [\text{Mg}]_T - [\text{EGTA}]_T + K_{\text{Ca-app}} + K_{\text{Mg-app}})}{\alpha}$$

$$C = \frac{([\text{Ca}]_T K_{\text{Mg-app}} + [\text{Mg}]_T K_{\text{Ca-app}} - [\text{EGTA}]_T (K_{\text{Ca-app}} + K_{\text{Mg-app}}) + K_{\text{Ca-app}} K_{\text{Mg-app}})}{\alpha^2}$$

$$D = \frac{-([\text{EGTA}]_T K_{\text{Ca-app}} K_{\text{Mg-app}})}{\alpha^3}$$

The concentrations of the other protonated forms of EGTA are as follows:

$$5) [\text{H-EGTA}^{3-}] = [\text{EGTA}^{4-}] 10^{(\text{p}K_{\text{H-1}} - \text{pH})} \quad [247]$$

$$6) [\text{H}_2\text{-EGTA}^{2-}] = [\text{EGTA}^{4-}] 10^{(\text{p}K_{\text{H-1}} + \text{p}K_{\text{H-2}} - 2\text{pH})} \quad [248]$$

$$7) [\text{H}_3\text{-EGTA}^{1-}] = [\text{EGTA}^{4-}] 10^{(\text{p}K_{\text{H-1}} + \text{p}K_{\text{H-2}} + \text{p}K_{\text{H-3}} - 3\text{pH})} \quad [249]$$

$$8) [\text{H}_4\text{-EGTA}] = [\text{EGTA}^{4-}] 10^{(\text{p}K_{\text{H-1}} + \text{p}K_{\text{H-2}} + \text{p}K_{\text{H-3}} + \text{p}K_{\text{H-4}} - 4\text{pH})} \quad [250]$$

9) Bound calcium: $[\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}]$ or $[\text{Ca}]_B$
equation [213], namely

$$[EGTA]_T = [EGTA]_F + [Ca-EGTA^{2-}] + [Ca-H-EGTA^{-1}] + [Mg-EGTA^{2-}] + [Mg-H-EGTA^{-1}]$$

can be written:

$$[EGTA]_T = [EGTA]_F + [Ca]_B + ([Mg]_T - [Mg^{2+}]) \quad [251]$$

[EGTA]_F in terms of [Ca]_B and equilibrium constants

from equation [209] it follows that:

$$[Ca^{2+}] = [Ca]_T - [Ca]_B \text{ and } [Ca]_T - [Ca^{2+}] = [Ca]_B \quad [252][253]$$

substituting in equation [217] gives:

$$[EGTA]_F = \frac{K_{Ca-app}[Ca]_B}{([Ca]_T - [Ca]_B)} \quad [254]$$

([Mg]_T - [Mg²⁺]) in terms of [Ca]_B and equilibrium constants

substituting in equation [222] gives:

$$([Mg]_T - [Mg^{2+}]) = \frac{K_{Ca-app}[Mg]_T[Ca]_B}{K_{Ca-app}[Ca]_B + K_{Mg-app}([Ca]_T - [Ca]_B)} \quad [255]$$

thus it follows that:

$$[EGTA]_T = \frac{K_{Ca-app}[Ca]_B}{([Ca]_T - [Ca]_B)} + [Ca]_B + \frac{K_{Ca-app}[Mg]_T[Ca]_B}{K_{Ca-app}[Ca]_B + K_{Mg-app}([Ca]_T - [Ca]_B)} \quad [256]$$

on simplification this gives:

$$A[Ca]_B^3 + B[Ca]_B^2 + C[Ca]_B + D = 0 \quad [257]$$

where:

$$A = (K_{Mg-app} - K_{Ca-app})$$

$$B = -[EGTA]_T * A - [Ca]_T * A - K_{Ca-app} * A - [Mg]_T K_{Ca-app} - [Ca]_T K_{Mg-app}$$

$$C = [EGTA]_T [Ca]_T * A + [EGTA]_T [Ca]_T K_{Mg-app} + [Ca]_T K_{Ca-app} K_{Mg-app} + [Ca]_T^2 K_{Mg-app} + [Ca]_T [Mg]_T K_{Ca-app}$$

$$D = -[EGTA]_T [Ca]_T^2 K_{Mg-app}$$

where $A = (K_{\text{Mg-app}} - K_{\text{Ca-app}})$ in equations B and C.

10) $[\text{Ca-EGTA}^{2-}]$ and $[\text{Ca-H-EGTA}^{1-}]$

The concentrations of $[\text{Ca-EGTA}^{2-}]$ and $[\text{Ca-H-EGTA}^{1-}]$ can be calculated from $[\text{Ca}]_B$ as follows:

$$[\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}] = [\text{Ca}]_B \quad [258]$$

and

$$[\text{Ca-EGTA}^{2-}](1 + \frac{[\text{Ca-H-EGTA}^{1-}]}{[\text{Ca-EGTA}^{2-}]}) = [\text{Ca}]_B \quad [259]$$

$$[\text{Ca-H-EGTA}^{1-}](1 + \frac{[\text{Ca-EGTA}^{2-}]}{[\text{Ca-H-EGTA}^{1-}]}) = [\text{Ca}]_B \quad [260]$$

and it follows from [13] that:

$$[\text{Ca-EGTA}^{2-}] = \frac{[\text{Ca}]_B}{(1 + \frac{K_{\text{Ca-1}}}{K_{\text{Ca-2}}} 10^{(\text{pK}_{\text{H-1}} - \text{pH})})} \quad [261]$$

$$[\text{Ca-H-EGTA}^{1-}] = \frac{[\text{Ca}]_B}{(1 + \frac{K_{\text{Ca-2}}}{K_{\text{Ca-1}}} 10^{(\text{pH} - \text{pK}_{\text{H-1}})})} \quad [262]$$

11) Bound magnesium: $[\text{Mg-EGTA}^{2-}] + [\text{Mg-H-EGTA}^{1-}]$: $[\text{Mg}]_B$

With a very similar method to above $[\text{Mg}]_B$ can be calculated. Equation [213], namely

$$[\text{EGTA}]_T = [\text{EGTA}]_F + [\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}] + [\text{Mg-EGTA}^{2-}] + [\text{Mg-H-EGTA}^{1-}]$$

can be written:

$$[\text{EGTA}]_T = [\text{EGTA}]_F + [\text{Ca-EGTA}^{2-}] + [\text{Ca-H-EGTA}^{1-}] + [\text{Mg}]_B \quad [263]$$

$[\text{EGTA}]_F$ in terms of $[\text{Mg}]_B$ and equilibrium constants

from equation [210] it follows that:

$$[\text{Mg}^{2+}] = [\text{Mg}]_T - [\text{Mg}]_B \quad \text{and} \quad [\text{Mg}]_T - [\text{Mg}^{2+}] = [\text{Mg}]_B \quad [264][265]$$

substituting in equation [217] gives:

$$[\text{EGTA}]_F = \frac{K_{\text{Mg-app}} [\text{Mg}]_B}{([\text{Mg}]_T - [\text{Mg}]_B)} \quad [266]$$

$([\text{Ca}]_T - [\text{Ca}^{2+}])$ in terms of $[\text{Mg}]_B$ and equilibrium constants
from equation [231]:

$$([\text{Ca}]_T - [\text{Ca}^{2+}]) = \frac{K_{\text{Mg-app}} [\text{Ca}]_T [\text{Mg}]_B}{K_{\text{Mg-app}} [\text{Mg}]_B + K_{\text{Ca-app}} ([\text{Mg}]_T - [\text{Mg}]_B)} \quad [267]$$

thus it follows that:

$$[\text{EGTA}]_T = \frac{K_{\text{Mg-app}} [\text{Mg}]_B}{([\text{Mg}]_T - [\text{Mg}]_B)} + \frac{K_{\text{Mg-app}} [\text{Ca}]_T [\text{Mg}]_B}{K_{\text{Mg-app}} [\text{Mg}]_B + K_{\text{Ca-app}} ([\text{Mg}]_T - [\text{Mg}]_B)} + [\text{Mg}]_B \quad [268]$$

on simplification this gives:

$$A[\text{Mg}]_B^3 + B[\text{Mg}]_B^2 + C[\text{Mg}]_B + D = 0 \quad [269]$$

where:

$$A = (K_{\text{Ca-app}} - K_{\text{Mg-app}})$$

$$B = -[\text{EGTA}]_T * A - [\text{Mg}]_T * A - K_{\text{Mg-app}} * A - [\text{Mg}]_T K_{\text{Ca-app}} - [\text{Ca}]_T K_{\text{Mg-app}}$$

$$C = [\text{EGTA}]_T [\text{Mg}]_T * A + [\text{EGTA}]_T [\text{Mg}]_T K_{\text{Ca-app}} + [\text{Mg}]_T K_{\text{Ca-app}} K_{\text{Mg-app}} + [\text{Mg}]_T^2 K_{\text{Ca-app}} + [\text{Ca}]_T [\text{Mg}]_T K_{\text{Mg-app}}$$

$$D = -[\text{EGTA}]_T [\text{Mg}]_T^2 K_{\text{Ca-app}}$$

12) $[\text{Mg-EGTA}^{2-}]$ and $[\text{Mg-H-EGTA}^{1-}]$

These concentrations can be calculated exactly as for calcium:

$$[\text{Mg-EGT A}^{2-}] = \frac{[\text{Mg}]_B}{(1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} 10^{(\text{pK}_{\text{H-1}} - \text{pH})})} \quad [270]$$

$$[\text{Mg-H-EGT A}^{1-}] = \frac{[\text{Mg}]_B}{(1 + \frac{K_{\text{Mg-2}}}{K_{\text{Mg-1}}} 10^{(\text{pH} - \text{pK}_{\text{H-1}})})} \quad [271]$$

BINDING OF H⁺-IONS TO EGTA IN THE PRESENCE OF Ca²⁺ AND Mg²⁺

The concentration of H^+ bound to EGTA is as follows:

$$[H^+]_B = [Ca-H-EGTA^{1-}] + [Mg-H-EGTA^{1-}] + [H-EGTA^{3-}] + 2*[H_2-EGTA^{2-}] + 3*[H_3-EGTA^{1-}] + 4*[H_4-EGTA] \quad [272]$$

similarly to equation [166] this can be written.

$$[H^+]_B = [EGTA^{4-}] \left(\frac{[Ca^{2+}][H^+]}{K_{Ca-2} K_{H-1}} + \frac{[Mg^{2+}][H^+]}{K_{Mg-2} K_{H-1}} + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + 2 \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + 3 \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + 4 \frac{[H_4-EGTA]}{[EGTA^{4-}]} \right) \quad [273]$$

to simplify let:

$$\varepsilon = \left(\frac{[Ca^{2+}][H^+]}{K_{Ca-2} K_{H-1}} + \frac{[Mg^{2+}][H^+]}{K_{Mg-2} K_{H-1}} + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + 2 \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + 3 \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + 4 \frac{[H_4-EGTA]}{[EGTA^{4-}]} \right) \quad [274]$$

or in terms of pH and pK:

$$\varepsilon = \{10^{(pK_{Ca-2} - pH)} + 10^{(pK_{Mg-2} - pH)} + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} - pH)} + \dots + 4*10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)}\} \quad [275]$$

$$[H^+]_B = [EGTA^{4-}] \varepsilon \quad [276]$$

[EGTA⁴⁻] in terms of [Ca²⁺], [Mg²⁺] and equilibrium constants

Equation [211] namely:

$$[EGTA]_T = [EGTA^{4-}] + [H-EGTA^{3-}] + [H_2-EGTA^{2-}] + [H_3-EGTA^{1-}] + [H_4-EGTA] + ([Ca-EGTA^{2-}] + [Ca-H-EGTA^{1-}]) + ([Mg-EGTA^{2-}] + [Mg-H-EGTA^{1-}])$$

can also be written:

$$[EGTA]_T = [EGTA^{4-}] \left(1 + \frac{[H-EGTA^{3-}]}{[EGTA^{4-}]} + \frac{[H_2-EGTA^{2-}]}{[EGTA^{4-}]} + \frac{[H_3-EGTA^{1-}]}{[EGTA^{4-}]} + \frac{[H_4-EGTA]}{[EGTA^{4-}]} \right) + ([Ca]_T - [Ca^{2+}]) + ([Mg]_T - [Mg^{2+}]) \quad [277]$$

whence:

$$[EGTA^{4-}] = \frac{\{[EGTA]_T - ([Ca]_T - [Ca^{2+}]) - ([Mg]_T - [Mg^{2+}])\}}{\alpha} \quad [278]$$

substituting in equation [273] gives:

$$[H]_B = \{[EGTA]_T - ([Ca]_T - [Ca^{2+}]) - ([Mg]_T - [Mg^{2+}])\} \frac{\varepsilon}{\alpha} \quad [279]$$

where:

$$\alpha = (1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)})$$

and “ ε ” as defined in equation [275].

[H⁺] IONS RELEASED WHEN EGTA BINDS Ca²⁺ AND Mg²⁺

The concentration of H⁺-ions released when EGTA binds Ca²⁺ and Mg²⁺ can be calculated as follows:

$$\Delta[H^+] = [H^+]_B([Ca]_T = 0; [Mg]_T = 0) - [H^+]_B([Ca]_T = [Ca]_T; [Mg]_T = [Mg]_T) \quad [280]$$

Since $(10^{(pK_{Ca-2} - pH)})$ and $(10^{(pK_{Mg-2} - pH)})$ tends to zero as $[Ca]_T$ and $[Mg]_T$ tend to zero, it follows from equation [273] in pK and pH terminology:

$$\frac{[H^+]_B([Ca]_T = 0; [Mg]_T = 0)}{[EGTA]_T \{ 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)} \}} = \alpha \quad [281]$$

and

$$[H^+]_B([Ca]_T = [Ca]_T; [Mg]_T = [Mg]_T) = \{ [EGTA]_T - ([Ca]_T - 10^{-pCa}) - ([Mg]_T - 10^{-pMg}) \} * \frac{\varepsilon}{\alpha} \quad [282]$$

subtracting equation [282] from [281] and simplifying gives:

$$\Delta[H^+] = \frac{\{ ([Ca]_T - 10^{-pCa}) + ([Mg]_T - 10^{-pMg}) \} \varepsilon - [EGTA]_T \{ 10^{(pK_{H-1} - pH) + (pK_{Ca-2} - pCa)} + 10^{(pK_{H-1} - pH) + (pK_{Mg-2} - pMg)} \}}{\alpha} \quad [283]$$

PART III: EQUILIBRIUM CONSTANTS: CHANGE OF TEMPERATURE AND IONIC STRENGTH

EQUILIBRIUM CONSTANTS/BINDING CONSTANTS

In what follows the constants are expressed as equilibrium constants, but in Martell & Smith (1974), the constants are tabulated as binding constants. The definition of the two constants is as follows:

Equilibrium constant:

$$\frac{[\text{Ca}^{2+}][\text{Ligand}^{n-}]}{[\text{Ca-Ligand}^{(n-2)-}]} = K_{\text{Equilibrium}} \quad [284]$$

Binding constant

$$\frac{[\text{Ca-Ligand}^{(n-2)-}]}{[\text{Ca}^{2+}][\text{Ligand}^{n-}]} = K_{\text{Binding}} \quad [285]$$

It follows that:

$$K_{\text{Equilibrium}} = \frac{1}{K_{\text{Binding}}} \quad [286]$$

and

$$\log(K_{\text{Equilibrium}}) = -\log(K_{\text{Binding}}) \quad [287]$$

The consequences of equation [287] is that when using the constants tabulated in Martell & Smith (1974) which tabulates the log(binding constants), equation [287] *has to be* used to convert to log(equilibrium constants).

TABULATED CONSTANTS

Tabulated constants (e.g., Martell & Smith, 1974) are the stoichiometric binding constants, for a given temperature, 20°C or 25°C and a given ionic strength. Depending on the equilibrium constant, temperature and ionic strength used in the experimental situation, the tabulated constants have to be corrected to the conditions that are actually being used. A comprehensive method to do this is in Harrison & Bers (1989). The following is similar, but the equations were derived by Professor Paul Schindler, Institute of Chemistry, University of Berne, Switzerland. In using the tabulated constants when working with equilibrium constants, equation [287] has to be used.

CONSTANTS

There are three ways of defining an equilibrium constant

1) Thermodynamic constants (K_T)

$$\frac{a_{H^+} a_{L^{n-}}}{a_{H-L^{(n-1)-}}} = K_T \quad [288]$$

This is in terms of activity and the constant is independent of ionic strength.

2) Stoichiometric constants (K_S)

$$\frac{[H^+][L^{n-}]}{[H-L^{(n-1)-}]} = K_S \quad [289]$$

It is this constant that is tabulated in Martell & Smith (1974). This is in terms of concentration and K_S is a function of the ionic strength

3) Mixed constant (K_M):

This constant refers specifically to the protonation constants.

$$\frac{[a_{H^+}][L^{n-}]}{[H-L^{(n-1)-}]} = K_M \quad [290]$$

Mixed, because it is a combination of hydrogen ion activity and concentrations of the L^{n-} and $H-L^{(n-1)-}$. This arises because pH as measured, measures activity and not concentration (Section-I, page 4). K_M is also a function of ionic strength.

RELATIONSHIPS AMONGST THE THREE DIFFERENT CONSTANTS

1) K_T and K_S

Equation [288] can also be written;

$$\frac{[H^+][L^{n-}]}{[H-L^{(n-1)-}]} * \frac{\gamma_{H^+}\gamma_{L^{n-}}}{\gamma_{H-L^{(n-1)-}}} = K_T \quad [291]$$

Where γ is the single ion activity coefficient for H^+ , L^{n-} and $H-L^{(n-1)-}$ respectively.

Rearranging gives:

$$K_S * \frac{\gamma_{H^+}\gamma_{L^{n-}}}{\gamma_{H-L^{(n-1)-}}} = K_T \quad [292]$$

and

$$\log K_S + \log \gamma_{H^+} + \log \gamma_{L^{n-}} - \log \gamma_{H-L^{(n-1)-}} = \log K_T \quad [293]$$

2) K_M and K_S

From the definition of K_M , equation [290]:

$$\frac{[H^+]\gamma_{H^+}[L^{n-}]}{[H-L^{(n-1)-}]} = K_M \quad [294]$$

since $a_H = [H^+]\gamma_H$

Rearranging gives:

$$\frac{[H^+][L^{n-}]}{[H-L^{(n-1)-}]} = \frac{K_M}{\gamma_{H^+}} \quad [295]$$

from the definition of K_S , it follows that:

$$K_S = \frac{K_M}{\gamma_{H^+}} \text{ and } \log K_S = \log K_M - \log \gamma_{H^+} \quad [296][297]$$

or

$$\log K_M = \log K_S + \log \gamma_{H^+} \quad [298]$$

In both cases the single ion activity coefficient for H^+ -ions (γ_{H^+}) has to be calculated.

3) Conversion of pH_a to pH_c and vice-versa

pH_a and pH_c are related as follows:

$$pH_a = -\log[H^+]\gamma_{H^+} \text{ and } pH_a = -\log[H^+] - \log \gamma_{H^+} \quad [299][300]$$

whence:

$$pH_c = pH_a + \log \gamma_{H^+} \quad [301]$$

Thus to convert from pH_a to pH_c it is necessary to calculate the single ion activity coefficient for H^+ at the given ionic strength and temperature. Three ways of doing this are described below (see also page 4, Section-I).

a) Davies equation and the Debye-Hückel convention

From Davies equation (see section on correction for ionic strength, equation [311], page, 49):

$$-\log \gamma_{\text{HCl}^\pm} = A\phi(I)$$

and from Debye-Hückel convention, equations [312] and [313], page 50

$$-\log \gamma_{\text{H}^+} = A\phi(I) \text{ and } \gamma_{\text{H}^+} = 10^{-A\phi(I)}$$

b) Empirical fit to the measured mean activity coefficient

Hamer & Wu (1972) list the measured mean activity coefficients for uni-valent electrolytes at 25°C. The following equation was used to fit the measured data for HCl.

$$\log \gamma_{\text{HCl}^\pm} = -\frac{A\sqrt{I}}{1 + 1.525\sqrt{I}} + 1.0494 * 10^{-1} * I + 6.5360 * 10^{-3} * I^2 - 4.2058 * 10^{-4} * I^3 - 4.0700 * 10^{-6} * I^4 + 5.2580 * 10^{-7} * I^5$$

[302]

c) Empirical fit of Bers, Patton & Nuccietelli (1994)

These authors provide an empirical equation for the mean H^+ activity coefficient:

at

$$\gamma_{\text{HCl}^\pm} = 0.145045 * \exp^{-B*I} + 0.063546 * \exp^{-43.97704 * I} + 0.695634$$

[303]

where

$$B = 0.522932 * \exp^{0.0327016 * t} + 4.015942$$

[304]

I and t are ionic strength, mol/l and temperature in °C respectively.

If γ_{H^+} , then it is possible to K_M calculated from K_S using equation [298].

TEMPERATURE CORRECTION:

1) Enthalpy method (Δh as tabulated in Martell & Smith, 1974)

From Atkins, (1990), equation [12] page 221:

$$\ln K(T_2) = \ln K(T_1) - \frac{\Delta H}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right)$$

[305]

or in terms of log

$$\log K(T_2) = \log K(T_1) - \frac{\Delta H}{R * \ln(10)} \left(\frac{1}{T_2} - \frac{1}{T_1} \right)$$

[306]

ΔH is the standard enthalpy (kJ/mol), R gas constant (8.31441 J/Kmol) and T the absolute temperature (K). With ΔH in kJ/mol this equation simplifies to:

$$\log K(T_2) = \log K(T_1) - 52.23395 \Delta H \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad [307]$$

In some of the older literature ΔH is given in terms of kcal/mol. This value has to be multiplied by 4.184 to convert to kJ/mol. The corresponding equation for ΔH in terms of kcal/mol is:

$$\log K(T_2) = \log K(T_1) - 218.5492 \Delta H \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad [308]$$

2) $\Delta pK/^\circ C$ METHOD

Especially for hydrogen buffers the temperature dependence is often given as a change in pK for a one degree change in temperature (cf. Burton, 1975, Table 1, page 40), If

$\frac{\Delta pK}{^\circ C} = x$ then for a change in temperature from $t_1^\circ C$ to $t_2^\circ C$:

$$pK(t_2) = pK(t_1) + x(t_2 - t_1) \quad [309]$$

If x is negative an increase in temperature causes a decrease in the pK value.

IONIC STRENGTH CORRECTIONS (EQUILIBRIUM CONSTANTS)

Strategy

- 1) The K_S at each ionic strength is expressed in terms of K_T . Since K_T is by definition independent of ionic strength the two expressions for K_S can then be equated with each other and $K_S(I_2)$ expressed in terms of $K_S(I_1)$ and activity coefficients.
- 2) The Davies equation (Davies, 1962) is taken as a reasonable approximation to calculate the mean activity coefficients γ_{\pm} .
- 3) The Debye-Hückel convention (see Ammann, 1986) is used to calculate the single ion activity coefficient

Implicit in this method is that activity coefficients calculated for pure solutions are also valid in solutions of mixed electrolytes.

Calculation

- 1) Express $K_S(I_1)$ in terms of K_T

Using equation [293] gives:

$$\log K_S(I_1) + \log \gamma_{H^+}(I_1) + \log \gamma_{L^{n-}}(I_1) - \log \gamma_{H-L^{(n-1)-}}(I_1) = \log K_T \quad [310]$$

2) The Davies equation (1962) is used to calculate γ_{\pm}

$$-\log \gamma_{\pm} = A \left| z^+ z^- \right| \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2 I \right) \quad [311]$$

where A is a constant defined as follows (see Baumgarten, 1981):

$$A = \frac{1.8246 * 10^6}{(DT)^{\frac{3}{2}}} \quad [312]$$

T is the absolute temperature, D is the dielectric constant of water. The temperature dependence of D is given by the following equation:

$$D = 78.54 \{ 1 + (-4.579 * 10^{-3})(t - 25) + (1.19 * 10^{-5})(t - 25)^2 - (2.8 * 10^{-8})(t - 25)^3 \} \quad [313]$$

to simplify let:

$$\phi = \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2 I \right) \quad [314]$$

Substituting in equation [311] gives:

$$-\log \gamma_{\pm} = A \left| z^+ z^- \right| \phi \quad [315]$$

3) Debye-Hückel convention for the single ion activity coefficient is:

$$\log \gamma_+ = \left| \frac{z^+}{z^-} \right| \log \gamma_{\pm} \text{ and } \log \gamma_- = \left| \frac{z^-}{z^+} \right| \log \gamma_{\pm} \quad [316][317]$$

Protonation constants

Substituting equations [315], [316] and [317] in equation [310] gives:

$$\log K_s(I_1) - A \left| z_{H^+}^2 \right| \phi(I_1) - A \left| z_L^2 \right| \phi(I_1) + A \left| z_{H-L}^2 \right| \phi(I_1) = \log K_T \quad [318]$$

since $z_{H^+} = 1$ it follows that

$$\log K_s(I_1) - A \phi(I_1) - A \phi(I_1) n^2 + A(n-1)^2 \phi(I_1) = \log K_T \quad [319]$$

which on simplification gives:

$$\log K_s(I_1) - 2nA\phi(I_1) = \log K_T \quad [320]$$

and similarly for $K_s(I_2)$:

$$\log K_s(I_2) - 2nA\phi(I_2) = \log K_T \quad [321]$$

Equating equations [320] and [321] gives:

$$\log K_s(I_2) = \log K_s(I_1) - 2nA\phi(I_1) + 2nA\phi(I_2) \quad [322]$$

or

$$\log K_s(I_2) = \log K_s(I_1) + 2nA\{\phi(I_2) - \phi(I_1)\} \quad [323]$$

Protonation constants K_{H-1} to K_{H-4}

$$1) K_{H-1} = \frac{[H^+][EGT A^{4-}]}{[H-EGT A^{3-}]}, n = 4, \text{ so it follows that:}$$

$$\log K_s(I_2) = \log K_s(I_1) + 8A\{\phi(I_2) - \phi(I_1)\} \quad [324]$$

$$2) K_{H-2} = \frac{[H^+][H-EGT A^{3-}]}{[H_2-EGT A^{2-}]}, n = 3, \text{ so it follows that:}$$

$$\log K_s(I_2) = \log K_s(I_1) + 6A\{\phi(I_2) - \phi(I_1)\} \quad [325]$$

$$3) K_{H-3} = \frac{[H^+][H_2-EGT A^{2-}]}{[H_3-EGT A^{1-}]}, n = 2, \text{ so it follows that:}$$

$$\log K_s(I_2) = \log K_s(I_1) + 4A\{\phi(I_2) - \phi(I_1)\} \quad [326]$$

$$4) K_{H-4} = \frac{[H^+][H_3-EGT A^{1-}]}{[H_4-EGT A]}, n = 1, \text{ so it follows that:}$$

$$\log K_s(I_2) = \log K_s(I_1) + 2A\{\phi(I_2) - \phi(I_1)\} \quad [327]$$

Calcium constant

A similar procedure for the calcium constants gives:

$$K_s(I_1) * \frac{\gamma_{Ca^{2+}} \gamma_{L^{n-}}}{\gamma_{Ca-L^{(n-2)-}}} = K_T \quad [328]$$

and

$$\log K_s(I_1) + \log \gamma_{Ca^{2+}}(I_1) + \log \gamma_{L^{n-}}(I_1) - \log \gamma_{Ca-L^{(n-2)-}}(I_1) = \log K_T \quad [329]$$

Substituting equations [315], [316] and [3174] in equation [329] gives:

$$\log K_s(I_1) - A \left| z_{Ca^{2+}}^2 \right| \phi(I_1) - A \left| z_{L^{n-}}^2 \right| \phi(I_1) + A \left| z_{Ca-L^{(n-2)-}}^2 \right| \phi(I_1) = \log K_T \quad [330]$$

and similarly for $K_s(I_2)$:

$$\log K_s(I_2) - A \left| z_{Ca^{2+}}^2 \right| \phi(I_2) - A \left| z_{L^{n-}}^2 \right| \phi(I_2) + A \left| z_{Ca-L^{(n-2)-}}^2 \right| \phi(I_2) = \log K_T \quad [331]$$

equating equations [330] and [331]

$$\log K_s(I_2) = \log K_s(I_1) + 4nA \{ \phi(I_2) - \phi(I_1) \} \quad [332]$$

Calcium constants K_{Ca-1} and K_{Ca-2}

1) $K_{Ca-1} = \frac{[Ca^{2+}][EGT A^{4-}]}{[Ca-EGT A^{2-}]}$, $n = 4$, so it follows that:

$$\log K_s(I_2) = \log K_s(I_1) + 16A \{ \phi(I_2) - \phi(I_1) \} \quad [333]$$

2) $K_{Ca-2} = \frac{[Ca^{2+}][H-EGT A^{3-}]}{[Ca-H-EGT A^{1-}]}$, $n = 3$, so it follows that:

$$\log K_s(I_2) = \log K_s(I_1) + 12A \{ \phi(I_2) - \phi(I_1) \} \quad [334]$$

CONVERSION OF K_M AT I_1 , $K_M(I_1)$ TO K_M AT I_2 , $K_M(I_2)$

Combining equations [293] and [319], namely:

$$\log K_s = \log K_M - \log \gamma_{H^+} \text{ and } \log K_s(I_2) = \log K_s(I_1) + 2nA \{ \phi(I_2) - \phi(I_1) \}$$

gives:

$$\log K_M(I_2) - \log \gamma_{H^+}(I_2) = \log K_M(I_1) - \log \gamma_{H^+}(I_1) + 2nA \{ \phi(I_2) - \phi(I_1) \} \quad [335]$$

and

$$\log K_M(I_2) = \log K_M(I_1) + \{ \log \gamma_{H^+}(I_2) - \log \gamma_{H^+}(I_1) \} + 2nA \{ \phi(I_2) - \phi(I_1) \} \quad [336]$$

Substituting equation [311], [312] and [313] into equation [332] gives:

$$\log K_M(I_2) = \log K_M(I_1) + A\{\phi(I_2) - \phi(I_1)\} + 2nA\{\phi(I_2) - \phi(I_1)\} \quad [337]$$

$$\log K_M(I_2) = \log K_M(I_1) + (2n + 1)A\{\phi(I_2) - \phi(I_1)\} \quad [338]$$

IONIC STRENGTH CORRECTIONS (BINDING CONSTANTS)

Introduction: As emphasised on page 47, the tabulated constants are binding constants *not* equilibrium constant and since the equations [324] to [327] and equations [334] and [335] have been developed using equilibrium constants, the tabulated binding constants *must* be converted to equilibrium constants before use, by using equation [287] i.e.

$$\log(K_{\text{Equilibrium}}) = -\log(K_{\text{Binding}})$$

The consequences this has for the calculation of the protonation and calcium constants is shown below.

Use of binding constants instead of equilibrium constants: The general solution for the ionic strength correction for the protonation constants is:

$$\log K_{\text{Equilibrium}}(I_2) = \log K_{\text{Equilibrium}}(I_1) + 2nA\{\phi(I_2) - \phi(I_1)\} \quad [339]$$

where n is the net charge on EGTA.

Using equation [287] to convert from equilibrium constants to binding constants in equation [339] gives:

$$-\log K_{\text{Binding}}(I_2) = -\log K_{\text{Binding}}(I_1) + 2nA\{\phi(I_2) - \phi(I_1)\} \quad [340]$$

simplification gives:

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 2nA \{\phi(I_2) - \phi(I_1)\} \quad [341]$$

When using binding constants this is the equation that has to used.

A similar argument for the Ca^{2+} constants gives:

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 4nA \{\phi(I_2) - \phi(I_1)\} \quad [3429]$$

Calculation of Protonation constants K_{H-1} to K_{H-4} using Binding constants

$$1) K_{H-1} = \frac{[H^+][\text{EGT A}^{4-}]}{[\text{H-EGT A}^{3-}]}, n = 4, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 8A \{\phi(I_2) - \phi(I_1)\} \quad [343]$$

$$2) K_{H-2} = \frac{[H^+][\text{H-EGT A}^{3-}]}{[\text{H}_2\text{-EGT A}^{2-}]}, n = 3, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 6A \{\phi(I_2) - \phi(I_1)\} \quad [344]$$

$$3) K_{H-3} = \frac{[H^+][\text{H}_2\text{-EGT A}^{2-}]}{[\text{H}_3\text{-EGT A}^{1-}]}, n = 2, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 4A \{\phi(I_2) - \phi(I_1)\} \quad [345]$$

$$4) K_{H-4} = \frac{[H^+][\text{H}_3\text{-EGT A}^{1-}]}{[\text{H}_4\text{-EGT A}]}, n = 1, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 2A \{\phi(I_2) - \phi(I_1)\} \quad [346]$$

Calculation of calcium constants $K_{\text{Ca-1}}$ and $K_{\text{Ca-2}}$ using Binding constants

$$1) K_{\text{Ca-1}} = \frac{[\text{Ca}^{2+}][\text{EGT A}^{4-}]}{[\text{Ca-EGT A}^{2-}]}, n = 4, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 16A \{\phi(I_2) - \phi(I_1)\} \quad [347]$$

$$2) K_{\text{Ca-2}} = \frac{[\text{Ca}^{2+}][\text{H-EGT A}^{3-}]}{[\text{Ca-H-EGT A}^{1-}]}, n = 3, \text{ so it follows that:}$$

$$\log K_{\text{Binding}}(I_2) = \log K_{\text{Binding}}(I_1) - 12A \{ \phi(I_2) - \phi(I_1) \} \quad [348]$$

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