SECTION-III

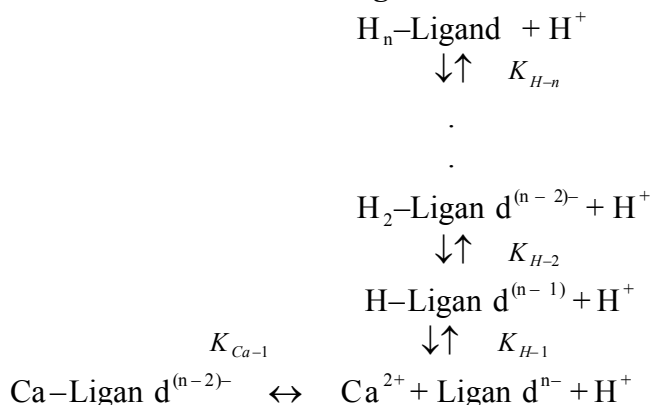## Ca^{2+} BINDING TO BAPTA, Br$_2$BAPTA, CDTA, EDTA, HEDTA AND NTA: BUFFER CHARACTERISTICS OF NTA

CONTENTS

INTRODUCTION

In Section-II the equations for the binding of Ca^{2+} and Mg^{2+} to the ligand EGTA were derived. However, it is also of interest to derive the equations for the binding of Ca^{2+} and H^+ to other ligands, that cover different pCa ranges than EGTA. Such ligands are BAPTA, Br_2BAPTA, CDTA, EDTA, HEDTA and NTA. EDTA is similar in its behaviour to EGTA and the equations derived in Section-II also apply to this ligand. This is not the case with the remaining ligands BAPTA, Br_2BAPTA, CDTA, and HEDTA and the Ca^{2+} and H^+ binding to these ligands is considered in PART I. Ca^{2+} binds to one and to two molecules of NTA and PART II derives the equations for this Ca^{2+} binding. In Part III, these equations are use to explore the extent of Ca^{2+} binding to NTA as a function of pH, and as pCa and total Ca^{2+} concentrations are varied at different pH values. Part IV, discusses the buffering characteristics of NTA

PART I: BINDING OF Ca^{2+} AND H^+ TO CDTA, BAPTA, Br_2BAPTA, AND HEDTA

EQUILIBRIUM CONSTANTS

The binding of Ca^{2+} and H^+ to these compounds is very similar to EGTA and EDTA and as an example, the binding of both these ions to the ligands is illustrated below.

$$H_n\text{–Ligand} + H^+$$
$$\downarrow\uparrow \quad K_{H-n}$$
$$.$$
$$.$$
$$H_2\text{–Ligand}^{(n-2)-} + H^+$$
$$\downarrow\uparrow \quad K_{H-2}$$
$$H\text{–Ligand}^{(n-1)} + H^+$$
$$K_{Ca-1} \qquad \downarrow\uparrow \quad K_{H-1}$$
$$Ca\text{–Ligand}^{(n-2)-} \quad \leftrightarrow \quad Ca^{2+} + \text{Ligand}^{n-} + H^+$$

In contrast to EGTA/EDTA, Ca^{2+} only binds to Ligand^{n-} and not to H-Ligand^{1-n-}.

APPARENT EQUILIBRIUM CONSTANT (K_{app})

As shown above, there is no binding of Ca^{2+} to the protonated form of the ligands *i.e.*

$$\frac{[Ca^{2+}]([\text{Ligand}^n] + [H\text{–Ligand}^{(n-1)-}] + [H_2\text{–Ligand}^{(n-2)-}] + ... + [H_n\text{–Ligand}])}{([Ca\text{–Ligand}^{(n-2)-}])} = K_{app} \qquad [1]$$

$$\frac{[Ca^{2+}][\text{Ligand}^n]}{([Ca\text{–Ligand}^{(n-2)-}])}\left(1 + \frac{[H\text{–Ligand}^{(n-1)-}]}{[\text{Ligand}^n]} + \frac{[H_2\text{–Ligand}^{(n-2)-}]}{[\text{Ligand}^n]} + ... + \frac{[H_n\text{–Ligand}])}{[\text{Ligand}^n]}\right) = K_{app} \qquad [2]$$

Similar to equation [19] on page 8 of Section-II, this gives:

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

or

$$K_{Ca-1}(1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + ... + 10^{(pK_{H-1} + pK_{H-2} + ... + pK_{H-n} - npH)}) = K_{app} \quad [4]$$

In other words β = 1 (see page 8, Section-II, equation [22])

H+-BOUND TO THE LIGANDS

$$[H^+]_B = [H-Ligand^{(n-1)-}] + 2*[H_2-Ligand^{(n-2)-}] + ... + n*[H_n-Ligand] \quad [5]$$

$$[H^+]_B = [Ligand^{n-}](\frac{[H-Ligand^{(n-1)-}]}{[Ligand^{n-}]} + 2*\frac{[H_2-Ligand^{(n-2)-}]}{[Ligand^{n-}]} + ... + n\frac{*[H_n-Ligand]}{[Ligand^{n-}]}) \quad [6]$$

and

$$[H^+]_B = [Ligand^{n-}](\frac{[H^+]}{K_{H-1}} + 2*\frac{[H^+]^2}{K_{H-1}K_{H-2}} + ... + n*\frac{[H^+]^n}{K_{H-1}K_{H-2} .... K_{H-n}}) \quad [7]$$

[Ligand^{n-}] in terms of $[Ca]_T$, $[Ca^{2+}]$ and $[Ligand]_T$

$$[Ligand]_T = [Ligand^{n-}] + [H-Ligand^{(n-1)-}]) + [H_2-Ligand^{(n-2)-}] + ... + [H_n-Ligand] + [Ca-Ligand^{(n-2)-}] \quad [8]$$

and

$$[Ligand]_T = [Ligand^{n-}]\alpha + [Ca-Ligand^{(n-2)-}] \quad [9]$$

$$[Ca]_T = [Ca-Ligand^{(n-2)-}] + [Ca^{2+}] \quad [10]$$

substitution gives:

$$[Ligand^{n-}] = \frac{\{[Ligand]_T - ([Ca]_T - [Ca^{2+}])\}}{\alpha} \quad [11]$$

substitution for [Ligand^{n-}] gives:

$$[H^+]_B = \{[Ligand]_T - ([Ca]_T - [Ca^{2+}])\}\frac{(\frac{[H^+]}{K_{H-1}} + 2*\frac{[H^+]^2}{K_{H-1}K_{H-2}} + ... + n*\frac{[H^+]^n}{K_{H-1}K_{H-2} .... K_{H-n}})}{\alpha} \quad [12]$$

$$\text{let} \quad \partial^| = (\frac{[H^+]}{K_{H-1}} + 2*\frac{[H^+]^2}{K_{H-1}K_{H-2}} + ... + n*\frac{[H^+]^n}{K_{H-1}K_{H-2} .... K_{H-n}}) \quad [13]$$

and it follows that:

$$[H^+]_B = \{[\text{Ligand }]_T - ([Ca]_T - [Ca^{2+}])\}\frac{\partial^|}{\alpha} \qquad [14]$$

H+ RELEASED WHEN THE LIGANDS BIND Ca²⁺

$$\Delta[H^+] = [H^+]_B([Ca]_T = 0) - [H^+]_B([Ca]_T = [Ca]_T) \qquad [15]$$

This gives:

$$\Delta[H^+] = [\text{Ligand }]_T\frac{\partial^|}{\alpha} - \{[\text{Ligand }]_T - ([Ca]_T - [Ca^{2+}])\}\frac{\partial^|}{\alpha} \qquad [16]$$

or

$$\Delta[H^+] = ([Ca]_T - [Ca^{2+}])\frac{\partial^|}{\alpha} \qquad [17]$$

H+-released per mole Ca²⁺ bound is:

$$\frac{\Delta[H^+]}{([Ca]_T - [Ca^{2+}])} = \frac{\partial^|}{\alpha} \text{ or } N = \frac{\partial^|}{\alpha} \qquad [18][19]$$

Ca²⁺ BINDING TO THE LIGANDS

Since a K_{app} can be calculated for the ligands the equations derived in Section-II for EGTA (Part I) also apply.
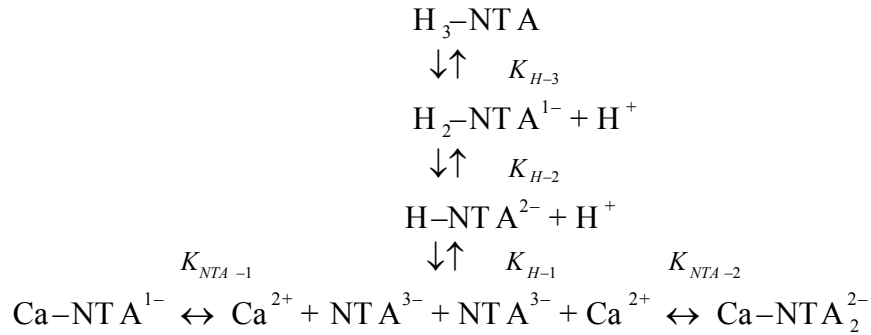
PART II: Ca²⁺ AND H⁺ BINDING TO NTA.

Ca²⁺ AND H⁺ BINDING

This is complicated by the fact that Ca^{2+} binds to one and two molecules of NTA, namely Ca-NTA and Ca-NTA$_2$ (Martell & Smith, 1974) and the binding is illustrated below. The equilibrium constants K_{NTA-1} and K_{NTA-2} are defined in equations [20] and [21]. The equilibrium constants for H^+ are as in equations [2] to [5] on page [6] of Section-II.

$$K_{NTA-1} = \frac{[Ca^{2+}][NTA^{3-}]}{[Ca-NTA^{1-}]} \quad \text{and} \quad K_{NTA-2} = \frac{[Ca^{2+}][NTA^{3-}]^2}{[Ca-NTA_2^{2-}]} \qquad [20][21]$$

EQUILIBRIUM CONSTANTS

The binding of Ca^{2+} and H^+ is illustrated below:

$$
\begin{array}{c}
H_3-NTA \\
\downarrow\uparrow \quad K_{H-3} \\
H_2-NTA^{1-} + H^+ \\
\downarrow\uparrow \quad K_{H-2} \\
H-NTA^{2-} + H^+ \\
\end{array}
$$

$$K_{NTA-1} \qquad \downarrow\uparrow \quad K_{H-1} \qquad K_{NTA-2}$$

$$Ca-NTA^{1-} \leftrightarrow Ca^{2+} + NTA^{3-} + NTA^{3-} + Ca^{2+} \leftrightarrow Ca-NTA_2^{2-}$$

TOTAL Ca²⁺([Ca]$_T$)

This is defines as follows:

$$[Ca]_T = [Ca^{2+}] + [Ca-NTA^{1-}] + [Ca-NTA_2^{2-}] \qquad [22]$$

TOTAL NTA ([NTA]$_T$)

NTA also binds H^+-ions so [NTA]$_T$ is given by:

$$[NTA]_T = [NTA^{3-}] + [H-NTA^{2-}] + [H_2-NTA^{1-}] + [H_3-NTA] + [Ca-NTA^{1-}] + 2[Ca-NTA_2^{2-}]$$

or $\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad$ [23]

$$[NTA]_T = [NTA^{3-}](1 + \frac{[H-NTA^{2-}]}{[NTA^{3-}]} + \frac{[H_2-NTA^{1-}]}{[NTA^{3-}]} + \frac{[H_3-NTA]}{[NTA^{3-}]}) + [Ca-NTA^{1-}] + 2[Ca-NTA_2^{2-}]$$

$$[24]$$

Let:

$$\alpha = (1 + \frac{[H-NTA^{2-}]}{[NTA^{3-}]} + \frac{[H_2-NTA^{1-}]}{[NTA^{3-}]} + \frac{[H_3-NTA]}{[NTA^{3-}]})$$

and equation [24] becomes

$$[NTA]_T = [NTA^{3-}]\alpha + [Ca-NTA^{1-}] + 2[Ca-NTA_2^{2-}] \qquad [25]$$

This simplification for $[NTA]_T$ will be used throughout. α can be calculated using equation [21] on page 8 of Section-II.

INDIRECT ESTIMATION OF [Ca²⁺] BY FIRST CALCULATING [NTA³⁻]
1) [NTA³⁻]
Substituting for [Ca-NTA¹⁻] and [Ca-NTA₂] using equations [20] and [21] in equation [25], gives:

$$[NTA]_T = [NTA^{3-}]\alpha + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + 2\frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}} \qquad [26]$$

[Ca²⁺] can be eliminated using equation [22] for [Ca]$_T$ as follows:

$$[Ca]_T = [Ca^{2+}] + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + \frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}} \qquad [27]$$

or

$$[Ca]_T K_{NTA-1} K_{NTA-2} = [Ca^{2+}]K_{NTA-1}K_{NTA-2} + K_{NTA-2}[Ca^{2+}][NTA^{3-}] + K_{NTA-1}[Ca^{2+}][NTA^{3-}]^2 \quad [28]$$

it follows that:

$$[Ca^{2+}] = \frac{[Ca]_T K_{NTA-1} K_{NTA-2}}{K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2} \qquad [29]$$

substituting in equation [26] gives:

$$[NTA]_T = [NTA^{3-}]\alpha + \frac{[Ca]_T K_{NTA-1} K_{NTA-2}}{K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2}*\frac{[NTA^{3-}]}{K_{NTA-1}}$$
$$+ \qquad\qquad [30]$$
$$2\frac{[Ca]_T K_{NTA-1} K_{NTA-2}}{K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2}*\frac{[NTA^{3-}]^2}{K_{NTA-2}}$$

Simplification gives a cubic equation:

$$A[NTA^{3-}]^3 + B[NTA^{3-}]^2 + C[NTA^{3-}] + D = 0 \qquad [31]$$

where:

$$A = 1$$

$$B = \frac{-K_{NTA-1}[NTA]_T + \alpha K_{NTA-2} + 2K_{NTA-1}[Ca]_T}{\alpha K_{NTA-1}}$$

$$C = \frac{-K_{NTA-2}[NTA]_T + \alpha K_{NTA-1}K_{NTA-2} + K_{NTA-2}[Ca]_T}{\alpha K_{NTA-1}} \qquad [32]$$

$$D = \frac{-K_{NTA-2}[NTA]_T}{\alpha}$$

2) Protonated forms of NTA^{3-}

The protonated forms of NTA can be calculated exactly as for the protonated forms of EGTA, page 18, Section-II:

$$1) \; [H-NTA^{2-}] = [NTA^{3-}]\frac{[H^+]}{K_{H-1}} \qquad [33]$$

$$2) \; [H_2-NTA^{1-}] = [NTA^{3-}]\frac{[H^+]^2}{K_{H-1}K_{H-2}} \qquad [34]$$

$$3) \; [H_3-NTA] = [NTA^{3-}]\frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \qquad [35]$$

Having calculated $[NTA^{3-}]$ it is possible to calculate the concentrations of $[Ca^{2+}]$, $[Ca-NTA^{1-}]$ and $[Ca-NTA_2]$ as follows:

3) $[Ca^{2+}]$

This follows directly from equation [29], namely:

$$[Ca^{2+}] = \frac{[Ca]_T K_{NTA-1}K_{NTA-2}}{K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2}$$

4) $[Ca-NTA^{1-}]$

Substituting in equation [22] for $[Ca-NTA_2]$ with equation [21] gives:

$$[Ca]_T = [Ca^{2+}] + [Ca-NTA^{1-}] + \frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}} \qquad [36]$$

and substituting for $[Ca^{2+}]$ with equation [20] gives:

$$[Ca]_T = \frac{K_{NTA-1}[Ca-NTA^{1-}]}{[NTA^{3-}]} + [Ca-NTA^{1-}] + \frac{K_{NTA-1}[Ca-NTA^{1-}]}{[NTA^{3-}]} * \frac{[NTA^{3-}]^2}{K_{NTA-2}} \qquad [37]$$

or

$$[Ca]_T = \frac{K_{NTA-1}[Ca-NTA^{1-}]}{[NTA^{3-}]} + [Ca-NTA^{1-}] + \frac{K_{NTA-1}[Ca-NTA^{1-}][NTA^{3-}]}{K_{NTA-2}} \qquad [38]$$

Simplifying for [Ca-NTA1-] gives:

$$[Ca-NTA^{1-}] = \frac{K_{NTA-2}[Ca]_T[NTA^{3-}]}{(K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2)} \qquad [39]$$

5) [Ca-NTA$_2$]

This is similar to above. Substituting in equation [22] for [Ca-NTA] with equation [20] gives:

$$[Ca]_T = [Ca^{2+}] + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + [Ca-NTA_2^{2-}] \qquad [40]$$

and substituting for [Ca^{2+}] with equation [21] gives:

$$[Ca]_T = \frac{K_{NTA-2}[Ca-NTA_2^{2-}]}{[NTA^{3-}]^2} + \frac{K_{NTA-2}[Ca-NTA_2^{2-}]}{[NTA^{3-}]^2} * \frac{[NTA^{3-}]}{K_{NTA-1}} + [Ca-NTA_2^{2-}] \qquad [41]$$

or

$$[Ca]_T = \frac{K_{NTA-2}[Ca-NTA_2^{2-}]}{[NTA^{3-}]^2} + \frac{K_{NTA-2}[Ca-NTA_2^{2-}]}{K_{NTA-1}[NTA^{3-}]} + [Ca-NTA_2^{2-}] \qquad [42]$$

Simplifying for [Ca-NTA$_2$] gives:

$$[Ca-NTA_2^{2-}] = \frac{K_{NTA-1}[Ca]_T[NTA^{3-}]^2}{(K_{NTA-1}K_{NTA-2} + K_{NTA-2}[NTA^{3-}] + K_{NTA-1}[NTA^{3-}]^2)} \qquad [43]$$

DIRECT ESTIMATION OF [Ca^{2+}]

1) [Ca^{2+}]

It is also possible to estimate [Ca^{2+}] directly. From equation [20] and [21] and equation [22]:

$$[Ca]_T = [Ca^{2+}] + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + \frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}} \qquad [44]$$

The next step is to derive [NTA3-] in terms of [Ca^{2+}] and [Ca]$_T$. Equation [22] can be rewritten as follows:

$$[Ca-NTA_2^{2-}] = ([Ca]_T - [Ca^{2+}] - [Ca-NTA^{1-}]) \qquad [45]$$

substituting in equation [25] gives:

$$[NTA]_T = [NTA^{3-}]\alpha + [Ca-NTA^{1-}] + 2([Ca]_T - [Ca^{2+}] - [Ca-NTA^{1-}]) \qquad [46]$$

or

$$[NTA]_T = [NTA^{3-}]\alpha + 2([Ca]_T - [Ca^{2+}]) - [Ca-NTA^{1-}] \qquad [47]$$

substituting for [Ca-NTA^{1-}] with equation [20] gives:

$$[NTA]_T = [NTA^{3-}]\alpha + 2([Ca]_T - [Ca^{2+}]) - \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} \qquad [48]$$

Simplifying in terms of [NTA^{3-}] gives:

$$[NTA^{3-}] = \frac{K_{NTA-1}\{[NTA]_T - 2([Ca]_T - [Ca^{2+}])\}}{(\alpha K_{NTA-1} - [Ca^{2+}])} \qquad [49]$$

and this can be substituted in equation [40] to give:

$$[Ca]_T = [Ca^{2+}] + \frac{[Ca^{2+}]}{K_{NTA-1}} * \frac{K_{NTA-1}\{[NTA]_T - 2([Ca]_T - [Ca^{2+}])\}}{(\alpha K_{NTA-1} - [Ca^{2+}])}$$
$$+$$
$$\frac{[Ca^{2+}]}{K_{NTA-2}} * \frac{K_{NTA-1}^2\{[NTA]_T - 2([Ca]_T - [Ca^{2+}])\}^2}{(\alpha K_{NTA-1} - [Ca^{2+}])^2} \qquad [50]$$

Equation [50] can be solved for [Ca^{2+}] to give the following cubic:

$$A[Ca^{2+}]^3 + B[Ca^{2+}]^2 + C[Ca^{2+}] + D = 0 \qquad [51]$$

where

$A = 1$

$$B = \frac{(K_{NTA-2} - 8K_{NTA-1}^2)[Ca]_T + (4K_{NTA-1}^2 - K_{NTA-2})[NTA]_T}{(4K_{NTA-1}^2 - K_{NTA-2})}$$

$$C = \frac{\alpha^2 K_{NTA-1}^2 K_{NTA-2} + 4K_{NTA-1}^2[Ca]_T^2 - 4K_{NTA-1}^2[Ca]_T[NTA]_T + K_{NTA-1}^2[NTA]_T^2 + \alpha K_{NTA-1}K_{NTA-2}[NTA]_T}{(4K_{NTA-1}^2 - K_{NTA-2})}$$

$$D = \frac{-\alpha^2 K_{NTA-1}^2 K_{NTA-2}[Ca]_T}{(4K_{NTA-1}^2 - K_{NTA-2})}$$

[52]

Once [Ca²⁺] has been calculated the other concentrations follow.

2) [NTA³⁻]
This follows from equation [49]:

$$[NTA^{3-}] = \frac{K_{NTA-1}\{[NTA]_T - 2([Ca]_T - [Ca^{2+}])\}}{(\alpha K_{NTA-1} - [Ca^{2+}])}$$

3) [Ca-NTA¹⁻]
This can be derived from equation [20]:

$$[Ca-NTA^{1-}] = \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}}$$

[53]

4) [Ca-NTA₂]
This follows from equation [21]

$$[Ca-NTA_2^{2-}] = \frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}}$$

[54]

ESTIMATION OF [NTA³⁻] AND [Ca]_T AT A GIVEN [Ca²⁺] or pCa:
1) [NTA³⁻]
The [NTA]_T is given by equation [26]

$$[NTA]_T = [NTA^{3-}]\alpha + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + 2\frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}}$$

solving for [NTA³⁻] gives:

$$2K_{NTA-1}[Ca^{2+}][NTA^{3-}]^2 + (K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}[Ca^{2+}])[NTA^{3-}] - K_{NTA-1}K_{NTA-2}[NTA]_T = 0$$

[55]

The solution is:

$$[NTA^{3-}] = \frac{-A + \sqrt{A^2 + 8K_{NTA-1}^2 K_{NTA-2}[NTA]_T[Ca^{2+}]}}{4K_{NTA-1}[Ca^{2+}]}$$

[56]

where:

$$A = (K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}[Ca^{2+}])$$

[57]

2) $[Ca]_T$

The $[Ca]_T$ is given by equation [27]:

$$[Ca]_T = [Ca^{2+}] + \frac{[Ca^{2+}][NTA^{3-}]}{K_{NTA-1}} + \frac{[Ca^{2+}][NTA^{3-}]^2}{K_{NTA-2}}$$

or

$$[Ca]_T = [Ca^{2+}](1 + \frac{[NTA^{3-}]}{K_{NTA-1}} + \frac{[NTA^{3-}]^2}{K_{NTA-2}})$$

[58]

It is possible to eliminate [NTA3-] from equation [58] by substituting equation [56]. (see equations [78] for [Ca-NTA1-] and equation [83] for [Ca-NTA$_2$]). However, this leads to complicated algebraic expressions and it is much easier to solve equation [58] by solving initially for [NTA3-] (equation [56]) and using this concentration to solve equation [58].

PART III: CALCULATION OF Ca²⁺ BINDING TO NTA

CONSTANTS

The constants were taken from Martell & Smith (1974), were for 25°C and an ionic strength of 0.1 mol/l. These were:

Constant	pK	K (concentrations in mol/l)	K (concentrations in mmol/l)
H-1	9.65	$2.2387*10^{-10}$ mol/l	$(2.2387*10^{-10})*1000$ mmol/l
H-2	2.48	$3.3113*10^{-3}$ mol/l	$(3.3113*10^{-3})*1000$ mmol/l
H-3	1.80	$1.5848*10^{-2}$ mol/l	$(1.5848*10^{-2})*1000$ mmol/l
NTA-1	6.39	$4.0738*10^{-7}$ mol/l	$(4.0738*10^{-7})*1000$ mmol/l
NTA-2	8.76	$1.7378*10^{-9}$ (mol/l)²	$(1.7378*10^{-9})*1000^2$ (mmol/l)²

When converting from units in mol/l to mmol/l which has the advantage that the concentrations are usually stated as mmol/l, it has to be born in mind that the conversion for the protonation constants and K_{NTA-1} is to multiply K by 1000. However, for K_{NTA-2} the conversion factor is 1000^2.

This arises, because if the concentrations are in mmol/l then from equation [20] and [21]:

$$K_{NTA-1} = \frac{\frac{[Ca^{2+}]}{1000}*\frac{NTA^{3-}]}{1000}}{\frac{[Ca-NTA_2^{2-}]}{1000}} \quad \text{and} \quad K_{NTA-2} = \frac{\frac{[Ca^{2+}]}{1000}*\frac{NTA^{3-}]}{1000}*\frac{NTA^{3-}]}{1000}}{\frac{[Ca-NTA_2^{2-}]}{1000}} \qquad [59][60]$$

which simplify to:

$$K_{NTA-1} = \frac{[Ca^{2+}][NTA^{3-}]}{[Ca-NTA_2^{2-}]*1000} \quad \text{and} \quad K_{NTA-2} = \frac{[Ca^{2+}][NTA^{3-}]^2}{[Ca-NTA_2^{2-}]*1000^2} \qquad [61][62]$$

This means, when concentrations are in mmol/l K_{NTA-1} (and protonation constants) have to multiplied by 1000 and K_{NTA-2} by 1000^2.

The $[NTA]_T$ was 5 mmol/l and the cubic equations were solved with the Newton-Raphson method.

BINDING AND K_{app} FOR NTA: APPROXIMATION

It is usual to calculate the binding of Ca^{2+} or Mg^{2+} to a ligand by estimating K_{app}. For NTA K_{app} is defined as follows:

$$K_{app} = \frac{[Ca^{2+}]([NTA^{3-}] + [H-NTA^{2-}] + [H_2-NTA^{1-}] + [H_3-NTA])}{([Ca-NTA^{1-}] + [Ca-NTA_2^{2-}])} \quad [63]$$

or

$$K_{app} = \frac{[Ca^{2+}][NTA^{3-}]\alpha}{([Ca-NTA^{1-}] + [Ca-NTA_2^{2-}])} \quad [64]$$

where

$$\alpha = (1 + [H-NTA^{2-}] + [H_2-NTA^{1-}] + [H_3-NTA])$$

and

$$K_{app} = \frac{[Ca^{2+}][NTA^{3-}]\alpha}{[Ca-NTA^{1-}](1 + \frac{[Ca-NTA_2^{2-}]}{[Ca-NTA^{1-}]})} \quad [65]$$

or

$$K_{app} = \frac{K_{NTA-1}\alpha}{(1 + \frac{[Ca-NTA_2^{2-}]}{[Ca-NTA^{1-}]})} \quad [66]$$

From equations [20] and [21]:

$$\frac{[Ca-NTA_2^{2-}]}{[Ca-NTA^{1-}]} = \frac{K_{NTA-1}}{K_{NTA-2}}[NTA^{3-}] \quad [67]$$

(cf. equations [12] and [13] page 6 of the EGTA Appendix)

Substituting equation [67] in equation [66] gives:

$$K_{app} = \frac{[Ca^{2+}][NTA^{3-}]\alpha}{[Ca-NTA^{1-}](1 + \frac{K_{NTA-1}}{K_{NTA-2}}[NTA^{3-}])} \quad [68]$$

or

$$K_{app} = \frac{K_{NTA-1}\alpha}{(1 + \frac{K_{NTA-1}}{K_{NTA-2}}[NTA^{3-}])} \quad [69]$$

Since $[NTA^{3-}]$ is a function of both $[Ca]_T$ and $[Ca^{2+}]$, K_{app} is not a constant and K_{app} cannot be used to calculate $[Ca^{2+}]$. However, if $[NTA^{3-}] \approx 0$, then equation [69] becomes:

$$K_{app} = K_{NTA-1}\alpha \quad [70]$$

which means K_{app} is a constant at a given pH and ionic strength. The implicit assumption in equation [70] is that Ca^{2+} binding to two NTA^{3-} molecules can be ignored.

VALIDITY OF THE K_{app} APPROXIMATION

If in the calculation of $[Ca^{2+}]$ both K_{NTA-1} and K_{NTA-2} are considered, a cubic equation has to be solved. If K_{NTA-2} is ignored, the $[Ca^{2+}]$ can be calculated using a quadratic equation, greatly simplifying the calculations. To investigate under what conditions equation [70] was a reasonable approximation, $[NTA^{3-}]$ and $[Ca^{2+}]$ were calculated using equations [31] and [29]. The $[Ca-NTA]$ and $[Ca-NTA_2]$ could then be calculated from equations [20] and [21]. These values could then be compared with $[NTA^{3-}]$, $[Ca^{2+}]$ and $[Ca-NTA]$ calculated using the appropriate quadratic equations (Appendix to Lüthi et al., 1999) and equation [70].

1) pH effect

A comparison between $[NTA^{3-}]$ calculated using the cubic equation [31] and $[NTA^{3-}]$ calculated on the basic of only Ca-NTA is illustrated in Figure 1 as a function of pH. There is little or no difference between the two calculated concentrations until around pH 9 when the two curves deviate from each other; the quadratic calculation predicting a higher concentration of $[NTA^{3-}]$ than actually present. The Figure also shows that the $[Ca-NTA_2]$ increases as the pH becomes alkaline, reaching concentrations of around 30 µmol/l at pH values greater than 10.

The fraction of Ca^{2+} bound calculated using both methods, as well as the difference between the two, is illustrated in Figure 2. Bound Ca^{2+}, calculated using the quadratic simplification is slightly greater than the bound Ca^{2+} calculated using equations [29] and [31]. The difference is minor, some 20 µmol/l at bound Ca^{2+} of around 5 mmol/l at pH values greater than 10. It may at first seem odd, that binding is greater on the assumption that Ca binds only to NTA and not to NTA and NTA_2. However, at pH values greater than 10, the concentration of Ca-NTA_2 is some 35 µmol/l. Since two molecules of NTA^{3-} are bound, some 70 µmol/l of NTA are bound as Ca-NTA_2. Making the simplification that there is only one to one binding, means at alkaline pH values, there is no formation of Ca-NTA_2 and an additional 35 µmol/l of NTA^{3-} are available for further Ca^{2+} binding, thus explaining the difference.
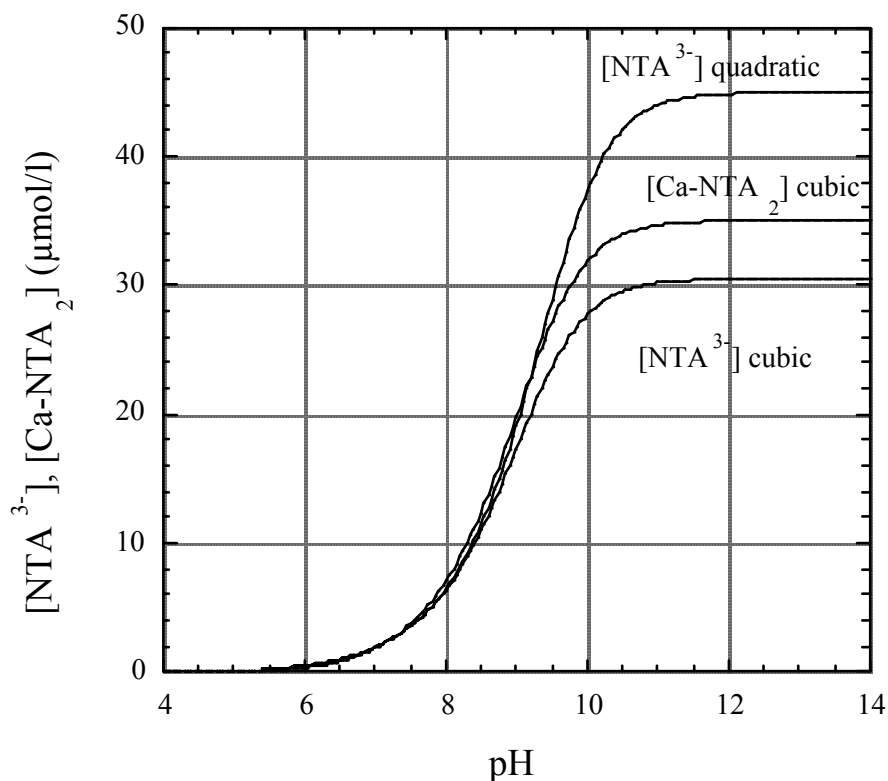
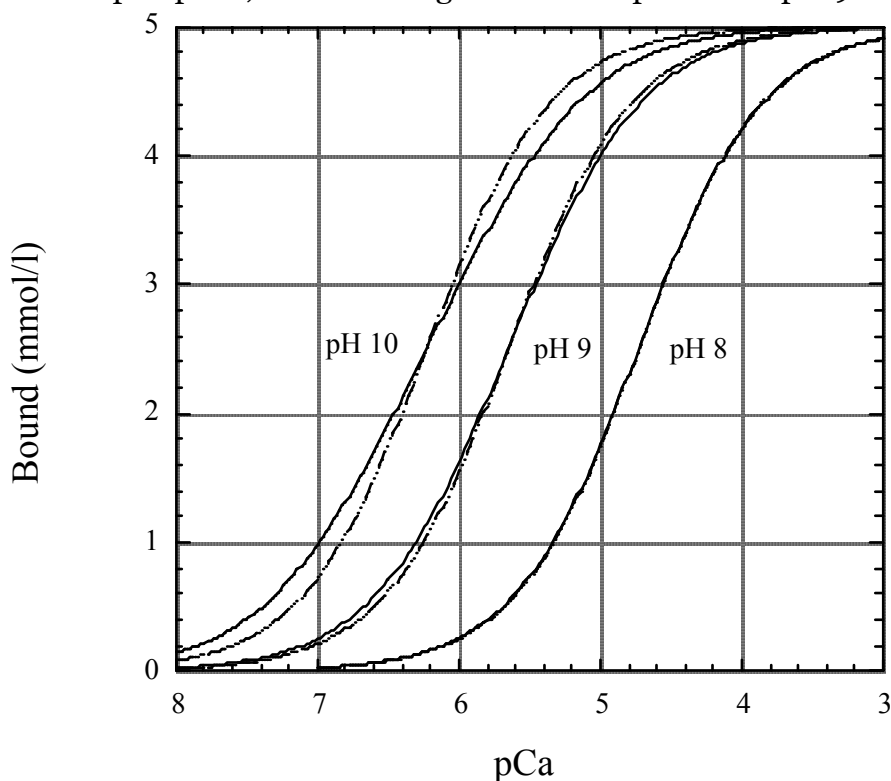Figure 1. [NTA³⁻] calculated from the cubic equation [31] and using the simplification of equation [70] and the corresponding quadratic. The [Ca-NTA₂] is also illustrated.
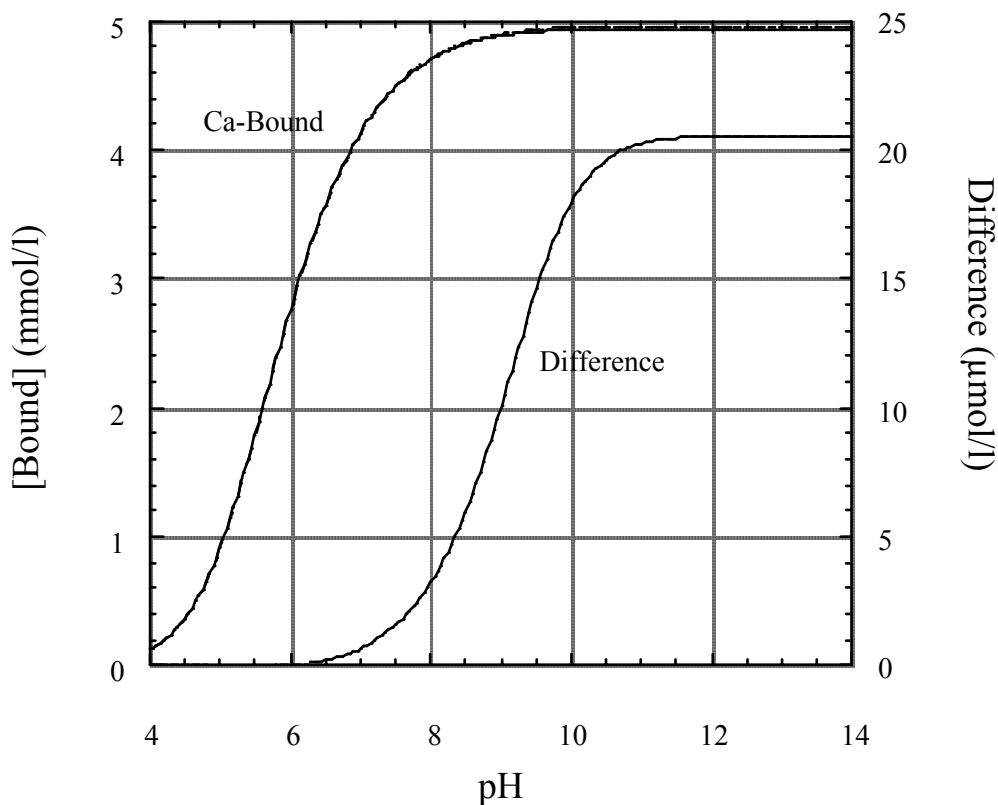
Figure 2. [Ca-Bound] as a function of pH calculated using equation [31] and then equation [29] (continuous line) and using equation [70] and the appropriate quadratic (dashed line). The difference between the two curves (µmol/l) is also shown.

2) [Ca²⁺-Bound] as a function of pCa at various pH values

The results of these calculations are illustrated in Figure 3. Deviation between the two methods is minimal up to pH 8, but as the Figure shows is present at pH 9 and 10.


Figure 3. [Ca-Bound] as a function of pCa at three different pH values, namely, 8, 9 and 10. The [Ca-Bound] was calculated from the cubic equation(continuous lines) or using the quadratic simplification (dashed lines).

3) [Ca^{2+}] at [Ca]$_T$ over a range of pH values

The [Ca^{2+}] at [Ca]$_T$ ranging from 0.5 to 4.5 mmol/l calculated by both methods is shown in Figure 4. The difference between the two methods at pH values less than 8.8 is minimal, However at pH 10, the difference amounts to 3 μmol/l at a [Ca]$_T$ of 4.5 and a [Ca^{2+}] of 8.4 μmol/l.

4) Conclusions:
Up to a pH of 8.5 the simplification that the only binding is Ca-NTA is more than adequate. In other words the binding, Ca-NTA$_2$ can be ignored and [Ca^{2+}] in NTA buffer solutions can be calculated as for other Ca^{2+} binding ligands. At more alkaline pH values the simplification breaks down, and [Ca^{2+}] can only be calculated when Ca-NTA$_2$ binding is also incorporated.
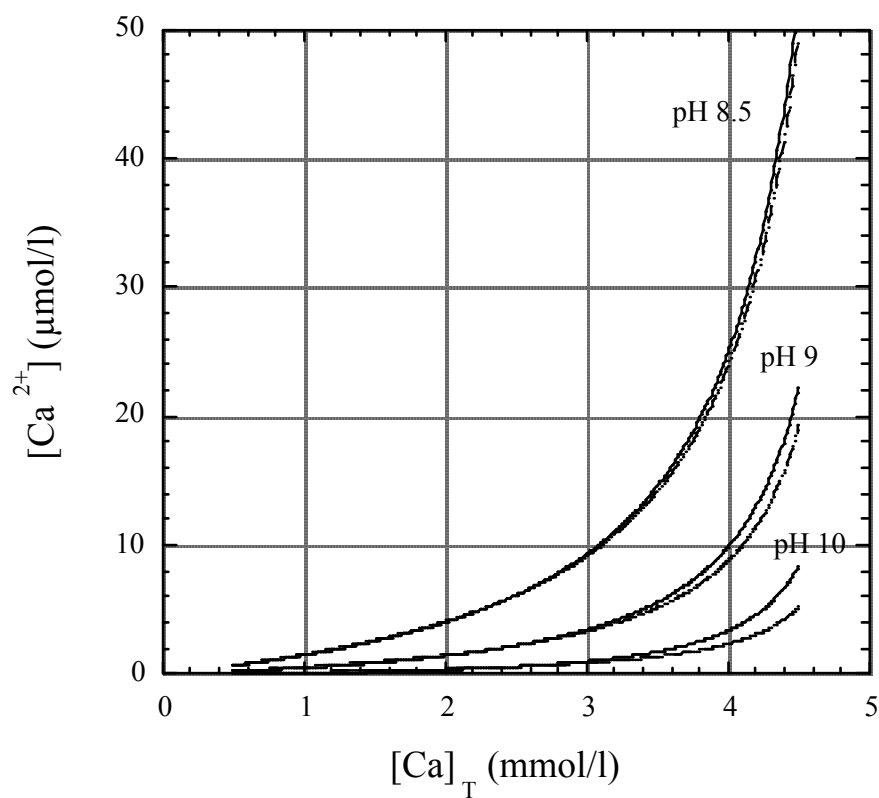
Figure 4. Comparison between $[Ca^{2+}]$ calculated from $[Ca]_T$ using either the cubic equation (continuous line) or the quadratic simplification (dashed line) at pH values of 8.5, 9 and 10.

PART IV: NTA-BUFFER CHARACTERISTICS

DEFINITION: BUFFERING POWER

As shown in Table 3 of Lüthi, Günzel & McGuigan, (1999), there are various ways of expressing buffering characteristics. The simplest method is to use "buffering power" (π) defined as:

$$\pi = -\frac{d[Ca-bound\ ]}{dpCa} \qquad [71]$$

This can be calculated as follows (Lüthi et al., 1999, Table 4):

$$\pi = \frac{[Ligand\ ]_T K_{app}[Ca^{2+}]\ln(10)}{(K_{app} + [Ca^{2+}])^2} \qquad [72]$$

How far this can be applied to NTA is considered below.

BUFFERING BY NTA: DERIVATION OF BUFFERING POWER

Ca²⁺ bound to NTA is given by following equation:

$$[Ca-Bound\ ] = [Ca-NTA^{1-}] + [Ca-NTA_2^{2-}] \qquad [73]$$

From equations [20] and [21] it follows that:

$$[Ca-Bound\ ] = \frac{[Ca^{2+}]}{K_{NTA-1}}[NTA^{3-}] + \frac{[Ca^{2+}]}{K_{NTA-2}}[NTA^{3-}]^2 \qquad [74]$$

For simplicity, each term on the RHS is expanded independently.

1) [Ca-NTA¹⁻]
Substituting for [NTA³⁻] with equation [56] gives:

$$[Ca-NTA^{1-}] =$$
$$\frac{[Ca^{2+}]}{K_{NTA-1}}(\frac{-(K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}[Ca^{2+}]) + \sqrt{(K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}[Ca^{2+}])^2 + 8K_{NTA-1}^2 K_{NTA-2}[NTA]_T[Ca^{2+}]}}{4K_{NTA-1}[Ca^{2+}]})$$

$$[75]$$

which simplifies to:

$[Ca-NTA^{1-}] =$
$$\frac{-(K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}[Ca^{2+}]) + \sqrt{K_{NTA-1}^2 K_{NTA-2}^2 \alpha^2 + (2K_{NTA-1}K_{NTA-2}^2\alpha + 8K_{NTA-1}^2 K_{NTA-2}[NTA]_T)[Ca^{2+}] + K_{NTA-2}^2[Ca^{2+}]^2}}{4K_{NTA-1}^2}$$

[76]

and in terms of pCa:

$[Ca-NTA^{1-}] =$
$$\frac{-(K_{NTA-1}K_{NTA-2}\alpha + K_{NTA-2}10^{-pCa}) + \sqrt{K_{NTA-1}^2 K_{NTA-2}^2 \alpha^2 + (2K_{NTA-1}K_{NTA-2}^2\alpha + 8K_{NTA-1}^2 K_{NTA-2}[NTA]_T)10^{-pCa} + K_{NTA-2}^2 10^{-2pCa})}}{4K_{NTA-1}^2}$$

To simplify let [77]

$A = K_{NTA-1}K_{NTA-2}\alpha$ $\qquad$ $B = K_{NTA-2}$

$C = 8K_{NTA-1}^2 K_{NTA-2}[NTA]_T$ $\qquad$ $D = 4K_{NTA-1}^2$

Substituting in equation [76] gives:

$$[Ca-NTA^{1-}] = \frac{-(A + B[Ca^{2+}]) + \sqrt{A^2 + (2AB + C)[Ca^{2+}] + B^2[Ca^{2+}]^2}}{D}$$

[78]

or in pCa terms

$$[Ca-NTA^{1-}] = \frac{-(A + B*10^{-pCa}) + \sqrt{A^2 + (2AB + C)10^{-pCa} + B^2 10^{-2pCa}}}{D}$$

[79]

Note: For the differentiation, the following are useful:

$$\frac{d}{dx}(A*10^{-npCa}) = -nA*\ln(10)10^{-npCa} \quad \text{and} \quad \frac{d}{dx}(A\sqrt{u}) = \frac{A}{2\sqrt{u}}du$$

$$\frac{d}{dx}(\frac{u}{v}) = \frac{vdu - udv}{v^2} \quad \text{and} \quad \frac{d}{dx}(uv) = vdu + udv$$

Differentiation of equation [79] gives:

[80]

$$\frac{d[Ca-NTA^{1-}]}{dpCa} = -\ln(10)(\frac{B10^{-pCa}}{D} - \frac{\{(2AB + C)10^{-pCa} + 2B^2 10^{-2pCa}\}}{2D\sqrt{A^2 + (2AB + C)10^{-pCa} + B^2 10^{-2pCa}}})$$

2) Ca-NTA₂

From equation [74]

$$[Ca-NTA_2^{-2}] = \frac{[Ca^{2+}]}{K_{NTA-2}}[NTA^{3-}]^2$$

and substituting for [NTA³⁻] from equation [56] gives:

$$[Ca-NTA_2^{-2}] = \frac{[Ca^{2+}]}{K_{NTA-2}} * \{\frac{-(A+B[Ca^{2+}])+\sqrt{(A+B[Ca^{2+}])^2+C(A+B[Ca^{2+}])}}{4K_{NTA-1}[Ca^{2+}]}\}^2 \quad [81]$$

which simplifies to:

$$[Ca-NTA_2^{-2}] = \frac{2(A+B[Ca^{2+}])^2+C[Ca^{2+}]-2(A+B[Ca^{2+}])\sqrt{(A+B[Ca^{2+}])^2+C[Ca^{2+}]}}{E[Ca^{2+}]} \quad [82]$$

where:

$$E = 16K_{NTA-1}^2 K_{NTA-2}$$

or in terms of pCa

$$[Ca-NTA_2^{-2}] = \frac{2(A+B10^{-pCa})^2+C10^{-pCa}-2(A+B10^{-pCa})\sqrt{(A+B10^{-pCa})^2+C10^{-pCa}}}{E\,10^{-pCa}}$$

$$[83]$$

Differentiation of equation [82] gives:

$$\frac{d[Ca-NTA_2^{2-}]}{dpCa} = \ln(10)(\frac{10^{-pCa}\{\frac{2A^3}{10^{-3pCa}}+\frac{A}{10^{-2pCa}}(2AB+C)-\frac{B}{10^{-2pCa}}(2AB+C)-2B^3\}}{E\sqrt{(\frac{A^2}{10^{-2pCa}}+\frac{(2AB+C)}{10^{-pCa}}+B^2)}}$$

$$-\frac{2A^2}{E*10^{-pCa}}+\frac{2B^2*10^{-pCa}}{E})$$

$$[84]$$

3) Second derivative

Both equation [80] ($\frac{d[Ca-NTA^{1-}]}{dpCa}$) and equation [84] ($\frac{d[Ca-NTA_2^{2-}]}{dpCa}$) were differentiated using a mathematical computer program (Derive). However, the results were so unwieldy that the matter was not pursued further.

GENERAL CONCLUSIONS NTA

With NTA there are two forms of bound Ca^{2+} namely, Ca-NTA^{1-} and Ca-NTA$_2$. However, Ca-NTA$_2$ binding is only significant at pH values greater than 8.5 and because of this, Ca-NTA$_2$ binding at pH values less than 8.5 can be ignored. This valid approximation greatly simplifies the calculation of the [Ca^{2+}] in NTA buffer solutions as the calculations involve quadratic equations and not cubics. This simplification means that up to pH values of 8.5 NTA can be treated like any other buffer ligand. At more alkaline pH values this is *not* the case and the cubic equations developed in this section have to be applied.

REFERENCES

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