

SECTION-I

THE BINDING OF H^+ , Mg^{2+} , K^+ , Na^+ , Ca^{2+} AND POLYAMINES TO ATP;
ACID/BASE TITRATION AND DEFINITIONS OF BUFFERING

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ATP AND RELATED TOPICS

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ATP AND RELATED TOPICS

pH (ACTIVITY) AND pH (CONCENTRATION)

pH as measured, is defined as minus the logarithm of the hydrogen ion activity (a_{H^+}), not concentration

$$pH_a = -\log a_{H^+} \quad [1]$$

It is also possible to define pH in terms of concentration:

$$pH_c = -\log[H^+] \quad [2]$$

The relationship between the two is as follows:

$$pH_a = -\log[H^+]\gamma_{H^+} \quad [3]$$

and

$$pH_a = -\log[H^+] - \log\gamma_{H^+} \quad [4]$$

where γ_{H^+} is the single ion activity coefficient of the hydrogen ions.

$$pH_a + \log\gamma_{H^+} = pH_c, \text{ or } pH_c - \log\gamma_{H^+} = pH_a \quad [5][5a]$$

In other words, the measured value of pH_a has to be decreased by the factor $|\log\gamma_{H^+}|$ to convert it to pH_c , since $\log\gamma_{H^+}$ is negative. It also follows that:

$$[H^+] = 10^{-pH_c} \text{ and } a_{H^+} = 10^{-pH_a} \quad [6]/[7]$$

Measured pH values are in terms of H^+ activity, but equilibrium constants are usually tabulated in terms of H^+ concentration, necessitating the conversion of activity to concentration (McGuigan, Lüthi & Buri, 1991). One way of doing this is to use the equation in Harrison & Bers (1989) to calculate the H^+ activity coefficient at 20°C. This is:

$$\gamma_{H^+} = 0.144 \exp^{-5.147 * I} + .06352 \exp^{-44.168 * I} + 0.6971 \quad [8]$$

where I is the ionic strength. This activity coefficient can be converted to the desired temperature by the following equation from Baumgarten (1981):

$$\log\gamma_t = \frac{A_t I^{1/2} B_{20} \log\gamma_{20}}{A_{20} I^{1/2} B_t + \log\gamma_{20} B_t - B_{20} \log\gamma_{20}} \quad [9]$$

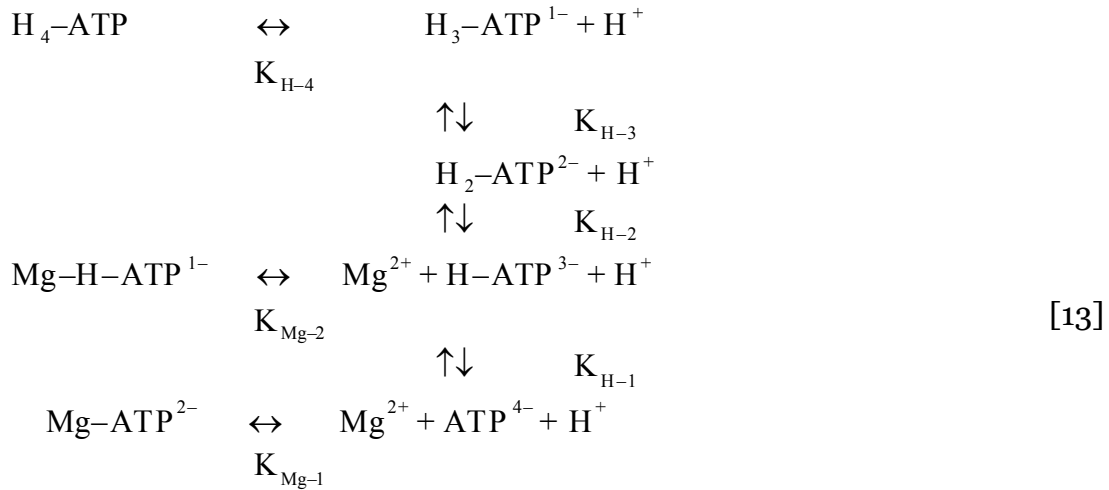
$$\text{where } A = \frac{1.8246 \cdot 10^6}{(DT)^{3/2}} \text{ and } B = \frac{50.29 \cdot 10^8}{(DT)^{1/2}} \quad [10]/[11]$$

and

$$D = 78.54 \{ 1 - 4.579 \cdot 10^{-3}(t - 25) + 1.19 \cdot 10^{-5}(t - 25)^2 - 2.8 \cdot 10^{-8}(t - 25)^3 \} \quad [12]$$

where t is temperature in °C. It should be noted that this calculates the mean activity coefficient. However, the Debye-Hückel convention sets the mean activity coefficient of a univalent ion equal to the single ion activity coefficient (McGuigan, Buri, Chen et al., 1993). Other methods to calculate the H^+ activity coefficient are described in Section II, page 49.

ATP H^+ AND Mg^{2+} BINDING (GENERAL)



EQUILIBRIUM BETWEEN ATP AND H^+

ATP binds H^+ and at equilibrium:

$$\frac{[H^+][ATP^{4-}]}{[H\text{-ATP}^{3-}]} = K_{H-1} \quad \frac{[H^+][H\text{-ATP}^{3-}]}{[H_2\text{-ATP}^{2-}]} = K_{H-2} \quad [14]/[15]$$

$$\frac{[H^+][H_2\text{-ATP}^{2-}]}{[H_3\text{-ATP}^{1-}]} = K_{H-3} \quad \frac{[H^+][H_3\text{-ATP}^{1-}]}{[H_4\text{-ATP}]} = K_{H-4} \quad [16]/[17]$$

Since H^+ -ions are expressed in concentration, these are stoichiometric constants.

STOICHIOMETRIC AND MIXED CONSTANTS

If a_{H^+} is used instead of $[H^+]$ this gives from equation [14]

$$\frac{a_{H^+}[ATP^{4-}]}{[H-ATP^{3-}]} = K_{H-1(M)} \quad \text{or} \quad \frac{10^{-pH_a}[ATP^{4-}]}{[H-ATP^{3-}]} = K_{H-1(M)} \quad [18]/[19]$$

and similarly with equations [15] to [17]. Since the proton concentration is in activity this is a mixed constant (denoted M); “mixed” because it contains both activity and concentrations.

K_{H-1} and $K_{H-1(M)}$ are related as follows:

$$\frac{([H^+]\gamma_{H^+})[ATP^{4-}]}{[H-ATP^{3-}]} = K_{H-1(M)} \quad [20]$$

$$\frac{[H^+][ATP^{4-}]}{[H-ATP^{3-}]} = \frac{K_{H-1(M)}}{\gamma_{H^+}} \quad [21]$$

so

$$K_{H-1} = \frac{K_{H-1(M)}}{\gamma_{H^+}} \quad [22]$$

pH as explained above is defined as H^+ -ion activity. In order to use tabulated stoichiometric constants, either pH has to be converted to concentration or stoichiometric constants have to be converted to mixed constants.

EQUILIBRIUM BETWEEN Mg^{2+} AND ATP

Two forms of ATP bind Mg^{2+} namely, ATP^{4-} and $H-ATP^{3-}$. At equilibrium this gives:

$$\frac{[Mg^{2+}][ATP^{4-}]}{[Mg-ATP^{2-}]} = K_{Mg-1} \quad [23]$$

$$\frac{[Mg^{2+}][H-ATP^{3-}]}{[Mg-H-ATP^{1-}]} = K_{Mg-2} \quad [24]$$

EQUILIBRIUM BETWEEN ATP AND OTHER CATIONS

In intracellular solutions, ATP can also bind univalent cations such as K⁺ and Na⁺. Assume binding of ATP and H-ATP to K⁺ to give the following equilibrium states:

$$\frac{[K^+][ATP^{4-}]}{[K-ATP^{3-}]} = K_{K-1} \quad [25]$$

$$\frac{[K^+][H-ATP^{3-}]}{[K-H-ATP^{2-}]} = K_{K-2} \quad [26]$$

and similarly for Na⁺.

ATP also binds Ca²⁺, to give the following equilibrium states:

$$\frac{[Ca^{2+}][ATP^{4-}]}{[Ca-ATP^{2-}]} = K_{Ca-1} \quad [27]$$

$$\frac{[Ca^{2+}][H-ATP^{3-}]}{[Ca-H-ATP^{1-}]} = K_{Ca-2} \quad [28]$$

However, in an intracellular-like physiological solution where the [Ca²⁺] is around 100 nmol/l this binding can be neglected.

DEFINITION OF pH_c AND pK_{eq} (K AS EQUILIBRIUM CONSTANT)

Since pH_c = -log[H⁺] a similar definition can be introduced for the equilibrium constant K_{Eq} namely,

$$pK_{eq} = -\log K_{eq} \quad \text{and} \quad 10^{-pK_{eq}} = K_{eq} \quad [29]/[30]$$

Note: A binding constant (K_{bind}) is defined as the reciprocal of the equilibrium constant ($\frac{1}{K_{eq}}$).

pK_{bind} = +log K_{bind} or $\log(\frac{1}{K_{eq}})$ or {log(1) - logK_{eq}}. Since log(1) is zero both pK_{eq} and pK_{bind} have the same *numerical* value.

FORMS OF ATP AS A FUNCTION OF pH

Introduction: From equations [14] to [17] and in the absence of Mg^{2+} and Ca^{2+} , the binding of H^+ to ATP can be expressed as follows:

$$[H-ATP^{3-}] = [ATP^{4-}] \frac{[H^+]}{K_{H-1}} \quad [31]$$

$$[H_2-ATP^{2-}] = [H-ATP^{3-}] \frac{[H^+]}{K_{H-2}} \text{ or } [H_2-ATP^{2-}] = [ATP^{4-}] \frac{[H^+]^2}{K_{H-1}K_{H-2}} \quad [32]/[33]$$

$$[H_3-ATP^{1-}] = [H_2-ATP^{2-}] \frac{[H^+]}{K_{H-3}} \text{ or } [H_3-ATP^{1-}] = [ATP^{4-}] \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \quad [34]/[35]$$

$$[H_4-ATP] = [H_3-ATP^{1-}] \frac{[H^+]}{K_{H-4}} \text{ or } [H_4-ATP] = [ATP^{4-}] \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \quad [36]/[37]$$

Since, $[ATP]_T = ([ATP^{4-}] + [H-ATP^{3-}] + [H_2-ATP^{2-}] + [H_3-ATP^{1-}] + [H_4-ATP])$

it follows from equations [31], [33], [35] and [37]:

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

and [38]

$$[ATP]_T = [ATP^{4-}] \left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right) \quad [39]$$

or

$$[ATP]_T = [ATP^{4-}] (1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)}) \quad [40]$$

It follows that:

1)

$$[ATP^{4-}] = \frac{[ATP]_T}{(1 + 10^{(pK_{H-1} - pH)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)})} \quad [41]$$

$$2) [H-ATP^{3-}] = [ATP^{4-}] 10^{(pK_{H-1} - pH)} \quad [42]$$

$$3) [H_2-ATP^{2-}] = [H-ATP^{3-}] 10^{(pK_{H-2} - pH)} \quad [43]$$

$$4) [H_3-ATP^{1-}] = [H_2-ATP^{2-}] 10^{(pK_{H-3} - pH)} \quad [44]$$

$$5) [H_4-ATP] = [H_3-ATP^{1-}] 10^{(pK_{H-4} - pH)} \quad [45]$$

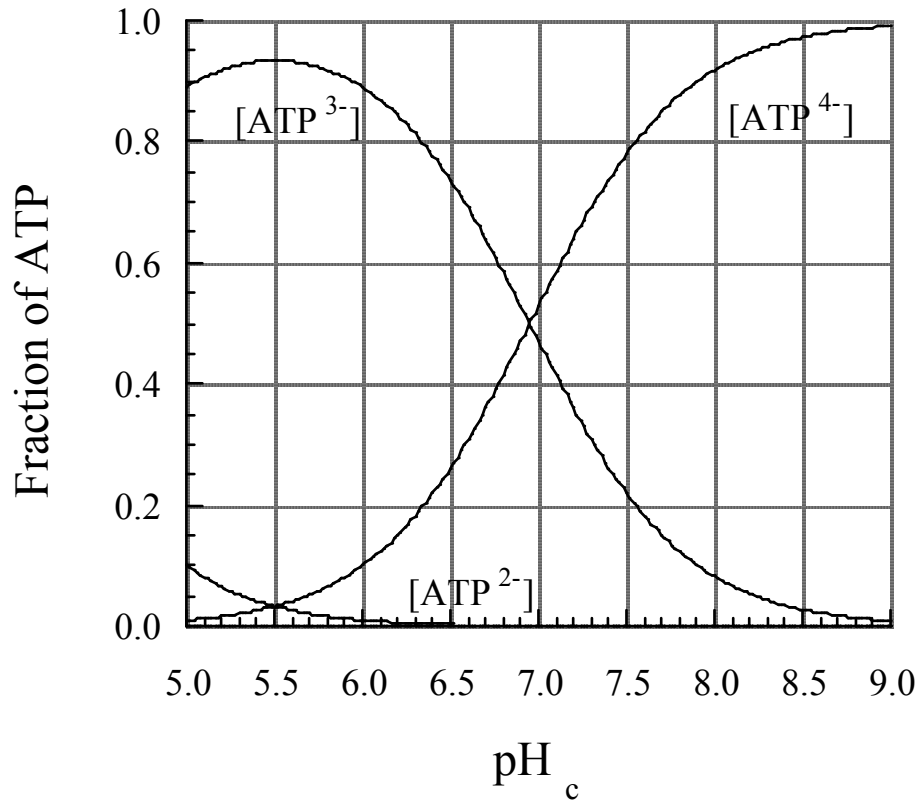
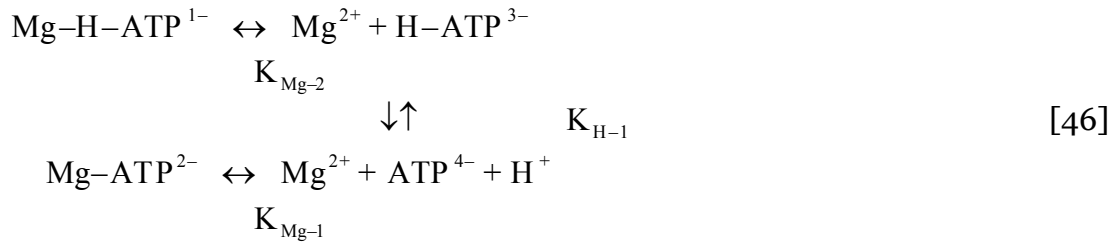


Figure 1: Calculated forms of ATP as the pH_c is varied from 5 to 9.

Equations [41] to [45] can be used to calculate the fraction of the various forms of ATP that are present at various pH_c values. Using the constants tabulated in Fabiato & Fabiato (1979); ionic strength 0.16 mol/l, temperature 22 °C, $\text{pK}_{\text{H-1}}$ 6.95 and $\text{pK}_{\text{H-2}}$ 4.05 the fraction of the various forms has been calculated. This is illustrated in Figure 1 and it is clear that at pH_c values from 5 to 9 the ATP is present in the forms ATP^{4-} and H-ATP^{3-} . This being so, equation [13] can be simplified to:



FORMS OF Mg^{2+} -ATP AS A FUNCTION OF pH

Equations [41] to [45] calculate the percentage of the forms of ATP present as the pH is varied. However, since the affinity of ATP^{4-} for Mg^{2+} is greater than that of H-ATP^{3-} it is of interest to calculate the amount of Mg^{2+} bound to each as the pH changes.

1) Total ATP Concentration

$$[\text{ATP}]_T = [\text{ATP}^{4-}] + [\text{H-ATP}^{3-}] + [\text{H}_2\text{-ATP}^{2-}] + [\text{H}_3\text{-ATP}^{1-}] + [\text{H}_4\text{-ATP}] + [\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}] \quad [47]$$

and

$$[\text{ATP}]_T = [\text{ATP}^{4-}] \left(1 + \frac{[\text{H-ATP}^{3-}]}{[\text{ATP}^{4-}]} + \frac{[\text{H}_2\text{-ATP}^{2-}]}{[\text{ATP}^{4-}]} + \frac{[\text{H}_3\text{-ATP}^{1-}]}{[\text{ATP}^{4-}]} + \frac{[\text{H}_4\text{-ATP}]}{[\text{ATP}^{4-}]} \right) + [\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}] \quad [48]$$

from equations [31] to [37]:

$$[\text{ATP}]_T = [\text{ATP}^{4-}] \alpha + [\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}] \quad [49]$$

where:

$$\alpha = \left(1 + \frac{[\text{H}^+]}{K_{\text{H-1}}} + \frac{[\text{H}^+]^2}{K_{\text{H-1}}K_{\text{H-2}}} + \frac{[\text{H}^+]^3}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}} + \frac{[\text{H}^+]^4}{K_{\text{H-1}}K_{\text{H-2}}K_{\text{H-3}}K_{\text{H-4}}} \right) \quad [50]$$

2) Total Magnesium Concentration

$$[\text{Mg}]_T = ([\text{Mg}^{2+}] + [\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}]) \quad [51]$$

3) Ratio of $[\text{Mg-H-ATP}]$ to $[\text{Mg-ATP}^{2-}]$

Eliminating $[\text{H-ATP}^{3-}]$ from equation [24] by use of equation [14] gives:

$$\frac{[\text{Mg}^{2+}][\text{H}^+][\text{ATP}^{4-}]}{K_{\text{H-1}}[\text{Mg-H-ATP}^{1-}]} = K_{\text{Mg-2}} \quad [52]$$

Taking the ratio of equation [23] and [52] gives:

$$\frac{[\text{Mg}^{2+}][\text{ATP}^{4-}]}{[\text{Mg-ATP}^{2-}]} \frac{[\text{Mg-H-ATP}^{1-}]K_{\text{H-1}}}{[\text{Mg}^{2+}][\text{H}^+][\text{ATP}^{4-}]} = \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \quad [53]$$

which gives:

$$\frac{[\text{Mg-H-ATP}^{1-}]}{[\text{Mg-ATP}^{2-}]} = \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}} \quad [54]$$

or in terms of pH and pK

$$\frac{[\text{Mg-H-ATP}^{1-}]}{[\text{Mg-ATP}^{2-}]} = 10^{(pK_{\text{Mg-2}} - pK_{\text{Mg-1}})} 10^{(pK_{\text{H-1}} - p\text{H}_o)} \quad [55]$$

4) $[\text{Mg-ATP}^{2-}]$

From equations [51] and [54] we get:

$$[\text{Mg}]_{\text{T}} = ([\text{Mg}^{2+}] + [\text{Mg-ATP}^{2-}] + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}} [\text{Mg-ATP}^{2-}]) \quad [56]$$

Using equation [23] to eliminate $[\text{Mg}^{2+}]$ and rearranging gives:

$$[\text{Mg-ATP}^{2-}](1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}}) + \frac{K_{\text{Mg-1}}[\text{Mg-ATP}^{2-}]}{[\text{ATP}^{4-}]} - [\text{Mg}]_{\text{T}} = 0 \quad [57]$$

but from equation [49]

$$[\text{ATP}^{4-}]\alpha = [\text{ATP}]_{\text{T}} - ([\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}]) \quad [58]$$

Using equation [54] gives:

$$[\text{ATP}^{4-}]\alpha = [\text{ATP}]_{\text{T}} - [\text{Mg-ATP}^{2-}](1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}}) \quad [59]$$

Let:

$$\beta = (1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}}) \quad [60]$$

and

$$[\text{ATP}^{4-}] = \frac{([\text{ATP}]_{\text{T}} - [\text{Mg-ATP}^{2-}]\beta)}{\alpha} \quad [61]$$

and substituting for $[\text{ATP}^{4-}]$ in [57] gives:

$$[\text{Mg-ATP}^{2-}]^2 - (\frac{[\text{ATP}]_{\text{T}}}{\beta} + \frac{[\text{Mg}]_{\text{T}}}{\beta} + \frac{K_{\text{Mg-1}}\alpha}{\beta^2})[\text{Mg-ATP}^{2-}] + \frac{[\text{ATP}]_{\text{T}}[\text{Mg}]_{\text{T}}}{\beta^2} = 0 \quad [62]$$

which on solution gives

$$[\text{Mg-ATP}^{2-}] = \frac{A - \sqrt{A^2 - 4 \frac{[\text{ATP}]_{\text{T}}[\text{Mg}]_{\text{T}}}{\beta^2}}}{2} \quad [63]$$

where

$$A = \left(\frac{[ATP]_T}{\beta} + \frac{[Mg]_T}{\beta} + \frac{K_{Mg-1}\alpha}{\beta^2} \right) \quad [64]$$

5) $[Mg-H-ATP^{1-}]$

From equations [51] and [54] we get:

$$[Mg]_T = [Mg^{2+}] + [Mg-H-ATP^{1-}]\left(1 + \frac{K_{Mg-2}}{K_{Mg-1}} \frac{K_{H-1}}{[H^+]}\right) \quad [65]$$

Let:

$$\gamma = \left(1 + \frac{K_{Mg-2}}{K_{Mg-1}} \frac{K_{H-1}}{[H^+]}\right) \quad [66]$$

and

$$[Mg]_T = [Mg^{2+}] + [Mg-H-ATP^{1-}]\gamma \quad [67]$$

or

$$[Mg^{2+}] + [Mg-H-ATP^{1-}]\gamma - [Mg]_T = 0 \quad [68]$$

From equations [23] and [24]:

$$[Mg^{2+}] = \frac{K_{H-1}}{[H^+]} \frac{K_{Mg-2}}{[ATP^{4-}]} [Mg-H-ATP^{1-}] \quad [69]$$

From equation [58]:

$$[ATP^{4-}]\alpha = [ATP]_T - [Mg-H-ATP^{1-}]\gamma \quad [70]$$

and

$$[ATP^{4-}] = \frac{[ATP]_T - [Mg-H-ATP^{1-}]\gamma}{\alpha} \quad [71]$$

substituting equations [69] in [68] gives:

$$\frac{K_{H-1}}{[H^+]} \frac{K_{Mg-2}}{[ATP^{4-}]} [Mg-H-ATP^{1-}] + [Mg-H-ATP^{1-}]\gamma - [Mg]_T = 0 \quad [72]$$

substituting [71] in [72] gives:

$$\frac{K_{H-1}}{[H^+]} \frac{K_{Mg-2}}{([ATP]_T - [Mg-H-ATP^{1-}])\alpha} [Mg-H-ATP^{1-}] + [Mg-H-ATP^{1-}]\gamma - [Mg]_T = 0 \quad [73]$$

this simplifies to:

$$[Mg-H-ATP^{1-}]^2 - \left(\frac{[ATP]_T}{\gamma} + \frac{[Mg]_T}{\gamma} + \frac{K_{H-1}K_{Mg-2}\alpha}{[H^+]\gamma^2} \right) [Mg-H-ATP^{1-}] + \frac{[ATP]_T[Mg]_T}{\gamma^2} = 0 \quad [74]$$

and the solution to the quadratic is similar to equation [62], namely

$$[Mg-H-ATP^{2-}] = \frac{B - \sqrt{B^2 - 4 \frac{[ATP]_T[Mg]_T}{\gamma^2}}}{2} \quad [75]$$

where

$$B = \left(\frac{[ATP]_T}{\gamma} + \frac{[Mg]_T}{\gamma} + \frac{K_{H-1}K_{Mg-2}\alpha}{[H^+]\gamma^2} \right) \quad [76]$$

6) $[Mg^{2+}]$

$$[Mg^{2+}] = ([Mg]_T - [Mg-ATP^{2-}] - [Mg-H-ATP^{1-}]) \quad [77]$$

using equation [54] gives:

$$[Mg^{2+}] = ([Mg]_T - [Mg-ATP^{2-}]) \left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}} \right) \quad [78]$$

or

$$[Mg^{2+}] = ([Mg]_T - [Mg-ATP^{2-}])\beta \quad [79]$$

From equations [23] and [61]:

$$[Mg-ATP^{2-}] = \frac{[Mg^{2+}][ATP]_T}{(K_{Mg-1}\alpha + [Mg^{2+}]\beta)} \quad [80]$$

substituting in equation [79] and rearranging gives the following:

$$[Mg^{2+}]^2 + \left(\frac{K_{Mg-1}\alpha}{\beta} + [ATP]_T - [Mg]_T \right) [Mg^{2+}] - \frac{[Mg]_T K_{Mg-1}\alpha}{\beta} = 0 \quad [81]$$

The solution of the quadratic is:

$$[\text{Mg}^{2+}] = \frac{-C + \sqrt{C^2 + 4 \frac{[\text{Mg}]_T K_{\text{Mg-1}} \alpha}{\beta}}}{2} \quad [82]$$

where

$$C = \left(\frac{K_{\text{Mg-1}} \alpha}{\beta} + [\text{ATP}]_T - [\text{Mg}]_T \right) \quad [83]$$

where:

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

and

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

equations [50] and [60] respectively.

Since $\frac{K_{\text{Mg-1}} \alpha}{\beta} = K_{\text{app}}$ (equation [119]) equation [81] becomes on substitution

$$[\text{Mg}^{2+}] = \frac{-(K_{\text{app}} + [\text{ATP}]_T - [\text{Mg}]_T) + \sqrt{(K_{\text{app}} + [\text{ATP}]_T - [\text{Mg}]_T)^2 + 4[\text{Mg}]_T K_{\text{app}}}}{2} \quad [84]$$

K_{app} is the apparent equilibrium constant. Since $K_{\text{app}} = \frac{1}{K_{\text{app-bind}}}$ substitution in equation [84] gives the following:

$$[\text{Mg}^{2+}] = \frac{-\left(\frac{1}{K_{\text{app-bind}}} + [\text{ATP}]_T - [\text{Mg}]_T\right) + \sqrt{\left(\frac{1}{K_{\text{app-bind}}} + [\text{ATP}]_T - [\text{Mg}]_T\right)^2 + 4[\text{Mg}]_T \frac{1}{K_{\text{app-bind}}}}}{2} \quad [85]$$

which on simplification gives:

$$[\text{Mg}^{2+}] = \frac{-D + \sqrt{D^2 + 4[\text{Mg}]_T K_{\text{app-bind}}}}{2K_{\text{app-bind}}} \quad [86]$$

where

$$D = (1 + [\text{ATP}]_T K_{\text{app-bind}} - [\text{Mg}]_T K_{\text{app-bind}}) \quad [87]$$

This solution is similar to that in McGuigan, Buri, Chen et al. (1993).

7, $[\text{ATP}^{4-}]$, $[\text{H}-\text{ATP}^{3-}]$, $[\text{H}_2-\text{ATP}^{2-}]$, $[\text{H}_3-\text{ATP}^{1-}]$, $[\text{H}_4-\text{ATP}]$

From equations [59] and [60]

$$[\text{ATP}^{4-}]\alpha = [\text{ATP}]_{\text{T}} - [\text{Mg}-\text{ATP}^{2-}]\beta \quad [88]$$

and from equation [56] and [60]

$$[\text{Mg}]_{\text{T}} = [\text{Mg}^{2+}] + [\text{Mg}-\text{ATP}^{2-}]\beta \quad [89]$$

or

$$[\text{Mg}^{2+}] = [\text{Mg}]_{\text{T}} - [\text{Mg}-\text{ATP}^{2-}]\beta \quad [90]$$

Substituting [90] in equation [23] gives:

$$\frac{([\text{Mg}]_{\text{T}} - [\text{Mg}-\text{ATP}^{2-}]\beta)[\text{ATP}^{4-}]}{[\text{Mg}-\text{ATP}^{2-}]} = K_{\text{Mg-1}} \quad [91]$$

which on simplification gives:

$$[\text{Mg}-\text{ATP}^{2-}] = \frac{[\text{Mg}]_{\text{T}}[\text{ATP}^{4-}]}{(K_{\text{Mg-1}} + \beta[\text{ATP}^{4-}])} \quad [92]$$

Substitution in equation [88] gives:

$$[\text{ATP}^{4-}]\alpha = [\text{ATP}]_{\text{T}} - \frac{[\text{Mg}]_{\text{T}}[\text{ATP}^{4-}]}{(K_{\text{Mg-1}} + \beta[\text{ATP}^{4-}])}\beta \quad [93]$$

Solving for $[\text{ATP}^{4-}]$ gives:

$$[\text{ATP}^{4-}]^2 + \left(\frac{K_{\text{Mg-1}}}{\beta} + \frac{[\text{Mg}]_{\text{T}}}{\alpha} - \frac{[\text{ATP}]_{\text{T}}}{\alpha}\right)[\text{ATP}^{4-}] - \frac{K_{\text{Mg-1}}[\text{ATP}]_{\text{T}}}{\alpha\beta} = 0 \quad [94]$$

Since $\frac{K_{\text{Mg-1}}\alpha}{\beta} = K_{\text{app}}$ (see equation [119]) it follows that:

$$[\text{ATP}^{4-}]^2 + \left(\frac{K_{\text{app}}}{\alpha} + \frac{[\text{Mg}]_{\text{T}}}{\alpha} - \frac{[\text{ATP}]_{\text{T}}}{\alpha}\right)[\text{ATP}^{4-}] - \frac{K_{\text{app}}[\text{ATP}]_{\text{T}}}{\alpha^2} = 0 \quad [95]$$

The solution to the quadratic is:

$$[ATP^{4-}] = \frac{-E + \sqrt{E^2 + 4 \frac{K_{app} [ATP]_T}{\alpha^2}}}{2} \quad [96]$$

where

$$E = \frac{(K_{app} + [Mg]_T - [ATP]_T)}{\alpha} \quad [97]$$

The protonated forms follow from [42] to [45],

$$[H-ATP^{3-}] = [ATP^{4-}] * 10^{(pK_{H-1} - pH)} \quad [98]$$

$$[H_2-ATP^{2-}] = [ATP^{4-}] * 10^{(pK_{H-1} + pK_{H-2} - 2pH)} \quad [99]$$

$$[H_3-ATP^{1-}] = [ATP^{4-}] * 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} - 3pH)} \quad [100]$$

$$[H_4-ATP] = [ATP^{4-}] * 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH)} \quad [101]$$

8) $[Mg-ATP^{2-}] + [Mg-H-ATP^{1-}]$ or $[Mg-ATP]_B$ in terms of K_{app}

From equation [51]:

$$[Mg-ATP]_B = [Mg]_T - [Mg^{2+}] \quad [102]$$

From equation [115]:

$$[Mg^{2+}] = \frac{K_{app} [Mg-ATP]_B}{[ATP]_F} \quad [103]$$

Substituting equation [103] in equation [102] gives:

$$[Mg-ATP]_B = [Mg]_T - \frac{K_{app} [Mg-ATP]_B}{[ATP]_F} \quad [104]$$

but

$$[ATP]_F = [ATP]_T - [Mg-ATP]_B \quad [105]$$

Substituting in equation [105] in [104] gives:

$$[Mg-ATP]_B = [Mg]_T - \frac{K_{app} [Mg-ATP]_B}{([ATP]_T - [Mg-ATP]_B)} \quad [106]$$

solving for $[\text{Mg-ATP}]_B$ gives:

$$[\text{Mg-ATP}]_B^2 - ([\text{ATP}]_T + [\text{Mg}]_T + K_{\text{app}})[\text{Mg-ATP}]_B + [\text{Mg}]_T[\text{ATP}]_T = 0 \quad [107]$$

The solution is

$$[\text{Mg-ATP}]_B = \frac{F - \sqrt{F^2 - 4[\text{Mg}]_T[\text{ATP}]_T}}{2} \quad [108]$$

where

$$F = ([\text{ATP}]_T + [\text{Mg}]_T + K_{\text{app}}) \quad [109]$$

APPARENT CONSTANT (K_{app})

Introduction: Because of the binding of H^+ and possibly also other cations such as K^+ to ATP^{4-} and $H-ATP^{3-}$, and because Mg^{2+} binds to both ATP^{4-} and $H-ATP^{3-}$ the concept of an apparent constant has been introduced. In this constant all forms of ATP that bind Mg^{2+} are lumped together as “bound” Mg^{2+} , $[Mg-ATP]_B$. All forms of ATP not bound to Mg^{2+} are regarded as the “free form” of ATP or $[ATP]_F$. This gives the following equilibrium

$$\frac{[Mg^{2+}][ATP^{4-}] + [H-ATP^{3-}] + [H_2-ATP^{2-}] + [H_3-ATP^{1-}] + [H_4-ATP] + [K-ATP^{3-}] + [K-H-ATP^{2-}]}{([Mg-ATP^{2-}] + [Mg-H-ATP^{1-}])} = K_{app} \quad [110]$$

but from equations [48] and [50]:

$$[ATP^{4-}] + [H-ATP^{3-}] + [H_2-ATP^{2-}] + [H_3-ATP^{1-}] + [H_4-ATP] = [ATP^{1-}]\alpha \quad [111]$$

it follows that equation [110] simplifies to:

$$\frac{[Mg^{2+}]([ATP^{4-}]\alpha + [K-ATP^{3-}] + [K-H-ATP^{2-}])}{([Mg-ATP^{2-}] + [Mg-H-ATP^{1-}])} = K_{app} \quad [112]$$

but, by definition

$$[ATP]_F = ([ATP^{4-}]\alpha + [K-ATP^{3-}] + [K-H-ATP^{2-}]) \quad [113]$$

and

$$[Mg-ATP]_B = ([Mg-ATP^{2-}] + [Mg-H-ATP^{1-}]) \quad [114]$$

Substituting equations [113] and [114] in equation [112] gives:

$$\frac{[Mg^{2+}][ATP]_F}{[Mg-ATP]_B} = K_{app} \quad [115]$$

and this is the constant that is measured in the appropriate solutions.

CALCULATION OF K_{app} FROM THE VARIOUS EQUILIBRIUM CONSTANTS

From equation [112] it follows that:

$$\frac{[\text{Mg}^{2+}]}{[\text{Mg-ATP}^{2-}]} \frac{([\text{ATP}^{4-}]\alpha + [\text{K-ATP}^{3-}] + [\text{K-H-ATP}^{2-}])}{(1 + \frac{[\text{Mg-H-ATP}^{1-}]}{[\text{Mg-ATP}^{2-}]})} = K_{\text{app}} \quad [116]$$

or

$$\frac{[\text{Mg}^{2+}][\text{ATP}^{4-}]}{[\text{Mg-ATP}^{2-}]} * \frac{(\alpha + \frac{[\text{K-ATP}^{3-}]}{[\text{ATP}^{4-}]} + \frac{[\text{K-H-ATP}^{2-}]}{[\text{ATP}^{4-}]})}{(1 + \frac{[\text{Mg-H-ATP}^{1-}]}{[\text{Mg-ATP}^{2-}]})} = K_{\text{app}} \quad [117]$$

From equation [23] and [54], it follows that

$$\frac{K_{\text{Mg-1}}(\alpha + \frac{[\text{K-ATP}^{3-}]}{[\text{ATP}^{4-}]} + \frac{[\text{K-H-ATP}^{2-}]}{[\text{ATP}^{4-}]})}{(1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}})} = K_{\text{app}} \quad [118]$$

or

$$\frac{K_{\text{Mg-1}}(\alpha + \frac{[\text{K}^+]}{K_{\text{K-1}}} + \frac{[\text{K}^+]}{K_{\text{K-2}}})}{(1 + \frac{K_{\text{Mg-1}}}{K_{\text{Mg-2}}} \frac{[\text{H}^+]}{K_{\text{H-1}}})} = K_{\text{app}} \quad [119]$$

Equation [119] follows from the definition of the equilibrium constants (equations [14], [25], [26] and [54]). Equation [119] can also be written as:

$$\frac{10^{-pK_{\text{Mg-1}}} \{\alpha + [\text{K}^+](10^{pK_{\text{K-1}}} + 10^{pK_{\text{K-2}}})\}}{(1 + 10^{(pK_{\text{Mg-2}} - pK_{\text{Mg-1}})}) 10^{(pK_{\text{H-1}} - \text{pH}_c)})} = K_{\text{app}} \quad [120]$$

COMMENTS ON THE K_{app} CALCULATIONS

1) Including binding to cations such as K⁺ (equation [120]): This is the basic equation and it predicts the change of the apparent constant at each pH_c. The full equation contains the binding to cations such as K⁺. In the equation the term, ($[K^+]10^{pK_{K-1}} + [K^+]10^{pK_{K-2}}$) can as a first approximation be regarded as a constant. This arises because the binding to K⁺, normal concentration around 140 mmol/l is low and the free concentration of K⁺ can be regarded as a constant over the range of [Mg]_T used in buffer solution. This means that equation [120] can be written as:

$$\frac{10^{-pK_{Mg-1}}(\alpha + A)}{(1 + 10^{(pK_{Mg-2} - pK_{Mg-1})}10^{(pK_{H-1} - pH_c)})} = K_{app} \quad [121]$$

where A is a constant. The constant A also includes the binding to other cations other than K⁺ such as Na⁺.

2) No binding to cations such as K⁺: If there is no binding to K⁺ and/or Na⁺, or it is so small that it can be ignored the equation simplifies to:

$$\frac{K_{Mg-1}\alpha}{\beta} = K_{app} \quad [122]$$

This arises because if there is no binding then K_{K-1} and K_{K-2} tend to infinity and the K⁺ terms tends to zero (see equation [119]).

3) No binding of Mg²⁺ to H-ATP³⁻: The third simplification is if K_{Mg-2} also tends to infinity i.e. there is no binding of Mg²⁺ to H-ATP³⁻. Under these conditions the equation becomes:

$$K_{Mg-1}\alpha = K_{app} \quad [123]$$

PREDICTION OF K_{app} AT DIFFERENT pH_c VALUES

If K_{app} has been measured at pH_c of 7.2, then from equation [123] and ignoring H₂-ATP²⁻, H₃-ATP¹⁻ and H₄-ATP:

$$K_{Mg-1}(1 + 10^{(pK_{H-1} - 7.2)}) = K_{app}(7.2) \quad [124]$$

and

$$K_{Mg-1}(1 + 10^{(pK_{H-1} - pH_c)}) = K_{app}(pH_c) \quad [125]$$

it follows:

$$\frac{K_{Mg-1}(1 + 10^{(pK_{H-1} - 7.2)})}{K_{Mg-1}(1 + 10^{(pK_{H-1} - pH_c)})} = \frac{K_{app}(7.2)}{K_{app}(pH_c)} \quad [126]$$

and

$$K_{app}(7.2) \frac{(1 + 10^{(pK_{H-1} - pH_c)})}{(1 + 10^{(pK_{H-1} - 7.2)})} = K_{app}(pH_c) \quad [127]$$

This is the Bock equation (Bock, Wenz & Gupta, 1985). However, it is only valid if H-ATP³⁻ does not bind Mg²⁺ and H₂-ATP²⁻, H₃-ATP¹⁻ and H₄-ATP can be ignored. Moreover, this assumes no binding of K⁺ to ATP. To account for the binding of Mg²⁺ to H-ATP³⁻, K⁺ to ATP⁴⁻ and H₂-ATP²⁻, H₃-ATP¹⁻ and H₄-ATP the following equation has to be used:

$$K_{app}(7.2) \frac{(\alpha_{pH_c} + [K^+] 10^{pK_{K-1}})}{(\alpha_{7.2} + [K^+] 10^{pK_{K-1}})} * \frac{(1 + \frac{K_{Mg-1}}{K_{Mg-2}} 10^{(pK_{H-1} - 7.2)})}{(1 + \frac{K_{Mg-1}}{K_{Mg-2}} 10^{(pK_{H-1} - pH_c)})} = K_{app}(pH_c) \quad [128]$$

Since these equations are in terms of pH_c it necessitates the conversion of pH_a to pH_c.

[Mg]_T AND [ATP]_T AT A GIVEN [Mg²⁺] IN TERMS OF K_{app}

Equation [115] defines K_{app} as follows:

$$\frac{[\text{Mg}^{2+}][\text{ATP}]_F}{[\text{Mg-ATP}]_B} = K_{\text{app}}$$

where $[\text{ATP}]_F$ represents all forms of ATP not bound to Mg and $[\text{Mg-ATP}]_B$ represents all forms of Mg bound to ATP. From this definition it follows that:

$$[\text{ATP}]_F + [\text{Mg-ATP}]_B = [\text{ATP}]_T \quad \text{and} \quad [\text{Mg-ATP}]_B + [\text{Mg}^{2+}] = [\text{Mg}]_T \quad [129]/[130]$$

$$[\text{Mg-ATP}]_B = [\text{Mg}]_T - [\text{Mg}^{2+}] \quad [131]$$

$$[\text{ATP}]_F = [\text{ATP}]_T - [\text{Mg-ATP}]_B \quad [132]$$

$$[\text{ATP}]_F = [\text{ATP}]_T - ([\text{Mg}]_T - [\text{Mg}^{2+}]) \quad [133]$$

Substituting equation [133] in equation [115] gives:

$$\frac{[\text{Mg}^{2+}]([\text{ATP}]_T - [\text{Mg}]_T + [\text{Mg}^{2+}])}{([\text{Mg}]_T - [\text{Mg}^{2+}])} = K_{\text{app}} \quad [134]$$

From equation [134] the following can be derived.

1) $[\text{Mg}]_T$

$$[\text{Mg}]_T = \frac{[\text{Mg}^{2+}]([\text{ATP}]_T + K_{\text{app}} + [\text{Mg}^{2+}])}{(K_{\text{app}} + [\text{Mg}^{2+}])} \quad [135]$$

This can also be written:

$$[\text{Mg}]_T = \frac{[\text{Mg}^{2+}][\text{ATP}]_T}{(K_{\text{app}} + [\text{Mg}^{2+}])} + \frac{[\text{Mg}^{2+}](K_{\text{app}} + [\text{Mg}^{2+}])}{(K_{\text{app}} + [\text{Mg}^{2+}])} \quad [136]$$

which gives:

$$[\text{Mg}]_T = \frac{[\text{Mg}^{2+}][\text{ATP}]_T}{(K_{\text{app}} + [\text{Mg}^{2+}])} + [\text{Mg}^{2+}] \quad [137]$$

since $K_{\text{app}} = 10^{-pK}$ and $[\text{Mg}^{2+}] = 10^{-pMg}$ and in mmolar concentrations,

$$[\text{Mg}]_T = \frac{1000 * 10^{-pMg} [\text{ATP}]_T}{(1000 * 10^{-pK} + 1000 * 10^{-pMg})} + 1000 * 10^{-pMg} \quad [138]$$

$$[\text{Mg}]_T = \frac{10^{-pMg} [\text{ATP}]_T}{(10^{-pK} + 10^{-pMg})} + 1000 * 10^{-pMg} \quad [139]$$

which is similar to equation [1.71] in McGuigan, Lüthi & Buri (1991).

2) $[Mg-ATP]_B$

The amount of Mg^{2+} bound to ATP, $([Mg-ATP]_B)$ is:

$$[Mg]_T - 1000 * 10^{-pMg} = [Mg-ATP]_B \quad [140]$$

it follows that:

$$[Mg-ATP]_B = \frac{10^{-pMg} [ATP]_T}{(10^{-pK} + 10^{-pMg})} \quad [141]$$

3) $[ATP]_T$

$$[ATP]_T = \frac{([Mg]_T - [Mg^{2+}])(K_{app} + [Mg^{2+}])}{[Mg^{2+}]} \quad [142]$$

4) K_{app} at a given $[Mg]_T$ and $[ATP]_T$

This is simply equation [134], namely:

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

TOTAL H^+ BOUND TO ATP

From equation [197], page 30, for the binding of four H^+ ions to ATP

$$[H]_T = \frac{[ATP]_T \left(\frac{[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{3[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{4[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right)}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right)} + [H^+] \quad [143]$$

or in terms of pH_c and pK

$$[H]_T = \frac{[ATP]_T (10^{(pK_{H-1} - pH_c)} + 2 * 10^{(pK_{H-1} + pK_{H-2} - 2pH_c)} + \dots + 4 * 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH_c)})}{(1 + 10^{(pK_{H-1} - pH_c)} + 10^{(pK_{H-1} + pK_{H-2} - 2pH_c)} + \dots + 10^{(pK_{H-1} + pK_{H-2} + pK_{H-3} + pK_{H-4} - 4pH_c)})} + 10^{-pH_c} \quad [144]$$

SIMULTANEOUS BINDING OF Mg^{2+} AND A CATION TO ATP

Introduction: ATP binds not only Mg^{2+} , but can bind K^+ , Na^+ and polyamines. The solution for the simultaneous binding of Mg^{2+} and one other cation ion, X^{n+} , to ATP gives

a cubic equation. The derivation is similar to the binding of Ca^{2+} , Mg^{2+} and H^+ to EGTA and the equations are derived in Section II, page 35 and only the results are presented here.

Definitions:

$$\frac{[\text{Mg}^{2+}][\text{ATP}]_F}{[\text{Mg-ATP}]_B} = K_{\text{Mg-app}} \quad [145]$$

and

$$\frac{[\text{X}^{n+}][\text{ATP}]_F}{[\text{X-ATP}]_B} = K_{\text{X-app}} \quad [146]$$

In equations [145] and [146], $[\text{ATP}]_F$ represents ATP that is neither bound to Mg^{2+} or to X^{n+} . It is defined as follows:

$$[\text{ATP}]_F = ([\text{ATP}^{4-}] + [\text{H-ATP}^{3-}] + \dots + [\text{H}_4\text{-ATP}] + \dots [\text{Y-ATP}^{n-4}]) \quad [147]$$

$[\text{Y-ATP}^{n-4}]$ represents ATP binding to cations that are neither Mg^{2+} nor X^{n+} .

$[\text{Mg-ATP}]_B$, represents the sum of $([\text{Mg-ATP}^{2-}] + [\text{Mg-H-ATP}^{1-}])$ and $[\text{X-ATP}]_B$ the X^{n+} bound to ATP.

Equations

1) $[\text{Mg}^{2+}]$

$$A[\text{Mg}^{2+}]^3 + B[\text{Mg}^{2+}]^2 + C[\text{Mg}^{2+}] + D = 0 \quad [148]$$

where

$$A = 1$$

$$B = \frac{[\text{ATP}]_T(K_{\text{Mg-app}} - K_{\text{X-app}}) + [\text{Mg}]_T(K_{\text{X-app}} - 2K_{\text{Mg-app}}) - [\text{X}]_T K_{\text{Mg-app}} - K_{\text{Mg-app}} K_{\text{X-app}} + K_{\text{Mg-app}}^2}{(K_{\text{Mg-app}} - K_{\text{X-app}})}$$

$$C = \frac{[\text{Mg}]_T K_{\text{Mg-app}} ([\text{Mg}]_T + [\text{X}]_T) + [\text{Mg}]_T K_{\text{Mg-app}} (K_{\text{X-app}} - 2K_{\text{Mg-app}}) - [\text{Mg}]_T [\text{ATP}]_T K_{\text{Mg-app}}}{(K_{\text{Mg-app}} - K_{\text{X-app}})}$$

$$D = \frac{[\text{Mg}]_T^2 K_{\text{Mg-app}}^2}{(K_{\text{Mg-app}} - K_{\text{X-app}})}$$

2) $[\text{X}^{n+}]$

$$A[\text{X}^{n+}]^3 + B[\text{X}^{n+}]^2 + C[\text{X}^{n+}] + D = 0 \quad [149]$$

where:

$$A = 1$$

$$B = \frac{[ATP]_T(K_{X-app} - K_{Mg-app}) + [X]_T(K_{Mg-app} - 2K_{X-app}) - [Mg]_T K_{X-app} - K_{Mg-app} K_{X-app} + K_{X-app}^2}{(K_{X-app} - K_{Mg-app})}$$

$$C = \frac{[X]_T K_{X-app} ([X]_T + [Mg]_T) + [X]_T K_{X-app} (K_{Mg-app} - 2K_{X-app}) - [X]_T [ATP]_T K_{X-app}}{(K_{X-app} - K_{Mg-app})}$$

$$D = \frac{K_{X-app}^2 [X]_T^2}{(K_{X-app} - K_{Mg-app})}$$

3) [ATP]_F

$$A[ATP]_F^3 + B[ATP]_F^2 + C[ATP]_F + D = 0 \quad [150]$$

where:

$$A = 1$$

$$B = [Mg]_T + [X]_T - [ATP]_T + K_{Mg-app} + K_{X-app}$$

$$C = [Mg]_T K_{X-app} + [X]_T K_{Mg-app} - [ATP]_T (K_{Mg-app} + K_{X-app}) + K_{Mg-app} K_{X-app}$$

$$D = -[ATP]_T K_{Mg-app} K_{X-app}$$

From these estimations the following can be calculated.

4) [Mg-ATP]_B

$$[Mg-ATP]_B = \frac{[Mg^{2+}][ATP]_F}{K_{Mg-app}} \quad [151]$$

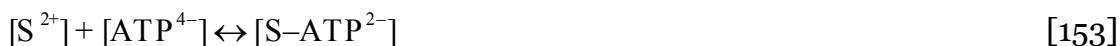
5) [X-ATP]_B

$$[X-ATP]_B = \frac{[X^{n+}][ATP]_F}{K_{X-app}} \quad [152]$$

BINDING OF POLYAMINES TO ATP IN THE PRESENCE OF Mg

Binding of Spermine to ATP

If the assumption is made that spermine ([S²⁺]) binds like Mg²⁺, then:



and

$$\frac{[S^{2+}][ATP^{4-}]}{[S-ATP^{2-}]} = K_{S-1} \quad [154]$$

From equation [121] and assuming K^+ binding only to ATP^{4-} gives:

$$\frac{K_{Mg-1} \left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[K^+]}{K_{K-1}} + \frac{[S^{2+}]}{K_{S-1}}\right)}{\left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}}\right)} = K_{app} \quad [155]$$

or

$$\frac{10^{-pK_{Mg-1}} \left(1 + 10^{(pK_{H-1} - pH_0)} + [K^+] 10^{pK_{K-1}} + [S^{2+}] 10^{pK_{S-1}}\right)}{\left(1 + 10^{(pK_{Mg-2} - pK_{Mg-1})} 10^{(pK_{H-1} - pH_0)}\right)} = K_{app} \quad [156]$$

Relationship between K_{app} and $[S^{2+}]$

From equation [155]

$$K_{app}(S) = \frac{K_{Mg-1} \left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[K^+]}{K_{K-1}}\right)}{\left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}}\right)} + \frac{K_{Mg-1} \frac{[S^{2+}]}{K_{S-1}}}{\left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}}\right)} \quad [157]$$

which simplifies to:

$$K_{app}(S) = K_{app}([S] = 0) + \frac{K_{Mg-1}}{\left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}}\right) K_{S-1}} [S^{2+}] \quad [158]$$

Thus a plot of K_{app} against $[S]$ will give a straight line, with intercept of $K_{app}([S] = 0)$ on the y-axis and the slope:

$$s(\text{slope}) = \frac{K_{Mg-1}}{\left(1 + \frac{K_{Mg-1}}{K_{Mg-2}} \frac{[H^+]}{K_{H-1}}\right) K_{S-1}} \quad [159]$$

If the other parameters are known, then K_{S-1} can be calculated from the slope “s”.

ACID-BASE CHEMISTRY

Introduction: General references to this section are Koppel & Spiro (1914) or the translation from the original German by Ross & Boron (1980).

1) Total [H] ([H]_T) in a H⁺ buffer solution

a) One H⁺ binding site:



$$\frac{[H^+][B^-]}{[H-B]} = K_{H-1} \quad [161]$$

$$[B]_T = [B^-] + [H-B] \quad [162]$$

$$[H]_T = \text{Bound} + \text{Free, or } [H]_T = [H-B] + [H^+] \quad [163][164]$$

from equation [161]

$$[H-B] = \frac{[H^+][B^-]}{K_{H-1}} \quad \text{and} \quad [H-B] = \frac{[H^+]([B]_T - [H-B])}{K_{H-1}} \quad [165][166]$$

and

$$[H-B] = \frac{[B]_T [H^+]}{(K_{H-1} + [H^+])} \quad \text{or} \quad [H-B] = \frac{[B]_T \frac{[H^+]}{K_{H-1}}}{(1 + \frac{[H^+]}{K_{H-1}})} \quad [167][168]$$

and in terms of pK_{H-1} and pH

$$[H-B] = \frac{[B]_T 10^{(pK_{H-1} - pH)}}{(1 + 10^{(pK_{H-1} - pH)})} \quad [169]$$

substitution equation [168] in [169],

$$[H]_T = \frac{[B]_T \frac{[H^+]}{K_{H-1}}}{(1 + \frac{[H^+]}{K_{H-1}})} + [H^+] \quad [170]$$

b) Two H⁺ binding sites



$$\frac{[H^+][B^-]}{[H-B]} = K_{H-1} \quad \text{and} \quad \frac{[H^+][H-B]}{[H_2-B]} = K_{H-2} \quad [173][174]$$

$$[B]_T = [B^-] + [H-B] + [H_2-B] \quad [175]$$

$$[H]_T = [H-B] + 2[H_2-B] + [H^+] \quad [176]$$

from equation [165]:

$$[H-B] = \frac{[H^+][B]}{K_{H-1}} \quad [177]$$

[B] in terms of [B]_T and K_{H-1}, K_{H-2} and [H⁺].

Substitution for [H₂-B] (equation [174]) in equation [175] gives:

$$[B]_T = [B] + [H-B] + \frac{[H^+][H-B]}{K_{H-2}} \quad [178]$$

and

$$[B]_T = [B] + \frac{[H^+][B]}{K_{H-1}} + \frac{[H^+]^2[B]}{K_{H-1}K_{H-2}} \quad [179]$$

whence

$$[B] = \frac{[B]_T}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}}\right)} \quad [180]$$

substituting equation [180] in equation [177] gives:

$$[H-B] = \frac{[B]_T \frac{[H^+]}{K_{H-1}}}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}}\right)} \quad [181]$$

From equation [179]:

$$[H_2-B] = \frac{[H^+]^2[B]}{K_{H-1}K_{H-2}} \quad [182]$$

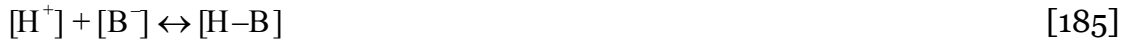
substituting equation [180] in equation [182] gives:

$$[H_2-B] = \frac{[B]_T \frac{[H^+]^2}{K_{H-1}K_{H-2}}}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}}\right)} \quad [183]$$

Substituting for $[H-B]$ and $[H_2-B]$ in equation [179] gives:

$$[H]_T = \frac{[B]_T \left(\frac{[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} \right)}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} \right)} + [H^+] \quad [184]$$

c) Three H^+ binding sites .



and

$$\frac{[H^+][B]}{[H-B]} = K_{H-1} \text{ and } \frac{[H^+][H-B]}{[H_2-B]} = K_{H-2} \text{ and } \frac{[H^+][H_2-B]}{[H_3-B]} = K_{H-3} \quad [188][189][190]$$

$$[B]_T = [B] + [H-B] + [H_2-B] + [H_3-B] \quad [191]$$

and

$$[H]_T = [H^+] + [H-B] + 2[H_2-B] + 3[H_3-B] \quad [192]$$

The same procedure as for the two H^+ binding sites gives:

$$[H-B] = \frac{[B]_T \frac{[H^+]}{K_{H-1}}}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right)} \quad [193]$$

$$[H_2-B] = \frac{[B]_T \frac{[H^+]^2}{K_{H-1}K_{H-2}}}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right)} \quad [194]$$

$$[H_3-B] = \frac{[B]_T \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}}}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right)} \quad [195]$$

and from equation [192] it follows that:

$$[H]_T = \frac{[B]_T \left(\frac{[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{3[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right)}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} \right)} + [H^+] \quad [196]$$

d) Four H⁺ binding sites.

$$[H]_T = \frac{[B]_T \left(\frac{[H^+]}{K_{H-1}} + \frac{2[H^+]^2}{K_{H-1}K_{H-2}} + \frac{3[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{4[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right)}{\left(1 + \frac{[H^+]}{K_{H-1}} + \frac{[H^+]^2}{K_{H-1}K_{H-2}} + \frac{[H^+]^3}{K_{H-1}K_{H-2}K_{H-3}} + \frac{[H^+]^4}{K_{H-1}K_{H-2}K_{H-3}K_{H-4}} \right)} + [H^+] \quad [197]$$

and so on.

2) Bound H⁺ in a H⁺ buffer solution

Since, $[H]_T = [\text{Bound}] + [H^+]$ it follows that:

$$[\text{Bound}] = [H]_T - [H^+] \quad [198]$$

This can be derived directly from equations, [170], [184], [196] and [197] respectively.

3) [H⁺] at a given [H]_T.

In general this can be derived from equations [170], [184], [196] and [197] by solving for [H⁺]. Only the solutions for one and two binding sites is given.

a) Solution for one H⁺ binding site is:

$$[H^+]^2 + (K_{H-1} + [B]_T - [H]_T)[H^+] - K_{H-1}[H]_T = 0 \quad [199]$$

The solution is similar to equation [84], namely

$$[H^+] = \frac{-(K_{H-1} + [B]_T - [H]_T) + \sqrt{(K_{H-1} + [B]_T - [H]_T)^2 + 4K_{H-1}[H]_T}}{2} \quad [200]$$

b) Solution for two binding is a cubic equation:

$$A[H^+]^3 + B[H^+]^2 + C[H^+] + D = 0 \quad [201]$$

where:

$$A = 1$$

$$B = K_{H-2} + 2[B]_T - [H]_T$$

$$C = (K_{H-1} + [B]_T - [H]_T) K_{H-2}$$

$$D = - [H]_T K_{H-1} K_{H-2}$$

TITRATION OF A BUFFER SOLUTION FROM pH 12 TO pH 2 WITH HCl

Introduction: Take as an example a 5 mmol/l potassium buffer (K-B), with a pK_{H-1} of 6.5. It is titrated to pH 12 with NaOH, the initial pH for the titration. In such a solution the following three conditions have to be fulfilled:

1) Buffer equilibrium:

$$[H^+] + [B^-] = [H-B] \text{ and } \frac{[H^+][B^-]}{[H-B]} = K_{H-1} \quad [202][203]$$

2) Ionic product of water (also known as autoprotolysis constant), K_w

$$[H^+][OH^-] = K_w \text{ or } [OH^-] = \frac{K_w}{[H^+]} \quad [204][205]$$

3) Electrical neutrality of the solution

Cations:

$$[H^+] + [K^+] \text{ (from K-B)} + [Na^+] \text{ (from NaOH, to set initial pH)} \quad [206]$$

Anions

$$[B^-] \text{ (from buffer)} + [Cl^-] \text{ (from HCl)} + [OH^-] \text{ (from NaOH and from water, see 2 above)} \quad [207]$$

Because of electrical neutrality they are equal:

$$[H^+] + [K^+] + [Na^+] = [B^-] + [Cl^-] + [OH^-] \quad [208]$$

4) Basic equation

In equation [204] the $[K^+]$ is equal to $[K-B]$ or to the total buffer concentration $[B]_T$, the $[Cl^-]$ is equivalent to the concentration of added acid $[HCl]_{\text{added}}$ and $[Na^+]$ is the concentration of the added alkali [Alkali]. Using these concentrations gives the basic equation:

$$[H^+] + [B]_T + [\text{Alkali}] = [B^-] + [HCl]_{\text{added}} + [OH^-] \quad [209]$$

Amount of NaOH that has to be added to reach a given pH

In the absence of HCl and rearranging equation [209] gives;

$$[\text{Alkali}] = [OH^-] - \{([B]_T - [B^-]) - [H^+]\} \quad [210]$$

but $([B]_T - [B^-]) = [H-B]$ and $[OH^-] = \frac{K_w}{[H^+]}$, so equation [210] becomes:

$$[\text{Alkali}] = \frac{K_w}{[H^+]} - ([H-B] + [H^+]) \quad [211]$$

Substituting for $[H-B]$ from equation [168] gives:

$$[\text{Alkali}] = \frac{K_w}{[H^+]} - \left(\frac{[B]_T \frac{[H^+]}{K_{H-1}}}{\left(1 + \frac{[H^+]}{K_{H-1}}\right)} + [H^+] \right) \quad [212]$$

Equation [212] can also be solved for the $[H^+]$ to give:

$$A[H^+]^3 + B[H^+]^2 + C[H^+] + D = 0 \quad [213]$$

where:

$$A = 1$$

$$B = [\text{Alkali}] + [B]_T + K_{H-1}$$

$$C = [\text{Alkali}]K_{H-1} - K_w$$

$$D = -K_{H-1}K_w$$

Equation [212] predicts the amount of alkali added to reach a given pH, and equation [213] can be used to predict the pH after the addition of a given quantity of alkali.

Titration of the buffer solution with HCl

The basic equation [209] can be solved for $[\text{HCl}]_{\text{added}}$:

$$[\text{HCl}]_{\text{added}} = ([B]_T - [B^-]) + [H^+] + [\text{Alkali}] - [\text{OH}^-]$$

The same substitution as for equation [210] gives:

$$[\text{HCl}]_{\text{added}} = \frac{[B]_T \frac{[H^+]}{K_{H-1}}}{\left(1 + \frac{[H^+]}{K_{H-1}}\right)} + [H^+] + [\text{Alkali}] - \frac{K_w}{[H^+]} \quad [214]$$

The equation can also be solved for $[H^+]$ to give:

$$A[H^+]^3 + B[H^+]^2 + C[H^+] + D = 0 \quad [215]$$

where

$$A = 1$$

$$B = [\text{Alkali}] + [B]_T + K_{H-1} - [HCl]_{\text{added}}$$

$$C = [\text{Alkali}]K_{H-1} - K_w - [HCl]_{\text{added}}K_{H-1}$$

$$D = -K_{H-1}K_w$$

Results of the titration

The concentration of NaOH, necessary to bring the buffer solution to pH 12 can be calculated using equation [212]. With the initial conditions, stated in the Introduction this was, 10.116 mmol/l NaOH. For the calculation, a pK_w at 25°C of 13.995 was used. This value is in mol/l; in mmol/l this value is 7.995 as:

$$\frac{[H^+]}{1000} * \frac{[OH^-]}{1000} = 10^{-13.995} \text{ or } [H^+](\text{mmol/l}) * [OH^-] = 10^{-7.995} \quad [216][217]$$

Either equation [214] or [215] can be used to calculate the titration curve. Equation [214] calculates the [HCl] necessary to acquire a given pH and equation [215] the pH change as the [HCl] is increased. Both give the same result. In the calculations the dilution effect of the titration has been neglected. Such a theoretical titration is illustrated in Figure 2. Figure 3 illustrates that the curve consists of three components, 1) titration of NaOH, 2) titration of the buffer and 3) increase of the $[H^+]$.

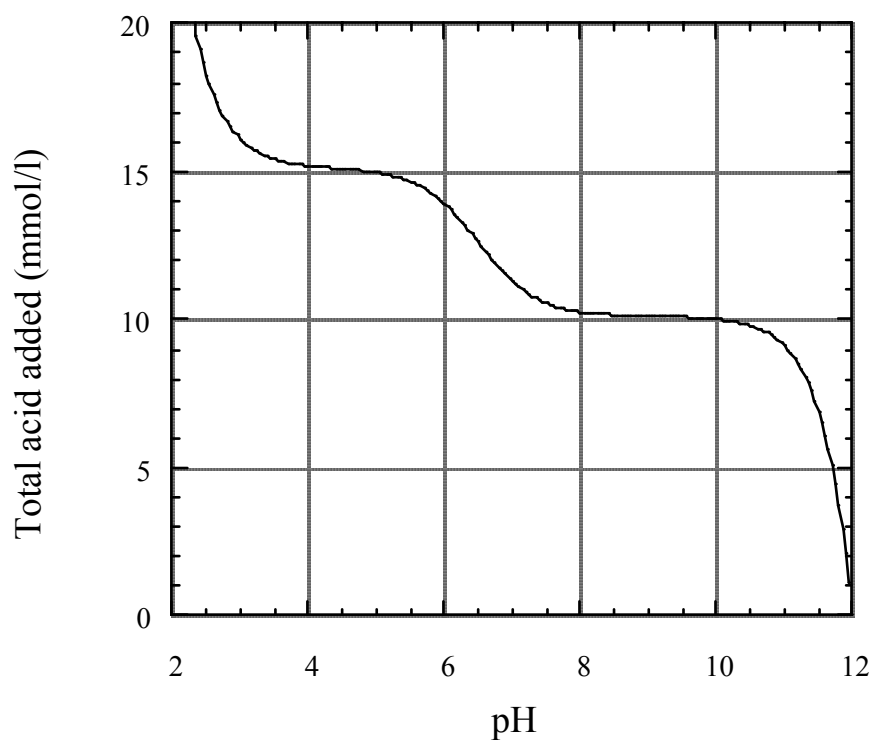


Figure 2: Calculated from equation [215], a $[B]_T$ of 5 mmol/l, and a pK_{H-1} of 6.5. The initial pH was 12 due to an addition of 10.116 mmol/l of NaOH.

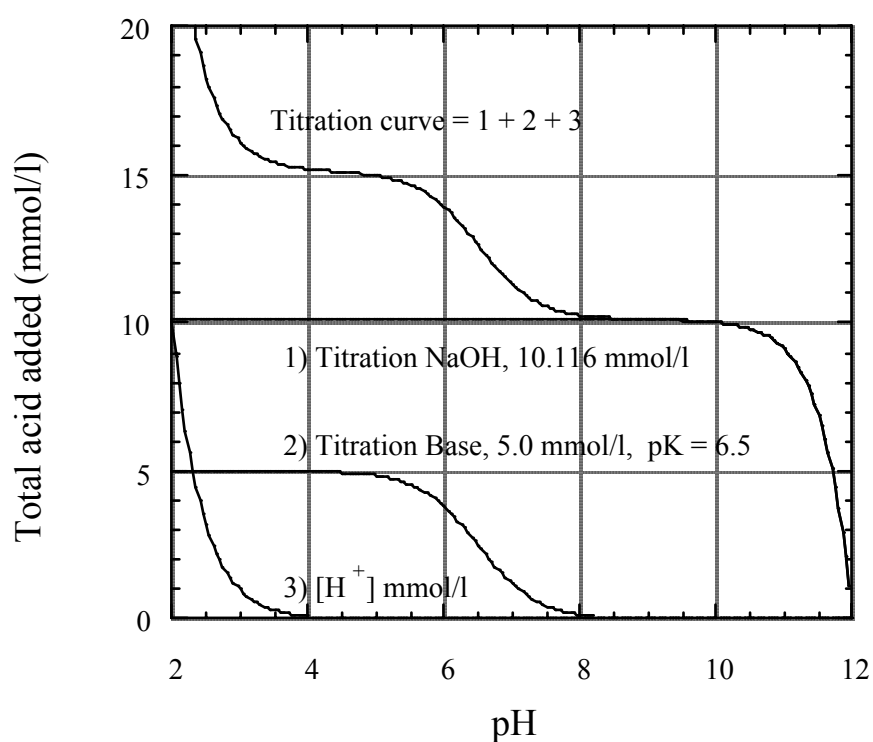


Figure 3. The titration curve consists of three components, namely, 1) initial titration with NaOH, 2) H^+ binding to the buffer and 3) an increase in the $[H^+]$.

DEFINITIONS AND DERIVATIONS OF BUFFERING

The following are the definitions used to define intracellular buffering

| Quantity | Symbol | Definition | Reference |
|---------------------------------------|----------|--|--|
| Buffer Capacity | β | $\frac{d[Mg]_T}{dpMg}$ | Michaelis, 1922 |
| Buffer Capacity | β | $\frac{d[Mg]_T}{dpMg}(\text{Buffer}) - \frac{d[Mg]_T}{dpMg}(\text{No Buffer})$ | Koppel & Spiro, 1914 |
| Buffer Power | π | $\frac{d[Mg]_B}{dpMg}$ | Klabusay & Blinks, 1996 |
| Binding capacity or Binding ratio | κ | $\frac{d[Mg]_B}{d[Mg^{2+}]}$ | Neher, 1995 |
| Buffer coefficient or Buffer ratio | B_x | $\frac{d[Mg]_T}{d[Mg^{2+}]}$ | Koss et al., 1993 Schwiening & Thomas, 1996 |

Where, $[Mg]_T$, $[Mg]_B$ and pMg are the total, bound and $-\log[Mg^{2+}]$ respectively.

Let the binding of Mg^{2+} be to ATP. It follows that

$$\frac{[Mg^{2+}][ATP^{4-}]}{[Mg-ATP^{2-}]} = K_{app} \quad [218]$$

$$[Mg-ATP^{2-}] + [Mg^{2+}] = [Mg]_T \quad [219]$$

$$[Mg-ATP^{2-}] + [ATP^{4-}] = [ATP]_T \quad [220]$$

and

$$[ATP^{4-}] = [ATP]_T - ([Mg]_T - [Mg^{2+}]) \quad [221]$$

For differentiation of a quotient, the following rule applies:

$$\frac{d}{dx} \left(\frac{u}{v} \right) = \frac{vdu - udv}{v^2}$$

1) Buffer capacity (Michaelis)

This is defined as $\frac{d[Mg]_T}{dpMg}$

$[Mg]_T$ can be calculated from equations [218] to [220]

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

this gives:

$$[Mg]_T(K_{app} + [Mg^{2+}]) = [ATP]_T[Mg^{2+}] + (K_{app} + [Mg^{2+}])[Mg^{2+}] \quad [223]$$

$$[Mg]_T = \frac{[ATP]_T[Mg^{2+}]}{(K_{app} + [Mg^{2+}])} + [Mg^{2+}] \quad [224]$$

$$[Mg]_T = \frac{[ATP]_T 10^{-pMg}}{(K_{app} + 10^{-pMg})} + 10^{-pMg} \quad [225]$$

differentiating

$$\frac{d[Mg]_T}{dpMg} = -\left\{ \frac{(K_{app} + 10^{-pMg})[ATP]_T \ln(10) 10^{-pMg} - [ATP]_T 10^{-pMg} \ln(10) 10^{-pMg}}{(K_{app} + 10^{-pMg})^2} + \ln(10) 10^{-pMg} \right\} \quad [226]$$

simplification gives:

$$\frac{d[Mg]_T}{dpMg} = -\left\{ \frac{[ATP]_T K_{app} 10^{-pMg} \ln(10)}{(K_{app} + 10^{-pMg})^2} + \ln(10) 10^{-pMg} \right\} \quad [227]$$

or

$$\frac{d[Mg]_T}{dpMg} = -\left\{ \frac{[ATP]_T K_{app} [Mg^{2+}] \ln(10)}{(K_{app} + [Mg^{2+}])^2} + \ln(10) [Mg^{2+}] \right\} \quad [228]$$

since $\frac{d}{dpMg}(10^{-pMg}) = -\ln(10) 10^{-pMg}$

Differentiating each term in equation [226] separately for the second differential gives:

$$\frac{(K_{app} + 10^{-pMg})^2 [ATP]_T K_{app} \{\ln(10)\}^2 10^{-pMg} - [ATP]_T K_{app} \{\ln(10)\}^2 10^{-pMg} 2(K_{app} + 10^{-pMg}) 10^{-pMg}}{(K_{app} + 10^{-pMg})^4} \quad [229]$$

and

$$(\ln(10))^2 10^{-pMg} \quad [230]$$

which gives on simplification:

$$\frac{d^2[Mg]_T}{dpMg^2} = \frac{[ATP]_T K_{app} (\ln(10))^2 [Mg^{2+}] (K_{app} - [Mg^{2+}])}{(K_{app} + [Mg^{2+}])^3} + (\ln(10))^2 [Mg^{2+}] \quad [231]$$

As seen from equation [228] the buffering capacity consists of two terms, namely a term due to the binding of Mg^{2+} to the buffer:

$$\frac{(K_{app} + 10^{-pMg}) [ATP]_T \ln(10) 10^{-pMg} - [ATP]_T 10^{-pMg} \ln(10) 10^{-pMg}}{(K_{app} + 10^{-pMg})^2} \quad [232]$$

and a term due to ionized Mg^{2+} in the solution, namely,

$$\ln(10) 10^{-pMg} \quad [233]$$

It is this second term that causes the spurious rise in the buffer capacity at lower pMg values (see McGuigan et al. 1991, Figure 2A). This was realised by Koppel & Spiro (1914) who subtracted it in their definition of the buffer capacity (see below).

2) Buffer capacity (Koppel & Spiro)

This is defined as $\frac{d[Mg]_T}{dpMg}(\text{Buffer}) - \frac{d[Mg]_T}{dpMg}(\text{No Buffer})$

The second term is the total Mg^{2+} in a solution without the buffer and is thus equivalent to the ionized concentration of Mg^{2+} . The buffer capacity β becomes:

$$\beta = \frac{d}{dpMg} \left(\frac{[ATP]_T 10^{-pMg}}{(K_{app} + 10^{-pMg})} + 10^{-pMg} \right) - \frac{d}{dpMg} (10^{-pMg}) \quad [234]$$

or

$$\beta = \frac{d}{dpMg} \left(\frac{[ATP]_T 10^{-pMg}}{(K_{app} + 10^{-pMg})} \right) \quad [235]$$

The solution to this is similar to equation [228]

$$\beta = \frac{[ATP]_T K_{app} [Mg^{2+}] \ln(10)}{(K_{app} + [Mg^{2+}])^2} \quad [236]$$

and

$$\frac{d}{dpMg}(\beta) = \frac{[ATP]_T K_{app} (\ln(10))^2 [Mg^{2+}] (K_{app} - [Mg^{2+}])}{(K_{app} + [Mg^{2+}])^3} \quad [237]$$

which is similar to equation [231].

3) Buffering power

This is defined as $\frac{d[Mg]_B}{dpMg}$

The total Mg^{2+} consists of two parts namely: 1) bound to ATP and 2) ionized Mg^{2+} . The definition of Koppel & Spiro (1914) removes this second term, leaving only the bound. Thus the definition of the Buffering power is equivalent to definition of Koppel & Spiro (1914).

4) Binding capacity/ratio

By definition $[Mg]_B = [Mg-ATP^{2-}]$ It follows from equations [218] to [221] that

$$[Mg-ATP^{2-}] = \frac{([ATP]_T - [Mg-ATP^{2-}])[Mg^{2+}]}{K_{app}} \quad [238]$$

simplification gives:

$$[Mg-ATP^{2-}] = \frac{[ATP]_T [Mg^{2+}]}{(K_{app} + [Mg^{2+}])} \text{ or } [Mg]_B = \frac{[ATP]_T [Mg^{2+}]}{(K_{app} + [Mg^{2+}])} \quad [239]/[240]$$

differentiation gives:

$$\frac{d[Mg]_B}{d[Mg^{2+}]} = \frac{(K_{app} + [Mg^{2+}])[ATP]_T - [ATP]_T [Mg^{2+}]}{(K_{app} + [Mg^{2+}])^2} \quad [241]$$

or

$$\frac{d[Mg^{2+}]_B}{d[Mg^{2+}]} = \frac{K_{app}[ATP]_T}{(K_{app} + [Mg^{2+}])^2} \quad [242]$$

the second derivative is:

$$\frac{d^2[Mg]_B}{d[Mg^{2+}]^2} = \frac{(K_{app} + [Mg^{2+}])^2 * 0 - 2K_{app}[ATP]_T(K_{app} + [Mg^{2+}])}{(K_{app} + [Mg^{2+}])^4} \quad [243]$$

which simplifies to

$$\frac{d^2[Mg]_B}{d[Mg^{2+}]^2} = \frac{-2K_{app}[ATP]_T}{(K_{app} + [Mg^{2+}])^3} \quad [244]$$

5) Buffer coefficient/ratio

This is defined as $\frac{d[Mg]_T}{d[Mg^{2+}]}$

from equation [224]

$$[Mg]_T = \frac{[ATP]_T[Mg^{2+}]}{(K_{app} + [Mg^{2+}])} + [Mg^{2+}]$$

differentiation gives:

$$\frac{d[Mg]_T}{d[Mg^{2+}]} = \frac{(K_{app} + [Mg^{2+}])[ATP]_T - [ATP]_T[Mg^{2+}]}{(K_{app} + [Mg^{2+}])^2} + 1 \quad [245]$$

which simplifies to

$$\frac{d[Mg]_T}{d[Mg^{2+}]} = \frac{K_{app}[ATP]_T}{(K_{app} + [Mg^{2+}])^2} + 1 \quad [246]$$

The second derivative is similar to that of equation [244]

$$\frac{d^2[Mg]_T}{d[Mg^{2+}]^2} = \frac{-2 * K_{app}[ATP]_T}{(K_{app} + [Mg^{2+}])^3}$$

since the derivative of 1 is zero.

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