

CALCIUM AND MAGNESIUM BUFFER SYSTEMS

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DEFINITIONS

BUFFER SYSTEMS

$[Ligand]_N$	Nominal ligand total concentration, mol/l
F	Ligand purity expressed as a fraction
$[Ligand]_T$	Actual or true ligand concentration, mol/l; $[Ligand]_T = [Ligand]_N \cdot F$
$[X]_{T-L}$	Total X^{2+} concentration in X-ligand solution; equal to $[Ligand]_N$, mol/l
$[X]_{T-M}$	Total X^{2+} concentration in mixtures, mol/l
$[X^{2+}]$	Ionised concentration of X^{2+} , mol/l
$[Ligand]_F$	Concentration of ligand not bound to X^{2+} , mol/l
$[X-Ligand]_B$	Concentration of all forms of X^{2+} bound to ligand, mol/l

R_{sol}	Solution Ratio, expressed as $\frac{x \text{ ml X-Ligand solution}}{y \text{ ml Ligand solution}}$
R_{con}	Concentration Ratio, $\frac{[X - \text{Ligand}]_B}{[\text{Ligand}]_F}$
K_{app}	Apparent equilibrium constant, mol/l
pK_{app}	$-\log K_{app}$

TRUE/ESTIMATED PARAMETERS

$[X^{2+}]_{True}$	True ionised concentration, mol/l
$[X^{2+}]_{Est}$	Estimated ionised concentration, mol/l
$R_{E/T}$	Ratio of estimated to true concentration, $\frac{[X^{2+}]_{Est}}{[X^{2+}]_{True}}$
$pK_{app-true}$	True pK_{app}
$pK_{app-est}$	Estimated pK_{app}
ΔpK_{app}	Difference between estimated and true pK_{app} , $(pK_{app-est} - pK_{app-true})$
$\Delta pK_{app}(\%)$	Difference between estimated and true pK_{app} expressed as a percentage, $\Delta pK_{app}(\%) = \frac{\Delta pK_{app}}{pK_{app-true}} * 100$
$K_{app-true}$	True K_{app} , mol/l
$K_{app-est}$	Estimated K_{app} , mol/l
ΔK_{app}	Difference between estimated and true K_{app} , $(K_{app-est} - K_{app-true})$
$[X]_{T-true}$	True total concentration, mol/l
$[X]_{T-est}$	Estimated total concentration, mol/l
Δ_{X-T}	Difference between estimated and true total concentrations, $([X]_{T-est} - [X]_{T-true})$
$\Delta_{X-T}(\%)$	Difference between estimated and true total concentrations as a percentage, $\Delta_{X-T}(\%) = \frac{\Delta_{X-T}}{[X]_{T-true}} * 100$
$[\text{Ligand}]_{T-true}$	True total ligand concentration, mol/l
$[\text{Ligand}]_{T-est}$	Estimated ligand concentration, mol/l
Δ_L	Difference between estimated and true ligand concentrations, $([\text{Ligand}]_{T-est} - [\text{Ligand}]_{T-true})$
$\Delta_L(\%)$	Difference between estimated and true ligand concentrations as a percentage, $\Delta_L(\%) = \frac{\Delta_L}{[\text{Ligand}]_{T-true}} * 100$

RANGE

$\Delta\mu$	\pm deviation from the true ionised mean concentrations in buffer solutions, % $\text{mean} \pm \frac{\Delta\mu}{100} * \text{mean}$
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RATIO METHOD

INTRODUCTION

When making up buffer solutions the ratio method is the most straightforward. It has however, the disadvantage that because the purity of the ligands is not 100% the $[Ca]_T$ or $[Mg]_T$ ($[X]_T$) is slightly greater than $[Ligand]_T$. This has consequences for the calculated $[X^{2+}]$, which are greater than they would be, if $[X]_T$ and $[Ligand]_T$ were equal. In the ratio method, Ligand and X-Ligand are mixed in set proportions to give a range of $[X^{2+}]$. The normal buffer range is $pK_{app} \pm 1$ which implies either a 10 fold increase or decrease of the $[X^{2+}]$, from the value at pK_{app} . This corresponds to a change from $10^{-pK_{app}}$ to either $10^{-(pK_{app} - 1)}$ or to $10^{-(pK_{app} + 1)}$ and such changes in $[X^{2+}]$ are achieved by altering the solution

ratio $\frac{x \text{ ml X-Ligand solution}}{y \text{ ml Ligand solution}}$. In the following section, the influence of ligand purity on the calculated $[X^{2+}]$ using the ratio method is considered.

$[X^{2+}]$ AT A GIVEN RATIO: INFLUENCE OF PURITY

From the above definitions, $[X]_{T-M}$ can be calculated as follows:

$$[X]_{T-M} = \frac{x [X]_{T-L}}{x + y} \text{ or } [X]_{T-M} = \frac{x [\text{Ligand}]_N}{x + y} \quad [1][2]$$

and it follows that:

$$[X]_{T-M} = \frac{R_{sol} [\text{Ligand}]_N}{(R_{sol} + 1)} \quad [3]$$

$[X]_{T-M}$ in the mixture is:

$$[X]_{T-M} = [X^{2+}] + [X - \text{Ligand}]_B \quad [4]$$

and

$$[X]_{T-M} = [X^{2+}] + \frac{[X^{2+}][\text{Ligand}]_N * F}{([X^{2+}] + K_{app})} \quad [5]$$

(see equation [141] page 23, Section-I)

Substituting equation [3] for $[X]_{T-M}$ in equation [5] gives:

$$\frac{R_{sol} [\text{Ligand}]_N}{(R_{sol} + 1)} = [X^{2+}] + \frac{[X^{2+}][\text{Ligand}]_N * F}{([X^{2+}] + K_{app})} \quad [6]$$

solving for R_{sol} :

$$R_{sol} = \frac{[X^{2+}][\text{Ligand}]_N * F + [X^{2+}]([X^{2+}] + K_{app})}{[\text{Ligand}]_N K_{app} - [X^{2+}]([X^{2+}] + K_{app}) + [X^{2+}][\text{Ligand}]_N (1 - F)} \quad [7]$$

The accepted range of buffer solutions is $pK_{app} \pm 1$ unit, which corresponds to a change in $[X^{2+}]$ equal to $10^{-pK_{app}}$ or K_{app} to $10 * K_{app}$ or $\frac{K_{app}}{10}$. It is possible to express $[X^{2+}]$ in terms of K_{app} and the concentration ratio R_{con} , and hence R_{sol} in terms of R_{con} . From equation [111] page 18, Section-I:

$$\frac{[X^{2+}][\text{Ligand}]_F}{[X - \text{Ligand}]_B} = K_{app} \quad [8]$$

it follows that:

$$[X^{2+}] = K_{app} \frac{[X - \text{Ligand}]_B}{[\text{Ligand}]_F} \quad [9]$$

and from the definition of R_{con} :

$$[X^{2+}] = K_{app} R_{con} \quad [10]$$

R_{sol} in terms of the R_{con} then becomes:

$$R_{sol} = \frac{K_{app} R_{con} [\text{Ligand}]_N * F + K_{app} R_{con} (K_{app} R_{con} + K_{app})}{[\text{Ligand}]_N K_{app} - K_{app} R_{con} (K_{app} R_{con} + K_{app}) + K_{app} R_{con} [\text{Ligand}]_N (1 - F)} \quad [11]$$

which simplifies to:

$$R_{sol} = \frac{R_{con} \{[\text{Ligand}]_N * F + K_{app} (R_{con} + 1)\}}{[\text{Ligand}]_N - K_{app} R_{con} (R_{con} + 1) + R_{con} [\text{Ligand}]_N (1 - F)} \quad [12]$$

A plot of equation [12] is illustrated in Figure 1 for a $[\text{Ligand}]_N$ of 4 mmol/l and a R_{con} of 7 for three values of F namely, 1.0, 0.95 and 0.90. At values of pK_{app} over 6 in each case the value of R_{sol} reaches a maximum because $R_{con} K_{app} \ll [\text{Ligand}]_N * F$.

Equation [12] becomes:

$$R_{sol} = \frac{R_{con} [\text{Ligand}]_N * F}{[\text{Ligand}]_N + R_{con} [\text{Ligand}]_N (1 - F)} \quad [13]$$

or

$$R_{sol} = \frac{R_{con} * F}{1 + R_{con} (1 - F)} \quad [14]$$

which means that R_{sol} is independent of both K_{app} , $[X]_T$ and $[\text{Ligand}]_T$.

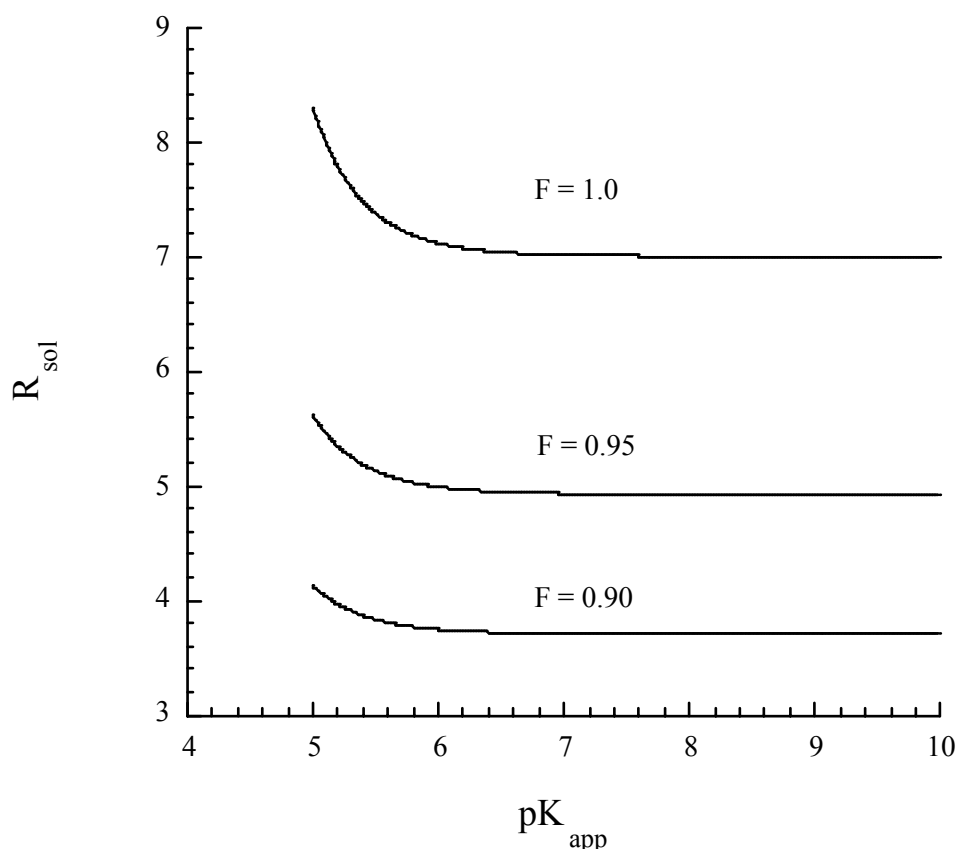


Figure 1. Value of R_{sol} as pK_{app} is increased from 5 to 10 for three different values of F .

The effect that ligand purity has on the ratio method is illustrated in Figure 2, where the solution ratio R_{sol} , necessary to cause a given change in $[X^{2+}]$ from an initial value of $10^{-pK_{\text{app}}}$ is illustrated. If the ligand was 100% pure then there is for practical purposes a one to one relationship between a given change in the $[X^{2+}]$ and the ratio necessary to cause it. This relationship breaks down as the purity decreases. With a purity of 0.95, a tenfold increase in the $[X^{2+}]$ is caused by a ratio of around 6.5, instead of 10. A further decrease of the purity to 0.90 means that a 10 fold increase in the concentration occurs at a ratio of 4.5. For a decrease in concentration the deviation as the purity decreases is much less. The explanation is that as the solution ratio increases, the excess $[X^{2+}]$ becomes relatively more important and the actual $[X^{2+}]$ is higher than would be expected from the nominal ratio.

CONCLUSIONS

With the ratio method, to achieve a range of buffers to cover the range $pK \pm 1$, it is necessary to choose the correct mixtures of X-ligand to ligand. If the ligand has a purity of 100% this varied, as would be expected from 10 to 1 to 1 to 10. However, as illustrated in Figure 2 as the purity decreases from 1 to 0.95, the largest ratio decreases from 10 to 1 to just less than 7 to 1. This was indeed found to be the case by McGuigan, Lüthi & Buri, (1991) for Ca^{2+} buffers where measurement showed that the ratios of 9 to 1 and 8 to 1 lay outside the range of $pK \pm 1$. At the other extreme, for the largest pK values, it was still possible to employ a ratios of 1 to 9 and 1 to 10. This means that in designing buffer mixtures with the ratio

method, the chosen mixtures should range from 7 to 1 to 1 to 10. In future buffer mixtures this has to be taken account of.

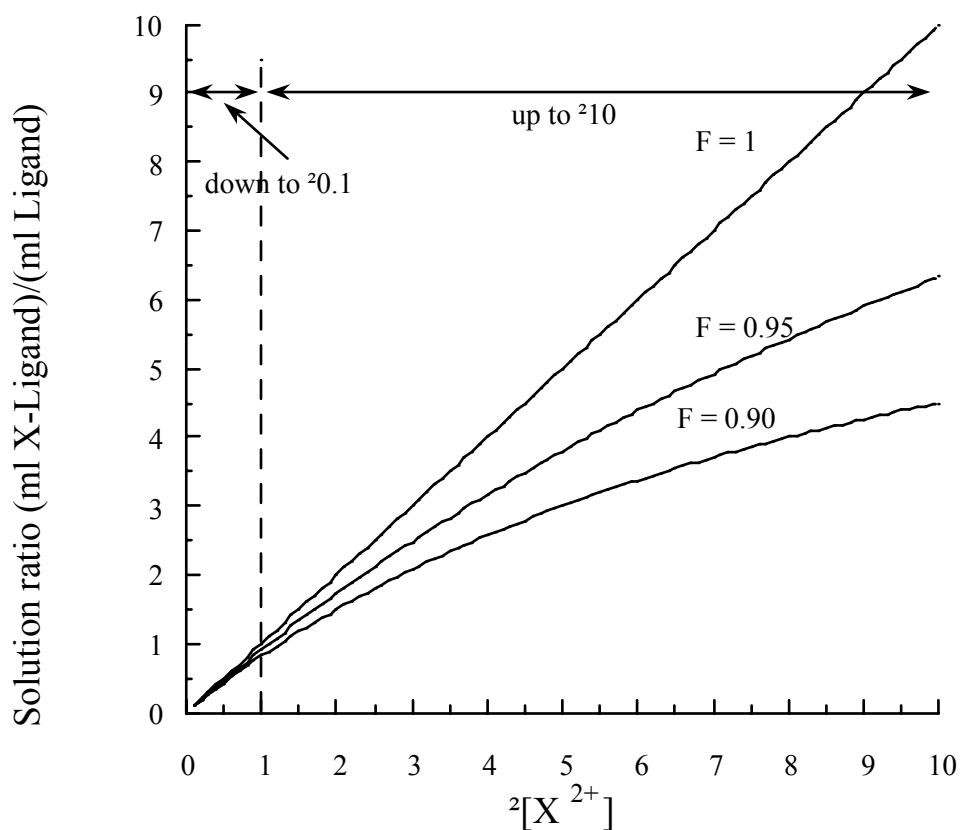


Figure 2. Relationship between the solution ratio and the change in $[X^{2+}]$ from 1 down to 0.1 or from 1 up to 10 times the $[X^{2+}]$, which has been set to equal K_{app} .

BUFFER RANGE

INTRODUCTION

When working with $\text{Ca}^{2+}/\text{Mg}^{2+}$ -macroelectrodes it has been common practice to use EGTA Ca^{2+} buffers in the range from approximately, pCa 8.0 to pCa 6.0 (from approximately, 10 nmol/l to 1 $\mu\text{mol/l}$) or EDTA Mg^{2+} buffers in the range from pMg 6.6 to 4.7 (from approximately 0.25 $\mu\text{mol/l}$ to 20 $\mu\text{mol/l}$), combined with calibration solutions in the range from 0.25 mmol/l to 10 mmol/l (pX, 3.6020 to 2). There is a gap in the calibration in both cases, but for electrode calibration this is not a problem, as the characteristics, such as the slope and the constant of the recording system can be determined using the calibration solutions. In the buffer range the iteration method of Lüthi, Spichiger, Forster & McGuigan (1997) allows the estimation of both the K_{app} and ligand purity and as a consequence, the lumped interference “ Σ ” of macroelectrode. The potential/pX relationship of the macroelectrode can then be described using the Nicolsky-Eisenman equation.

BUFFER LIGANDS

There are buffer ligands other than EGTA and EDTA such as CDTA, EDTA, HEDTA, NTA, citrate and malate as well as the Ca^{2+} buffer ligands, BAPTA and Dibromo-BAPTA (Br_2BAPTA) introduced by Tsien (1980). Each of these ligands can span a different buffer range and in order to make a comparison the tabulated constants in Martell & Smith (1974, 1977) and those for BAPTA and Br_2BAPTA (Tsien, 1980) have been corrected from an ionic strength of 0.1 mol/l to an ionic strength of 0.15 mol/l (see page 49 of Section-II). In Martell & Smith (1974, 1977) the temperature was 25°C and in Tsien (1980) 22°C. The K_{app} was calculated for a pH_c of 7.2.

Table 1. K_{app} of Ca- Buffer ligands

Ligand	pK_{app}	pCa Range $\pm 1 \text{ pK}_{\text{app}}$	Range as concentration		
			minimum	K_{app}	maximum
CDTA	7.9015	6.9-8.9	0.0012 $\mu\text{mol/l}$	0.012 $\mu\text{mol/l}$	0.13 $\mu\text{mol/l}$
EDTA	7.4937	6.5-8.5	0.0031 $\mu\text{mol/l}$	0.032 $\mu\text{mol/l}$	0.32 $\mu\text{mol/l}$
EGTA	7.0367	6.0-8.0	0.010 $\mu\text{mol/l}$	0.09 $\mu\text{mol/l}$	1.00 $\mu\text{mol/l}$
BAPTA	6.6883	5.7-7.7	0.020 $\mu\text{mol/l}$	0.20 $\mu\text{mol/l}$	2.00 $\mu\text{mol/l}$
Br_2BAPTA	5.5561	4.5-6.5	0.25 $\mu\text{mol/l}$	2.7 $\mu\text{mol/l}$	25.0 $\mu\text{mol/l}$
HEDTA	5.4984	4.5-5.5	0.32 $\mu\text{mol/l}$	3.2 $\mu\text{mol/l}$	31.6 $\mu\text{mol/l}$
NTA	3.8498	2.8-4.8	15.9 $\mu\text{mol/l}$	141.3 $\mu\text{mol/l}$	1.6 mmol/l
Citrate	3.3130	2.3-4.3	50.1 $\mu\text{mol/l}$	0.49 mmol/l	-
Malate	1.8411	0.8-2.8	1.5 mmol/l	14.4 mmol/l	-
Calibration	-	3.6 - 2.0	0.25 mmol/l	-	10 mmol/l

The calculated constants for Ca^{2+} binding are shown in Table 1 and for Mg^{2+} binding in Table 2. As well as the concentration range covered by these buffer ligands the Tables also

include the range of the calibration solutions, 0.25 mmol/l to 10 mmol/l (pX, 3.6020 to 2.0).

Table 2. K_{app} of Mg Buffer ligands

Ligand	pK_{app}	pMg Rang $\pm 1 pK_{app}$	Range as concentration		
			minimum	K_{app}	maximum
CDTA	5.8237	4.8-6.8	0.15 $\mu\text{mol/l}$	1.50 $\mu\text{mol/l}$	15.8 $\mu\text{mol/l}$
EDTA	5.7174	4.7-6.7	0.19 $\mu\text{mol/l}$	1.9 $\mu\text{mol/l}$	19.9 $\mu\text{mol/l}$
HEDTA	4.2948	3.3-5.3	5.01 $\mu\text{mol/l}$	50.7 $\mu\text{mol/l}$	501.0 mmol/l
Citrate	3.1829	2.2-4.2	63.10 $\mu\text{mol/l}$	0.66 mmol/l	6.30 mmol/l
NTA	2.9298	1.9-3.9	125.9 $\mu\text{mol/l}$	1.18 mmol/l	12.5 mmol/l
EGTA	1.8884	0.9-2.9	1.26 mmol/l	12.93 mmol/l	-
Malate	1.5812	0.6-2.6	2.5 mmol/l	26.2 mmol/l	-
Calibration	-	3.6 - 2.0	0.25 mmol/l	-	10 mmol/l

BUFFER MIXTURES

As discussed above, buffer mixtures cover the range from 7 to 1, to 1 to 10. With this in mind, and to cover the buffer range shown in Tables 1 and 2, the mixtures used in McGuigan et al., (1991) have been updated and are illustrated in Table 3.

Table 3. Upgraded Buffer mixtures

Solution number	$\frac{[X\text{-Ligand}]}{[\text{Ligand}]}$	Volume (ml)		
		X-Ligand	Ligand	Total
1	7:1	70	10	80
2	6:1	120	20	140
3	5:1	100	20	120
4	4:1	100	25	125
5	3:1	60	20	80
6	2:1	100	50	150
7	1:1	50	50	100
8	1:2	50	100	150
9	1:4	25	100	125
10	1:9	10	90	100

Using these mixtures and the K_{app} values in Tables 1 and 2, the pCa and pMg values have calculated for the ligands and are shown in Tables 4 and 5. $[\text{Ligand}]_N$ for both Ca^{2+} and Mg^{2+} 4 mmol/l and purity in both cases was assumed to be 95%.

Table 4: Calculated pCa values for the mixtures shown in Table 3

No	Ratio	[Ca] _T (mmol/l)	pCa								
			CDTA	EDTA	EGTA	BAPTA	Br ₂ Bapta	HEDTA	NTA	Citrate	Malate
1	7:1	3.500	6.8370	6.4273	5.9714	5.6251	4.5333	4.4777	3.2870	3.0134	2.5422
2	6:1	3.428	6.938	6.5288	6.0726	5.7255	4.6210	4.5638	3.3139	3.0307	2.5515
6											
3	5:1	3.333	7.0499	6.6401	6.1835	5.8360	4.7220	4.6635	3.3500	3.0540	2.5641
4	4:1	3.200	7.1768	6.7668	6.3101	5.9622	4.8414	4.7818	3.4010	3.0875	2.5823
5	3:1	3.000	7.3297	6.9198	6.4629	6.1148	4.9891	4.9288	3.4779	3.1395	2.6111
6	2:1	2.666	7.5321	7.1221	6.6652	6.3170	5.1880	5.1273	3.6063	3.2309	2.6636
7	1:1	2.000	7.8579	7.4480	6.9910	6.6426	5.5118	5.4507	3.8663	3.4357	2.7913
8	1:2	1.333	8.1709	7.7609	7.3039	6.9555	5.8240	5.7628	4.1527	3.6875	2.9704
9	1:4	0.800	8.4777	8.0677	7.6107	7.2624	6.1306	6.0694	4.4487	3.9656	3.1946
10	1:9	0.400	8.8331	8.4231	7.9661	7.6177	6.4859	6.4247	4.7988	4.3060	3.4976

Table 5: Calculated pMg values for the mixtures shown in Table 3

No	Ratio	[Mg] _T (mmol/l)	pMg						
			CDTA	EDTA	HEDTA	Citrate	NTA	EGTA	Malate
1	7:1	3.500	4.7821	4.6821	3.5513	2.9543	2.8475	2.5498	2.5088
2	6:1	3.428	4.8755	4.7736	3.5912	2.9699	2.8608	2.5591	2.5179
3	5:1	3.333	4.980	4.8774	3.6438	2.9912	2.8788	2.5717	2.5303
8									
4	4:1	3.200	5.1034	4.9990	3.7156	3.0217	2.9048	2.5901	2.5482
5	3:1	3.000	5.2535	5.1482	3.8188	3.0692	2.9454	2.6190	2.5766
6	2:1	2.666	5.4540	5.3482	3.9791	3.1535	3.0181	2.6718	2.6282
7	1:1	2.000	5.7787	5.6726	4.2734	3.3454	3.1880	2.8000	2.7543
8	1:2	1.333	6.0913	5.9851	4.5754	3.5871	3.4103	2.9795	2.9315
9	1:4	0.800	6.398	6.2918	4.8780	3.8590	3.6691	3.2042	3.1542
0									
10	1:9	0.400	6.7533	6.6471	5.2314	4.1957	3.9976	3.5075	3.4560

In Figure 3 A and B, the range covered by the various buffers for Ca²⁺ and Mg²⁺ is plotted. The Figures shows the two extreme values taken from solutions 1 and 10, as well as the pK_{app} value for the ligand, where appropriate. In Figure 3A for clarity, the range covered by Br₂BAPTA is not shown because it is very similar to the range covered by HEDTA (see

Table 4). Malate lies within the range covered by the calibration solutions and has also been admitted from Figure 3A.

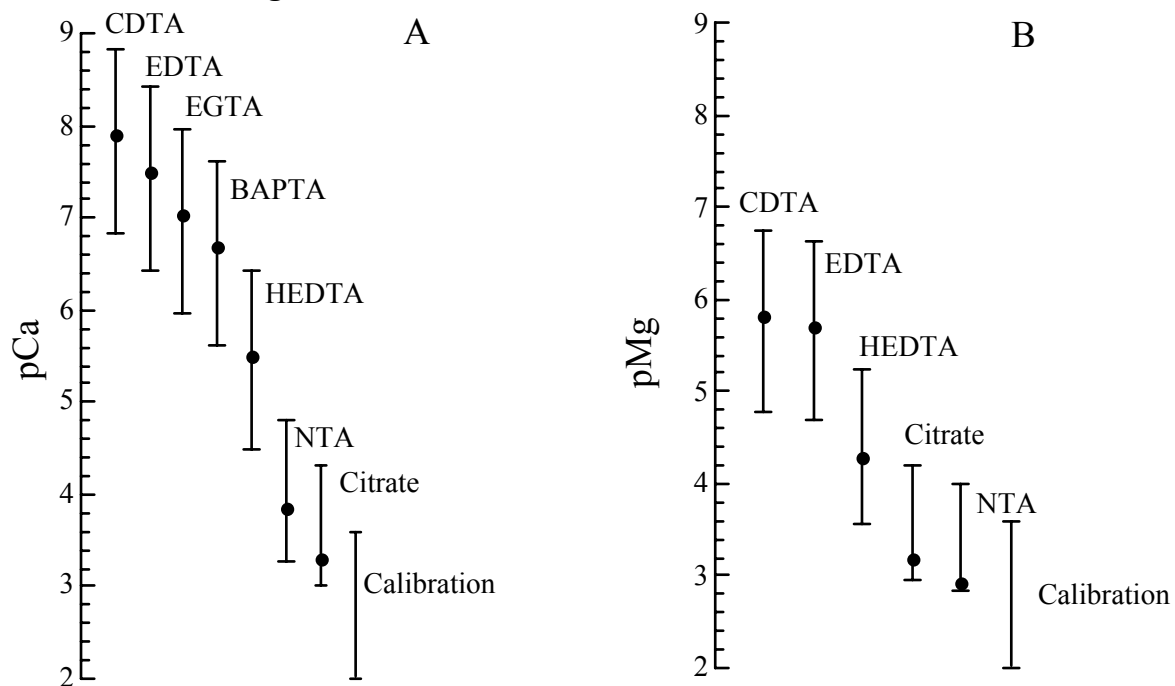


Figure 3. The range covered by the various buffer ligands. In each case the largest pCa value is that in solution 10 and the lowest that from solution 1. The filled circles are the pK_{app} values for the ligand. A, pCa values and B, pMg values.

In Figure 3B since the range covered by EGTA and malate also lie within the range covered by the calibration solutions they have also been admitted from the Figure. A striking feature of both Figure 3 A and B is that at pX values greater than 4 the pK_{app} values for the respective buffer ligands lie towards the higher end of the buffer range and not as would be expected in the middle of the range. This is a result of the ratio method. Taking as an example of Mg²⁺ buffering by NTA. The range of this buffer is from pMg of 1.9 to 2.9. However to achieve a pMg of 1.9 a [Mg]_T of 16.1 mmol/l would be necessary. The largest [Mg]_T in the ratio method is 3.5 mmol/l, thus explaining the skewing of the K_{app} to the higher pMg values.

As illustrated in Figure 3 A and B, since the ranges overlap, any desired buffer concentration can be achieved by a suitable choice of ligand(s). However, despite the degree of overlap for the ligands, there are jumps in the concentration ranges. For Ca²⁺, this occurs between the range covered by HEDTA and NTA and in the case of Mg²⁺ between EDTA and HEDTA. However, as will be shown in the next section, when using macroelectrodes this is not a major problem. Finally it has to be emphasised that Figure 3 is based on calculated K_{app} values, These do not always agree with the measured values (see Lüthi et al., 1997).

INFLUENCE OF CHANGES IN pK_{app} , $[X]_T$, AND $[Ligand]_T$ ON $[X^{2+}]$ IN BUFFER SOLUTIONS

INTRODUCTION

The fundamental question is “How much difference does a deviation in the estimated $pK_{app}/[Ligand]_T$ or $[X]_T$ from the true values, make to the estimated $[X^{2+}]$ in the buffer solutions?” The effect of a deviation of each of these parameters from its true value on the ratio $\frac{[X^{2+}]_{Est}}{[X^{2+}]_{True}}$ or $R_{E/T}$ is considered below. As will be shown later (page 27) an acceptable range (Δ_μ) for the $[X^{2+}]$ in buffer solutions is $\pm 10\%$ from the mean value of $[X^{2+}]$ which corresponds to a range of $R_{E/T}$ from 0.9 to 1.1. When considering the effect of changes in pK_{app} , $[X]_T$ and $[Ligand]_T$ on $R_{E/T}$, the changes are limited to this range in $R_{E/T}$ because this corresponds to a $\pm 10\%$ change in $[X^{2+}]$ in the buffer solutions.

CHANGES IN pK_{app}

The $pK_{app-true}$ can be estimated from $[X^{2+}]_{True}$, $[X]_T$ and $[Ligand]_T$. From equation [134] page 22, Section-I:

$$pK_{app-true} = \log \left\{ \frac{[X^{2+}]_{True} ([Ligand]_T - [X]_T + [X^{2+}]_{True})}{([X]_T - [X^{2+}]_{True})} \right\} \quad [15]$$

A change in K_{app} will cause a change in the $[X^{2+}]$. If the ratio between $[X^{2+}]_{Est}$ and $[X^{2+}]_{True}$ is $R_{E/T}$, then:

$$[X^{2+}]_{Est} = R_{E/T} [X^{2+}]_{True} \quad [16]$$

Substituting equation [16] in equation [15] gives:

$$pK_{app-est} = \log \left\{ \frac{R_{E/T} [X^{2+}]_{True} ([Ligand]_T - [X]_T + R_{E/T} [X^{2+}]_{True})}{([X]_T - R_{E/T} [X^{2+}]_{True})} \right\} \quad [17]$$

The change in pK_{app} , ΔpK_{app} for a given $R_{E/T}$ is the difference between equations [17] and [15] or:

$$\begin{aligned} \Delta pK_{app} = & \log \left\{ \frac{R_{E/T} [X^{2+}]_{True} ([Ligand]_T - [X]_T + R_{E/T} [X^{2+}]_{True})}{([X]_T - R_{E/T} [X^{2+}]_{True})} \right\} \\ & - \log \left\{ \frac{[X^{2+}]_{True} ([Ligand]_T - [X]_T + [X^{2+}]_{True})}{([X]_T - [X^{2+}]_{True})} \right\} \end{aligned} \quad [18]$$

or

$$pK_{app} = \log \left\{ \frac{R_{E/T} [X^{2+}]_{True} ([Ligand]_T - [X]_T + R_{E/T} [X^{2+}]_{True})}{([X]_T - R_{E/T} [X^{2+}]_{True})} * \frac{([X]_T - [X^{2+}]_{True})}{[X^{2+}]_{True} ([Ligand]_T - [X]_T + [X^{2+}]_{True})} \right\} \quad [19]$$

and

$$pK_{app} = \log \left\{ \frac{R_{E/T} ([Ligand]_T - [X]_T + R_{E/T} [X^{2+}]_{True})}{([X]_T - R_{E/T} [X^{2+}]_{True})} * \frac{([X]_T - [X^{2+}]_{True})}{([Ligand]_T - [X]_T + [X^{2+}]_{True})} \right\} \quad [20]$$

and

$$10^{\Delta pK_{app}} = \left\{ \frac{R_{E/T} ([Ligand]_T - [X]_T + R_{E/T} [X^{2+}]_{True})}{([X]_T - R_{E/T} [X^{2+}]_{True})} * \frac{([X]_T - [X^{2+}]_{True})}{([Ligand]_T - [X]_T + [X^{2+}]_{True})} \right\} \quad [21]$$

This can be solved for $R_{E/T}$ to give the following quadratic:

$$aR_{E/T}^2 + bR_{E/T} + c = 0 \quad [22]$$

where:

$$a = [X^{2+}]_{True}$$

$$b = [X^{2+}]_{True} \frac{([Ligand]_T - [X]_T + [X^{2+}]_{True}) 10^{\Delta pK_{app}}}{([X]_T - [X^{2+}]_{True})} + ([Ligand]_T - [X]_T) \quad [23]$$

$$c = - \frac{([Ligand]_T - [X]_T + [X^{2+}]_{True}) [X]_T 10^{\Delta pK_{app}}}{([X]_T - [X^{2+}]_{True})}$$

$$\text{The solution of the quadratic is: } R_{E/T} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

A plot of equation [22] for pK_{app} values from 1.5 to 10 and ΔpK_{app} values of ± 0.01 , ± 0.02 and ± 0.04 is illustrated in Figure 4. The calculations were carried out for a $[Ligand]_T$ of 3.8 mmol/l and a $[X]_T$ of 3.6 mmol/l. At each ΔpK_{app} $R_{E/T}$ reaches a constant value at pK_{app} values greater than 6 and the maximum change in ΔpK_{app} compatible with a $R_{E/T}$ range of 0.9 to 1.1 is ± 0.04 units.

$R_{E/T}$ (limit): At $pK_{app-true}$ values greater than 6, $[X^{2+}]_{True} \ll [X]_T$, $[X^{2+}]_{True}$ tends to zero and equation [22] becomes:

$$([Ligand]_T - [X]_T) R_{E/T} - ([Ligand]_T - [X]_T) 10^{\Delta pK_{app}} = 0 \quad [24]$$

and

$$R_{E/T}(\text{limit}) = 10^{\Delta pK} \quad [25]$$

i.e. $R_{E/T}$ is independent of $[X]_T$, $[Ligand]_T$ and pK_{app} .

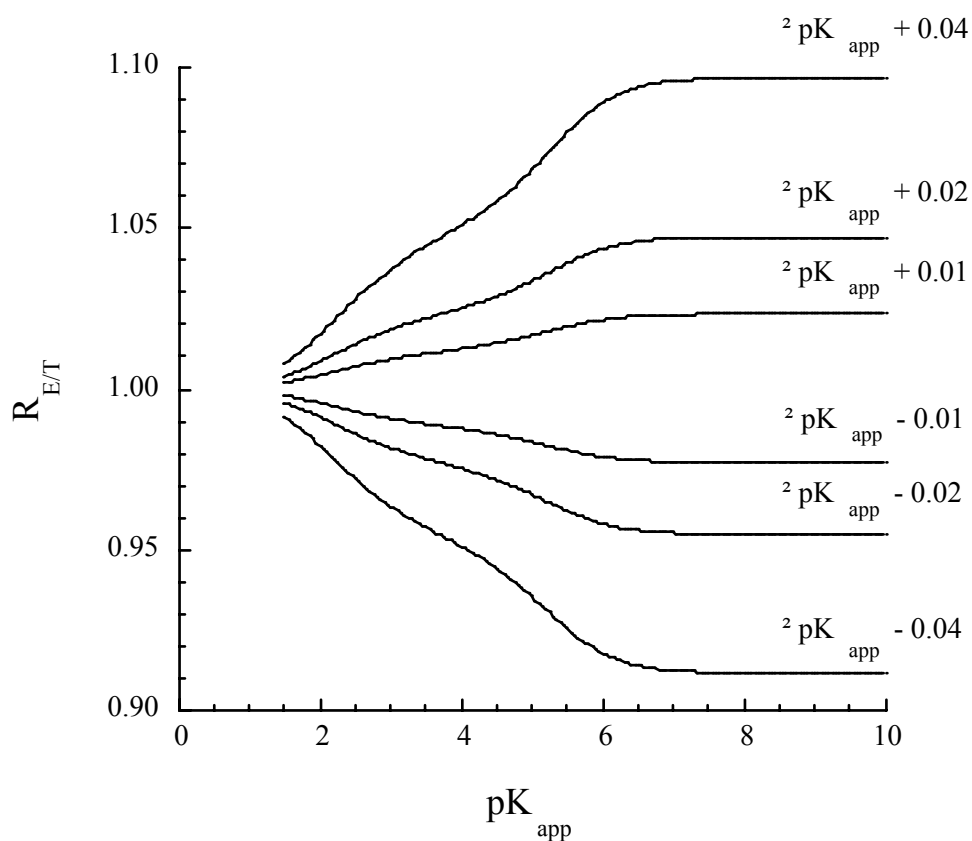


Figure 4: Calculation of the value of $R_{E/T}$, for an increase in pK_{app} of ± 0.01 , ± 0.02 and ± 0.04 units respectively.

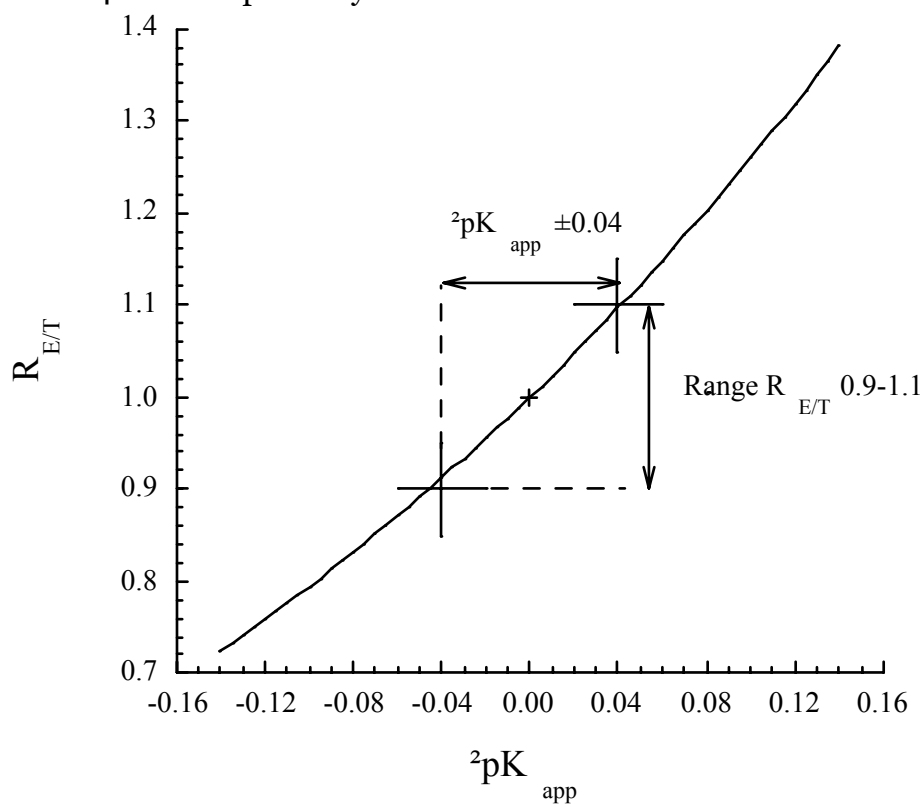


Figure 5: Relationship between ΔpK_{app} and the $R_{E/T}$ over the ΔpK_{app} range of ± 0.14

A plot of equation [25] for ΔpK_{app} over the range ± 0.14 is illustrated in Figure 5, which reinforces the fact that at pK_{app} values greater than 6, for $R_{E/T}$ to be within the range 0.9 to 1.1, ΔpK_{app} must be less than 0.04 units.

$R_{E/T}$ (limit) in terms of K_{app}

Since by definition

$$\Delta pK_{app} = pK_{app-est} - pK_{app-true}$$

it follows from equation [25]:

$$R_{E/T}(\text{limit}) = 10^{(pK_{app-est} - pK_{app-true})} \quad [26]$$

or

$$R_{E/T}(\text{limit}) = \frac{10^{-pK_{app-true}}}{10^{-pK_{app-est}}} \text{ or } R_{E/T}(\text{limit}) = \frac{K_{app-true}}{K_{app-est}} \quad [27][27a]$$

CHANGES IN $[X]_T$

From equation [135] page 22, Section-I:

$$[X]_T = \frac{[X^{2+}](\text{[Ligand]}_T + K_{app} + [X^{2+}])}{(K_{app} + [X^{2+}])} \quad [28]$$

since,

$$\Delta_{X-T} = [X]_{T-Est} - [X]_{T-True} \quad [29]$$

$$\Delta_{X-T} = \frac{R_{E/T}[X^{2+}](\text{[Ligand]}_T + K_{app} + R_{E/T}[X^{2+}])}{(K_{app} + R_{E/T}[X^{2+}])} - \frac{[X^{2+}](\text{[Ligand]}_T + K_{app} + [X^{2+}])}{(K_{app} + [X^{2+}])} \quad [30]$$

which can be solved for $R_{E/T}$ to give the following quadratic:

$$aR_{E/T}^2 + bR_{E/T} + c = 0 \quad [31]$$

where:

$$a = 1$$

$$b = \frac{\{K_{app}(\text{[Ligand]}_T + K_{app}) - [X^{2+}]^2\}}{[X^{2+}](K_{app} + [X^{2+}])} - \frac{\Delta_{X-T}}{[X^{2+}]} \quad [32]$$

$$c = -\frac{K_{app}(\text{[Ligand]}_T + K_{app} + [X^{2+}])}{[X^{2+}](K_{app} + [X^{2+}])} - \frac{\Delta_{X-T}K_{app}}{[X^{2+}]^2}$$

A plot of equation [32] is illustrated in Figure 6. The $[\text{Ligand}]_T$ was 3.8 mmol/l and the value of $R_{E/T}$ is a function not only of the deviation from $[X]_T$ but also of the initial value of

$[X]_T$. At an $[X]_T$ of 3.6 mmol/l a change of 0.45% in the value of $[X]_T$ gives a change in $R_{E/T}$ from approximately 0.9 to 1.1, However, at an $[X]_T$ of 1.0 mmol/l a change in 25% in $[X]_T$ changes $R_{E/T}$ from approximately 0.93 to 1.07.

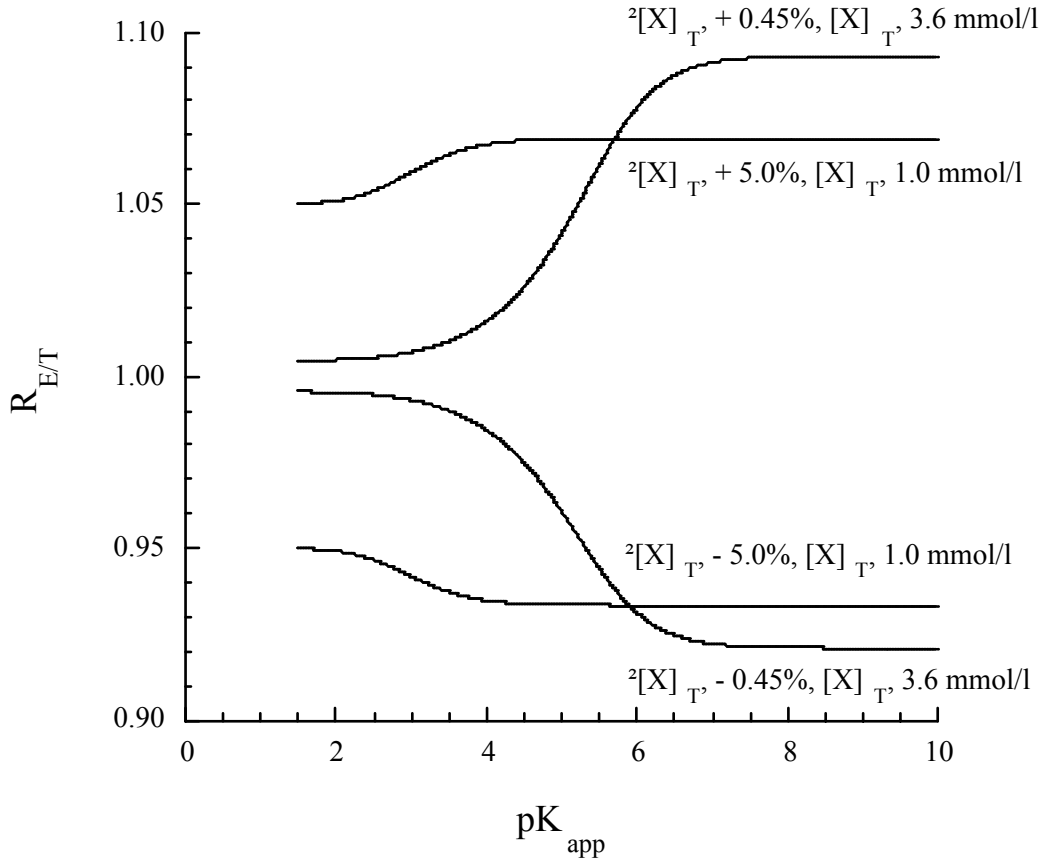


Figure 6. Change in the ratio $R_{E/T}$ as pK_{app} is varied from 1.5 to 10 for changes in $[X]_T$. The value of $R_{E/T}$ depends not only on the deviation of $[X]_T$ from the mean value but also on the initial value of $[X]_T$.

$R_{E/T}$ (limit): At pK_{app} values greater than 6, $R_{E/T}$ reaches a limit since $[X^{2+}] \ll [Ligand]_T$. Under these conditions, equation [28] becomes:

$$[X]_T = \frac{[X^{2+}][Ligand]_T}{(K_{app} + [X^{2+}])} \quad [33]$$

and

$$\Delta_{X-T} = \frac{R_{E/T}[X^{2+}][Ligand]_T}{(K_{app} + R_{E/T}[X^{2+}])} - \frac{[X^{2+}][Ligand]_T}{(K_{app} + [X^{2+}])} \quad [34]$$

or

$$\Delta_{X-T} = \frac{R_{E/T}[Ligand]_T}{(\frac{K_{app}}{[X^{2+}]} + R_{E/T})} - \frac{[Ligand]_T}{(\frac{K_{app}}{[X^{2+}]} + 1)} \quad [35]$$

since:

$$\frac{[X^{2+}][\text{Ligand}]_F}{[X - \text{Ligand}]_B} = K_{\text{app}} \quad [36]$$

(Equation [115] page 18, Section-I)

It follows that:

$$\frac{[X^{2+}]\{[\text{Ligand}]_T - ([X]_T - [X^{2+}])\}}{([X]_T - [X^{2+}])} = K_{\text{app}} \quad [37]$$

At $pK_{\text{app}} > 6$, since $[X^{2+}] \ll [X]_T$ equation [37] simplifies to:

$$\frac{[X^{2+}]([\text{Ligand}]_T - [X]_T)}{[X]_T} = K_{\text{app}} \quad [38]$$

and

$$\frac{([\text{Ligand}]_T - [X]_T)}{[X]_T} = \frac{K_{\text{app}}}{[X^{2+}]} \quad [39]$$

substituting equation [39] in equation [35] gives:

$$\Delta_{X-T} = \frac{R_{E/T} [\text{Ligand}]_T}{\left\{ \frac{([\text{Ligand}]_T - [X]_T)}{[X]_T} + R_{E/T} \right\}} - \frac{[\text{Ligand}]_T}{\left(\frac{([\text{Ligand}]_T - [X]_T)}{[X]_T} + 1 \right)} \quad [40]$$

and

$$\Delta_{X-T} = \frac{R_{E/T} [X]_T [\text{Ligand}]_T}{([\text{Ligand}]_T - [X]_T + R_{E/T} [X]_T)} - [X]_T \quad [41]$$

solving for $R_{E/T}$, gives:

$$R_{E/T} = \frac{([\text{Ligand}]_T - [X]_T)(\Delta_{X-T} + [X]_T)}{[X]_T \{([\text{Ligand}]_T - [X]_T) - \Delta_{X-T}\}} \quad [42]$$

This can also be written;

$$R_{E/T} = \frac{\left(\frac{[\text{Ligand}]_T}{[X]_T} - 1 \right) \left(\frac{\Delta_{X-T}}{[X]_T} + 1 \right)}{\left(\frac{([\text{Ligand}]_T - \Delta_{X-T})}{[X]_T} - 1 \right)} \quad [43]$$

Unlike ΔpK_{app} , $R_{E/T}$ for deviations in $[X]_T$ is a function of the ratio $\frac{[\text{Ligand}]_T}{[X]_T}$ and thus depends on both values (see Figure 6).

CHANGES IN [Ligand]_T

From equation [138] page 23, Section-I:

$$[\text{Ligand}]_T = \frac{([X]_T - [X^{2+}])(K_{\text{app}} + [X^{2+}])}{[X^{2+}]} \quad [44]$$

by definition:

$$\Delta_L = [\text{Ligand}]_{T-\text{est}} - [\text{Ligand}]_{T-\text{true}}$$

and it follows that:

$$\Delta_L = \frac{([X]_T - R_{E/T}[X^{2+}])(K_{\text{app}} + R_{E/T}[X^{2+}])}{R_{E/T}[X^{2+}]} - \frac{([X]_T - [X^{2+}])(K_{\text{app}} + [X^{2+}])}{[X^{2+}]} \quad [45]$$

or

$$\Delta_L R_{E/T}[X^{2+}] = ([X]_T - R_{E/T}[X^{2+}])(K_{\text{app}} + R_{E/T}[X^{2+}]) - R_{E/T}([X]_T - [X^{2+}])(K_{\text{app}} + [X^{2+}]) \quad [46]$$

solving for $R_{E/T}$ gives the following quadratic:

$$aR_{E/T}^2 + bR_{E/T} + c = 0 \quad [47]$$

where:

$$a = 1$$

$$b = \frac{\Delta_L}{[X^{2+}]} + \frac{[X]_T K_{\text{app}}}{[X^{2+}]^2} - 1 \quad [48]$$

$$c = -\frac{[X]_T K_{\text{app}}}{[X^{2+}]^2}$$

A plot of Equation [47] is illustrated in Figure 7. $R_{E/T}$ is a function of both $[X]_T$ and Δ_L . The calculations were carried out for a $[\text{Ligand}]_T$ of 3.8 mmol/l and two values of $[X]_T$ namely, 3.6 mmol/l and 1.0 mmol/l. At 3.6 mmol/l, for $R_{E/T}$ to remain within the range 0.9 to 1.1 the maximum variation in $[\text{Ligand}]_T$ was 0.45%, whereas this increased to 25% for an $[X]_T$ of 1.0 mmol/l.

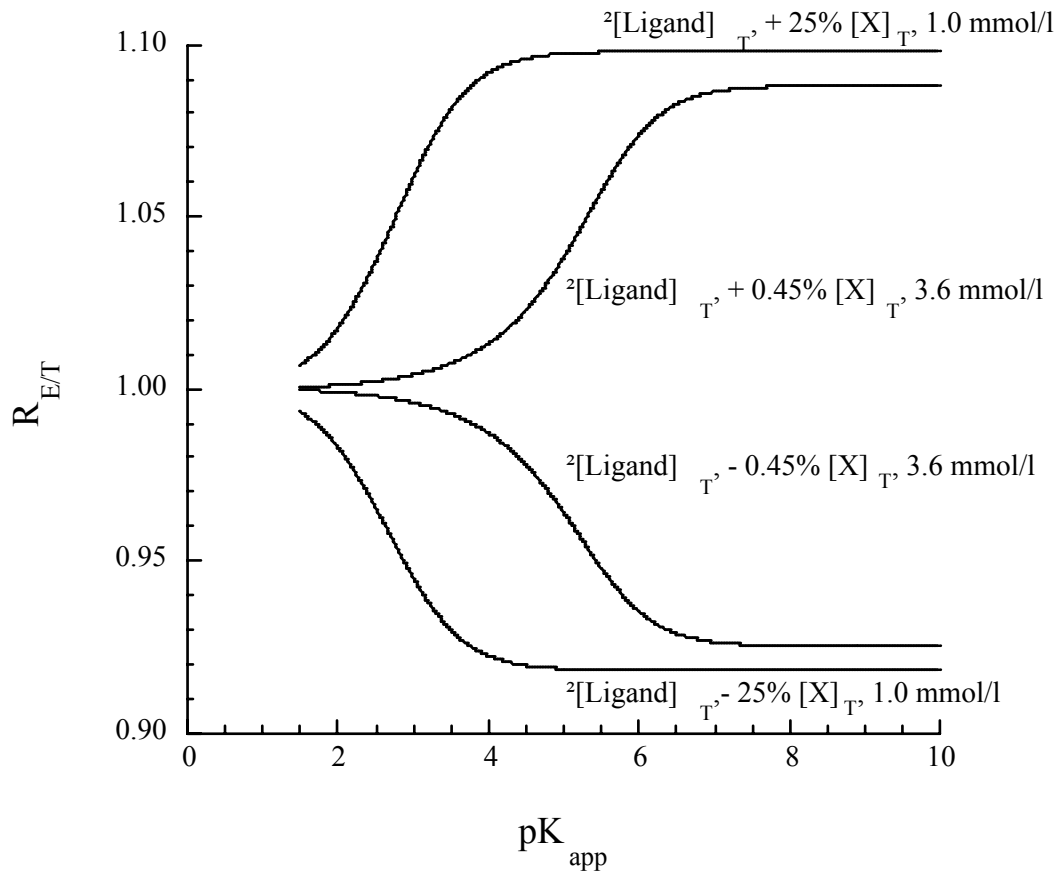


Figure 7. Plot of pK_{app} over the range 1.5 to 10 at a $[Ligand]_T$ of 3.8 mmol/l and at two values of $[X]_T$ namely, 3.6 mmol/l and 1.0 mmol/l, for changes in $[X]_T$ of 0.45% and 25% respectively.

$R_{E/T}(\text{limit})$: At pK_{app} values greater than 6 $[X^{2+}] \ll [Ligand]_T$ and equation [44] becomes:

$$[Ligand]_T = \frac{[X]_T(K_{app} + [X^{2+}])}{[X^{2+}]} \quad [49]$$

and

$$\Delta_L = \frac{[X]_T(K_{app} + R_{E/T}[X^{2+}])}{R_{E/T}[X^{2+}]} - \frac{[X]_T(K_{app} + [X^{2+}])}{[X^{2+}]} \quad [50]$$

it follows that:

$$\Delta_L R_{E/T}[X^{2+}] = [X]_T(K_{app} + R_{E/T}[X^{2+}]) - R_{E/T}[X]_T(K_{app} + [X^{2+}]) \quad [51]$$

solving for $R_{E/T}$ gives:

$$R_{E/T} = \frac{[X]_T K_{app}}{[X]_T K_{app} + \Delta_L [X^{2+}]} \quad [52]$$

and

$$R_{E/T} = \frac{[X]_T}{[X]_T + \Delta_L \frac{[X^{2+}]}{K_{app}}} \quad [53]$$

substituting equation [39] in equation [53] gives:

$$R_{E/T} = \frac{[X]_T}{[X]_T + \Delta_L \frac{[X]_T}{([Ligand]_T - [X]_T)}} \quad [54]$$

which simplifies to:

$$R_{E/T} = \frac{([Ligand]_T - [X]_T)}{([Ligand]_T - [X]_T + \Delta_L)} \quad [55]$$

which gives:

$$R_{E/T}(\text{limit}) = \frac{\left(\frac{[Ligand]_T}{[X]_T} - 1\right)}{\left\{\frac{([Ligand]_T + \Delta_L)}{[X]_T} - 1\right\}} \quad [56]$$

CHANGES EXPRESSED AS A PERCENTAGE

It follows from the definitions of the parameters that:

$$\Delta pK_{app} = pK_{app - true} * \frac{\Delta pK_{app} (\%)}{100} \quad [57]$$

$$\Delta K_{app} = K_{app - true} * \frac{\Delta K_{app} (\%)}{100} \quad [58]$$

$$\Delta_{X-T} = [X]_{T - true} * \frac{\Delta_{X-T} (\%)}{100} \quad [59]$$

$$\Delta_L = [Ligand]_{T - true} * \frac{\Delta_L (\%)}{100} \quad [60]$$

Equation 1

LEVELS OF IMPRECISION

RANGE: DEFINITIONS

There are several ways of expressing an acceptable range for a method. These are listed below.

- 1) Mean \pm 2SD. This is the most common method (Fraser, 2001)
- 2) Mean $\pm \frac{2CV}{100} * \text{mean}$. This follows form the definition of CV as $\frac{SD}{\text{mean}} * 100$.
- 3) Mean $\pm \frac{\Delta_{\mu}}{100} * \text{mean}$. Δ_{μ} is defined as the percentage variation from the mean.

From the definitions of range it follows, that $CV = \frac{\Delta_{\mu}}{2}$.

RELATIONSHIP BETWEEN Δ_{μ} AND $R_{E/T}$, ΔpK_{app} , ΔK_{app} , Δ_{X-T} and Δ_L

$R_{E/T}$

By definition:

$$\Delta_{\mu} = \frac{([X^{2+}]_{\text{Est}} - [X^{2+}]_{\text{True}})}{[X^{2+}]_{\text{True}}} * 100 \quad [61]$$

or

$$\Delta_{\mu} = \left(\frac{[X^{2+}]_{\text{Est}}}{[X^{2+}]_{\text{True}}} - 1 \right) * 100 \quad [62]$$

and

$$\Delta_{\mu} = (R_{E/T} - 1) * 100 \quad [63]$$

it follows that:

$$R_{E/T} = \left(\frac{\Delta_{\mu}}{100} + 1 \right) \quad [64]$$

$\Delta pK_{app}(\text{limit})$

Substituting equation [64] in equation [25] gives:

$$\left(\frac{\Delta_{\mu}}{100} + 1 \right) = 10^{\Delta pK_{app}} \quad [65]$$

it follows that:

$$\Delta pK_{app} = \log\left(\frac{\Delta_{\mu}}{100} + 1\right) \quad [66]$$

$\Delta K_{app}(\text{limit})$

Substituting equation [64] in equation [27a] gives:

$$\left(\frac{\Delta_{\mu}}{100} + 1 \right) = \frac{K_{app - \text{true}}}{K_{app - \text{est}}} \quad [67]$$

and

$$\left(\frac{\Delta_{\mu}}{100} + 1\right) = \frac{K_{\text{app} - \text{true}}}{(K_{\text{app} - \text{true}} + \Delta K_{\text{app}})} \quad [68]$$

solving for ΔK_{app} gives:

$$\Delta K_{\text{app}} = -K_{\text{app} - \text{true}} \frac{\Delta_{\mu}}{(100 + \Delta_{\mu})} \quad [69]$$

Δ_{X-T} (limit)

Substituting equation [64] in equation [42] gives:

$$\left(\frac{\Delta_{\mu}}{100} + 1\right) = \frac{([Ligand]_T - [X]_T)(\Delta_{X-T} + [X]_T)}{[X]_T ([Ligand]_T - [X]_T - \Delta_{X-T})} \quad [70]$$

Solving for Δ_{X-T} gives:

$$\Delta_{X-T} = \frac{\Delta_{\mu} [X]_T ([Ligand]_T - [X]_T)}{(100 * [Ligand]_T + \Delta_{\mu} [X]_T)} \quad [71]$$

Δ_L (limit)

Substituting equation [64] in equation [55] gives:

$$\left(\frac{\Delta_{\mu}}{100} + 1\right) = \frac{([Ligand]_T - [X]_T)}{([Ligand]_T - [X]_T + \Delta_L)} \quad [72]$$

Solving for Δ_L gives:

$$\Delta_L = \frac{\Delta_{\mu} ([X]_T - [Ligand]_T)}{(\Delta_{\mu} + 100)} \quad [73]$$

RELATIONSHIP BETWEEN Δ_{μ} AND $\Delta pK_{\text{app}}(\%)$, $K_{\text{app}}(\%)$, $\Delta_{X-T}(\%)$ and $\Delta_L(\%)$

$\Delta pK_{\text{app}}(\%)$

Substituting for ΔpK_{app} from equation [57] in equation [66] gives.

$$pK_{\text{app} - \text{true}} * \frac{\Delta pK_{\text{app}}(\%)}{100} = \log\left(\frac{\Delta_{\mu}}{100} + 1\right) \quad [74]$$

or

$$\Delta pK_{\text{app}}(\%) = \frac{100 * \log\left(\frac{\Delta_{\mu}}{100} + 1\right)}{pK_{\text{app} - \text{true}}} \quad [75]$$

since, $pK_{app} = -\log K_{app}$ equation [69] becomes:

$$\Delta pK_{app}(\%) = -\frac{100 * \log(\frac{\Delta_{\mu}}{100} + 1)}{\log K_{app - true}} \quad [76]$$

$\Delta K_{app}(\%)$

Substituting for pK_{app} from equation [58] in equation [69] gives:

$$K_{app - true} * \frac{\Delta K_{app}(\%)}{100} = -K_{app - true} \frac{\Delta_{\mu}}{(100 + \Delta_{\mu})} \quad [77]$$

it follows that:

$$\Delta K_{app}(\%) = -\frac{\Delta_{\mu}}{(100 + \Delta_{\mu})} * 100 \quad [78]$$

$\Delta_{X-T}(\%)$

Substituting for Δ_{X-T} from equation [59] in equation [71] gives:

$$[X]_T * \frac{\Delta_{X-T}(\%)}{100} = \frac{\Delta_{\mu} [X]_T ([Ligand]_T - [X]_T)}{(100 * [Ligand]_T - \Delta_{\mu} [X]_T)} \quad [79]$$

it follows that:

$$\Delta_{X-T}(\%) = \frac{\Delta_{\mu} ([Ligand]_T - [X]_T)}{(100 * [Ligand]_T + \Delta_{\mu} [X]_T)} * 100 \quad [80]$$

Equation [80] can also be written:

$$\Delta_{X-T}(\%) = \frac{\Delta_{\mu} (1 - \frac{[X]_T}{[Ligand]_T})}{(100 + \Delta_{\mu} \frac{[X]_T}{[Ligand]_T})} * 100 \quad [81]$$

4) $\Delta_L(\%)$

Substituting for Δ_L from equation [60] in equation [73] gives:

$$[Ligand]_T * \frac{\Delta_L(\%)}{100} = \frac{\Delta_{\mu} ([X]_T - [Ligand]_T)}{(\Delta_{\mu} + 100)} \quad [82]$$

it follows that:

$$\Delta_L(\%) = \frac{\Delta_\mu \left(\frac{[X]_T}{[\text{Ligand}]_T} - 1 \right)}{(\Delta_\mu + 100)} * 100 \quad [83]$$

ALLOWABLE IMPRECISION FOR $[X^{2+}]$ IN BUFFER SOLUTIONS

INTRODUCTION

In Ca^{2+} and Mg^{2+} buffer solutions the level of imprecision has to be defined for the ionised concentrations in the solutions. However, the $[\text{X}^{2+}]$ is calculated from the K_{app} , $[\text{Ligand}]_{\text{T}}$ and the total $\text{Ca}^{2+}/\text{Mg}^{2+}$ concentrations in each buffer solution. From these three parameters the initial two are estimated and the total cation concentration in the buffer solutions depends on the accuracy of the stock solutions and the pipetting *i.e.* the allowable imprecision of $[\text{X}^{2+}]$ depends on the accuracy of the estimations of K_{app} , $[\text{Ligand}]_{\text{T}}$ and on the precision of the $[\text{X}]_{\text{T}}$. Definition of an acceptable range for $[\text{X}^{2+}]$ has as a consequence the definition of levels of imprecision for K_{app} , $[\text{Ligand}]_{\text{T}}$ and $[\text{X}]_{\text{T}}$

DEFINED RANGES

Ionised concentrations, $[\text{X}^{2+}]$: There is at present no definition for an acceptable range for the $[\text{X}^{2+}]$ in $\text{Ca}^{2+}/\text{Mg}^{2+}$ buffer solutions and any definition has to be empirical. Experimentally, a within batch coefficient of variation (CV) of less than 5% for $[\text{Mg}^{2+}]$ in $\text{Mg}^{2+}/\text{EDTA}$ and $\text{Mg}^{2+}/\text{ATP}$ buffer solutions is attainable (McGuigan, Kay, Elder & Lüthi, 2005). Because of lack of other possible definitions of imprecision, it is proposed that this empirical definition *i.e.* a CV of less than 5% for $[\text{X}^{2+}]$ in the buffer solution be accepted. A CV of less than 5% means a range of $\pm 10\%$ from the mean values (see page 22). Defining the range, Δ_{μ} as $\pm 10\%$ has consequences for $R_{\text{E/T}}$, $\text{p}K_{\text{app}}$, $[\text{X}]_{\text{T}}$ and $[\text{Ligand}]_{\text{T}}$.

Ratio estimated/true $[\text{X}^{2+}]$, $R_{\text{E/T}}$: The relationship between $R_{\text{E/T}}$ and Δ_{μ} from equation [64] is:

$$R_{\text{E/T}} = \left(\frac{\Delta_{\mu}}{100} + 1 \right)$$

If Δ_{μ} is $\pm 10\%$, then the range for $R_{\text{E/T}}$ is from 0.9 to 1.1.

Change in $\text{p}K_{\text{app}}$, $\Delta \text{p}K_{\text{app}}$: At $\text{p}K_{\text{app}}$ values over 6, $\text{p}K_{\text{app}}$ tends to a constant value (equation [66]), namely:

$$\Delta \text{p}K_{\text{app}} = \log\left(\frac{\Delta_{\mu}}{100} + 1\right)$$

For a $\pm 10\%$ range for Δ_{μ} , this corresponds to a range of -0.045 to $+0.041$ or in round figures to a range of ± 0.04 $\text{p}K_{\text{app}}$ units. This is the range so it corresponds to a CV of 5%.

Changes in $[\text{X}]_{\text{T}}$ and $[\text{Ligand}]_{\text{T}}$; $\Delta_{\text{X-T}}(\%)$ and $\Delta_{\text{L}}(\%)$: As illustrated in equations [81] and [83] for $\Delta_{\text{X-T}}(\%)$ and $\Delta_{\text{L}}(\%)$ respectively:

$$\Delta_{X-T}(\%) = \frac{\Delta_{\mu} \left(1 - \frac{[X]_T}{[\text{Ligand}]_T}\right)}{\left(100 + \Delta_{\mu} \frac{[X]_T}{[\text{Ligand}]_T}\right)} * 100$$

and

$$\Delta_L(\%) = \frac{\Delta_{\mu} \left(\frac{[X]_T}{[\text{Ligand}]_T} - 1\right)}{(100 + \Delta_{\mu})} * 100$$

These equations illustrate that both $\Delta_{X-T}(\%)$ and $\Delta_L(\%)$ are functions of the ratio, $\frac{[X]_T}{[\text{Ligand}]_T}$ and because of this it is not possible to define set criteria for both these parameters as it was with both $R_{E/T}$ and ΔpK_{app} (see also McGuigan et al., 2005).

CONCLUSIONS

There is at present a complete lack of criteria for the allowable range of ionised concentrations of for both Ca^{2+} and Mg^{2+} in buffer solutions. Based on the experimental estimations of $[\text{Mg}^{2+}]$ in $\text{Mg}^{2+}/\text{EDTA}$ and $\text{Mg}^{2+}/\text{ATP}$ buffer solutions the empirical proposal was made that the range for $[\text{X}^{2+}]$ in Ca^{2+} and Mg^{2+} in buffer solutions should be less than $\pm 10\%$ of the mean values which corresponds to a CV of 5%. From this definition it follows that the ratio of estimated to true ionised concentrations must be within the range 0.9 to 1.1 and the range of ΔpK_{app} values at pK_{app} values greater than 6 must lie within ± 0.04 pK_{app} units. No such criteria can be set for changes in both $[X]_T$ and $[\text{Ligand}]_T$ as the changes for both depend on the ratio $\frac{[X]_T}{[\text{Ligand}]_T}$.

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