APPENDIX

This is the Appendix to the following three papers:

1) McGuigan, J. A. S., Kay, J. W. & Elder, H. Y (2005). Critical review of the methods used to measure the apparent equilibrium constant and ligand purity in Ca^{2+} and Mg^{2+} buffer solutions.
2) Kay, J. W. Steven Rachel J. McGuigan, J. A. S. & Elder, H. Y. (2005). Automatic determination of ligand purity and apparent equilibrium constant (K_{app}) in divalent cation buffer solutions and K_{app} on physiological solutions.
3) McGuigan, J. A. S., Kay, J. W. & Elder, H. Y & Lüthi, D. 2005). Lack of agreement between calculated ionised Ca^{2+} and Mg^{2+} concentrations and concentrations measured with macroelectrodes: the need for standardisation.

To make it easier to use the Appendix is divided into five Sections, each with an extensive list of contents.

SECTIONS

SECTION-I

This is the full Appendix to:

Lüthi, D., Günzel, D. & McGuigan, J. A. S. (1999). Mg-ATP binding: its modification by spermine, the relevance to cytosolic Mg^{2+} buffering, changes in the intracellular ionised Mg^{2+} concentration and the estimation of Mg^{2+} by ^{31}P-NMR. *Experimental Physiology*, **84**, 231-252.

Section-1 is included because the equations derived in this Appendix are referred to in the other Sections. Section-I covers the binding of H^{+}, Mg^{2+}, Na^{+}, Ca^{2+} and polyamines to ATP as well as acid base titration and definitions of buffering.

SECTION-II

This section derives the equations for the binding of Ca^{2+}, Mg^{2+} and H^{+} to EGTA. The number of H^{+}-ions released on Ca^{2+} binding is considered as well as the correction of the tabulated stoichiometric constants for ionic strength and temperature. The equations for the correction for ionic strength and temperature were derived by Professor (now emeritus) Paul Schindler, Department of Chemistry, University of Bern, Switzerland.

SECTION-III

This section covers the binding of Ca^{2+} to ligands other than EGTA. The ligands considered are: BAPTA, Br_2BAPTA, CDTA and EDTA. Also considered is the binding of Ca^{2+} to NTA, unique in that Ca^{2+} binds to one and to two molecules of NTA. The buffering characteristics of NTA are also considered.

SECTION-IV

The Section also considers the ratio method for the manufacture of buffer systems, as well as the buffer range for various ligands for both Ca^{2+} and Mg^{2+} buffer solutions. The influence of changes in pK_{app}, and in both total cation and ligand concentration is also considered. Finally the allowable imprecision for ionised concentrations in Ca^{2+} and Mg^{2+} buffer solutions is considered.

SECTION V

This section describes the Chelax partition method and is based on the Ph.D. Thesis of Professor R. E. Godt (1971):

Calcium-activated tension of skinned muscle fibres: dependence on Mg-ATP concentration. Ph.D. Thesis, University of Washington, Seattle, 128 pp.

UNITS

All units are in mol/l. *Failure to use such units will lead to errors.*

SOLUTION OF EQUATIONS

QUADRATIC EQUATIONS

The solution to the quadratic:

$$ax^2 + bx + c = 0$$

was:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

CUBIC EQUATIONS

The cubic equation of the form:

$$Ax^3 + Bx^2 + Cx + D = 0$$

Cubic equations were solved using the Newton-Raphson method:

$$x_{n+1} = x_n - \frac{Ax_n^3 + Bx_n^2 + Cx_n + D}{3Ax_n^2 + 2Bx_n + C}$$

A series of such equations can be readily solved using a spread sheet such as Excel or Kaleidagraph, provided a reasonable guess is made for the initial value x_n.

USE OF APPENDIX

The Appendix is intended as a reference. To find the required derivation it is advised to check the Content pages of the various Sections.

ACKNOWLEDGEMENTS

In the derivation of these equations my colleagues in the various papers involved, have offered help and advice and I would like to express my thanks to them. The equations in the various Sections have been derived over a period of years and while every effort has been made to insure the minimum of topographical errors it is almost inevitable that some will have been overlooked.

The responsibility for the accuracy of this Appendix is mine, and if errors are found please contact me at the following address:

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