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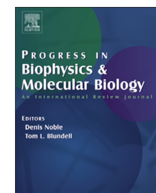

# Ionised concentrations in calcium and magnesium buffers: Standards and precise measurement are mandatory

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In $Ca^{2+}$ and $Mg^{2+}$ buffer solutions the ionised concentrations ($[X^{2+}]$) are either calculated or measured. Calculated values vary by up to a factor of seven due to the following four problems:

1) There is no agreement amongst the tabulated constants in the literature. These constants have usually to be corrected for ionic strength and temperature.
2) The ionic strength correction entails the calculation of the single ion activity coefficient, which involves non-thermodynamic assumptions; the data for temperature correction are not always available.
3) Measured pH is in terms of activity *i.e.* $pH_a$. $pH_a$ measurements are complicated by the change in the liquid junction potentials at the reference electrode making an accurate conversion from $H^+$ activity to $H^+$ concentration uncertain.
4) Ligands such as EGTA bind water and are not 100% pure. Ligand purity has to be measured, even when the $[X^{2+}]$ are calculated.

The calculated $[X^{2+}]$ in buffers are so inconsistent that calculation is not an option. Until standards are available, the $[X^{2+}]$ in the buffers must be measured. The Ligand Optimisation Method is an accurate and independently verified method of doing this (McGuigan and Stumpff, Anal. Biochem. 436, 29, 2013). Lack of standards means it is not possible to compare the published $[Ca^{2+}]$ in the nmolar range, and the apparent constant ($K'$) values for $Ca^{2+}$ and $Mg^{2+}$ binding to intracellular ligands amongst different laboratories. Standardisation of $Ca^{2+}/Mg^{2+}$ buffers is now essential. The parameters to achieve this are proposed.

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## 1. Introduction

Intracellular resting [$Ca^{2+}$] is of the order of 100 nmol/l (pCa 7.0) and the $pK^/$ values for calcium binding to intracellular ligands are also in this range (Bers, 2002, Table 10, p 46). The intracellular [$Mg^{2+}$] is around 0.8 mmol/l (McGuigan et al., 1993) and while $Mg^{2+}$ buffers are not necessary at this concentration, the $pK^/$ values for $Mg^{2+}$ binding to intracellular ligands such as ATP are around 4 (Lüthi et al., 1999). [$X^{2+}$] and the $pK^/$ values can be measured with electrodes (Lüthi et al., 1997), fluorochromes (Bers, 2002) or $^{31}P$-NMR (Iotti et al., 1996). In the range 0.5 mmol/l to 10 mmol/l (pX 3.301 to pX 2.000), concentrations can be set by dilution alone *i.e.* buffers are not required. However, at [$X^{2+}$] less than 0.5 mmol/l buffers are required, and are necessary down to the nmolar range for $Ca^{2+}$ and down to the μmolar range for $Mg^{2+}$; the full range can be covered by the correct choice of ligand (Fig. 10, McGuigan et al., 2006). Modelling of intracellular processes has become an important branch of physiology (see for instance, http://www.physiome.org; Michailova et al., 2007; Noble et al., 2012), but the accuracy of such modelling depends on a precise knowledge of the $pK^/$ values for all the $Ca^{2+}/Mg^{2+}$ intracellular interactions involved in the modelling processes, as well as the intracellular [$Ca^{2+}$]. Despite the need for accurate measurements, there are at present no internationally defined standard buffers for $Ca^{2+}$ and $Mg^{2+}$.

This paper considers first the physical chemical properties relevant to calculation of the [$X^{2+}$] in $Ca^{2+}/Mg^{2+}$ buffers before describing the results of the calculations. Methods of measuring the [$X^{2+}$] in the buffers are then considered and finally measured and

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