

THE THERMODYNAMIC ACTIVITY OF CALCIUM ION IN SODIUM CHLORIDE- CALCIUM CHLORIDE ELECTROLYTES

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ABSTRACT Experimental data on the mean activity coefficient of CaCl_2 in NaCl - CaCl_2 mixtures at ionic strengths below 1 m have been used to prepare a table of activity coefficients for Ca^{++} in solutions of physiological interest. The establishment of an empirical calcium ion activity scale is discussed, and a number of possible assumptions are examined. The assumption $\gamma_{++} = (\gamma_{\pm})^2$ is suggested as being the simplest with a theoretical basis.

INTRODUCTION

The activity of calcium ion in blood serum, urine, and other extracellular fluids is well known to be an important physiological parameter (1, 14). Direct measurement of this quantity until recently has been experimentally difficult, and also depends on nonthermodynamic assumptions to split experimentally accessible chemical potentials into parts attributable to single ions. Calcium ion concentration, a quantity directly measurable by ion exchange (18), titration (3), spectrophotometry (7, 13) or flame photometry (4, 12), has often been used as a measure of calcium ion activity, but the activity coefficient of calcium ion in physiological fluids is certainly not unity, and may vary considerably with the composition of the solution.

Recent interest in the measurement of calcium ion activity using specific ion electrodes (1, 17, 19, 20¹) has raised the question (8) of establishing an unambiguous calcium ion activity scale in a manner analogous to the establishment of the hydrogen ion activity (pH) scale. Quite apart from experimental difficulties, there are two important problems associated with establishing such a scale. The first is a choice of the method by which the chemical potential of a calcium salt in a mixed electrolyte shall be divided into contributions from its individual ions. The second is the establishment of standard solutions of known activity against which the spe-

¹ The reader's attention is called to a closely related paper by A. Shatkay which appeared in the August 1968 issue of the *Biophysical Journal*.

cific-ion electrode system may be calibrated in practice (2). To avoid systematic errors due to lack of complete specificity of the electrode and to effects of ionic composition on activity coefficients and liquid junction potentials, this calibration solution should be as similar as possible to the test solution.

The activity coefficients of the salts in NaCl-CaCl₂-H₂O mixtures have been the subject of a number of studies (5, 11, 14, 15) during the last three years, and sufficient data are now available to provide an accurate measure of the thermodynamic activity of calcium ion in solutions consisting primarily of NaCl. These solutions can be taken as calibration mixtures for direct measurements of calcium activity in serum and related solutions.

This paper presents a summary of experimental data on the NaCl-CaCl₂ electrolyte mixtures, discusses the possible calcium ion activity scales, and gives a table of activity coefficients for solutions of physiological interest.

EXPERIMENTAL DATA

All available experimental measurements of the activity coefficients of CaCl₂ in NaCl-CaCl₂ mixtures are indirect and depend on the use of thermodynamic relations to obtain the desired quantity. Cation-sensitive glass electrodes (11, 14) and sodium amalgam electrodes (5) measure the activity of NaCl in mixtures with CaCl₂. From the osmotic coefficients of the pure components (10, 16) the activity coefficients of CaCl₂ in the same mixtures can be calculated. An independent measurement has been made by the isopiestic method (15) which essentially measures the vapor pressure or activity of water in the mixed electrolyte by comparison with an electrolyte of known vapor pressure. Again, by using the osmotic coefficients of the pure components, the activity coefficients of NaCl or CaCl₂ in the mixture can be calculated.

In Fig. 1, the various experimental data are summarized in terms of the Harned rule coefficient α_{21} , defined by the equation (10, 16)

$$\log \gamma_{21} = \log \gamma_{20} - \alpha_{21}m_1 \quad (1)$$

where γ_{21} is the mean activity coefficient of CaCl₂ in the mixed electrolyte, γ_{20} is the mean activity coefficient of CaCl₂ (16) in a solution of the same ionic strength but containing only CaCl₂, and m_1 is the molal concentration of NaCl in the mixed electrolyte. The available data extend to ionic strength 7.0 (15), but we have presented data only for the range of ionic strength ($I < 1.0$) which is of physiological interest. The line on Fig. 1 represents our appraisal of the "best values" for α_{21} .

For the convenience of those wishing to avoid the graphical interpolation and subsequent calculations required to evaluate γ_{21} in a solution of interest we have prepared a summary, presented in Table I, of the activity coefficients of CaCl₂ in solutions containing various molal concentrations of NaCl (m_1) and CaCl₂ (m_2). To calculate this table, we used our "best values" of α_{21} from Fig. 1, together with activity coefficients for pure CaCl₂ solutions γ_{20} obtained from the tables of Robinson and Stokes (16). These tables cover only the range of ionic strength above 0.3. For the range below 0.3, we used the Guggenheim modification of

the Debye-Hückel equation (10)

$$\log \gamma_{20} = - \frac{2A\sqrt{I}}{1 + \sqrt{I}} + BI \quad (2)$$

with $A = 0.509$, $B = 0.2486$, and the total ionic strength given by

$$I = m_1 + 3 m_2 . \quad (3)$$

The value of B was obtained by making $\gamma_{20} = 0.518$ at $I = 0.3$, to correspond to the Robinson and Stokes tables. This resulted in a discontinuity in slope at $I = 0.3$, which was smoothed graphically, resulting in activity coefficient values slightly higher than those obtained by the Guggenheim equation in the range $I = 0.1$ to 0.3 . The largest discrepancy (0.004 in γ_{20}) was at $I = 0.2$.

All the activity coefficient values presented were obtained at 25°C instead of 37°C, the normal human body temperature. The effect of a 12° temperature change is not inordinately large. The predominant effect may be calculated from the temperature dependence of the coefficient A in the Debye-Hückel theory. At 37°C, $A = 0.476$, and at ionic strength 1.0, this results in a value of γ_{20} which is higher than the 25°C value by approximately 0.025. This discrepancy is relatively constant down to $I = 0.1$, but decreases to zero at $I = 0$, and may be calculated from equation 2, assuming B is independent of temperature:

$$\gamma_{20}^{(37^\circ)} = \gamma_{20}^{(25^\circ)} \text{ antilog } [0.050 \sqrt{I/(1 + \sqrt{I})}] . \quad (4)$$

Corrections calculated from this equation may be applied to the data in Table I. For intermediate temperatures, note that A varies inversely with the 3/2 power of the absolute temperature (10, 16). The effect of temperature on the reference electrode and liquid junction potential in practical measurements can be essentially eliminated by calibration at the temperature of measurement.

The influence of other ionic components of physiological fluids on the activity of calcium ion is probably small. Certainly potassium and magnesium ion, which comprise less than 6% of the total ionic strength of serum, have a negligible effect on the activity coefficient of Ca^{++} at ionic strengths less than 1. Calcium forms complexes with protein and other organic materials (13, 18) but complexed calcium species are not normally measured by a specific ion electrode, and because of their low charge density and low concentration probably do not affect the activity coefficient of free calcium ion. Because of the unavailability of complexed calcium for precipitation and membrane transport processes, it is best to consider the free ionic calcium as a separate species and to work out the equilibria with protein and other organic materials separately. These equilibria may, however, introduce substantial discrepancies between ionic calcium as measured with a specific ion electrode and total calcium as determined by other methods such as flame photometry (4, 12). Provisionally, then, we may suggest that NaCl-CaCl₂ solutions, of the same ionic strength (or less precisely, NaCl concentration) as the serum samples to be measured, be used as standards for calibration of specific ion electrodes.

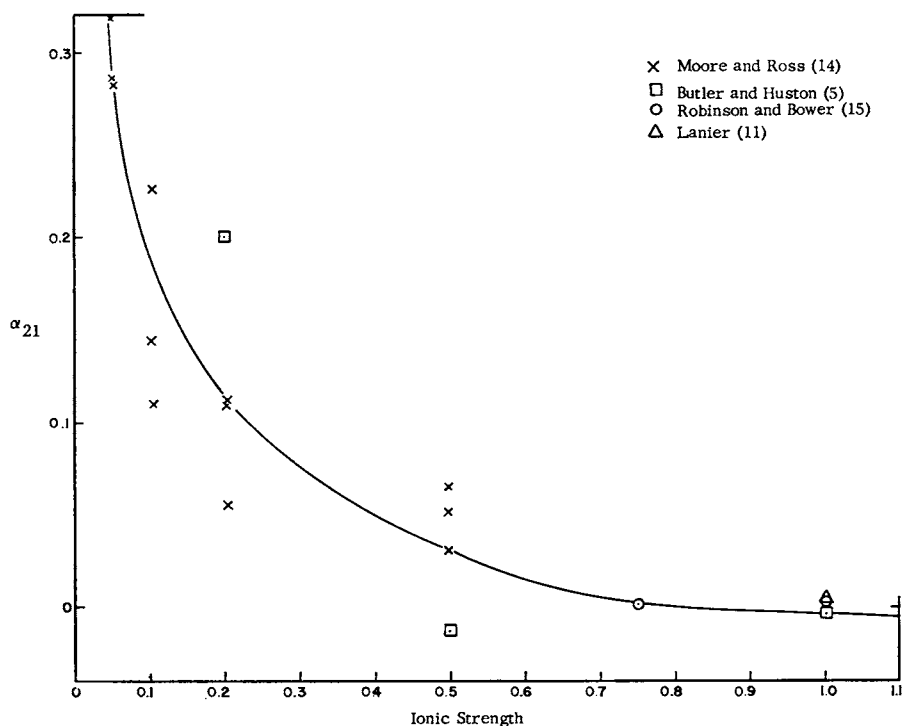


FIGURE 1 Harned rule coefficient for calcium ion in NaCl-CaCl₂ mixtures at 25°C. Points are experimental results of various workers, and the line represents the values used in preparing Table I.

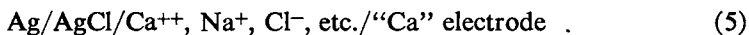
TABLE I
MEAN ACTIVITY COEFFICIENT OF CaCl₂ IN NaCl-CaCl₂ MIXTURES AT 25°C*

$m_1 \backslash m_2$	0	0.05	0.1	0.15	0.2	0.3	0.4	0.5	0.7	1.0
0	1.000	0.650	0.577	0.542	0.521	0.490	0.472	0.463	0.460	0.456
0.001	0.881	0.645	0.575	0.540	0.520	0.489	0.472	0.463	0.460	0.456
0.002	0.843	0.641	0.573	0.538	0.519	0.489	0.472	0.463	0.459	0.456
0.003	0.816	0.637	0.571	0.537	0.518	0.488	0.472	0.463	0.459	0.455
0.005	0.775	0.628	0.568	0.535	0.517	0.487	0.472	0.463	0.459	0.455
0.007	0.748	0.620	0.564	0.532	0.516	0.486	0.471	0.463	0.459	0.455
0.01	0.718	0.609	0.559	0.530	0.514	0.486	0.471	0.463	0.459	0.455
0.02	0.654	0.582	0.545	0.521	0.507	0.482	0.470	0.464	0.459	0.455
0.03	0.613	0.562	0.534	0.516	0.500	0.479	0.469	0.464	0.459	0.454
0.05	0.567	0.538	0.520	0.504	0.490	0.474	0.468	0.462	0.458	0.454

* m_1 = molal concentration of NaCl, m_2 = molal concentration of CaCl₂.

THE CALCIUM ION ACTIVITY SCALE

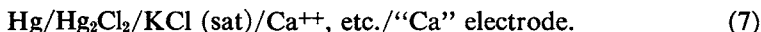
There are two important types of cells which can be used to measure calcium ion activity directly using a calcium-selective specific ion electrode. The first type is a cell without liquid junction:



where the reference electrode is reversible to chloride ion, and the potential is given by

$$E = E_s^0 + \frac{RT}{2F} \ln (m_{\text{Ca}^{++}}) + \frac{RT}{F} \ln (m_{\text{Cl}^-}) + \frac{3RT}{2F} \ln (\gamma_{\pm}) \quad (6)$$

where γ_{\pm} is the mean activity of CaCl_2 in the mixed electrolyte, approximated by values in Table I. The second type is more common, and employs a salt bridge and reference electrode of fixed potential:



The right-hand electrode, including the KCl salt bridge, is embodied in commercial saturated calomel reference electrodes. The potential of this cell is given by

$$E = E_7^0 + \frac{RT}{2F} \ln (m_{\text{Ca}^{++}}) + \frac{RT}{2F} \ln (\gamma_{++}) + E_j \quad (8)$$

where γ_{++} is the "single ion activity coefficient" of Ca^{++} , and E_j is the diffusion potential resulting from the liquid junction between the salt bridge and the test solution. The standard potentials E_s^0 and E_7^0 are independent of the composition of the test solution, provided the specific ion electrode does not respond to any ions in the solution besides Ca^{++} .

The first cell is thermodynamically well-defined, and the mean activity coefficient of CaCl_2 in a mixture of extreme complexity can in principle be obtained by any of the various methods used for simpler mixtures. The second cell is not at equilibrium because of the liquid junction between solutions, where mixing is taking place continuously. The diffusion potential is small if the difference between transport numbers of cation and anion in the salt bridge are small, the salt bridge concentration is large (e.g. saturated KCl), and the test solution concentration is small (<0.1 m). In practice this condition is obtained with good commercial reference electrodes, and E_j may vary by less than 5 mv over a considerable range of test solution compositions. Unfortunately, there is no unambiguous way to separate γ_{++} and E_j either thermodynamically or theoretically without making certain ad hoc assumptions; and an uncertainty in E_j of 5 mv corresponds to an uncertainty in

γ_{++} of 50 %. For this reason, various methods of eliminating the uncertainty in E_j have been proposed.

These methods are based on the same principle as the "operational" definitions of pH (2). Cell 7 is measured with a known solution whose composition approximates that of the unknown solution as closely as possible, but the known solution is prepared so that the thermodynamic activity coefficient γ_{\pm} is known from measurements in Cell 5 or by other measurements. The single ion activity coefficient γ_{++} is either calculated theoretically or taken to be some function of the quantity γ_{\pm} . Three simple choices of this function have been made, each of which has some validity.

Shatkay (19, 20) chose to set $\gamma_{++} = \gamma_{\pm}$, and justified this choice by comparison of experimental potential measurements made on Cell 7 with a theoretical curve calculated assuming $E_j = 0$. Garrels (9) assumed, following MacInnes, that the activity coefficient of chloride ion can be approximated by the mean activity coefficient of KCl in aqueous KCl solutions of the same ionic strength. This leads to the relation

$$\gamma_{++(\text{Ca})} = [\gamma_{\pm(\text{CaCl}_2)}]^3 / [\gamma_{\pm(\text{KCl})}]^2. \quad (9)$$

Still another assumption was suggested by Guggenheim on the basis of the various equations based on Debye-Hückel theory (10, 16). For a 1-2 electrolyte like CaCl_2 , the value of γ_{++} is given by

$$\log \gamma_{++} = -4A\sqrt{I}/(1 + \sqrt{I}) + \text{extended terms} \quad (10)$$

whereas the mean activity coefficient γ_{\pm} is given by

$$\log \gamma_{\pm} = -2A\sqrt{I}/(1 + \sqrt{I}) + \text{extended terms}. \quad (11)$$

If the extended terms are similar in both equations 10 and 11, the relationship

$$\gamma_{++} = (\gamma_{\pm})^2 \quad (12)$$

is obtained. Although this cannot be tested experimentally any more than the other two assumptions can, it has a stronger theoretical basis than Shatkay's assumption, and is simpler to calculate than Garrels' assumption (above) based on experimental γ_{\pm} values.

We have included in Fig. 2 two additional curves calculated by means of theoretical expressions; the Debye-Hückel equation for a divalent ion (10)

$$\log \gamma_{++} = - \frac{(0.509)(4)\sqrt{I}}{1 + 0.328a\sqrt{I}} \quad (13)$$

with the ion-size parameter $a = 6$; and the Davies (6) equation for a divalent ion

$$\log \gamma_{++} = -(0.509)(4) \left[\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2I \right]. \quad (14)$$

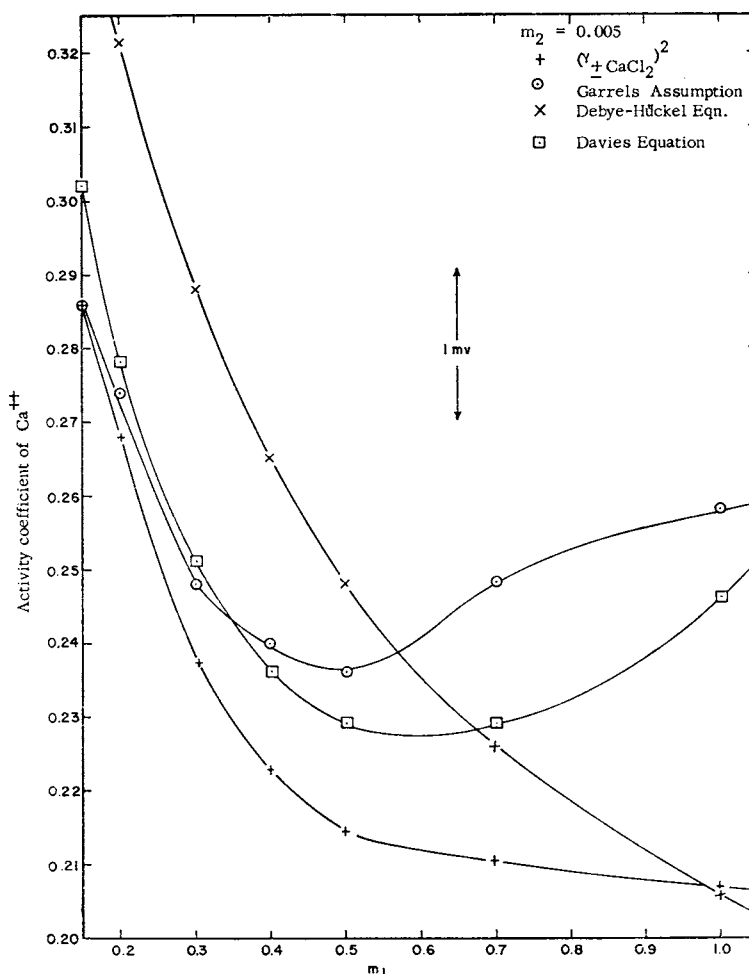


FIGURE 2 Estimates of the single-ion activity coefficient for Ca^{++} (at concentration $m_2 = 0.005$) in NaCl solutions of concentration m_1 . The various methods of calculation are described in the text.

The Davies equation has been shown to give a good fit to the mean activity coefficients of a large number of 1 - 1 and 1 - 2 electrolytes. In both of these equations I is the ionic strength, $m_1 + 3m_2$. The discrepancy between these four estimates corresponds to less than 2 mv in the potential of a specific ion electrode for m_1 less than 1.0. In contrast, $\gamma_{++} = \gamma_{\pm}$ (Shatkay's assumption) gives values between

0.45 and 0.55, which are off scale at the top of Fig. 2; and the difference from the group of four other estimates on Fig. 2 corresponds to as much as 10 mv in the potential of a specific ion electrode.

Because of the agreement with theoretical values for γ_{++} , we recommend that either the assumption $\gamma_{++} = (\gamma_{\pm})^2$ or the Garrels (9) assumption be used to estimate single-ion activity coefficients for calcium ion in standard mixtures. Either of these would result in a calcium ion activity scale with an uncertainty of less than 0.05 pCa units at ionic strengths less than 0.5. Although this is not as well-defined as the pH scale (2), it may form an interim basis for calcium ion activity measurements until international agreement is reached on activity standards.

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