CALCULATION OF THE CONCENTRATIONS OF FREE CATIONS AND CATION-LIGAND COMPLEXES IN SOLUTIONS CONTAINING MULTIPLE DIVALENT CATIONS AND LIGANDS

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ABSTRACT The method described permits the computation of the concentrations of free ions and ion-ligand complexes in a solution containing arbitrary numbers of divalent cations and ligands. It is required that the pH be known, along with appropriate sets of ligand-hydrogen and ligand-divalent cation concentration binding constants. It is assumed that these sets of constants are chosen to be consistent with the ionic strength of the complete solution which contains the divalent cations and ligands. The technique is an iterative one which provides upper and lower bounds for the values of the unknowns. The method does not require initial guesses at the values of the unknowns, and it gives correct answers even when the concentrations involved are many orders of magnitude apart. The present formulation of the problem is restricted to the case where only one cation can bind to a given ligand at any one time. The method is applicable to large molecules with multiple "sub-ligands" provided these sub-ligands are independent in their function as ion-binding sites. These sub-ligands need not all have the same properties. It is also shown that a simple modification of the method permits the determination of the subset of total ion concentrations that are required in order to produce a specified subset of free ion concentrations. The modifications required to include monovalent cation binding are presented in outline form.

INTRODUCTION

Investigators dealing with the effects of divalent cations in biological systems have long recognized that the free form of the ion may have a concentration that is significantly lower than its total concentration. It has also been recognized that the proper interpretation of experimental results often depends on an understanding of the value of the free ion concentrations and of the changes in free ion concentration that may occur during an experimental procedure. Such concerns have resulted in protocol or ligand-specific procedures for computing the level of two free ions with one or two ligands (Botts et al., 1966; Caldwell, 1970; Katz et al., 1970; Wolf, 1973). Consideration has also been given to the procedures for dealing with one ion and many ligands (Branegård and Österberg, 1974). More general approaches to the many-ion, many-ligand problem have been given by Perrin (1965), Perrin and Sayce (1967), and Fabiato and Fabiato (1978). These methods all use iterative numerical procedures to solve the appropriate set of equations. Such methods often give unsatisfactory results when the various concentrations of species are orders of magnitude apart. The method presented here is not sensitive to these considerations, and no attempt is made to actually solve

the equations. Instead, the upper and lower bounds of the value of each unknown are systematically made to approach each other until an appropriate precision is achieved. The formulation of the problem includes arbitrary numbers of divalent cations and ligands. Although thermodynamic binding constants are defined in terms of activities rather than concentrations, the calculation technique presented here is formulated in terms of concentration binding constants. Such a procedure is consistent with the literature as most of the measured values have been presented as concentration binding constants in salt solutions of varying ionic strength. The method is not limited by the values of the binding constants, but it is presumed that the ionic strength is known accurately enough that sets of concentration binding constants are obtainable. If the ionic strength is appreciably changed by the amounts of bound cations and ligands, then it will be very difficult to choose an appropriate set of binding constants. It is also assumed that the solution is adjusted to some desired pH value, and that no more than one divalent cation can be bound to a given ligand at any moment. It is presumed that no significant amount of monovalent cation binding occurs. The presumption that no monovalent cation binding occurs is not critical; it is easy to include such a possibility, and the procedures required to do so are given at the end of the Discussion. It makes no difference whether the ligands, which are assumed to behave independently, occur separately or in a macromolecule provided that the local charge densities do not rise to the point that it is inappropriate to use bulk solution concentrations for the hydrogen ion and the divalent cations.

The difficulties with the calculations involving macromolecules will be minimized if the binding constants used have been measured for the specific macromolecule at a pH and ionic strength close to the conditions for which the calculations are being made. Under these circumstances, we would expect that mass action principles would be satisfactory unless the values of the binding constants for one ligand depend on whether or not a neighboring ligand is bound. If such cooperative effects exist, the calculation method described in this communication is not appropriate. It will be found, for example, that the method will be satisfactory for some proteins, but not for others.

To present the development of this method in a clear manner, the following sections contain a set of required definitions; the conservation equations for ligands and cations, and the algorithms for computing the values of the unknown quantities.

DEFINITIONS

i Index for ligands: $1 \leqslant i \leqslant I$.

j Index for ligand or ligand complex charge.

k Dummy index.

m Index for divalent cations: $1 \leqslant m \leqslant M$.

(H^{+1}) Hydrogen ion concentration.

J_i Absolute value of the maximum charge on the ith ligand.

(L_i^{-j}) Concentration of the form of the ith ligand having charge $-j$; $j \leqslant J_i$ for the ith ligand.

(L_i^{-o}) Concentration of the neutral hydrogen form of the ith ligand.

(C_m^{+2}) Concentration of the free form of the mth divalent cation.

(C_m^{Tot}) Total concentration of the mth divalent cation in all forms.

$(C_mL_i^{-j+2})$ Concentration of the complex formed from the mth cation and the form of the ith ligand having charge $-j$: $(0 \leq j < J_i)$.

$\alpha'_{i,j}$ jth hydrogen binding constant for the ith ligand: $\alpha'_{i,j} = (L_i^{-j+1})/[(H^{+1})(L_i^{-j})]$.

$\alpha_{i,j}$ $(H^{+1})\,\alpha'_{i,j}$, for $j = 1, 2 \ldots J_i$; $\equiv 1$ for $j = J_i + 1$.

$\beta_{i,j,m}$ Concentration binding constant for the reaction of the ith ligand having charge $-j$:

$$\beta_{i,j,m} = (C_mL_i^{-j+2})/[(C_m^{2+})(L_i^{-j})].$$

EQUATION DEVELOPMENT

We begin by writing down the conservation equations for the I ligands and M divalent cations.

$$(L_i^{Tot}) = \sum_{j=o}^{J_i} (L_i^{-j}) + \sum_{m=1}^{M} \left\{ \sum_{j=o}^{J_i} (C_mL_i^{-j+2}) \right\}, i = 1, 2 \ldots I. \tag{1}$$

$$(C_m^{Tot}) = (C_m^{+2}) + \sum_{i=1}^{I} \left\{ \sum_{j=o}^{J_i} (C_mL_i^{-j+2}) \right\}, m = 1, 2 \ldots M. \tag{2}$$

Inasmuch as $\alpha_{i,j} = (L_i^{-j+1})/(L_i^{-j})$ for $j = 1, 2 \ldots J_i$ and $\alpha_{i,J_i+1} = 1$, we can write

$$\begin{aligned} \sum_{j=o}^{J_i} (L_i^{-j}) &= (L_i^{-J_i}) + (L_i^{-J_i+1}) + \cdots + (L_i^{-o}) \\ &= (L_i^{-J_i}) \left\{ 1 + \frac{(L_i^{-J_i+1})}{(L_i^{-J_i})} + \cdots + \frac{(L_i^{-o})}{(L_i^{-J_i})} \right\} \\ &= (L_i^{-J_i}) \left\{ \sum_{j=o}^{J_i} \prod_{k=-1}^{(J_i-j-1)} \alpha_{i,J_i-k} \right\}. \end{aligned} \tag{3}$$

Next, we can evaluate

$$\begin{aligned} \sum_{j=2}^{J_i} (C_mL_i^{-j+2}) &= (L_i^{-J_i})(C_m^{+2}) \sum_{j=o}^{J_i} \frac{(C_mL_i^{-j+2})}{(L_i^{-J_i})(C_m^{+2})} \\ &= (C_m^{+2})(L_i^{-J_i}) \left\{ \beta_{i,J_i,m} + \beta_{i,J_i-1,m} \frac{(L_i^{-(J_i-1)})}{(L_i^{-J_i})} + \cdots + \beta_{i,o,m} \frac{(L_i^{-o})}{(L_i^{-J_i})} \right\} \\ &= (C_m^{+2})(L_i^{-J_i}) \left\{ \sum_{j=o}^{J_i} \beta_{i,j,m} \prod_{k=-1}^{J_i-j-1} \alpha_{i,J_i-k} \right\}. \end{aligned} \tag{4}$$

Finally, we obtain

$$(L_i^{Tot}) = (L_i^{-J_i}) F_i[(C_1^{+2}), (C_2^{+2}), \ldots, (C_M^{+2})] \equiv F_i[(C_m^{+2})](L_i^{-J_i}) \tag{5}$$

and

$$(C_m^{Tot}) = (C_m^{+2})\, G_m[(L_1^{-J_1}), (L_2^{-J_2}), \ldots, (L_I^{-J_I})] \equiv G_m[(L_i^{-J_i})](C_m^{+2}), \tag{6}$$

where

$$F_i[(C_m^{+2})] \equiv \left\{ \sum_{j=o}^{J_i} \prod_{k=-1}^{J_i-j-1} \alpha_{i,J_i-k} + \sum_{m=1}^{M} (C_m^{+2}) \sum_{j=o}^{J_i} \beta_{i,j,m} \prod_{k=-1}^{J_i-j-1} \alpha_{i,J_i-k} \right\} \tag{7}$$

and

$$G_m[(L_i^{-J_i})] = \left\{1 + \sum_{2791=1}^{I} (L_i^{-J_i}) \sum_{j=o}^{J_i} \beta_{i,j,m} \prod_{k=-l}^{J_{i-j-l}} \alpha_{i,J_i-k}\right\}. \quad (8)$$

These equations show that

$$\frac{(L_i^{-J_i})}{(L_i^{Tot})} \text{ depends only on the concentrations } (C_m^{+2});$$

and

$$\frac{(C_m^{+2})}{(C_m^{Tot})} \text{ depends only on the ligand concentrations } (L_i^{-J_i}).$$

The algorithms given in the next two sections depend on the simplicity of this result. Note that both F and G represent the sums of positive terms.

ALGORITHM I

All (C_m^{Tot}) and (L_i^{Tot}) are known; the objective is to find the values of (C_m^{+2}) and $(L_i^{-J_i})$, from which the values of all other solution components can be calculated. This algorithm, in other words, can be used to compute the detailed composition of the solution when the pH and the total concentrations of the binding cations and ligands are known. The direct results of the computation are the concentrations of the free cation and of the most highly charged (free) ligand form. These results permit the calculation of the concentration of all other components in this solution.

Begin: Let $(C_m^{+2})_{\min} = 0$

$$(C_m^{+2})_{\max} = (C_m^{Tot})$$

Loop: $$(L_i^{-J_i})_{\min} = (L_i^{Tot})/F_i[(C_m^{+2})_{\max}]$$

$$(L_i^{-J_i})_{\max} = (L_i^{Tot})/F_i[(C_m^{+2})_{\min}]$$

$$(C_m^{+2})_{\min} = (C_m^{Tot})/G_m[(L_i^{-J_i})_{\max}]$$

$$(C_m^{+2})_{\max} = (C_m^{Tot})/G_m[(L_i^{-J_i})_{\min}].$$

Repeat from Loop until the minimum and maximum values of each unknown agree to the desired precision. Note that, by definition,

$$(L_i^{-J_i})_{\min} < (L_i^{-J_i}) < (L_i^{-J_i})_{\max}, \quad \text{and} \quad (C_m^{+2})_{\min} < (C_m^{+2}) < (C_m^{+2})_{\max}.$$

The successive estimates for the upper and lower bounds of the unknowns form monotonically decreasing and increasing sequences. In practice, these sequences will be limited by the precision of the computer used.

ALGORITHM II

The (L_i^{Tot}) are all known, as are the (C_m^{+2}) for $m = 1, 2 \ldots N \leq M$ and the (C_m^{Tot}) for $m = N + 1, \ldots M$. $(F_i[(C_m^{+2})])$ is now a function of $(M - N)$ unknown variables, but we use the same notation.) The objective is to calculate (C_m^{Tot}) for $1 \leq m \leq N$, and (C_m^{+2}) for $N + 1 \leq m \leq M$. The algorithm can be used to compute the detailed composition of the solution when the total concentrations of all ligands are known, but when some values of free binding cation concentration are specified. The total concentration of other binding cations may also be specified. The direct results of the computation are the concentrations of the most highly charged (free) ligand form and either the total or free concentration of binding cation. These results again permit the calculation of the concentrations of other components.

Begin: Let $(C_m^{+2})_{\min} = 0$ $\quad m = N + 1, \ldots M$

$(C_m^{+2})_{\max} = (C_m^{Tot})$ $\quad m = N + 1, \ldots M$

Loop: $(L_i^{-J_i})_{\min} = (L_i^{Tot})/F_i[(C_m^{+2})_{\max}]$

$(L_i^{-J_i})_{\max} = (L_i^{Tot})/F_i[(C_m^{+2})_{\min}]$

$(C_m^{Tot})_{\min} = (C_m^{+2})\,G_m[(L_i^{-J_i})_{\min}]$ $\quad m = 1, 2, \ldots N$

$(C_m^{Tot})_{\max} = (C_m^{+2})\,G_m[(L_i^{-J_i})_{\max}]$ $\quad m = 1, 2, \ldots N$

$(C_m^{+2})_{\min} = (C_m^{Tot})/G_m[(L_i^{-J_i})_{\max}]$ $\quad m = N + 1, \ldots M$

$(C_m^{+2})_{\max} = (C_m^{Tot})/G_m[(L_i^{-J_i})_{\min}]$ $\quad m = N + 1, \ldots M.$

Repeat from Loop until the minimum and maximum values of each unknown agree to the required precision. If $N = M$, the algorithm degenerates; the values of $(L_i^{-J_i})_{\min}$ and $(L_i^{-J_i})_{\max}$ are identical, and the expression for $(C_m^{Tot})_{\min}$ immediately yields the values of (C_m^{Tot}).

EXAMPLE

Suppose that we make the following identifications:

$L_1 \equiv$ EGTA; $(L_1^{Tot}) \equiv (E^{Tot})$; $J_1 = 4$; $(L_1^{-J_1}) \equiv (E^{-4})$

$L_2 \equiv$ ATP; $(L_2^{Tot}) \equiv (A^{Tot})$; $J_2 = 4$; $(L_2^{-J_2}) \equiv (A^{-4})$

$C_1 \equiv$ Ca; $(C_1^{Tot}) \equiv (Ca^{Tot})$; $(C_1^{+2}) \equiv (Ca^{+2})$

$C_2 \equiv$ Mg; $(C_2^{Tot}) \equiv (Mg^{Tot})$; $(C_2^{+2}) \equiv (Mg^{+2})$

Then: $(E^{Tot}) = (E^{-4})\,F_E[(\mathrm{Ca}^{+2}), (\mathrm{Mg}^{+2})]$

$(A^{Tot}) = (A^{-4})\,F_A[(\mathrm{Ca}^{+2}), (\mathrm{Mg}^{+2})]$

$(\mathrm{Ca}^{Tot}) = (\mathrm{Ca}^{+2})\,G_{\mathrm{Ca}}[(E^{-4}), (A^{-4})]$

$(\mathrm{Mg}^{Tot}) = (\mathrm{Mg}^{+2})\,G_{\mathrm{Mg}}[(E^{-4}), (A^{-4})]$

and, for example,

$$F_E \equiv \sum_{j=0}^{4} \sum_{k=-1}^{3-j} \alpha_{E,4-k} + (Ca^{+2}) \sum_{j=2}^{4} \beta_{E,j,Ca} \sum_{k=-1}^{3-j} \alpha_{E,4-k} + (Mg^{+2}) \sum_{j=2}^{4} \beta_{E,j,Mg} \sum_{k=-1}^{3-j} \alpha_{E,4-k}$$

$$= (\alpha_{E,4}\alpha_{E,3}\alpha_{E,2}\alpha_{E,1} + \alpha_{E,4}\alpha_{E,3}\alpha_{E,2} + \alpha_{E,4}\alpha_{E,3} + \alpha_{E,4} + 1) + (Ca^{+2})(\beta_{E,2,Ca}\alpha_{E,4}\alpha_{E,3} + \beta_{E,3,Ca}\alpha_{E,4} + \beta_{E,4,Ca}) + (Mg^{+2})(\beta_{E,2,Mg}\alpha_{E,4}\alpha_{E,3} + \beta_{E,3,Mg}\alpha_{E,4} + \beta_{E,4,Mg}).$$

Finally, referring to the definitions, we can identify some of the constants:

$$\alpha_{E,4} = (E^{-3})/(E^{-4}); \qquad \alpha_{E,3} = (E^{-2})/(E^{-3})$$
$$= (H^{+1})\alpha'_{E,4}; \qquad = (H^{+1})\alpha'_{E,3}$$
$$\beta_{E,3,Ca} = (CaE^{-1})/[(Ca^{2+})(E^{-3})]; \text{ and}$$
$$\beta_{E,4,Mg} = (MgE^{-2})/[(Mg^{2+})(E^{-4})].$$

These examples illustrate the method for identifying the parameters of the calculation technique with specific measurement in the literature. Also, from Table I:

$$\alpha_{E,4} = (H^{+1}) \cdot 10^{9.46} \text{ and } \beta_{E,3,Ca} = 10^{5.3}.$$

DISCUSSION

The techniques presented are simple to use and easy to program. They represent a straightforward method for estimating the solution of a set of equations obtained from

TABLE I
BINDING CONSTANTS FOR CATION-ATP AND CATION-EGTA COMPLEXES

Log (binding constant) at 20°C	Ligand									
	ATP					EGTA				
	1	2	3	4	Reference	1	2	3	4	Reference
Log α' for (H^{+1})	—	—	4.11	6.54	*	2.0	2.65	8.85	9.46	§
Log β for (Mg^{+2})	—	1.58	2.09	3.84	‡	—	—	3.4	5.2	§
Log β for (Ca^{+2})	—	—	2.13	3.98	*	—	—	5.3	10.97	§
Log β for (Sr^{+2})	—	—	2.07	3.58	*	—	—	—	8.05	¶
Log β for (Ba^{+2})	—	—	1.88	3.34	*	—	—	—	8.11	*
Log β for (Mn^{+2})	—	2.03	2.61	4.52	‡	—	—	7.02	12.28	§
Log β for (Zn^{+2})	—	2.09	2.78	4.75	‡	—	3.3	8.42	12.91	§

Columns for $j = 0$ have been left out because there are no data indicating the existence of these forms.
The data of Handschin and Brintzinger (1962) were measured in 0.1 M KCl. All other data were measured in 0.1 M KNO_3.
*Taqui Khan and Martell (1966).
‡Handschin and Brintzinger (1962).
§Anderegg (1964).
¶Wright et al. (1965).

thermodynamic considerations. They will give useful results to the extent that the equations written down accurately summarize the reactions occurring in a given solution. This means that the equations must account for all of the significant species present in the solution and that the values for the hydrogen (α') and cation (β) binding constants must be correct for the ionic strength used.

The use of these techniques, provided reliable values of α' and β are available, will make it possible to carry out experiments using media that are substantially more complex than those in common use at the present time. It may be possible, for example, to measure the values of α' and β for an enzyme that is activated by one or more divalent cations, and to correlate measured enzyme activity with a particular form of the cation-enzyme complex.

Table I summarizes some of the available data on the values of α' and β for several divalent cations and the ligands ATP and EGTA. Some of the values presented in the table have been converted from thermodynamic data, and all results were obtained at, or corrected to, 20°C.

Finally, it is appropriate to make a few comments about the algorithms. The nature of the procedures requires that the computations be repeated until the lower and upper bounds agree to the desired precision. The number of cycles required to achieve a desired level of precision can be quite variable. However, the computations are simple and convergence is very rapid on modern equipment. Perrin and Sayce (1967) used their method to solve a problem involving 10 metals, 10 ligands, and 195 constants. The computations required 7 min on an IBM 360/50 (IBM Corp., White Plains, N.Y.) Algorithm I was tested on a problem involving 10 metals, 10 ligands, and 333 constants. The results, to a precision of four figures, were obtained in 8 s of PR1ME P-500 (PR1ME Computer, Inc., Wellesley, Mass.) time. The Perrin and Sayce algorithm would have required about 140 s on the PR1ME machine, so algorithm I is at least an order of magnitude faster. In situations where the machine representation of real numbers is marginal, it would be wise to store each of the terms in each sum. The terms in a given sum can then be sorted and added together, beginning with the smallest value. It should be noted that it is trivial to extend the formulation to include the binding of monovalent cations, provided only one cation at a time (either monovalent or divalent) is bound to a given ligand. All that is necessary is to redefine

$$\beta_{i,j,m} \text{ as } \frac{(C_m L_i^{-j+Z_m})}{(C_m^{Z_m^+})(L_i^{-j})} \quad \text{for} \quad 0 < j < J_i,$$

where Z_m is the charge of the mth cation. Replacement of "2" by "Z_m" in the equations yields the appropriate results.

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