CHEMICAL METHODS FOR THE STUDY OF METAL-LIGAND INTERACTIONS IN AQUATIC ENVIRONMENTS

by

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Chemical Methods for the Study of Metal-Ligand Interactions in Aquatic Environments

by

John C. Westall

Submitted to the Department of Chemistry on January 13, 1977 in partial fulfillment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Part I. The behavior of the mixed copper sulfide/silver sulfide cupric ion-selective electrode in cuprous and argentous stabilizing media is explained by treating the electrode as if it were an elemental metallic electrode, with an activity of metal less than unity, reacting at its surface with cupric ion from the bulk of solution to form soluble complexes of Cu(I). The steady state potential of the electrode in unstirred solutions when the rate of the redox reaction becomes mass transport controlled is governed by equilibrium among the soluble cuprous, argentous and cupric species, and the hypothetical elemental copper and silver in the mixed sulfide phase. In general the mixed sulfide cupric ion-selective electrode is not useful for the measurement of cupric ion activity in media of high chloride concentration such as seawater.

Part II. A general method for the determination of metal-ligand complex stability constants from experimental data is based on the minimization of the weighted sum of squares of the residuals in the material balance equations of the chemical components for which both the total concentration and the free concentration is known. An estimate of the variance in the determined constants is made from estimates of the variance in the experimental data and analytical derivatives of the residuals in the material balance equations. Ancillary to this algorithm is a general method for the computation of the chemical equilibrium composition/speciation of aqueous systems, including gaseous and solid phases.

Part III. The interactions among trace metals, organic complexing agents, and phytoplankton are discussed with particular emphasis on the question whether substances exuded from pure phytoplankton cultures can significantly alter trace metal speciation in the culture medium. Titration methods based on the use of the glass electrode and the cupric ion-selective electrode are described which can detect a change on the order of micromolar in cupric ion speciation. No complexation of copper by extracellular organics was detected in cultures of T. pseudonana (3H) grown in a fresh water culture medium.

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Preface

This work comprises three parts, each of which is related to the characterization of metal-ligand interactions in aquatic environments. Part I, the study of the cupric ion-selective electrode in chloride media, was undertaken to assess the usefulness of the cupric ion-selective electrode for the measurement of cupric ion activity (and possible complexation of cupric ion) in seawater. Part II, the mathematical solution to the chemical equilibrium problem and the algorithm for the determination of metal-ligand complex stability constants, was developed for predictive purposes and for the analysis of experimental data from metal-ligand systems. Part III, the discussion of the interactions among phytoplankton, metals, and ligands considers possible effects of phytoplankton generated ligands on trace metal speciation in aquatic environments. Since each of these studies is complete within itself, all equations, figures, tables and references are labelled according to the part in which they are found.

Acknowledgement and thanks are due to Professor Francois Morel for providing a stimulating environment in which to work and to Professor David Hume for his advice and encouragement. I am indebted to a number of other people who have helped with this thesis in one way or another: Jean Coleman, Myra Kelly, Tom Lustig, Casey Swallow, and Neil Shifrin , who aided in preparation of the manuscript; John Rueter and Casey Swallow who contributed to the experimental work; my friends and colleagues, CA, DA, MA, DB, PC, HH, TDL, DMc, TMc, JR, NS, MsS, JY, who reviewed the manuscript and were always willing to have another at the Muddy Charles. Finally I acknowledge Gayle and my parents for their encouragement and support.

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Part I

The Interference of Chloride with the Cupric Ion-Selective Electrode

PART I
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Introduction

Solid state cupric ion-selective electrodes based on a mixed silver sulfide-copper sulfide phase have been used successfully for measurement of cupric ion activity in "uncomplicated" media (1,2). These electrodes respond with a slope of nominally 29.5 mV/decade change in cupric ion activity at 25°C, as predicted by the Nernst equation for a reaction involving a two electron transfer. However, difficulty is encountered when these electrodes are used in seawater or other media of high chloride ion activity. A relatively slow response and sensitivity to stirring are observed, along with a change in slope and apparent E° of the electrode. A slope of nominally 59 mV has been observed (3,4,5), which would correspond to a one electron transfer.

This behavior is not predicted by, nor can it be readily explained by the traditional view of the mixed cupric sulfide/silver sulfide electrode as an electrode of the third kind in equilibrium with Cu^{2+} and governed by the solubility equilibria of the metal sulfides. In this work we shall present a theory for the behavior of the mixed sulfide cupric ion-selective electrode in cuprous stabilizing media and discuss the analytical application of the electrode in such media.

THEORY

Analogy with Metallic Copper Electrode

The relatively slow response and sensitivity to stirring observed for the cupric ion-selective electrode in a cupric/chloride medium are reminiscent of the behavior of the copper metal electrode in a saturated cuprous chloride medium with a cupric ion impurity, as observed by Noyes

and Chow (6). They found the electrode potential to decrease slowly but steadily over several hours; when the solution was stirred, the potential increased, but declined again when stirring was ceased. After many hours the electrode potential reached a steady value and stirring no longer had any effect. This behavior was attributed to the reduction of the cupric ion impurity at the electrode surface to form cuprous ion. The potential of the electrode decreased as the supply of cupric ion at the electrode surface was depleted. Stirring temporarily restored the cupric ion concentration at the electrode surface to a higher value, but ultimately all of the cupric ion in solution was reduced, and a constant potential was reached.

The similarity between the behavior of the solid state copper electrode and the copper metal electrode in chloride media suggests that the solid state electrode is behaving as a copper metal electrode with an activity of elemental copper substantially lower than unity. Sato (7) first suggested that the potentials of semiconducting binary sulfide electrodes should be governed by the activities of the component elements in the solid phase. More recently Koebel (8) discussed the correspondence between the standard potentials of the mixed metal sulfide (Ag_2S - MeS) electrodes of Pb^{2+}, Cd^{2+}, and Cu^{2+} and the activity of the metal in the sensing material. The concept of the activity of the metal in mixed salt solid state electrodes influencing the potential is likewise an essential part of the more general theory of solid state electrode behavior developed by Buck and Shepard (9).

If the cupric ion-selective electrode functions as a metallic

copper electrode with an activity lower than that of the pure metal, the following behavior is to be expected: in a "non-complicated" medium the electrode potential depends only upon the activity of elemental copper in the electrode and the activity of cupric ion in solution; in a medium of high chloride concentration the electrode potential depends upon the activity of elemental copper in the electrode and the activity of cuprous ion at the electrode surface.

The apparent activity of the elemental copper in the mixed sulfide phase may be found experimentally from the electrode potential of the cupric ion-selective electrode in a solution of known cupric ion activity

$$E = E^\circ_{Cu^{2+}/Cu} + \frac{RT}{2F} \ln \frac{a_{Cu^{2+}}}{a_{Cu^\circ}} \qquad (1)$$

E is the potential of the ion-selective electrode vs. the normal hydrogen electrode, $E^\circ_{Cu^{2+}/Cu}$ the standard potential of the cupric/copper half reaction, $a_{Cu^{2+}}$ the activity of cupric ion in solution, and a_{Cu° the activity of Cu° in the electrode phase (the mixed sulfide phase and its contact to an external electron conducting circuit will be referred to as the "electrode phase," regardless of how many actual thermodynamic phases are present).

Mechanism of Interference

The response of the electrode in a chloride medium can be explained by the following formalism: cupric ion is reduced by elemental copper at the electrode surface to the cuprous ion

$$Cu^{2+} + Cu^\circ = 2Cu^+ \qquad (I)$$

and the cuprous ion is stabilized by chloride complexation

$$Cu^{+} + nCl^{-} = CuCl_n^{-(n-1)} \qquad \text{(II)}$$

Then at sufficiently high chloride concentrations in unstirred solutions, essentially all of the cupric ion in the immediate vicinity of the electrode is converted to the cuprous state and the response of the electrode is governed by the cuprous-copper half reaction

$$Cu^{+} + e^{-} = Cu^{\circ} \qquad \text{(III)}$$

This reaction involves a one electron transfer and gives an electrode response slope of 59 mV/decade.

Effect of Mass Transport

Since a chemical reaction does occur at the electrode surface, it is necessary to consider transport of chemical species to and from the electrode surface through the hydrodynamic boundary layer, in order to know the steady state concentration of potential determining species.

Under the experimental conditions normally employed, the thickness of the diffusion layer is small with respect to the area of the electrode (hence the approximation of linear diffusion applies), and the concentrations of species in the bulk of solution are unaffected by reactions at the electrode surface.

If the reactions at the electrode surface are reasonably fast compared to transport of material through the hydrodynamic boundary layer, quasi-equilibrium at the electrode surface will be approached within a reasonable period of time. (The "true" equilibrium potential may be

defined as the potential of the electrode when the flux of material through the hydrodynamic boundary layer is zero, i.e., when the solution at the electrode surface is equilibrated with the bulk of solution. The quasi-equilibrium potential is effectively equal to the true equilibrium potential, but the zero material flux condition through the hydrodynamic boundary layer is not strictly met. In each case, it is of course necessary that the exchange current be high enough to define a stable potential.)

The steady state condition may be approximated mathematically by the Nernst Diffusion Layer model of the electrode-solution interface (Figure 1): there is a stagnant layer of thickness δ in contact with the electrode on one side and the well mixed bulk of solution on the other; through the stagnant layer is a linear concentration gradient. The flux of a material through the hydrodynamic boundary layer is given by

$$J = \frac{D(C' - C)}{\delta} \qquad (2)$$

where J is the flux per unit area (mole sec^{-1} cm^{-2}), D is the diffusion coefficient (cm^2 sec^{-1}), C' is the concentration of the material at the electrode surface (mole cm^{-3}), C is the concentration in bulk and δ the thickness of the stagnant layer (cm). (The use of the Nernst Diffusion Layer Model here is only illustrative; the validity of the fundamental Equation 5 can be shown to depend solely on the approximate equality of the diffusion coefficients, not on the particular form of the concentration gradients.)

Figure 1. Nernst Diffusion Layer.

See text for explanation.

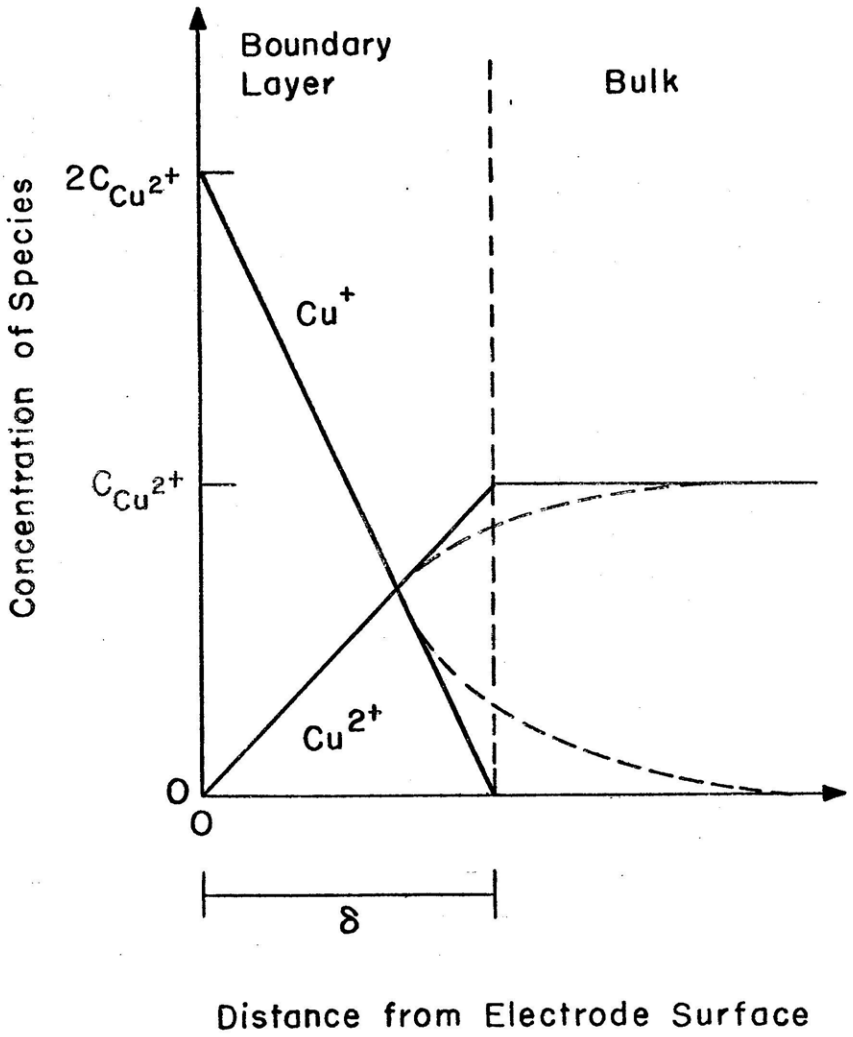

Boundary
Layer
Bulk
$2C_{Cu^{2+}}$
$C_{Cu^{2+}}$
Cu^{+}
Cu^{2+}
0
0
δ
Concentration of Species
Distance from Electrode Surface

Figure I

Predicted Electrode Behavior

The effects of total copper concentration and total chloride concentration on the potential of an electrode with an effective activity of copper a_{Cu° is demonstrated in the following example. (For simplicity of notation, the formation of only one cuprous species is considered and no solid species are considered. This is a good approximation of the computed equilibrium speciation for the domain $10^{-2}M < C_{Cl^-} < 10^{\circ}M$ and $10^{-6}M < C_{Cu^{2+}} < 10^{-4}M$ at $a_{Cu^\circ} = 10^{-8.5}$. The more general case is treated in a subsequent section). The initial concentration of the cuprous species is $C_{CuCl_2^-} = 0$. When the solution is brought into contact with a copper metal electrode of activity a_{Cu°, the dichloro cuprous complex forms at the electrode surface according to the reaction

$$Cu^{2+} + Cu^\circ + 4Cl^- = 2CuCl_2^- \qquad \text{(IV)}$$

The stoichiometry of this reaction imposes the boundary condition for flux of material at the electrode surface:

$$2\, J_{Cu^{2+}} + J_{CuCl_2^-} = 0 \qquad (3)$$

Substitution of Equation 2 into Equation 3 yields:

$$2\, D_{Cu^{2+}} \frac{(C'_{Cu^{2+}} - C_{Cu^{2+}})}{\delta} + D_{CuCl_2^-} \frac{(C'_{CuCl_2^-} - C_{CuCl_2^-})}{\delta} = 0 \quad (4)$$

If we assume that the diffusion coefficients of Cu^{2+} and $CuCl_2^-$ are approximately equal, $D_{Cu^{2+}} = D_{CuCl_2^-}$, substitution of the initial conditions into equation (4) gives a statement of the electrode surface concentrations of the species Cu^{2+} and $CuCl_2^-$ in terms of the initial (bulk) concentration of Cu^{2+}.

$$2\, C'_{Cu^{2+}} + C'_{CuCl_2^-} = 2\, C_{Cu^{2+}} \quad (5)$$

This is an important result because it defines the material balance condition at the electrode surface. Again it is emphasized that, although the quantitative conversion of cupric to cuprous may occur at the electrode surface, the actual amount of cupric ion reduced and elemental copper oxidized from the electrode surface is small compared to the amount of cupric ion in the bulk of solution.

Given the electrode surface concentrations of the species , we may compute the equilibrium potential of the electrode. The equilibrium potential will be observed experimentally only if the reactions at the electrode surface are fast compared to the rate of material transport by diffusion at the electrode surface. This condition is of course best realized in unstirred solutions. We assume that the quasi-equilibrium state is reached and return to

the material balance condition presented in Equation 5 ; there are two extreme cases:

1. If no chloride is present, then $C'_{Cu^{2+}} = C_{Cu^{2+}}$ and the electrode performs as a cupric ion electrode, with a slope of 29 mV/decade. The potential in this case is

$$E = E^{\circ\prime}_{Cu^{2+}/Cu^{\circ}} + \frac{.059}{2} \log \frac{C_{Cu^{2+}}}{a_{Cu^{\circ}}} \qquad (6)$$

where $E^{\circ\prime}$ is the effective standard potential of the electrode in the ionic medium being used.

2. If the chloride activity is sufficiently high, then Reaction IV proceeds quantitatively to the right and the dichloro complex is the predominant cuprous species of copper at the electrode surface:

$$C'_{CuCl_2^-} >> C'_{Cu^{2+}}$$

and Equation 5 becomes

$$C'_{CuCl_2^-} \cong 2\, C_{Cu^{2+}}$$

In this case the equilibrium electrode potential is given from Reaction IV

$$E = E^{\circ\prime}_{CuCl_2^-/Cu^{\circ}} + .059 \log \left[\frac{2 \cdot C_{Cu^{2+}}}{a_{Cu^{\circ}}\, C^2_{Cl^-}} \right] \qquad (7)$$

subject to the condition that chloride ion is in great excess over copper and $C'_{Cl^-} = C_{Cl^-}$.

Electrode Potential as a Function of Copper Concentration

The electrode potentials as given by Equations 6 or 7 are a function of three variables $C_{Cu^{2+}}$, a_{Cu°, C_{Cl^-}. It is instructive to hold two of these variables constant and plot potential as a function of the third. In Figure 2, C_{Cl^-} is fixed at 1M, and potential is plotted against $C_{Cu^{2+}}$ for $a_{Cu^\circ} = 1$ and $a_{Cu^\circ} = 10^{-8.5}$. ($a_{Cu^\circ} = 1$ is the activity of unionized copper in a pure copper metal electrode, and $a_{Cu^\circ} = 10^{-8.5}$ is approximately the activity of elemental copper for an electrode composed of sulfur rich cupric sulfide. An electrode of the latter type was used in the experimental part of this work.) To the left of the point of intersection ($C = 10^{-3.5}$M) of the two lines E_{Cu^{2+}/Cu° and $E_{CuCl_2^-/Cu^\circ}$ for $a_{Cu^\circ} = 10^{-8.5}$, the species $CuCl_2^-$ is more stable than Cu^{2+}, i.e., if Cu^{2+} is added to the system it will tend to be reduced by $Cu^\circ (a = 10^{-8.5})$ to $CuCl_2^-$; to the right of the point of intersection the situation is reversed, and added Cu^{2+} is stable with respect to reduction.

If Reaction IV is reasonably fast compared to transport of material to the electrode by diffusion, Reaction IV approaches quasi-equilibrium. Then to the left of the point of intersection of lines E_{Cu^{2+}/Cu° and $E_{CuCl_2^-}$ the electrode would respond with a slope of nominally 59 mV/ decade $C_{Cu^{2+}}$ and to the right of the point of intersection the electrode response slope would be a nominal 29.5mV/decade. For the higher activity of elemental copper $a_{Cu^\circ} = 1$, the $CuCl_2^-$ response dominates over the entire range; an equivalent statement of this fact is that the domain of 29mV/ decade response of the electrode in chloride media is extended by using

Figure 2. Calculated E vs. C_{Cu} for Two Values of a_{Cu°. Calculated potentials of the Cu^{2+}/Cu° and $CuCl_2^-/Cu^\circ$ couples as a function of $C_{Cu}{}^{2+}$ and $C_{CuCl_2^-}$ (Equations 6 and 7); $a_{Cu^\circ} = 10^{-8.5}$, $a_{Cu^\circ} = 1$.

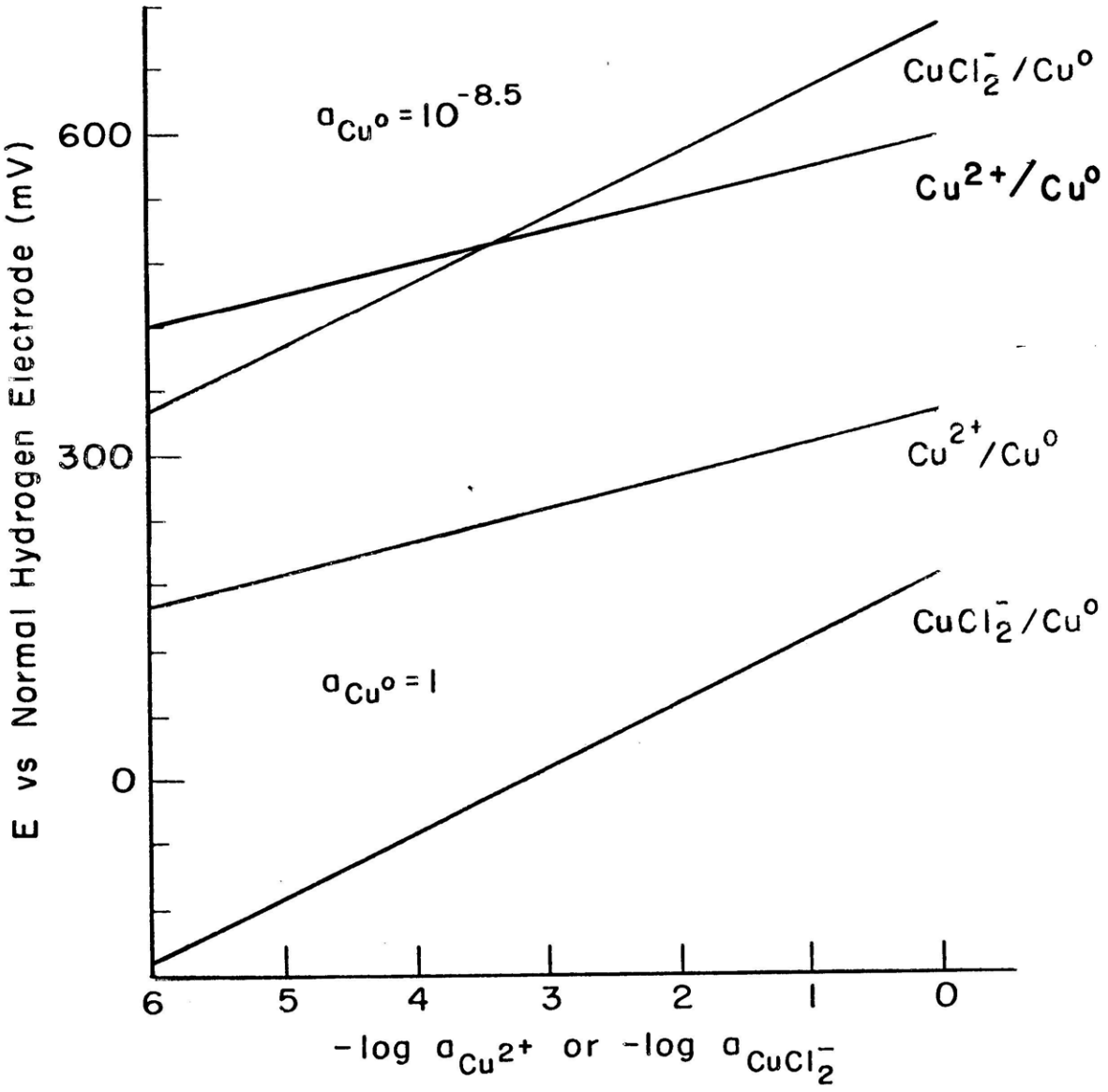

E vs Normal Hydrogen Electrode (mV)
600
300
0
$a_{Cu^o} = 10^{-8.5}$
$CuCl_2^- / Cu^o$
Cu^{2+} / Cu^o
Cu^{2+} / Cu^o
$CuCl_2^- / Cu^o$
$a_{Cu^o} = 1$
6
5
4
3
2
1
0
$-\log a_{Cu^{2+}}$ or $-\log a_{CuCl_2^-}$

Figure 2

an electrode with a lower a_{Cu°.

If the C_{Cl^-} were reduced by an order of magnitude the $E_{CuCl_2^-/Cu^\circ}$ line would be raised by 59 mV and the point of intersection of E_{Cu^{2+}/Cu° and $E_{CuCl_2^-/Cu^\circ}$ moved to the left, again extending the 29mV/decade response domain to lower values of $C_{Cu^{2+}}$.

Electrode Potential as a Function of Chloride Concentration

Another way to view the dependence of potential on the variables a_{Cu°, $C_{Cu^{2+}}$, C_{Cl^-} is by plotting E_{Cu^{2+}/Cu° and $E_{CuCl_2^-/Cu^\circ}$ as a function of C_{Cl^-} for several values of a_{Cu° and $C_{Cu^{2+}}$ (Figure 3). In this case, the domain of the 29 mV/decade response is to the left of the point of intersection of the lines $E_{Cu^{2+}}$ and $E_{CuCl_2^-/Cu^\circ}$; to the right of this point added Cu^{2+} would tend to be reduced to $CuCl_2^-$. The chloride concentration at the point of intersection of these lines, the onset of interference with increasing chloride concentration, will be termed the critical chloride concentration or $C_{Cl^-}^{crit}$.

It is seen that either increasing the total copper concentration or decreasing the activity of copper metal increases $C_{Cl^-}^{crit}$. This gives two means for increasing the 29mV/decade response domain of the cupric ion selective electrode in a chloride medium.

Electrode Potential as a Function of Chloride Concentration in a Cupric Ion Complexing Medium

The theoretical effect of the additon of a strong cupric ion complexing ligand on the behavior of the electrode in chloride medium is given in Figure 4. If cupric ion reacts with the ligand L according to the reaction:

$$Cu^{2+} + L = CuL \qquad K'_{CuL} \qquad (V)$$

Figure 3. Calculated E vs. C_{Cl^-} for Several Values of C_{Cu}. Calculated potentials of the $Cu^{2+}/Cu°$ and $CuCl_2^-/Cu°$ couples as a function of chloride concentration (Equations 6 and 7) for three different concentrations of Cu^{2+} and $CuCl_2^-$; the intersection of the lines is defined as $C_{Cl^-}^{crit}$; $a_{Cu°} = 10^{-8.5}$.

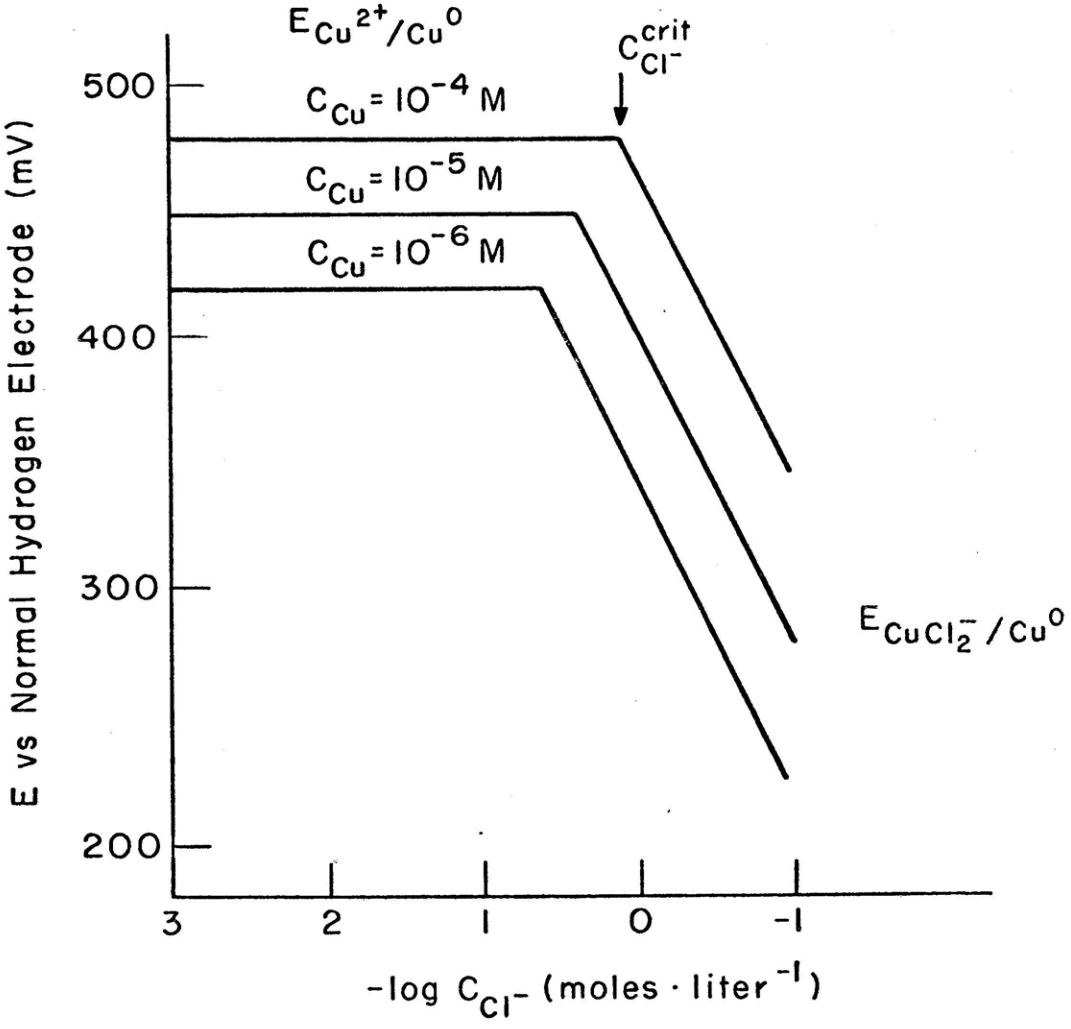

E_{Cu^{2+}/Cu^0}
$C_{Cl^-}^{crit}$
$C_{Cu} = 10^{-4}$ M
$C_{Cu} = 10^{-5}$ M
$C_{Cu} = 10^{-6}$ M
$E_{CuCl_2^-/Cu^0}$
E vs Normal Hydrogen Electrode (mV)
500
400
300
200
3
2
1
0
-1
$-\log C_{Cl^-}$ (moles · liter^{-1})

Figure 3

Figure 4. Calculated E vs. C_{Cl^-} for Several Values of C_L. Calculated potentials of the $Cu^{2+}/Cu°$ and $CuCl_2^-/Cu°$ couples as a function of chloride concentration (Equations 7 and 8) for three values of ligand concentration; $K_{CuL} = 10^4$, $C_{Cu^{2+}} = 10^{-5}M$, $a_{Cu°} = 10^{-8.5}$.

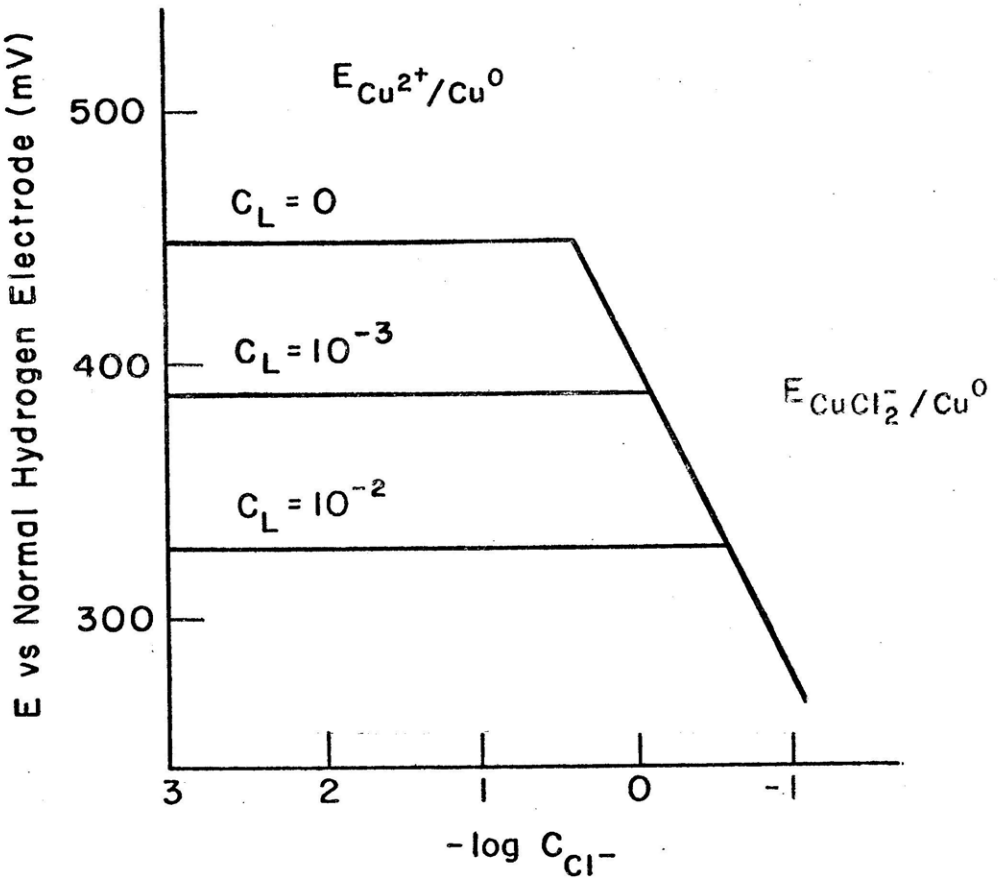

E vs Normal Hydrogen Electrode (mV)
500
400
300
E_{Cu^{2+}/Cu^0}
$C_L = 0$
$C_L = 10^{-3}$
$C_L = 10^{-2}$
$E_{CuCl_2^-/Cu^0}$
3
2
1
0
-1
$-\log C_{Cl^-}$

Figure 4

and the ligand is added in excess, the cupric ion concentration at the electrode surface becomes

$$C'_{Cu^{2+}} = \frac{C_{Cu^{2+}}}{C_L K_{CuL}} \qquad (8)$$

and the electrode potential becomes

$$E = E^{\circ\prime}_{Cu^{2+}/Cu^\circ} + \frac{.059}{2} \log \frac{\frac{C_{Cu^{2+}}}{C_L K_{CuL}}}{a_{Cu^\circ}} \qquad (9)$$

It is seen that $C^{crit}_{Cl^-}$ increases slightly with increasing stability of the metal ligand complex. The chloride interference may be viewed as competition between the ligand and chloride ion for complexation or reduction and complexation of the cupric ion.

General Theory

The preceding discussion considered only the chemical species Cu^{2+}, $CuCl_2^-$, Cu°, and Cl^-. In the study of the actual mixed sulfide ion-selective electrode there are additional factors to be considered due to the presence of silver and sulfur in the electrode phase. As is shown below, these factors became important only in extreme circumstances.

Silver and sulfur can be considered to be present in the electrode phase at some activity a_{Ag° and a_{S°. The chloro complexes of silver could form by reaction of the type

$$Cu^{2+} + 2Ag^{\circ} \underset{Cl}{=} 2AgCl_x^{(x-1)-} + Cu^{\circ} \qquad (VI)$$

The extent of these reactions, if they are not kinetically hindered, depends on the relative activities $a_{Cu^{\circ}}$ and $a_{Ag^{\circ}}$ in the electrode phase. Also the precipitation of the solid species AgCl could occur, depending as before on the activities of copper and silver.

The formation of soluble sulfide species could result from simple dissolution of the electrode (the dissolution process is different from the redox process necessary for argentous or cuprous formation); but unless the total copper concentration were exceedingly low, the concentration of sulfide species would be negligible.

One other effect which could occur in the mixed sulfide electrode is the concentration polarization of the electrode phase when a significant fraction of silver or copper at the electrode surface is oxidized. Polarization is most likely to occur in stirred solutions, or in solutions of high cupric ion and chloride ion activity when the redox processes at the electrode proceed most rapidly.

In view of all of these possible complications, it was deemed worthwhile to consider the hypothetical case in which every one of these possible side reactions had an effect, i.e., the case in which all of these reactions were at equilibrium with one another at the electrode surface. By generalization of Equation 3 and the approximation that the diffusion coefficients for all species are equal, the material balance equations at the electrode surface were

found, from which the equilibrium speciation at the electrode surface may be computed. For the initial concentration of species in the cupric state equal to $C_{Cu(II)}$ and initial concentration of other species

$$C_{Cu(I)} = C_{Ag(I)} = C_{S(-II)} = 0$$

the material balance equation at the electrode surface is

$$2C_{Cu(II)} = 2\,C'_{Cu(II)} + C'_{Cu(I)} + C'_{Ag(I)} - 2C'_{S(-II)}$$

where $C_{Cu(II)}$ represents the sum of the concentrations of all cupric species, etc. The chemical equilibrium computer program MINEQL (11) was used to compute equilibrium among all of the relevant reactions in Table I for several sets of values for a_{Cu°, a_{Ag°, C_{Cl^-} and $C_{Cu^{2+}}$. The summary result is that the chloro complexes of silver appear to be of approximately the same stability as the chloro cuprous complexes, and the potential of the electrode calculated considering the chloro argentous complexes and the trichloro cuprous complex is not greatly different from the potential determined by considering only the dichloro cuprous complex.

The computed equilibrium speciation and potential of the electrode as a function of chloride concentration for $a_{Cu^\circ} = 10^{-8.5}$, $a_{Ag^\circ} = 10^{-3.5}$ and $C_{Cu^{2+}} = 10^{-4}$M is given in Figure 5. (The values

Table I

Equilibrium Constants[1]

Ag^+/Cl^-		Ag^+/CH_3CH	
K_{SO}	-9.7	K_1	0.7
K_1	3.4	β_2	0.8
β_2	5.0		
β_3	5.3		
β_4	6.0		
Cu^+/Cl		Cu^+/CH_3CN	
K_{SO}	-6.7	β_2	3.9
β_2	5.5	β_3	4.1
β_3	5.1		
$K_{Cu2+/Cu°}$	11.4	$E°_{Cu2+/Cu°}$	338 mV
$K_{Cu2+/Cu+}$	2.7	$E°_{Cu2+/Cu+}$	159 mV
$K_{Ag+/Ag°}$	13.5	$E°_{Ag+/Ag°}$	799 mV

[1]Values are $\log_{10}K$, adapted from Reference 10.

Figure 5. Computed Equilibrium Potential and Speciation. Potential of hypothetical cupric ion selective electrode and equilibrium speciation at the electrode surface computed from general theory as a function of chloride concentration: $a_{Cu^\circ} = 10^{-8.5}$, $a_{Ag^\circ} = 10^{-3.5}$, $C_{Cu^{2+}} = 10^{-4.4}$ M.

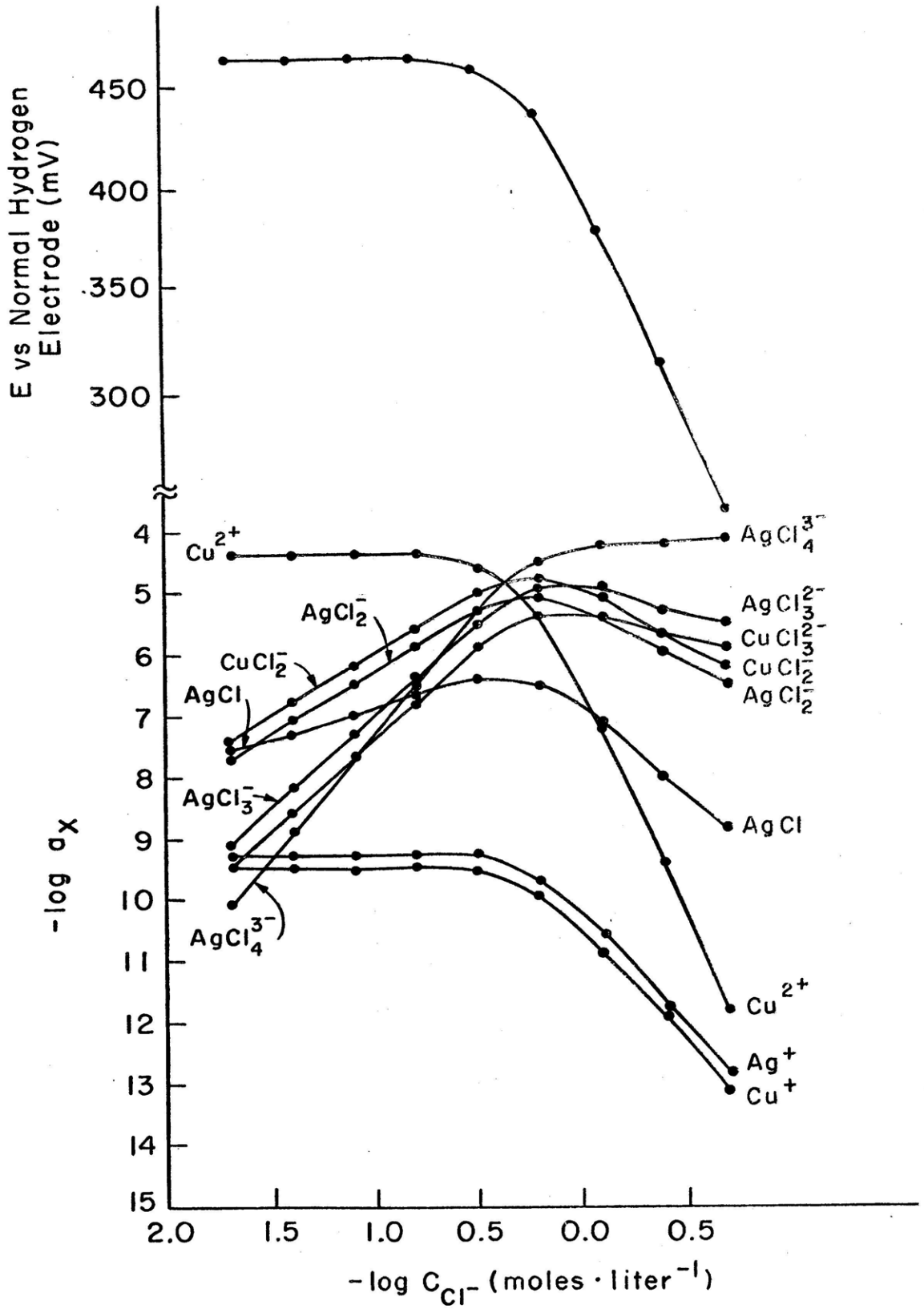

E vs Normal Hydrogen Electrode (mV)
450
400
350
300
4
5
6
7
8
9
10
11
12
13
14
15
-log a_X
Cu^{2+}
$AgCl_2^-$
$CuCl_2^-$
AgCl
$AgCl_3^-$
$AgCl_4^{3-}$
$AgCl_4^{3-}$
$AgCl_3^{2-}$
$CuCl_3^{2-}$
$CuCl_2^0$
$AgCl_2^-$
AgCl
Cu^{2+}
Ag^+
Cu^+
2.0
1.5
1.0
0.5
0.0
0.5
-log C_{Cl^-} (moles · liter^{-1})

Figure 5

for a_{Cu° and a_{Ag° are approximately those for an electrode composed of sulfur rich CuS and Ag_2S ; an electrode of this composition was used in the experimental part of this work). The electrode potential curve determined from the more general theory is strikingly similar to that found when only the species $CuCl_2^-$ was considered (Figure 3), although the computed cupric/cuprous/argentous speciation is much more complicated.

Other equilibrium computations predict that precipitation of AgCl should occur when the product $C_{Cl^-} \cdot C_{Cu^{2+}}$ is greater than the limit shown in Figure 6. The precipitation of CuCl is predicted not to occur. The computed limits of precipitation are dependent on the values used for a_{Ag° and a_{Cu°.

The general theory has allowed us to assess the possible roles of silver and sulfur vis á vis copper in the chloride effect on the electrode. It was found that, except at the extrema, the simple theory, Equations 6-8 and Figures 2-4 , predicted the same general behavior for the electrode as did the general theory. The lower limit to the applicability of the theory is $C_{Cu^{2+}} < 10^{-6}M$, the domain where the exchange current is too low for satisfactory electrode response, and the upper limit is the regime of silver chloride precipitation.

The experimental section of this paper gives verification of the theory as shown in Figures 2-4.

Figure 6. Computed regime of AgCl precipitation as a function of $C_{Cu^{2+}}$ and C_{Cl^-}; $a_{Cu^\circ} = 10^{-8.5}$ and $a_{Ag^\circ} = 10^{-3.5}$.

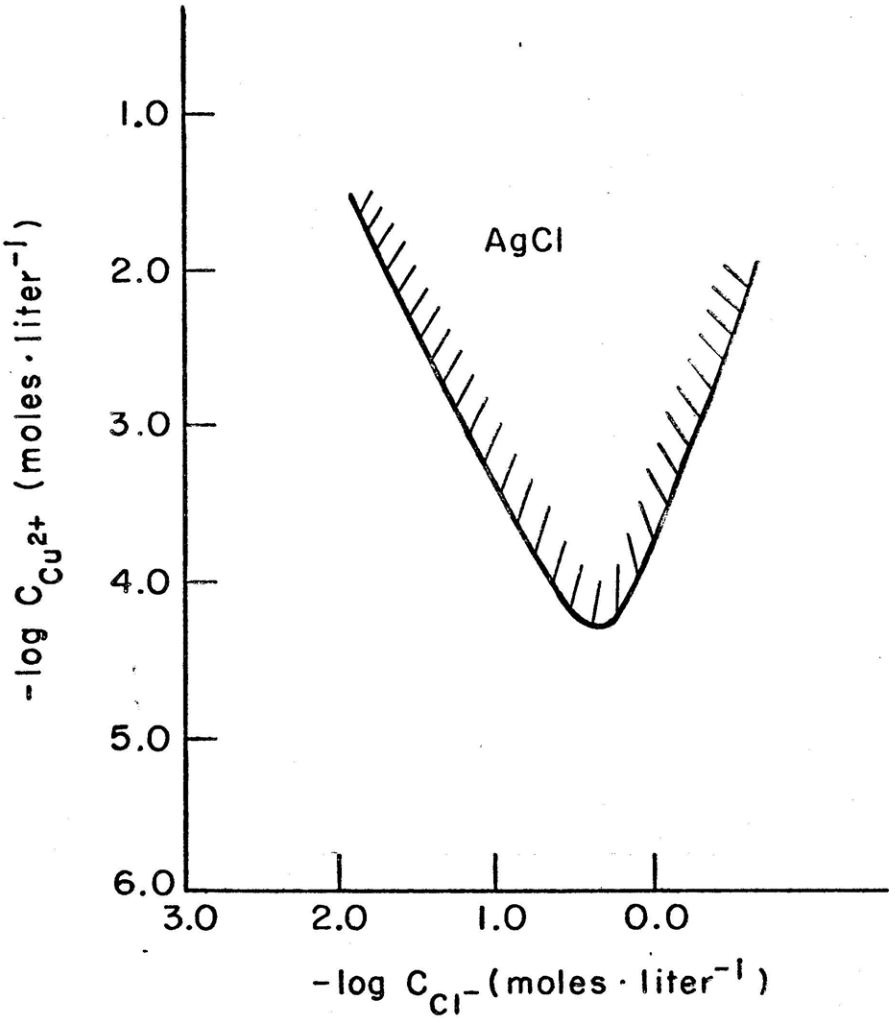

AgCl
1.0
2.0
3.0
4.0
5.0
6.0
3.0
2.0
1.0
0.0
-log $C_{Cu^{2+}}$ (moles · liter^{-1})
-log C_{Cl^-} (moles · liter^{-1})

Figure 6

EXPERIMENTAL

Reagents. The standard 0.1M Cu^{2+} solution was made by dissolution of copper shot in nitric acid; the concentrated KCl and KNO_3 solutions were filtered through 0.22μm Nucleopore filter prior to use; the other chemicals were used without prior treatment. The acetonitrile was Spectro grade from Eastman Organics, and the ethanol was 95%. All solutions were made with water which had been distilled, deionized, and redistilled.

Apparatus. All measurements were made at 25.0 ± 0.1°C in a thermostated cell isolated from the atmosphere. The cell was shielded from light due to the observed photosensitivity of the cupric ion-selective electrodes. Solutions were purged of oxygen with N_2 (or 1% CO_2 in N_2) prior to introduction of the cupric ion selective electrode, after which time nitrogen (or 1% CO_2 in N_2) was passed over the solution at a very slow flow rate to minimize gas flow induced circulation of the sample solution.

Potential measurements were made with the Orion 801A or Orion 701 Digital pH-meters against an Orion Model 90-01 Double Junction Electrode. Potential readings were taken in unstirred solutions when the rate of potential drift became less than 1mV/hour (as measured on a Valtec Model 1024 X-Y-t recorder); equilibration times greater than 2 hours were not uncommon. Two cupric ion-selective electrodes were used: the Radiometer Selectrode Model F-3000 and the Orion Model 94-29A cupric ion-selective electrode. The electroactive surface of the Radiometer electrode is the end of a Teflon impregnated graphite rod to which a mixed cupric sulfide/silver sulfide powder has been applied. The Radiometer electrode was used in all of the experiments except one because of the ease with which the chloride-poisoned electroactive element can be restored. The Orion electrode consists of a copper sulfide/silver sulfide pellet to which a silver wire is connected; it was used in one experiment to compare electrodes

of different construction.

Procedure. Four experiments were carried out to measure the response of the Radiometer cupric ion-selective electrode to various total concentrations of cupric ion, cuprous (and argentous) complexing agents, and cupric complexing agents. The experiments were:

I	$[Cu^{2+}]$	$[Cl^-]$	
	$10^{-3.4}$, $10^{-4.4}$, $10^{-5.4}$	$10^{-3.0}$ -$10^{0.3}$	
II	$[Cu^{2+}]$	$[Cl^-]$	[Oxalate]
	$10^{-4.4}$	$10^{-3.0}$ -$10^{0.3}$	$10^{-3.1}$, $10^{-3.4}$, $10^{-3.7}$
			[Tartrate] $10^{-2.3}$
III	$[Cu^{2+}]$	$[CH_3 CN]$	
	$10^{-5.4}$	$10^{-1.0}$ - $10^{0.6}$	
IV	$[Cu^{2+}]$	$[Cl^-]$	[NTA]
	50% - 150% of NTA	$10^{-0.3}$	$10^{-3.7}$, $10^{-4.4}$, $10^{-5.1}$

In each set the solutions were brought to a constant ionic strength and buffered: in Experiments I and II, an appropriate amount of KNO_3 was added to give a total ionic strength of 2.0 eq. l^{-1}. In Experiment III an appropriate amount of 95% ethanol was added to hold the dielectric of the medium approximately constant. Experiments I-III were buffered at pH 4.7 by a 10^{-3}M acetic acid/10^{-3}M sodium

acetate buffer. The medium for Experiment IV was water collected from Buzzards Bay, Massachusetts, filtered successively through a 0.45μm and a 0.22μm Nucleopore filter, refrigerated, and used within 48 hours of collection. The medium was buffered at pH 7.0 by the natural alkalinity and ∿ 1% CO_2 in N_2 passed over the top of the solution.

RESULTS AND DISCUSSION

Experiment I shows the effect on electrode response of increasing chloride concentration with three levels of total cupric ion concentration; the results of Experiment I, given in Figure 7, agree qualitatively with those predicted in Figure 3. The values of $C_{CL^-}^{crit}$ observed in Experiment I and the theoretical values are compared in Table II.A; good agreement (considering the uncertainty in the stability constants used for the cuprous and argentous chloride complexes, and the approximation that all diffusion coefficients are equal) of the observed and theoretical values of $C_{CL^-}^{crit}$ support the theory developed earlier.

Experiment II shows the effect of added cupric ion complexing ligand to the cupric/chloride medium, i.e., the effect of $K_{CuL} C_L$ in Equation 9 : The results of Experiment II, given in Figure 8, bear out the predictions of Figure 4: cupric ion stabilization by chelation pushes $C_{CL^-}^{crit}$ to values of C_{CL^-} higher than in the unstabilized case.

In Experiment III, an alternate cuprous/argentous stabilizing agent, acetonitrile, is introduced to confirm the notion that the interference is the result of cuprous or argentous complexation. The results given in Figure 9 and Table II.B again confirm the predictions. Experiment III is interesting from another point of view. In the previous experiments, the relative stabilities of the cuprous and argentous complexes were approximately equal, and it was

Figure 7. Experimental E vs. C_{Cl^-} for Several Values of C_{Cu}. Measured potential of cupric ion selective-electrode for various chloride concentrations at three different bulk cupric ion concentrations.

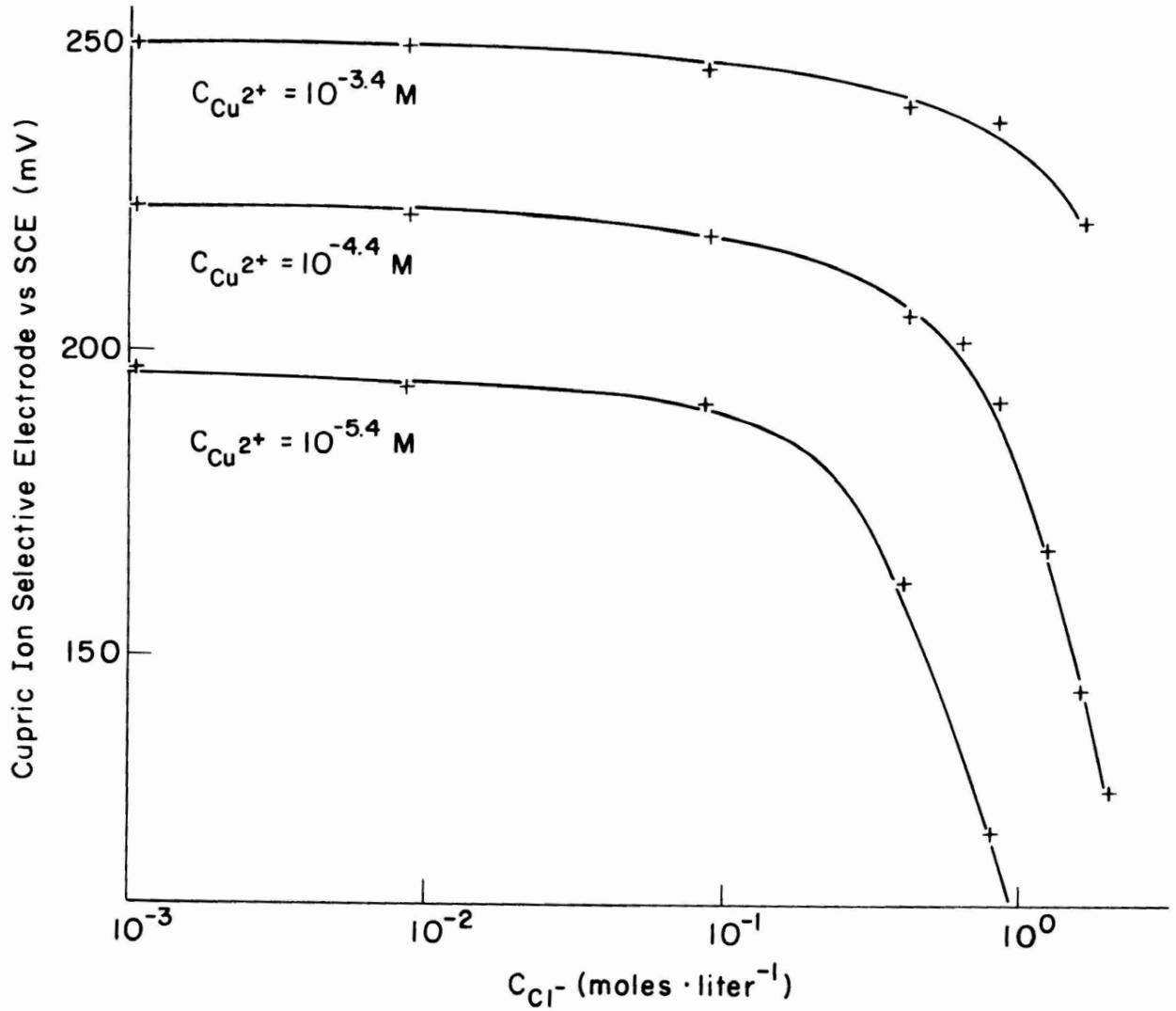

250
200
150
$C_{Cu^{2+}} = 10^{-3.4}$ M
$C_{Cu^{2+}} = 10^{-4.4}$ M
$C_{Cu^{2+}} = 10^{-5.4}$ M
Cupric Ion Selective Electrode vs SCE (mV)
10^{-3}
10^{-2}
10^{-1}
10^{0}
C_{Cl^-} (moles · liter^{-1})

Figure 7

TABLE II

Comparison of Experimental and Theoretical Values of $C^{crit}_{Cl^-}$ and $C^{crit}_{CH_3CN}$

A. Experiment I

$C_{Cu^{2+}}$	experimental $C^{crit}_{Cl^-}$	calculated[1] $C^{crit}_{Cl^-}$	computed[2] $C^{crit}_{Cl^-}$
$10^{-3.4}$	$10^{0.0}$	$10^{+0.2}$	$10^{-0.1}$
$10^{-4.4}$	$10^{-0.3}$	$10^{-0.1}$	$10^{-0.3}$
$10^{-5.4}$	$10^{-0.6}$	$10^{-0.3}$	$10^{0.5}$

B. Experiment II

$C_{Cu^{2+}}$	experimental $C^{crit}_{CH_3CN}$	calculated[3] $C^{crit}_{CH_3CN}$	computed[4] $C^{crit}_{CH_3CN}$
$10^{-4.4}$	$10^{0.3}$	$10^{0.2}$	$10^{0.2}$

Notes to Table II.

1. Calculated from the intersection of the lines given by Equations 6 and 7; $a_{Cu^\circ} = 10^{-8.5}$.
2. The electrode potential was computed as a function of chloride from all of the pertinent reactions in Table I; the potential vs. chloride concentration curve was plotted and the value of $C_{Cl^-}^{crit}$ obtained by extrapolation; $a_{Cu^\circ} = 10^{-8.5}$, $a_{Ag^\circ} = 10^{-3.5}$.
3. Calculated from the intersection of the lines given by Equation 6, and Equation 7 written for the reaction $Cu^{2+} + Cu^\circ + 6CH_3CN = 2\ Cu(CH_3CN)_3^+$; $a_{Cu^\circ} = 10^{-8.5}$.
4. Computed as in Note 2, except the reactions involving CH_3CN were considered.

Figure 8. Experimental E vs. C_{Cl^-} for Several Values of C_L. Measured potential of cupric ion-selective electrode for various chloride concentrations and various ligand concentrations; $C_{Cu^{2+}} = 10^{-4.4}$M.

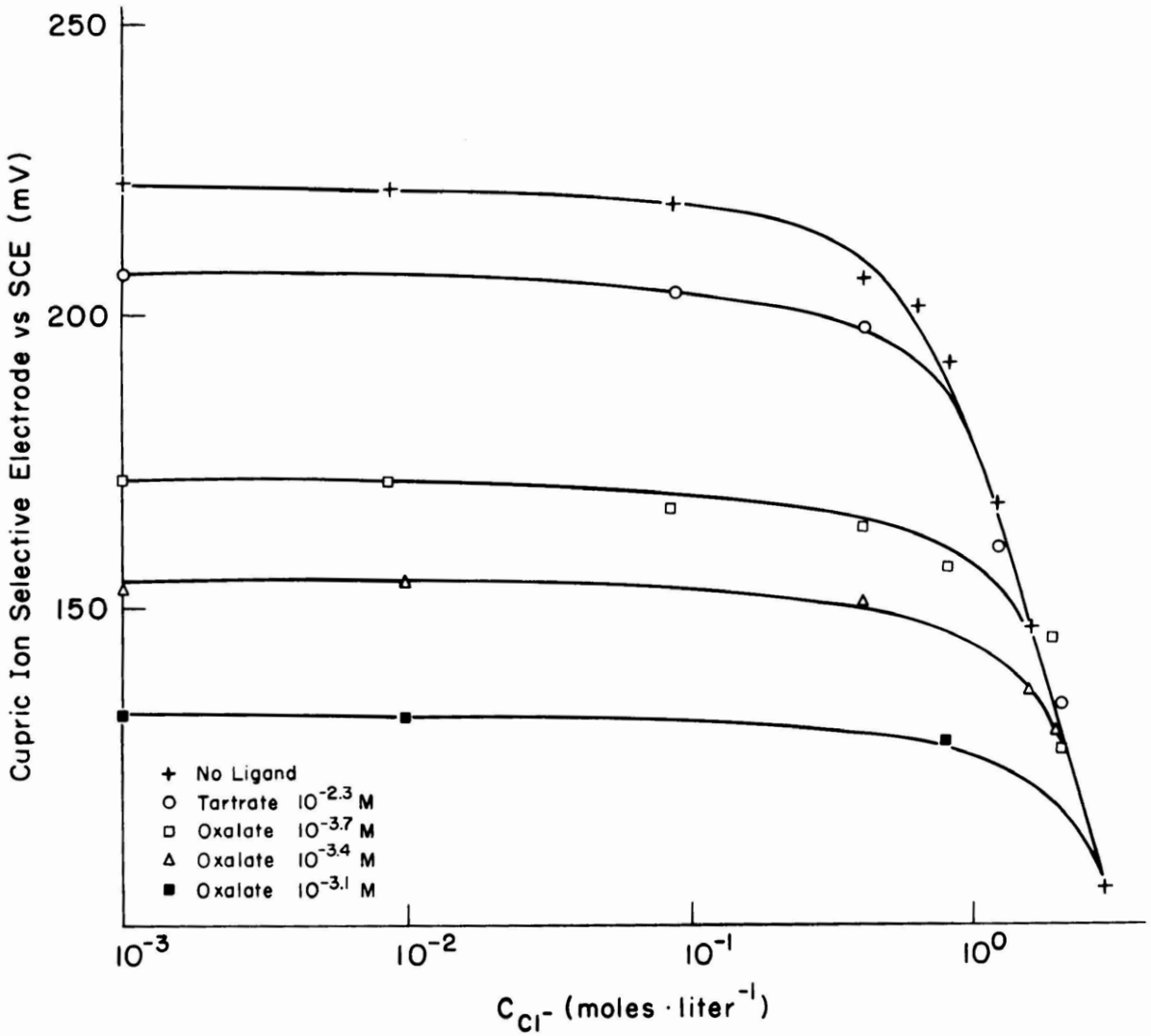

250
200
150
Cupric Ion Selective Electrode vs SCE (mV)
+ No Ligand
○ Tartrate $10^{-2.3}$ M
□ Oxalate $10^{-3.7}$ M
△ Oxalate $10^{-3.4}$ M
■ Oxalate $10^{-3.1}$ M
10^{-3}
10^{-2}
10^{-1}
10^{0}
C_{Cl^-} (moles · liter^{-1})

Figure 8

Figure 9. Experimental E vs. C_{CH_3CN}.
Measured potential of cupric ion-selective electrode for various acetonitrile concentrations; $C_{Cu^{2+}} = 10^{-5.4}$M.

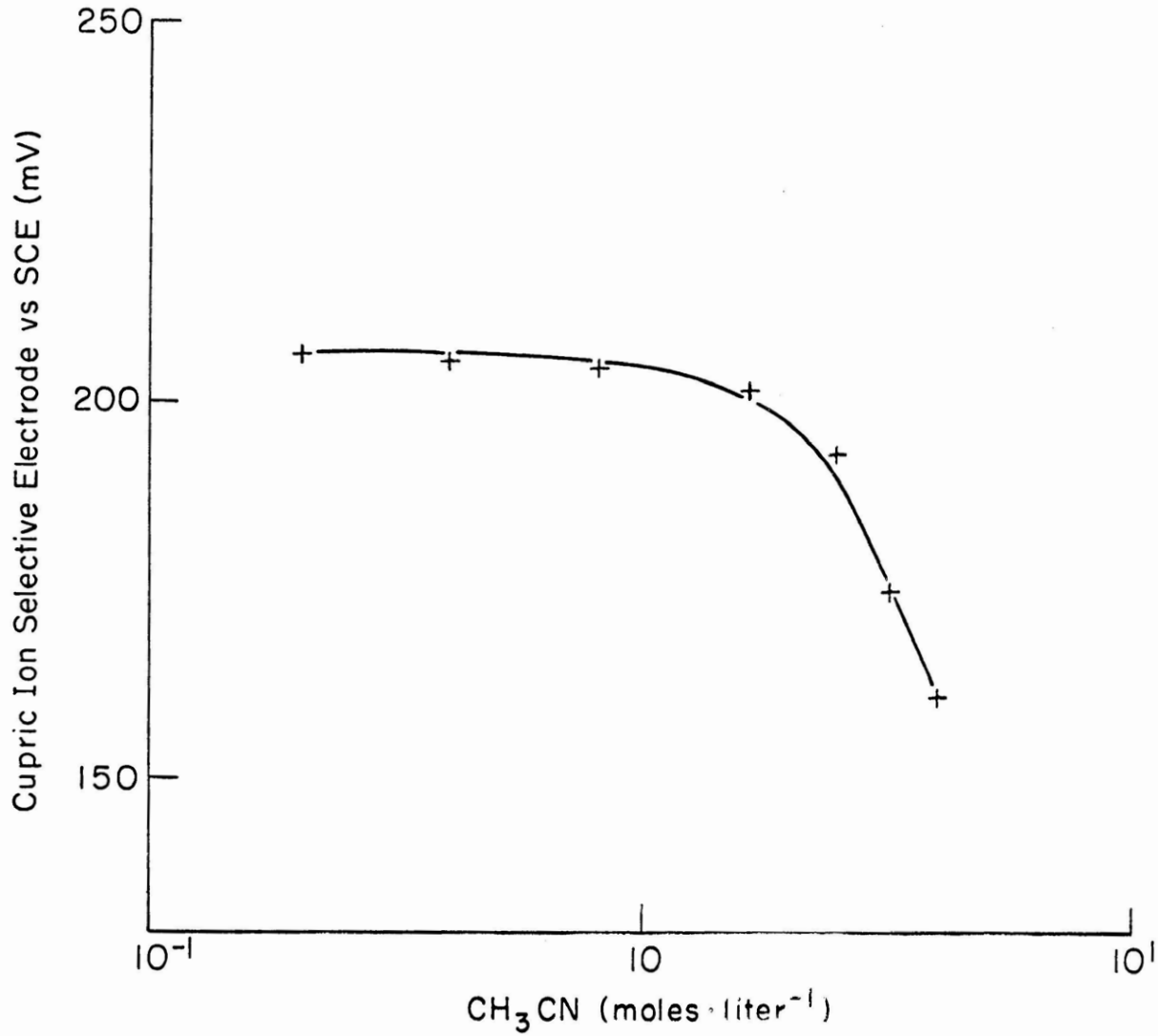

250
200
150
Cupric Ion Selective Electrode vs SCE (mV)
10^{-1}
10
10^{1}
CH_3CN (moles · liter^{-1})

Figure 9

impossible to tell by those experiments alone whether the reduction of cupric ion was coupled to the oxidation of Ag°, Cu°, or both (Reactions IV and V). However, since the cuprous acetonitrile complexes are several orders of magnitude more stable than the argentous acetonitrile complex, it is possible to separate the influence of cuprous and argentous acetonitrile complexes on the electrode potential. The $C^{crit}_{CH_3CN}$ in acetonitrile (Figure 9, Table II.B) occurs approximately where predicted by theory for formation of cuprous acetonitrile complexes: this result indicates that the reduction of cupric ion is coupled to the oxidation of Cu° at the electrode surface, and that the reaction proceeds at a reasonable rate in the acetonitrile medium (and presumably in the chloride medium as well). The result does not exclude the participation of Ag° in the reduction process in a chloride medium.

Experiment IV was performed to evaluate the feasibility of performing cupric complexiметric titrations in seawater for the purpose of determining "natural chelating capacity" as has been suggested previously (4,5). Samples of seawater spiked with various concentrations of NTA (a relatively strong chelator) were titrated with cupric ion from 50% to 150% of the total NTA. Figure 10 shows that for the titration of 2×10^{-4} M NTA, the effect of chloride is negligible, while for 8×10^{-6} M NTA the titration is completely dominated by the chloride interference and no titration break point is seen.

Figure 10. Titration of Seawater Spiked with NTA.
Measured potential of cupric ion-selective electrode during cupric ion titration of seawater spiked with NTA.

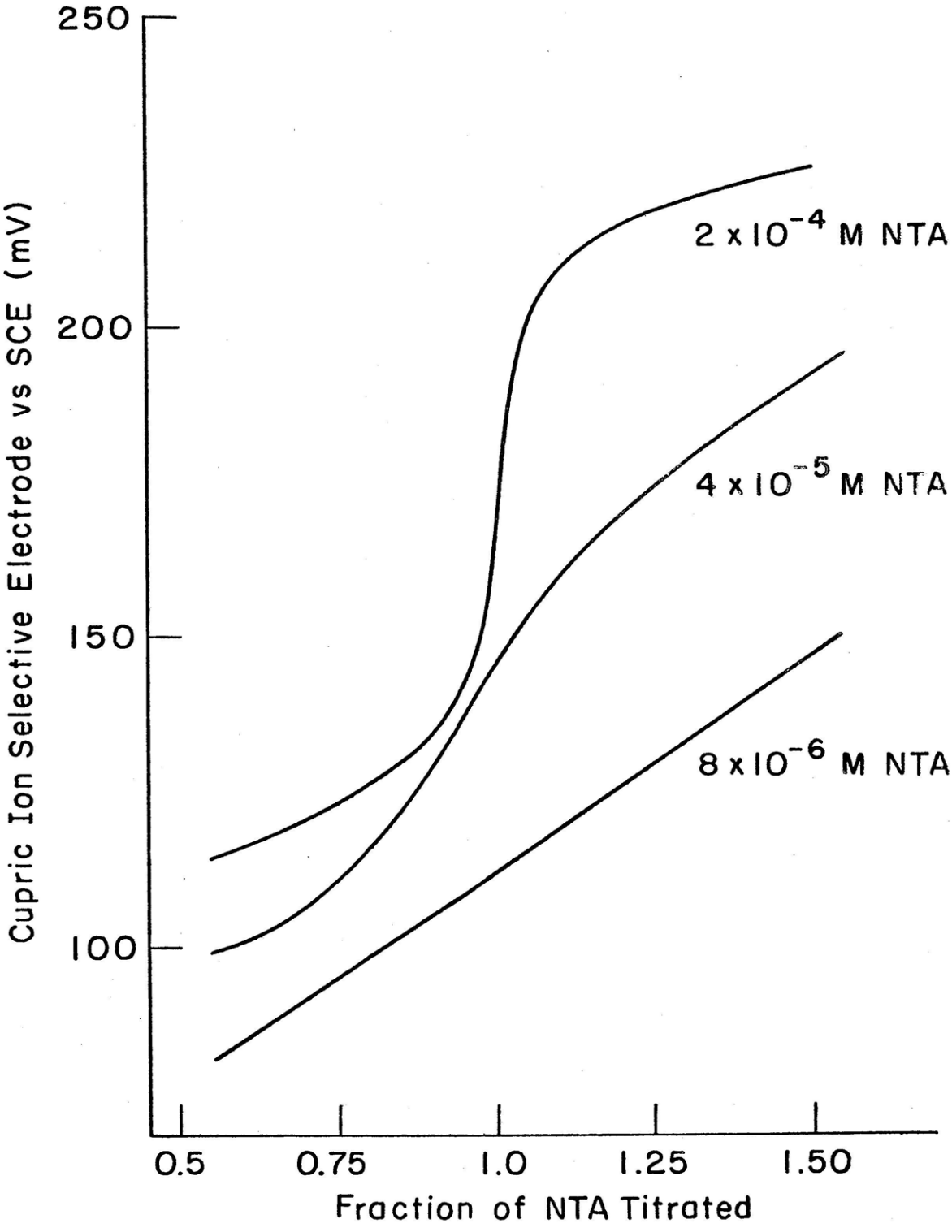

250
200
150
100
Cupric Ion Selective Electrode vs SCE (mV)
2 x 10⁻⁴ M NTA
4 x 10⁻⁵ M NTA
8 x 10⁻⁶ M NTA
0.5
0.75
1.0
1.25
1.50
Fraction of NTA Titrated

Figure 10

Actually this result was predictable from Figure 3, which shows that for copper equal to 4×10^{-5} molar, the critical chloride concentration, $C_{Cl^-}^{crit} = 0.5M$. Then in 0.5M chloride (or the choride concentration of seawater), one would expect to see a chloride interference with less than 4×10^{-5} M cupric ion in solution, and no interference with more than 4×10^{-5} M cupric ion in solution. Thus cupric chelometric titrations should be feasible for total chelator concentrations greater than 4×10^{-5}M, and the chloride interference should dominate for chelator concentrations less than 4×10^{-5}M. An additional upper limit to the cupric ion concentration is imposed by AgCl precipitation at $C_{Cu^{2+}} > 10^{-4.2}$M in 0.5M Cl^- (Figure 6).

An experiment was carried out to compare the behavior in chloride media of a cupric ion selective electrode of a different construction - hence a different $E°'_{Cu^{2+}/Cu°}$. An electrode based upon a pellet prepared from cupric sulfide and silver sulfide contacted to a silver metal wire was used in a solution similar to those of Experiment I. For this electrode the activity of elemental silver in the electrode phase should be on the order of $a_{Ag°} = 1$ (since silver in the electrode phase is presumably in equilibrium with the silver wire), and the apparent activity of elemental copper (as measured according to Equation 1) is $a_{Cu°} = 10^{-6.2}$. Since these values are substantially different from the values of $a_{Ag°} = 10^{-3.5}$ and $a_{Cu°} = 10^{-8.5}$ used in the theoretical discussion of the graphite contacted electrode, the theoretical speciation,

electrode response, and precipitation regimes predicted in Figures 5 and 6 are not strictly applicable to the silver contacted electrode. One may still predict that, because the activity of elemental copper and silver is much higher in the silver contacted electrode, the elemental metals are oxidized more easily, and $C_{Cl^-}^{crit}$ occurs at a lower chloride concentration. This expectation is borne out in Figure 11; the experimental value of $C_{Cl^-}^{crit} = 10^{-1.4}$M at $10^{-4.4}$M total copper is almost an order of magnitude lower than for the other electrode.

Finally, mention should be made of those experiments in which the theoretical predictions of Figures 2-4 were not seen, i.e., experiments in which precipitation of AgCl or polarization of the electrode did occur. At concentrations of copper and chloride greater than the limit of the precipitation region in Figure 6, electrode behavior was observed which was not continuous with behavior outside of this region. Whether this behavior should be attributed to electrode polarization or to AgCl precipitation or both, is uncertain.

Conclusion

The use of the cupric ion-selective electrode in chloride media (seawater) has been suggested for the monitoring of ionic cupric copper concentration (4) and the assessment of cupric ion acitivity with respect to organic complexation (3,5). These practices will be interpreted in light of the theory presented in this paper. The cupric ion-selective electrode has formally two modes of response: 29 mV/decade in the absence of

Figure 11. Experimental E vs. C_{Cl^-} for Silver-Contacted Cupric Ion Selective Electrode.

Measured potential of silver-contacted cupric ion-selective electrode for various chloride concentrations; $C_{Cu^{2+}} = 10^{-4.4}$M.

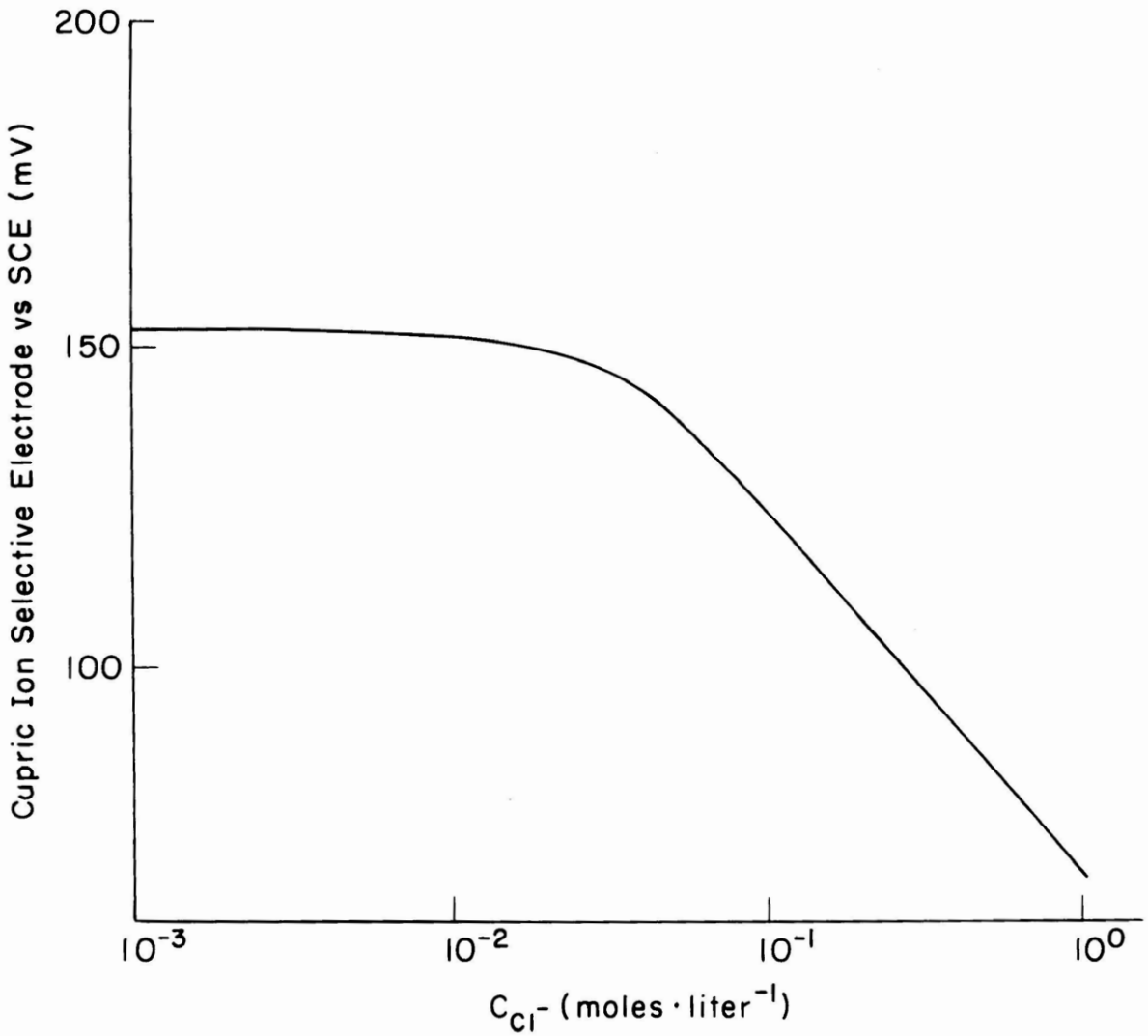

200
150
100
Cupric Ion Selective Electrode vs SCE (mV)
10^{-3}
10^{-2}
10^{-1}
10^{0}
C_{Cl^-} (moles · liter^{-1})

Figure 11

chloride, and 59 mV/decade in media of high chloride concentration. If the electrode is in the 29 mV/decade response domain, cupric ion activity may be measured normally as in a non-chloride medium. If the electrode is in the 59 mV/decade response domain, it is impossible to measure cupric ion activity; only the total copper concentration, as cuprous ion, can be measured, and this requires a painstaking calibration procedure. If the electrode response is in the transition zone between the two response domains, it is very difficult to interpret electrode potential measurements at all.

For a given chloride concentration, it has been shown that for the electrodes used in this study, several factors determine whether the electrode response is in the 29 mV/decade domain, the 59 mV/domain, or the transition zone between the two: the composition of the electrode, i.e. the value of a_{Cu° and a_{Ag°; the total (bulk) concentration of cupric ion, the concentration of complexing ligands, and the complex stability constants.

In the absence of cupric ion complexation, the following behavior is expected for the two types of electrodes. For the electrode based on the silver-contacted mixed sulfide pellet, (high relative a_{Cu° and a_{Ag°) the response of the electrode in seawater ($C_{Cl^-} = 0.5M$) will lie in the 59 mV/decade domain, in which it is possible to measure total copper but not cupric ion activity. For the electrode based on the graphite impregnated Teflon to which a mixed sulfide powder was applied (low relative

a_{Cu° and a_{Ag°), the transition from 59 mV/decade response to 29 mV/decade response occurs at 4 x 10^{-5}M total cupric ion. For bulk copper concentrations somewhat less than 4 x 10^{-5}M only total copper concentration (as cuprous ion at the electrode surface) can be measured, but for bulk copper concentrations somewhat greater than 4 x 10^{-5}M, cupric ion activity can be measured.

The addition of a cupric ion complexing ligand will affect the results given above only if the ligand is able to out-compete chloride ion for control of the cupric ion at the electrode surface. As shown in Figure 10 this does not occur with 5 x 10^{-6}M NTA at pH 7; in general it is unlikely that anything but an exceptionally strong cupric complexing ligand will bring the electrode out of the 59 mV/decade response domain. If the electrode remains in the 59 mV/decade response domain it is impossible to measure cupric ion activity.

In summary, extreme caution whould be exercised when interpreting measurements made with the solid state cupric ion selective electrode in chloride media, or any media which stabilizes cuprous or argentous ion.

The remarks made above pertain to unstirred solutions; in stirred solutions the situation becomes much more complicated. Since reaction rates at the electrode surface are not virtually infinitely fast, the electrode potential becomes mass transport controlled. As a result the measured potential is noisy and highly dependent on stirring rate. Also the rate of reduction of cupric ion at the electrode surface is increased, and the electrode is much more likely to become polarized.

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Part II

A General Algorithm for the Determination of Metal-Ligand Complex Stability Constants from Experimental Data

PART II
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Introduction

The problem of computing the chemical equilibrium composition/speciation in aqueous systems has long been discussed. There have been a number of approaches to the problem: graphical methods, approximation formulae, methods for specific systems, and general methods; but as was pointed out in a 1968 review article by Zeleznik and Gordon (1) it is just these variations which render a mathematical abstraction into an algorithm useful for solving a particular problem.

A pioneering work in the application of high speed digital computers to the chemical equilibrium problem was that of Sillén and Ingri in Stockholm with the programs HALTAFALL (2) and LETAGROP(3). Other major contributions to the field have been the program from the Rand Corporation (4-6), the program COMICS(7-8) of Perrin and Sayce, and REDEQL (9-10) of Morel and co-workers. Experience gained through the use of REDEQL was applied in the development of the most recent program on the subject, MINEQL (11). The basic algorithm of MINEQL is discussed in Appendices 1 and 2. In this work we present an extension to the basic algorithm of MINEQL for the determination of equilibrium constants from experimental data.

Historically, determination of equilibrium constants has been done by a combination of graphical and algebraic methods. Publications of Bjerrum (12), Schwarzenbach (13), Deford and Hume (14) describe some of these. They suffice for determining stability constants over a limited range of metal to ligand ratios, but are difficult to apply to large complexly interacting systems and lack the generality to determine stability constants which are consistent over extended ranges.

As an example, the determination of the stability constants for Copper and Tris (2-amino-(hydroxymethyl)-1,3-propanediol) by Bai and Martell (15) is cited (Figure 1). K_1 K_2 K_3 and K_4 were determined from the first part of solid curve 7; K's for 2 species involving the deprotonation of TRIS ($MH_{-1}L$, $(MH_{-1}L)_2$) were determined from curve 3 and the previously determined value K_1; K's for 2 other species($ML(H_{-1}L)$, $M(H_{-1}L)_2$) were determined from curve 4 between a = 1 and a = 2 with previously determined values of K_1 and K_2.

When the data was regenerated computationally with use of all K's simultaneously, the broken curves on the graph were obtained. The correspondence of the two curves is not bad, but the computed speciation does not reflect the presumed speciation from which the K's were determined. For example, in curve 7, the curve from which K_1 - K_4 were determined, the species ML_1 - ML_4 are present in only minor amounts (Figure 2).

It is important to interject that extensive mathematical manipulation of experimental data is not a panacea for the determination of equilibrium constants. In committing data to a computer we lose an intuitive feel for what is reasonable, and at the same time we often gain confidence in the result, simply because it came from a computer. Therefore the algorithm for equilibrium constant determination must be developed with some caution. Indeed an improperly designed algorithm can completely distort graphically determined constants. Such is the case for Cd thiocyanate constants which were originally determined graphically by Deford and Hume in 1951, and recalculated in 1960 by computer, incorrectly,

Figure 1. Titration curves for Tris and Copper (II). Solid lines are experimental data from Reference 15: T_{Cu} = 0.01 M for curves 3-6 and 0.001M for curve 7; molar metal-ligand ratios are (3) 1:1, (4) 1:2, (5) 1:3, (6) 1:4, and (7) 1:10; Fraction Titrated is number of moles of base added per mole of ligand. Broken lines are computed data from the same conditions and the constants from Reference 15.

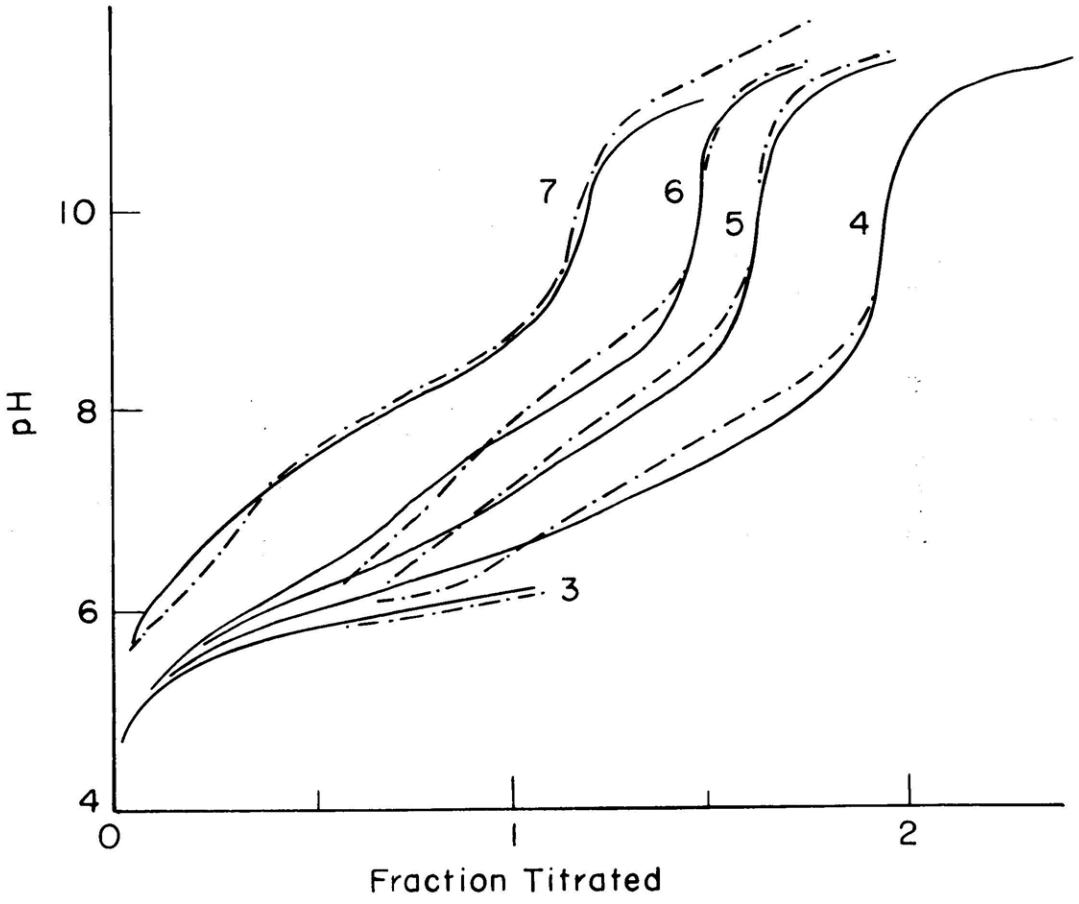
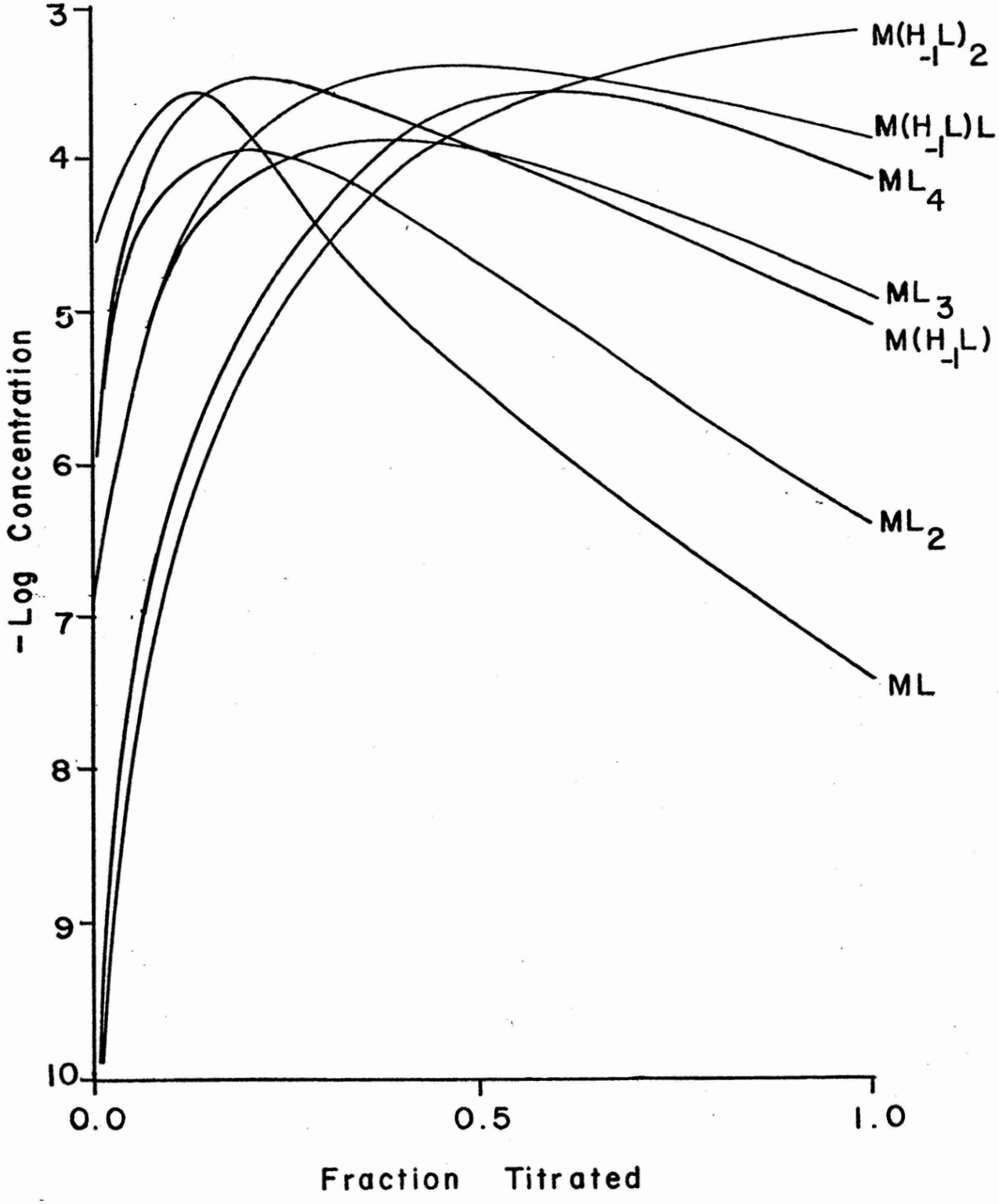


Figure 1

Figure 2. Computed speciation for Tris complexes of copper (II). Curves are computed for conditions of curve 7 in Figure 1: T_{Cu} = 0.001 M, T_{TRIS} = 0.01 M; K's were taken from Reference 15; Fraction Titrated represents moles of base added per mole of ligand.

Figure 2


3
4
5
6
7
8
9
10
-Log Concentration
0.0
0.5
1.0
Fraction Titrated
M(H-1L)2
M(H-1L)L
ML4
ML3
M(H-1L)
ML2
ML

by Irving et al., (16) using improper weighting. The original constants of Deford and Hume were substantiated in 1967 by an extensive computer fitting routine by Momoki, Sato, and Ogawa (17-18).

Example

To provide the conceptual basis for the determination of equilibrium constants, we shall work through a short example. Suppose we wish to determine the equilibrium constant for the reaction of a metal and a protonated ligand to form the metal-ligand complex.

$$M^{2+} + HL - H^{+} = ML^{+} \qquad K = ?$$

Experimentally we perform an acid/base titration on the metal/ligand solution and measure pH. We obtain the set of data

$T_M^1 = , \ldots T_M^M$	total metal
$T_L^1 = , \ldots T_L^M$	total ligand
$T_H^1 , \ldots T_H^M$	total hydrogen ion
$pX_H^1 , \ldots pX_H^M$	pH

for each of m = 1, ..M points on the titration curve. (In this particular experiment T_M and T_L happen to be constant).

At every point on the titration curve we can solve the equilibrium problem posed by total metal, total ligand, and pH, and an initial guess for the unknown constant, K (guess).

Experimental	Calculated	
T_M	pX_M	
T_L	pX_L	for each data point
pX_H	T_H	

Then we compute the residual Y_H between the experimentally observed

total hydrogen ion concentration and the calculated total hydrogen ion concentration

$$Y_H = T_H - T_H(T_M, T_L, pX_H, K) \text{ for each data point}$$

and finally minimize the sum of squares of the residual Y with respect to K:

$$\min_K \sum_m \left(\frac{Y_H^m}{W_H^m} \right)^2$$

(Here W_H is a weighting factor to be discussed later; for our present purposes it can be set equal to unity.)

The process of minimization of the weighted sum of squares involves taking the derivative with respect to K of the weighted sum of squares, setting it equal to zero, expanding in afirst order Taylor's Series about K (guess), solving for K, and iterating with the improved value of K. The final expression for K is of the form:

$$\Sigma \left(\frac{\partial Y}{\partial K} \right)^2 \Delta K = \Sigma \left(\frac{\partial Y}{\partial K} \cdot Y \right)$$

The method described above is effectively the Newton Raphson Method.

In summary, the procedure for the determination of equilibrium constants comprises the steps:

. Guess K

. Solve the equilibrium problem

. Compute the residual

. Minimize the sum of squares of the residuals with respect to K

. Iterate with improved value of K

The Function to be Minimized

Before examining the actual solution to the minimization problem it is helpful to consider the relationships among the number of variables and the number of equations in the algebraic problem. Because of the number of subscripts and superscripts involved in the following work, a notation has been devised and is presented in Table I.

In a general chemical equilibrium problem we deal with N components (the term "component" as used here is defined in Appendix 1); according to the notation of Table I, there are N members in the set N. For each component $n \varepsilon N$ there is an equation relating the total concentration of that component, T_n, to the free concentration of all of the other components

$$T_1 = T_1(X_1, X_2, \ldots X_N)$$

$$T_n = T_n(X_1, X_2, \ldots X_N)$$

$$T_N = T_N(X_1, X_2, \ldots X_N).$$

Thus there are a total of N equations and $2 \cdot N$ variables; if N of the variables are known the problem is exactly determined and can be shown to have a unique positive solution (5); if less than N of the variables are known the problem is under-determined (has an infinite number of solutions); and if more than N variables are known the problem is over-determined.

Using the definitions of Table I, we may consider chemical equilibrium problems with respect to the known and unknown variables (Figure 3). In the "basic" chemical equilibrium problem, the total concentration of all components are known and the free concentration of all components are

TABLE I
Notation

N = set of components

Subsets of N

I - set of components for which the free concentration, X_i, is known

J - set of components for which the total concentration T_j is known

$\bar{I}$ - set of components for which the free concentration $X_{\bar{i}}$ is unknown: $I \cup \bar{I} = N$

$\bar{J}$ - set of components for which the total concentration $T_{\bar{j}}$ is unknown: $J \cup \bar{J} = N$

P - set of components for which both the total concentration T_j and the free concentration X_i is known: $P = I \cap J$.

R = set of reactions

Subsets of R

U - set of reactions for which the equilibrium constant K_u is known

$\bar{U}$ - complement of U, set of reactions for which the equilibrium constant is $K_{\bar{u}}$ unknown $U \cup \bar{U} = R$

M = set of experimental observations T_i^m, X_j^m; in general each member of the set set M represents the observation of a distinct experimental condition; the set M does not represent replicate observations of the same experimental condition.

Upper case letters represent the name of the set and the number of members in that set. Unless otherwise specified, lower case letters represent any member of the set identified by the corresponding upper case letter. Thus:

$X_n (n \in N)$

is interpreted, "the free concentration of component n, where n is any one of the components in the set N".

Figure 3. Known and Unknown Variables in Chemical Equilibrium Problems

$j \in J$	$\bar{i} \in \bar{I}$
$i \in I$	$\bar{j} \in \bar{J}$

See text and Table I for explanation.

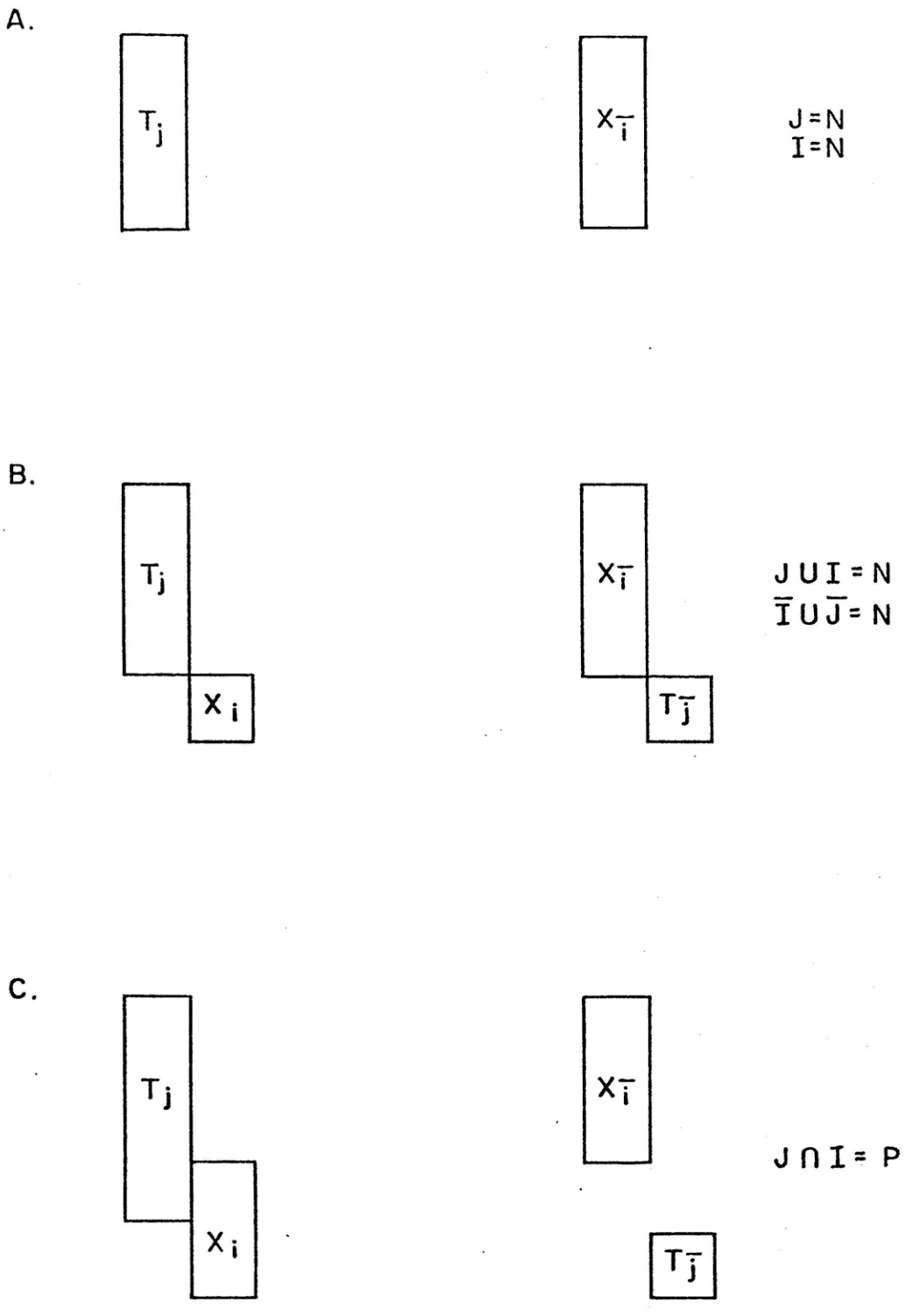

A.
T_j
$X_{\bar{i}}$
J=N
I=N
B.
T_j
X_i
$X_{\bar{i}}$
$T_{\bar{j}}$
J U I = N
$\bar{I}$ U $\bar{J}$ = N
C.
T_j
X_i
$X_{\bar{i}}$
$T_{\bar{j}}$
J ∩ I = P

Figure 3

to be calculated; there are N equations in N unknowns and the system is exactly determined (Figure 3A). In a slightly different chemical equilibrium problem the total concentration of some components are unknown, but for every component for which total concentration is unknown, the free concentration is known. Since in this case the union of the set of components for which the free concentration is known and the set of components for which the total concentration is known is equal to the set of all components, $J \cup I = N$, there are again N equations in N unknowns, and the system is exactly determined (Figure 3B).

Another case is the over-determined system: for some components $p \varepsilon P$, both the total concentration and the free concentration are known. Since there are P members in the intersection set P, there are N equations in N - P unknowns, and the system is overdetermined by P (Figure 3C). In this case up to P unknowns could be added to the problem - in the form of unknown equilibrium constants - and the problem would still be determined. If we make experimental observations over M sets of experimental conditions in the over-determined case, we have

$N \cdot M$ equations

$M \cdot (N+P)$ knowns

$M \cdot (N-P)$ unknowns

and the system is over-determined by $P \cdot M$.

With this background established we may pose the question of what function to minimize with respect to K_u ($u \varepsilon U$, the set of reactions for which constants are unknown) in order to obtain the "optimal fit" of the experimental data to the equilibrium model of the system. The term "optimal fit" is then defined by the choice of functions. We shall

discuss two functions appropriate for minimization.

Method 1. We may minimize with respect to K_u, $u \in U$, the weighted sum of squares of the residuals Y_p^m of the components in P, while the residuals of all other components $\bar{i} \in \bar{I}$ and $\bar{j} \in \bar{J}$ are set equal to zero (as in Figure 3C). This amounts to solving the chemical equilibrium problem posed by X_i^m ($i \in I$), and $T_{\bar{i}}^m$ ($\bar{i} \in \bar{I}$), for each group of data m ($m \in M$, the set of observations), then computing the residual $Y_p^m = T_p^m(\text{calc}) - T_p^m(\text{exp})$ for each component $p \in P$, and minimizing the weighted sum of squares of the residual.

$$\min_{K_u} \sum_{p,m} \left[\begin{pmatrix} Y_p^m \\ \hline W_p^m \end{pmatrix} \right] \qquad \text{subject to } Y_{\bar{j}}^m = 0$$

$u \in U$	the set of unknown constants
$p \in P$	set of components for which both total concentration T_p and free concentration X_p are known
$m \in M$	set of experimental observations (data points)
$\bar{j} \in \bar{J} \cup \bar{I}$	set of components for which either total concentration or free concentration, but not both, are known.

This is a general expression of the method used in the example given earlier. With this method it is necessary to solve at each iteration M(N×N) equilibrium problems followed by one (U×U) problem.

Method 2. There is no a priori reason why the residuals $Y_{\bar{j}}^{m}$ ($\bar{j} \in \bar{J} \cup \bar{I}$) should be set equal to zero. Then a more elegant, but unfortunately computationally more laborious method is to minimize simultaneously the residuals of all of the components $n \in N$ for every observation $m \in M$ with respect to all of the unknowns, K_u ($u \in U$), $T_{\bar{j}}$ ($\bar{j} \in \bar{J}$), and $X_{\bar{i}}$ ($\bar{i} \in \bar{I}$).

$$\min \sum_{n,m} \left(\frac{Y_n^m}{W_n^m} \right)^2$$

K_u,

$X_{\bar{i}}^m$,

$T_{\bar{j}}^m$

$u \in U$ the set of reactions for which equilibrium constants are unknown

$\bar{i} \in \bar{I}$ the set of components for which free concentration is unknown

$\bar{j} \in \bar{J}$ the set of components for which total concentration is unknown

$m \in M$ the set of experimental observations

$n \in N$ the set of components

This method requires the solution of one problem of dimension

$$(U + M \cdot (N - P)) \times (U + M \cdot (N - P))$$

at each step in the iteration.

The algorithm to be presented in the following pages follows Method 1, because it appears computationally more manageable. However, it is important to recognize that other methods are possible.

The Minimization Procedure

The minimization with respect to K_u of the weighted sum of squares from Method 1 in the previous section

$$\min_{K_u} \sum_{m,p} \left(\frac{Y_p^m}{W_p^m} \right)^2 \qquad \begin{array}{l} u \in U \\ m \in M \\ p \in P \end{array}$$

is described with the subscripts defined in Table I. The weighting factor W will be discussed later; here it will simply be carried as a constant. We take the partial derivative of the weighted sum of squares with respect to each K_u and set the result equal to zero. Since Y_p^m is a function of the known variables, the guesses for K_u , and T_p ,

$$Y_p^m = Y_p^m \left(T_{\bar{i}}, X_i, K_u, T_p\right) \qquad \begin{array}{l} m \in M \\ p \in P \\ \underline{i} \in \underline{I} \\ \bar{i} \in \bar{I} \\ u \in U \end{array}$$

the partial derivative is taken with these variables $(T_{\bar{i}}, X_i, K_u, T_p)$ held constant:

$$S_u = \sum_{m,p} \left\{ \frac{Y_p^m}{W_p^m} \cdot \frac{\left(\frac{\partial Y_p^m}{\partial K_u}\right)}{W_p^m} \right\} = 0 \qquad \begin{array}{l} m \in M \\ p \in P \\ u \in U \end{array}$$

We wish to solve the equation above for K_u. We can approach the solution iteratively by expanding the function S_u in a first order Taylor Series about K_u(guess) and solving for ΔK (this amounts to the well established

Newton-Raphson Method)

$$S_u + \sum_v \left[\left(\frac{\partial S_u}{\partial K_v}\right) \cdot \Delta K_v\right] = 0 \qquad \begin{matrix} u \in U \\ v \in U \end{matrix}$$

The derivative $(\partial S_u / \partial K_v)$ in the expression above is

$$\frac{\partial S_u}{\partial K_v} = \sum_{m,p} \left[\frac{\left(\frac{\partial Y_p^m}{\partial K_v}\right)}{W_p^m} \cdot \frac{\left(\frac{\partial Y_p^m}{\partial K_u}\right)}{W_p^m} + \frac{Y_p^m}{W_p^m} \cdot \frac{\left(\frac{\partial^2 Y_p^m}{\partial K_v \partial K_u}\right)}{W_p^m} \right]$$

If we maintain the approximation that second order derivatives of Y with respect to K are equal to zero, the equation in ΔK can be written in the form

$$\begin{aligned} D_{11}\ \Delta K_1 \ + \ . \ . \ . \ D_{1U}\ \Delta K_U &= -S_1 \\ &\vdots \\ D_{U1}\ \Delta K_1 \ + \ . \ . \ . \ D_{UU}\ \Delta K_U &= -S_U \end{aligned}$$

where the coefficients D are

$$D_{u,v} = \sum_{m,p} \left\{ \frac{\left(\frac{\partial Y_p^m}{\partial K_u}\right)}{W_p^m} \quad \frac{\left(\frac{\partial Y_p^m}{\partial K_v}\right)}{W_p^m} \right\}$$

The U linear equations in U unknowns may be solved by the usual methods, and values of ΔK_u used to compute improved values of K_u for the next iteration.

$$K_u(\text{improved}) = K_u(\text{guess}) + \Delta K_u$$

The computation of the coefficients $D_{u,v}$ and S_u is discussed in Appendix 3.

Weighting factor

The weighting factor W_p^m determines the relative importance of each residual in the sum of squares

$$SOS = \sum_{m,p} \left[\frac{Y_p^m}{W_p^m} \right]^2 \qquad \begin{matrix} m \in M \\ p \in P \end{matrix}$$

If the precision of the experimental data from which a particular residual is calculated is poor, then this residual should have less weight in the sum of squares than a residual calculated from more precisely known experimental data. In an ideal case, all of the experimental observations (X_i, T_j, $j \in J$, $i \in I$) would be known with infinite precision and the weighting factor could be ignored, i.e., all W_p^m could be set equal to one.

In reality there is experimental error in the observations $X_i (i \in I)$ and T_j $(j \in J)$; if the variance of the populations from which the X_i^m, T_j^m are sampled can be estimated, an estimate for the variance associated with Y_p^m can be computed; this variance $V_{Y_p^m}$ can then be used as the weighting factor in the sum of squares

$$SOS = \sum_{m,p} \left[\frac{[Y_p^m]^2}{V_{Y_p^m}} \right]$$

The equation for the propagation of error is used to compute $V_{Y_p^m}$ from the variances of the experimentally observed variables of which Y_p^m is a function:

$$Y_p^m = Y_p^m \left(T_{\bar{i}}, X_i, K_u, T_p\right)$$

$$V_{Y_p^m} = \left[\sum_{\bar{i}} \left(\frac{\partial Y_p^m}{\partial T_{\bar{i}}^m} \right) \left(V_{T_{\bar{i}}^m} \right)^{1/2} + \sum_{i} \left(\frac{\partial Y_p^m}{\partial X_i^m} \right) \left(V_{X_i^m} \right)^{1/2} + \left(\frac{\partial Y_p^m}{\partial T_p^m} \right) \left(V_{T_p^m} \right)^{1/2} \right]^2$$

The cross terms in the expression above cancel if the errors in T_j^m, X_i^m are uncorrelated, which we assume. Then

$$V_{Y_p^m} = \sum_{\bar{i}} \left(\frac{\partial Y_p^m}{\partial T_{\bar{i}}^m} \right)^2 V_{T_{\bar{i}}^m} + \sum_{i} \left(\frac{\partial Y_p^m}{\partial X_i^m} \right)^2 V_{X_i^m} + \left(\frac{\partial Y_p^m}{\partial T_p^m} \right)^2 V_{T_p^m}$$

Evaluation of the derivatives is given in Appendix 4.

Evaluation of V_X and V_T

Strictly speaking, to estimate $V_{X_i^m}$ and $V_{T_j^m}$ we should make t independent measurements of the variables X_i^m and T_j^m; then $V_{X_i^m}$ and $V_{T_j^m}$ are given by

$$V_{X_i^m} = \sum_t \frac{[{}^tX_i^m - \bar{X}_i^m]^2}{t - 1}$$

and

$$V_{T_j^m} = \sum_t \frac{[{}^tT_j^m - \bar{T}_j^m]^2}{t - 1}$$

In practice we might use a less rigorous method for the estimate of $V_{X_i^m}$ and $V_{T_j^m}$; for instance we could assume a constant relative standard deviation (the standard deviation equals the square root of the variance) on the order of .01 for T_p^m, then

$$V_{T_p^m} = [.01 \cdot T_p^m]^2$$

and we could assume a constant standard deviation on the order of .01 for $\log X_p^m$, since X_p^m may be measured from a logarithmic (e.g. potentiometric) response device, then

$$V_{\log X_p^m} = \frac{V_{\ln X_p^m}}{[2.303]^2} = \frac{V_{X_p^m}}{[2.303\ X_p^m]^2} = (.01)^2$$

Then with an estimate of the variance of the variables of which Y is a function, and the derivatives of Y with respect to these variables, it is possible to compute an estimate for the variance of Y, which can be

used as the weighting factor in the sum of squares.

A qualification is necessary about the derivation given above. Originally it was stated that Y_p^m was a function of X_i^m, T_j^m, K_u

$$Y_p^m = Y_p^m (X_i^m, T_j^m, K_u)$$

Actually Y_p^m is also a function of the "known" equilibrium constants, which are subject to experimental error as are X_i^m and T_j^m.

$$Y_p^m = Y_p^m (X_i^m, T_j^m, K_u, K_{\bar{u}}) \qquad \bar{u} \varepsilon \bar{U}$$

In this case, it is simplest to consider each of the "known" K's to be known with infinite precision. Then we may always express the determined constants relative to the other known constants, and the results will be self-consistent. Thus we set

$$V_{K_{\bar{u}}} = 0 \qquad \bar{u} \varepsilon \bar{U}$$

Estimate of Variance in K

Assuming that we have computed the "optimal" values for K_u using the variance of Y as the weighting factor as described above, we may use again the equation for the propagation of error to compute the variance of the equilibrium constants which have been determined, K_u. The elements of the D matrix from which K_u was calculated were given previously and are written here with $(W_p^m)^2 = V_{Y_p^m}$

$$D_{u,v} = \sum_{m,p} \left[\frac{\left(\frac{\partial Y_p^m}{\partial K_u}\right)\left(\frac{\partial Y_p^m}{\partial K_v}\right)}{V_{Y_p^m}} \right]$$

The inverse of the D matrix is the covariance matrix $V_{K_{u,v}}$.

The symmetric covariance matrix $V_{K_{u,v}}$ is almost as important as the value determined for K_u itself. The square root of an element $V_{K_{u,v}}$ of the covariance matrix is an estimate of the standard deviation in $K_{u,v}$ based on the estimated standard deviation in the experimentally observed variables. If the experimental data does reflect the influence of the reaction for which K_u was determined, the relative standard deviation in K_u will in general be of the same order of magnitude as the relative standard deviation of the experimentally observed variables.If the experimental data does not reflect the influence of the reaction for which K_u was determined, the relative standard deviation in general will be much greater than that for the experimentally observed variables. Thus the covariance matrix not only gives an idea of the precision with which K is known, but also aids in choosing reactions which reflect the experimental data.

Choice of Reactions to Describe Experimental Data

The mathematical aspects of the equilibrium constant problem have been discussed in some detail. Now we address some chemical problems: if we are given equilibrium data for a system, what criteria do we use to select reactions which reflect the data? The initial choice for the set of reactions should be all of those which are likely to occur. This judgement can be made by analogy of the system under study with other known systems. Then the correspondence of the experimental data and the theoretical predictions, as measured by the weighted sum of squares, can be compared for various combinations of reactions included in the theory. This comparison along with the computed variance of K should allow us to make a reasonable judgment about which reactions most adequately explain the experimental data, and what the equilibrium constants for these reactions are.

If the reactions which we choose are not at all appropriate for the experimental data, numerical problems may arise in the optimization procedure. The D matrix may become ill-conditioned, as illustrated in the following example.

Suppose we wish to determine the stepwise stability constants of a diprotic acid.

$$H + L = HL \qquad K_1$$

$$H + HL = H_2L \qquad K_2$$

The speciation of the acid as a function of pH is given in Figure 4. Suppose that all of the data are gathered on the far right hand side of this graph. The exact value of K is immaterial to the solution of the material balance equations and the derivative matrix $D_{u,v}$ is ill-conditioned. Even if we

Figure 4. Speciation of a Diprotic Acid.

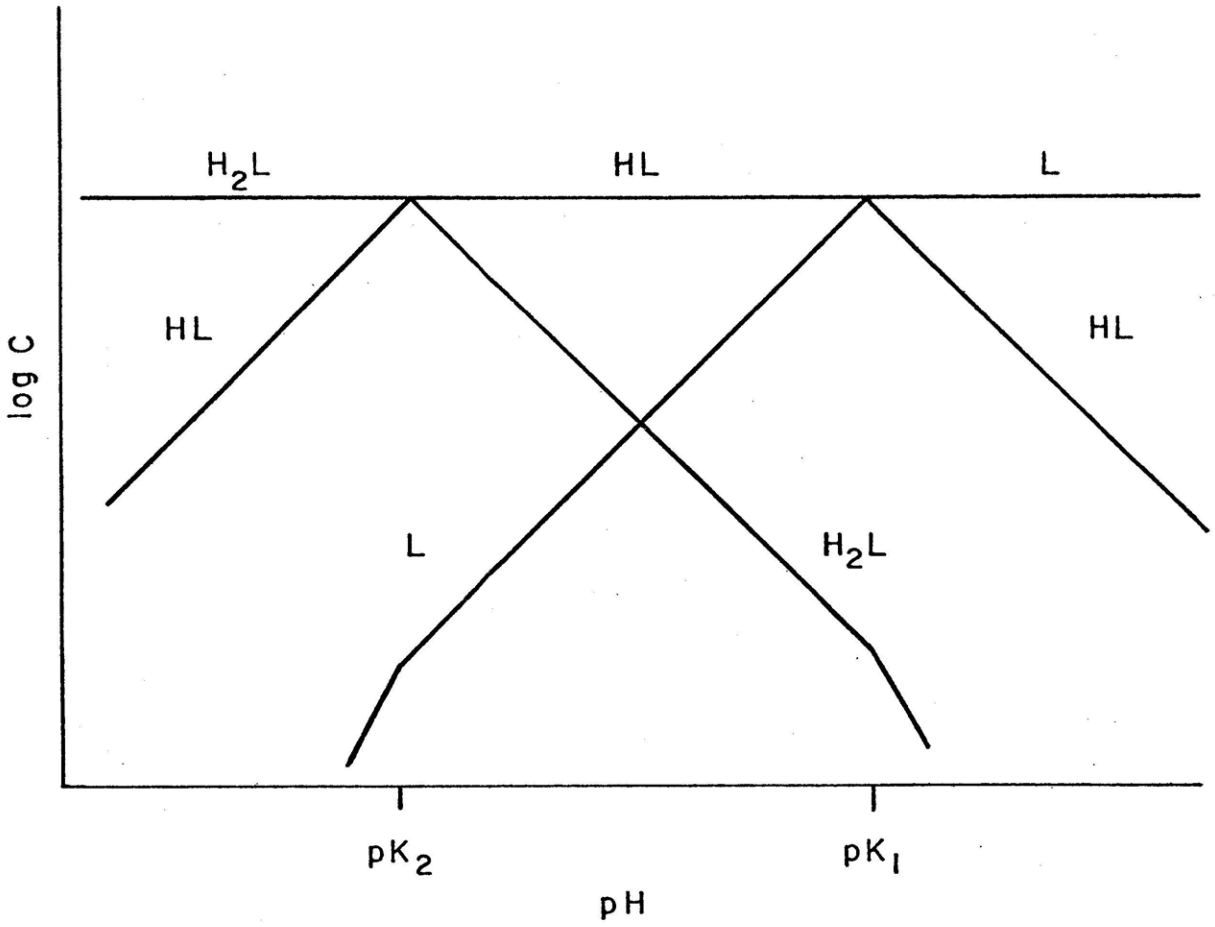


Figure 4

extend the range of data midway through the dominance range of HL, the $D_{u,v}$ matrix will still be rank deficient for K_2. However, if the range of data is extended to include the left hand side as well, the problem is well conditioned, with the terms from the region in which the constants K_1 and K_2 have an effect on the material balance equation predominating in the sums

$$\sum_{p,m} \left[\frac{\left(\frac{\partial Y_p^m}{\partial K_u}\right)\left(\frac{\partial Y_p^m}{\partial K_v}\right)}{\left(W_p^m\right)^2} \right]$$

which form the D matrix.

Computation of Equilibrium Constants from "Pseudo-Experimental" Data

We present a problem where the stability constants for the cupric mono- and di-glycinate species are determined from a simulated acid/base titration curve. The constants are not determined directly from cupric ion activity measurement, but indirectly by measurement of the pH and the hydrogen ion displacement from glycine by copper.

Equilibrium constants for the copper/hydrogen/glycine system were taken from the literature and the equilibrium speciation was computed at each step in a simulated acid/base titration curve. Then a random normal deviate was added to the computed data (as discussed in the section on weighting factors) to give pseudo-experimental data. The equilibrium constants for copper mono- and di-glycinate were then re-determined from the pseudo-experimental data and wrong guesses for the constants (Table II). This operation was carried out for various numbers of data points scattered uniformly over the titration range (Table IIA). The standard deviation computed for K_1 and β_2 is seen to become smaller as the number of data points increases, as expected. All computed values for K_1 and β_2 fall within the neighborhood of the correct values.

The determination of constants was also carried out with a fixed number of pseudo-experimental data points, but with different variances in the pseudo-experimental data (Table II B). For $e = 10^{-4}$ or 10^{-3} (e is the relative standard deviation in T_H and the standard deviation in log X_H, as described in Table II and previously in the text) the recomputed K's agree well with the initial correct K's, and the variance in the computed K's are quite small; for greater values of e,

TABLE II

Determination of Constants from
Pseudo-Experimental Data*

A.

Number of Data Points N	Computed Values			
	$\log K_1$	$\log \beta_2$	$V_{\log K_1 \log K_1}^{\frac{1}{2}}$	$V_{\log \beta_2 \log \beta_2}^{\frac{1}{2}}$
10	8.70	16.2	.12	.16
10	8.66	15.9	.12	.21
20	8.64	16.1	.08	.12
50	8.66	16.0	.05	.08
50	8.68	16.1	.05	.08

$$V_{T_H} = (.01)^2 \; T_H^2$$

$$V_{\log X_H} = (.01)^2$$

TABLE II

Determination of Constants from
Pseudo-Experimental Data *

B.

Variance in Experimental Measurements e	Computed Values			
	$\log K_1$	$\log \beta_2$	$V_{\log K_1 \log K_1}^{1/2}$	$V_{\log \beta_2 \log \beta_2}^{1/2}$
0.0001	8.70	16.0	.001	.001
0.001	8.70	16.0	.008	.013
0.005	8.67	16.0	.041	.062
0.01	8.74	16.3	.083	.12
0.05	8.06	16.3	.8	.47
0.10	9.32	17.1	.8	1.14
0.5	no convergence			

$V_{T_H} = (e)^2 T_H^2$

$V_{\log X_H} = (e)^2$

computed for N = 20 data points

*1. Generation of Titration Data

$T_{Cu} = 10^{-4}M$

$T_{GLY} = 10^{-3}M$

$T_H = 0.5 \times 10^{-3}M - 1.5 \times 10^{-3}M$ in $10^{-5}M$ increments

"Correct" values:

$\log K_1 = 8.70$ $\log \beta_2 = 16.00$

2. Creation of Pseudo-experimental Data

$V_{T_{Cu}} = 0$

$V_{T_{GLY}} = 0$

V_{T_H}, $V_{\log H}$ as described with each table

N, the number of data points, were uniformly distributed over the range of the titration; N is given with each table

3. Optimization

The "wrong" guesses

$\log K_1 = 8.00$ $\log \beta_2 = 15.00$

were used to initiate the optimization procedure. The recalculated values for $\log K_1$ and $\log \beta_2$ are given in the tables along with the square root of the covariance terms:

$$V_{\log K_1}, \log K_1, V_{\log \beta_2}, \log \beta_2$$

The covariance term $V_{\log K_1, \log K_2}$ is not given, but in every case is approximately one order of magnitude less than the other covariance terms.

the variances in the computed K's are correspondingly greater; for e = .5, the method does not converge.

The equilibrium speciation and the derivatives of the residual in hydrogen ion (Y_H) with respect to the K's ($\partial Y_H/\partial K$) are plotted against the amount of acid added (Figure 5). At high pH it is seen that copper diglycinate predominates and at the titration point there is a brief peak for the copper monoglycinate species. It is seen that the derivatives are important primarily at the titration point - interpreted algebraically this means that the D matrix, the elements of which are the sum of the products of the weighted derivatives, will depend only on the region of the curve $\pm$ 20% of the titration point and the other data are meaningless. Examination of the curve $\frac{\partial Y}{\partial K}$ then gives insight into the suitability of the data for the K's to be determined.

Figure 5. Titration of Copper (II)/Glycinate with Acid. $T_{GLY} = 10^{-3}$ M, $T_{Cu} = 10^{-4}$ M, $T_H = .5 \times 10^{-3}$ M - 1.5×10^{-3} M; the relative weighted values of the derivatives are plotted to allow comparison of the two; i.e., the curve labelled $\left(\frac{\partial Y}{\partial K}\right)$ is actually $\frac{\left(\frac{\partial Y}{\partial K}\right) \cdot K}{(V_Y)^{\frac{1}{2}}}$.

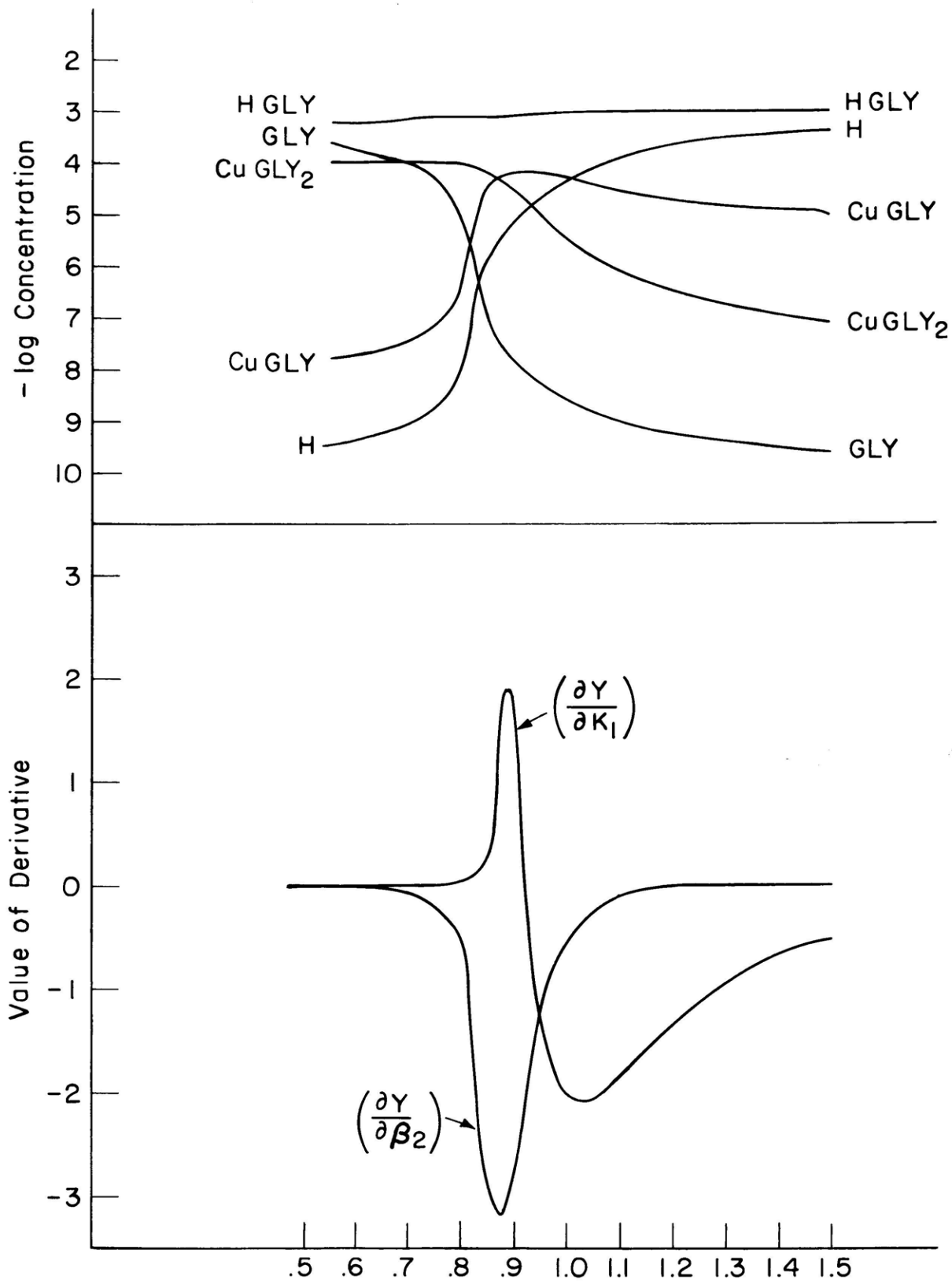

- log Concentration
2
3
4
5
6
7
8
9
10
H GLY
GLY
Cu GLY2
Cu GLY
H
H GLY
H
Cu GLY
Cu GLY2
GLY
Value of Derivative
3
2
1
0
-1
-2
-3
(∂Y/∂K1)
(∂Y/∂β2)
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
1.5

Figure 5

Determination of Stability Constants for Copper (II)-Ammine Complexes

The utility of an algorithm such as described in this paper lies not so much in cases where one or two constants are determined as in cases where many constants are determined, and it becomes unwieldy to manipulate the data by hand. The following example demontrates the determination of stability constants of four consequetive copper(II) ammine complexes. The data of Bjerrum(12) for copper ammine species in 2N NH_4NO_3 at 30° C is used. Liberal (compared to Bjerrum's description of the data) estimates of the standard deviations in the experimentally observed variables were made ($\sim$0.5% for total concentration, and 0.5 mV for the potetiometric measurements) and guesses for the stability constants were $\log K_1 = 3$, $\log \beta_2 = 6$, $\log \beta_3 = 9$, and $\log \beta_4 = 12$.

The fitting routine converged after five iterations (Table III) to values similar to those of Bjerrum determined by hand. The computed standard deviation in the logarithms of the constants ($V^{\frac{1}{2}}$) confirm that the constants adequately describe the experimental data.

TABLE III

Determination of Constants for the

Copper(II)-Ammine System

Iteration	$\log K_1$	$\log \beta_2$	$\log \beta_3$	$\log \beta_4$	SOS
1	3.00	6.00	9.00	12.00	1.77×10^{4}
2	4.08	5.00	10.07	12.34	5.97×10^{2}
3	4.20	7.35	10.30	12.62	6.32×10^{1}
4	4.15	7.64	10.47	12.69	1.98×10^{0}
5	4.14	7.66	10.53	12.68	5.92×10^{-2}
6	4.14	7.66	10.53	12.68	5.72×10^{-2}
7	4.14	7.66	10.53	12.68	5.72×10^{-2}
Bjerrum	4.15	7.65	10.54	12.67	
$V^{\frac{1}{2}}$	0.013	0.027	0.035	0.037	

Conclusion

The availability of a sound, general method for the determination of stability constants from experimental data is a definite asset to research in analytical chemistry. The method presented here has three principal advantages: it is general; it takes into account explicitly the mathematics of chemical equilibrium, whereas an all purpose regression routine might not; and it allows an estimate of the precision of the computed equilibrium constants to be made. In addition, information on the appropriateness of the data for the constants to be determined (c.f. Figure 5) is available, and can be helpful in the planning of experiments.

The theoretical capabilities of this method for the determination of stability constants are in practice somewhat limited by difficulties with experimental technique: whereas in theory an accurate estimate for the variance of an experimentally observed variable is possible, in practice such an estimate requires much work - then instead of a statistical estimate of variance in an observed variable, an order of magnitude guess might be used. This is as much a problem of analytical technique as a computational problem, but it does slightly limit the theoretical capabilities of the computational method. Finally we emphasize that the finest computational scheme possible is useless without some chemical understanding of the system under investigation.

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Appendix 1

Mathematical Description

Methodology for Chemical Equilibrium Problems

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Methodology for Chemical Equilibrium Problems

Two basic approaches exist to the chemical equilibrium problem. One which has been developed extensively in a series of reports from the Rand Corporation (4-6), involves the following procedure: from an initial guess of the solution, a positive feasible solution to the mass balance equations is found without consideration of the Gibbs free energy of the system; then subject to the constraint of the mass balance equations the Gibbs free energy is minimized. Another approach, the so-called equilibrium constant approach, begins with an initial guess for the concentrations of components, whence the minimum Gibbs free energy composition is readily calculated from equilibrium constants, then the mass balance equations are solved by iteration. This is the approach used in the programs of Sillén (2-3), Perrin (7), and Morel (9-10), and others.

Thus in the first case, mass balance equations are the constraints as the Gibbs free energy function is minimized, and in the second case, the Gibbs free energy function is directly minimized and the mass balance equations are solved. Zeleznik and Gordon present a complete discussion of this topic (1).

While the former "direct minimization of the Gibbs free energy function" method seems more elegant in a thermodynamic sense, the latter "equilibrium constant" method appears to be more an extension of chemists' intuition, and thereby more useful and tractable in

many ways. The equilibrium constant method is used in MINEQL.

One may view the equilibrium constant approach in the following way: at every step in the iteration towards the final solution a chemical equilibrium problem is solved (i.e., the computed composition represents the minimum Gibbs free energy for a set of mass balance constraints); the task is to iterate to the correct chemical equilibrium problem (i.e., the chemical equilibrium problem with the desired set of mass balance constraints).

Similarly, one may consider a computation involving solid phases as an iteration towards the correct problem, but at a higher level of hierarchy: for a given set of solid phases in equilibrium with the aqueous phase, a chemical equilibrium problem can be solved, as described above. But then it is necessary to find the correct set of solid phases in equilibrium with the aqueous phase, (i.e., that set for which no solubility products are exceeded and no mass balance conditions are less than zero). This is achieved by successively redefining the set of solid phases considered to be in equilibrium with the aqueous phase and solving the resulting equilibrium problem until the condition specified above for solid phases are met.

Definitions

There are terms used in this appendix which have a particular significance outside of their normal English language, chemical, or mathematical use. These terms are defined.

1. Components - in a mathematical sense this is the independent basis set from which every species can be defined and upon which the mass balance equations are based. In a chemical sense, the components are a set of chemical entities such that every species can be represented as the product of a reaction involving only these components, and no component can be represented as the product of a reaction involving only the other components. The particular set of components for a given problem is certainly not unique, but once a set of components has been chosen, every species has a unique representation in terms of this set. There has been a tendency to consider only neutral entities for the set of components; however, there is no reason for the exclusion of ionic entities from this set, (nor are there any such restrictions in Gibbs original definition of components (19) and in this discussion ionic entities will be included as components.

2. Species - mathematically, a species represents a log linear combination of the components; chemically, a species is the product of a chemical reaction involving the components as reactants. The species include every chemical entity to be considered in a chemical equilibrium problem. Species are of two kinds: soluble complexes and solids.

1.1 General Description of the Problem

The problem of chemical equilibrium in an aqueous system can be described in mathematical terms as the set of equations

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}$$

for i=1,m

$$Y_j = \sum_{i=1}^{m} a(i,j)\, C_i - T_j$$

for j = 1,n

for which the solution is the set $\{X_j:\ j=1,n\}$ such that $\{Y_j:\ j=1,n\} = \{0\}$ given $\{K_i,\ a(i,j),\ T_j:\ j=1,n,\ i=1,m\}$. The significance of X,Y,K,a & T will be discussed below.

The solution to the problem is found by making an initial guess for the $\{X_j\}$ and iterating by the multidimentional Newton Raphson method to the root of the equation. The method converges over a wide range of initial guesses.

1.2 Computation of Complexes

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}$$

C_i is the concentration of the complex of stoichiometry

$$X_1{}_{a(i,1)} \ldots X_n{}_{a(i,n)}$$

K_i is the stability constant of the complex C_i

X_j is the concentration of component C_j

a(i,j) is the stoichiometric coefficient of X_j in C_i

1.3 Computation of Mass Balance Equation

$$Y_j = \sum_{i=1}^{m} a(i,j)\ (C_i) - T_j$$

T_j is the total analytical concentration of component j

Y_j is the difference between the imposed total analytical concentration of component j (T_j), and the amount of component j in all of the complexes as computed from the X_j's. The solution to the problem is, of course, the set of X_j's such that all Y_j's = 0.

1.4 Computation of the Jacobian

$$Z(j,k) = \sum_{i=1}^{m} a(i,j)\ a(i,k)\ C_i/X_k$$

Z(j,k) is the element of the Jacobian representing $(\partial Y_j/\partial X_k)$. It is derived as follows:

$$\frac{\partial Y_j}{\partial X_k} = \sum_{i=1}^{m} \left(\frac{\partial Y_j}{\partial C_i}\right)\left(\frac{\partial C_i}{\partial X_k}\right)$$

(1) $$\left(\frac{\partial Y_j}{\partial C_i}\right) = a(i,j)$$

(2) $$\left(\frac{\partial C_i}{\partial X_k}\right) = (a(i,k)\ X_k^{a(i,k)-1})\ K_i \prod_{\substack{\ell=1 \\ \ell\neq k}}^{n} X_\ell^{a(i,\ell)}$$

$$= \frac{a(i,k)}{X_k}\ (K_i\ X_k^{a(i,k)} \prod_{\substack{\ell=1 \\ \ell\neq k}}^{n} X_\ell^{a(i,\ell)}$$

$$= a(i,k)\ \frac{C_i}{X_k}$$

$$\left(\frac{\partial Y_j}{\partial X_k}\right) = \sum_{i=1}^{m} a(i,j)\ a(i,k)\ C_i/X_k$$

1.5 Iteration with the Newton Raphson Method

The Newton Raphson method for the determination of the root of the equation

$$y(x) = 0$$

yields the following iterative formula for the N + 1 iteration for x, given the value of x and $\frac{dy}{dx}$ at the N^{th} iteration

$$x^{N+1} = x^N - y^N \left[\left(\frac{dy}{dx}\right)\Big|_{x^N}\right]^{-1}$$

1.5.1 Application to matrix equations

In order to avoid matrix inversion the equation above is modified to:

$$y^N = \left[\left(\frac{dy}{dx}\right)\Big|_{x^N}\right](x^N - x^{N+1})$$

and the matrix equation

$$\bar{Y}^N = \bar{\bar{Z}}^N(\bar{X}^N - \bar{X}^{N+1})$$

(where $\bar{\bar{Z}}$ is the Jacobian of $\bar{Y}$ with respect to $\bar{X}$) is solved for $(\bar{X}^N - \bar{X}^{N+1})$ by Gaussian elimination. Then

$$\bar{X}^{N+1} = \bar{X}^N - (\bar{X}^N - \bar{X}^{N+1})$$

1,5.2 Avoidance of Negative Convergence

If for some X_j, $X_j^{N+1} = X_j^N - (X_j^N - X_j^{N+1}) < 0$ (i.e. the equation begins to converge to a physically meaningless negative solution), X_j^{N+1} is set to $X_j^N/10$.

1.6 Test for Convergence

In theory, the system of equations

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}$$

$$i = 1,m$$

$$Y_j = \sum_{i=1}^{m} a(i,j)\, C_i - T_j$$

$$j = 1,n$$

is "solved" when the set $\{X_j: \ j=1,n\}$ is found such that $\{Y_j: \ j=1,n\} \equiv 0$. In practice some criterion must be established such that when, by iteration, each Y_j becomes sufficiently close to zero, the system of equations is said to be "solved". Since each Y_j is the sum of terms which vary widely in order of magnitude, the convergence criterion is chosen to reflect the magnitude of Y_j relative to the maximum of the terms of which Y_j is the sum. Thus, the criterion for convergence is

$$\frac{|Y_j|}{\max(Y_j)} < \varepsilon \qquad \text{for } j=1,n$$

where $\max(Y_j)$ is the maximum of the absolute values of the set of terms $\{(a(i,j)\ C_i),\ i=1,m;\ T_j\}$ of which Y_j is the sum.

In the case where $a(i,j) \geq 0$ for all i,j, $\max(Y_j)$ is clearly equal to T_j, and the convergence criterion

$$\frac{Y_j}{\max(Y_j)} < \varepsilon$$

has the physical interpretation that all of element j within $\varepsilon \times 100\%$ can be accounted for; in cases where some $a(i,j) < 0$ (as is often encountered when solid phases are present, see Appendix 1) this physical interpretation is no longer valid, but the criterion for convergence certainly is.

1.7 Summary

The problem can be visualized in the following way

	X_1	. . .	X_i	. . .	X_n	
C_1	$a_{1,1}$					K_1
.						.
.						.
.						.
C_i			$a_{i,j}$			K_i
.						.
.						.
.						.
C_m					$a_{m,m}$	K_m
	T_1	. . .	T_j	. . .	T_n	

1. The C_i's are a function of the X_j's

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}: \quad I=1,m \text{ or } \bar{C} = \bar{f}(X_1,\ldots,X_n)$$

2. The Y_j's are a function of the C_i's

$$Y_j = \sum_{i=1}^{m} a(i,j)\, C_i - T_j: \quad j=1,n \text{ or } \bar{Y} = \bar{g}(C_1,\ldots,C_m)$$

3. Then $\bar{Y}$ is a composite function of $\bar{X}$.

$$\bar{Y} = \bar{g}(\bar{f}(x))$$

or

$$\begin{bmatrix} Y_1 = Y_1(X_1,\ldots X_N) \\ \\ Y_N = Y_N(X_1,\ldots X_N) \end{bmatrix}$$

4. Then the Jacobian of $\bar{Y}$ with respect to $\bar{X}$ is

$$\bar{\bar{Z}} = \begin{bmatrix} \left(\frac{\partial Y_1}{\partial X_1}\right) & \cdots & \\ \vdots & \ddots & \\ \left(\frac{\partial Y_N}{\partial X_1}\right) & & \left(\frac{\partial Y_N}{\partial X_N}\right) \end{bmatrix}$$

5. And the value of $\bar{X}^{N+1}$ (at the N+1 iteration) is found from the solution (by Gaussian elimination) to the matrix equation

$$\begin{bmatrix} Y_1^N \\ \vdots \\ Y_n^N \end{bmatrix}^t = \begin{bmatrix} \left.\frac{\partial Y_1}{\partial X_1}\right|_{\bar{X}^N} & & \\ & \ddots & \\ & & \left.\frac{\partial Y_N}{\partial X_N}\right|_{\bar{X}^N} \end{bmatrix} \begin{bmatrix} X_1^N - X_1^{N+1} \\ \vdots \\ X_n^N - X_n^{N+1} \end{bmatrix}$$

2. Transformation of Basis

In order to improve the efficiency of computation, the basis $\{X_1,\ldots,X_n\}$ can be transformed such that the resulting Z matrix appraoches a diagonal matrix. This transformation is carried out by substitution of one of the C_i, say C_{ii}, for one of the X_j, say X_{jj}, in the basis. C_{ii} then becomes basis element X_{n+1}.

2.1 Transformation of C_i's

In the basic problem

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}$$

Now solve for X_{jj} in terms of C_{ii}, and substitute this expression for X_{jj} in the C_i's.

a) Separate terms in C_{ii}

$$C_{ii} = K_{ii} \prod_{j=1}^{n} X_j^{a(ii,j)} = K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(ii,j)} \; X_{jj}^{a(ii,jj)}$$

b) Solve for X_{jj}

$$X_{jj} = C_{ii}^{\frac{1}{a(ii,jj)}} \cdot \left[K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(ii,j)} \right]^{\frac{-1}{a(ii,jj)}}$$

c) Substitute for X_{jj} in the expressions for the C_i's.

$$C_i = K_i \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(i,j)} \cdot \left[C_{ii}^{\frac{1}{a(ii,jj)}} \left[K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(ii,j)} \right]^{\frac{-1}{a(ii,jj)}} \right]^{a(i,jj)}$$

2.2 Transformation of the Y_j's

In the basic problem

$$Y_j = \sum_{i=1}^{m} a(i,j)\ C_i - T_j$$

Now solve the equation $Y_{jj} = 0$ for C_{ii} and substitute the expression for C_{ii} into the other Y_j's.

a) Separate terms in $Y_{jj} = 0$

$$Y_{jj} = \sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj)\ C_i + a(ii,jj)C_{ii} - T_{jj} = 0$$

b) Solve for C_{ii}

$$C_{ii} = -\left(\sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj)C_i - T_{jj}\right)/a(ii,jj)$$

c) Substitute for C_{ii} in the expressions for the Y_j's

$$Y_{\substack{j \\ j\neq jj}} = \sum_{\substack{i \\ i\neq ii}}^{m} a(i,j)C_i - T_j - \left[\frac{a(ii,j)}{a(ii,jj)}\left(\sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj)C_i - T_{jj}\right)\right]$$

2.3 Computation of Mass Balance for $C_{ii} \equiv X_{n+1}$

In the place of Y_{jj} (which is now trivial), a new Y_{n+1} which is based on the new basis element, $C_{ii} \equiv X_{n+1}$, must be computed:

$$Y_{n+1} = \frac{Y_{jj}}{a(ii,jj)} = \sum_{\substack{i=1 \\ i\neq ii}}^{m} \frac{a(i,jj)}{a(ii,jj)}\ C_i + C_{ii} - \frac{T_{jj}}{a(ii,jj)}$$

2.4 Formulation of Transformation

When the expressions of 2.1(c), 2.2(c) and 2.3 are rearranged, a similarity is seen which suggests a simple formula for the modification of the a , T, and K matrices.

eqn. 2.1(c)

$$C_i = K_i \cdot K_{ii}^{-\frac{a(i,jj)}{a(ii,jj)}} \cdot \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{\left(a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)}\right)} \cdot C_{ii}^{\frac{a(i,jj)}{a(ii,jj)}}$$

eqn. 2.2(c)

$$Y_{\substack{j \\ j\neq jj}} = \sum_{\substack{i=1 \\ i\neq ii}}^{m} \left(a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)}\right) C_i - \left(T_j - \frac{a(ii,j)}{a(ii,jj)}\, T_{jj}\right)$$

eqn. 2.3

$$Y_{n+1} = \sum_{i=1}^{m} \frac{a(i,jj)}{a(ii,jj)}\, C_i - \frac{T_{jj}}{a(ii,jj)}$$

Now consider the following transformation

New	Old
$a'(i,j)$	$a(i,j) - \frac{a(ii,j)\ a(i,jj)}{a(ii,jj)}$
T_j'	$T_j - \frac{a(ii,j)}{a(ii,jj)} T_{jj}$
K_i'	$K_i \cdot K_{ii}^{-\frac{a(i,jj)}{a(ii,jj)}}$

and for the new basis element $X_{n+1} \equiv C_{ii}$

$a'(i,n+1)$	$\frac{a(i,jj)}{a(ii,jj)}$
T'_{n+1}	$\frac{T_{jj}}{a(ii,jj)}$

Then equations 2.1(c), 2.2(c) and 2.3 now read, in terms of the transformed variables,

$$C_{i\ i=1,m} = K_i' \prod_{\substack{j=1 \\ j\neq jj}}^{n+1} X_j^{a'(i,j)}$$

$$Y_{j\ j\neq jj\ j=1,n+1} = \sum_{i=1}^{m} a'(i,j)\ C_i - T'_j$$

The above two equations are of course similar in form to the equations for the basic problem discussed in section I. Then it can be shown by induction that these transformations can be carried out successively any number of times.

3. Computation of Solids

The method for computing chemical equilibrium in the presence of solid phases will be shown as follows: Computation where one solid phase is present, introduction of a generalized notation, computation for two solid phases, and extension to an arbitrary number of solid phases.

Again we will denote the solid phase as C_{ii} (where C_{ii} = the activity of the solid phase $\equiv 1$), the amount of solid present as S_{ii}, and will eliminate basis element X_{jj} as the constraint $C_{ii} = 1$ is put on the system.

3.1 One Solid Phase

3.1.1 Eliminate X_{jj} via solid C_{ii}

In the basic problem

$$C_i = K_i \prod_{j=1}^{n} X_j^{a(i,j)}$$

Now since solid C_{ii} exists

$$1 = C_{ii} = K_{ii} \prod X_j^{a(ii,j)} = K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(i,j)} \cdot X_{jj}^{a(ii,jj)}$$

Solve for X_{jj}

$$X_{jj} = \left[K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(ii,j)} \right]^{-\frac{1}{a(ii,jj)}}$$

and substitute into the C_i's

$$C_i = K_i \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(i,j)} \cdot \left[K_{ii} \prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(ii,j)} \right]^{-\frac{a(i,jj)}{a(ii,jj)}}$$

or rearranging terms (compare Section 2.4)

$$C_i = K_i \cdot K_{ii}^{-\frac{a(i,jj)}{a(ii,jj)}} \left[\prod_{\substack{j=1 \\ j\neq jj}}^{n} X_j^{a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)}} \right]$$

3.1.2 Eliminate Y_{jj} via C_{ii}

In the basic problem

$$Y_j = \sum_{i=1}^{m} a(i,j)\, C_i - T_j$$

Now, since there exists an amount of solid S_{ii} then

$$Y_j = \sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,j) C_i + a(ii,j) S_{ii} - T_j$$

and in specific for Y_{jj},

$$Y_{jj} = \sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj) C_i + a(ii,jj) S_{ii} - T_{jj}$$

Set $Y_{jj} = 0$ and solve for S_{ii}

$$S_{ii} = -\left(\sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj) C_i - T_{jj} \right) / a(ii,jj)$$

Substitute S_{ii} into the Y_j's

$$Y_j = \sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,j)C_i - T_j$$

$$- a(ii,j) \{ \sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj)\, C_i - T_{jj} \}/a(ii,jj)$$

And rearranging terms (compare Section 2.4)

$$Y_j = \sum_{\substack{i=1 \\ i\neq ii}}^{m} \left(a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)} \right) C_i - \left(T_j - \frac{a(ii,j)}{a(ii,jj)}\, T_{jj}\right)$$

Now all of the Y_j's $\underset{j\neq jj}{}$ are independent of S_{ii}.

The problem has been reduced to an

$(n\text{-}1 \times n\text{-}1)$ problem in X and Y and a

$(\ 1 \times 1\ )$ problem in S and Y.

3.1.3 Solution for S_{ii}

After the $(n\text{-}1 \times n\text{-}1)$ problem has been solved for $\bar{X}$, $\bar{C}$ can be computed and S_{ii} is readily found from

$$S_{ii} = -\left(\sum_{\substack{i=1 \\ i\neq ii}}^{m} a(i,jj)C_i - T_{jj} \right)/a(ii,jj)$$

3.2 Solution for Numerous Solid Phases

The solution for numerous solid phases is simply an extension of that for one solid phase. With the computation of each solid phase the matrices are transformed successively, each time as before.

3.2.1 Review

To review, in the basic problem (no solids) a solution to the following set of equations was found:

$$Y_j = \sum_{i=1}^{m} a(i,j)\, C_i - T_j$$

where

$$C_i = K_i \prod_{j=1}^{n} X^{a(i,j)}$$

3.2.2 One Solid Phase

In the case of one solid phase the set of equations below ($n-1 \times n-1$, 1×1) was solved:

$$\left[Y_{\substack{j \\ j \neq jj}} = \sum_{\substack{i=1 \\ i \neq ii}}^{m} \left(a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)} \right) C_i - \left(T_j - \frac{a(ii,j)}{a(ii,jj)}\, T_{jj} \right) \right]$$

where

$$C_{\substack{i \\ i \neq ii}} = K_i \cdot K_i^{-\frac{a(i,jj)}{a(ii,jj)}} \prod_{\substack{j=1 \\ j \neq jj}}^{n} X_j^{\,a(i,j) - \frac{a(ii,j)\, a(i,jj)}{a(ii,jj)}}$$

and

$$\left[S_{ii} = -\left(\sum_{\substack{i=1 \\ i \neq ii}}^{m} a(i,jj)\, C_i - T_{jj} \right) / a(ii,jj) \right]$$

3.2.3 Notation

For the case of many solids, it is necessary to introduce a new condensed notation:

3.2.4 Condensed Notation: One Solid

Then the set of equations in part 3.2.2 (one solid) can be written in the new notation:

$$\left[\begin{array}{l} Y_j = \sum_{\substack{i=1 \\ i\neq ii'}}^{m} a'(i,j)\, C_i - T_j' \\ j\neq jj' \\ \text{where} \\ C_i = K_i' \prod_{\substack{j=1 \\ j\neq jj'}}^{n} X_j^{a'(i,j)} \\ i\neq ii' \end{array}\right] \qquad (n-1 \times n-1)$$

and

$$S_{ii'} = -\left(\sum_{\substack{i=1 \\ i\neq ii}}^{m} a^\circ(i,jj')\, C_i - T^\circ_{jj'} \right) / a^\circ(ii',jj')$$

3.2.5 Condensed Notation: Two Solids

The argument used in Section 3.1 can be used to consider two solids

$$\left[\begin{array}{l} Y_j = \sum_{\substack{i=1 \\ i\neq ii^1,ii^2}}^{m} a^2(i,j)\, C_i - T_j^2 \\ y\neq jj^1,jj^2 \\ \text{where} \\ C_i = K_i^2 \prod_{\substack{j=1 \\ j\neq jj^1,jj^2}}^{n} X_j^{a^2(i,j)} \\ i\neq ii^1,ii^2 \end{array}\right] \qquad (n-2 \times n-2)$$

for no solids:

new old

$$a^{\circ}(i,j) = a(i,j)$$

$$T^{\circ}_{j} = T_{j}$$

$$K_{i}^{\circ} = K_{i}$$

for one solid

$$a'(i,j) = a^{\circ}(i,j) - \frac{a^{\circ}(ii',j)\, a^{\circ}(i,jj')}{a^{\circ}(ii',jj')}$$

$$T_{j}' = T_{j}^{\circ} - \frac{a^{\circ}(ii',j)}{a^{\circ}(ii',jj')}\, T_{jj'}^{\circ}$$

$$K_{i}' = K_{i}^{\circ} - \frac{a^{\circ}(i,jj')}{a^{\circ}(ii',jj')}\, K_{ii'}^{\circ}$$

and in general

$$a^{\ell+1}(i,j) = a^{\ell}(i,j) - \frac{a^{\ell}(ii^{\ell+1},j)\, a^{\ell}(i,jj^{\ell+1})}{a^{\ell}(ii^{\ell+1}, jj^{\ell+1})}$$

$$T_{j}^{\ell+1} = T_{j}^{\ell} - \frac{a^{\ell}(ii^{\ell+1},j)}{a^{\ell}(ii^{\ell+1}, jj^{\ell+1})}\, T_{jj^{\ell+1}}^{\ell}$$

$$K_{i}^{\ell+1} = K_{i}^{\ell} - \frac{a^{\ell}(i,jj^{\ell+1})}{a^{\ell}(ii^{\ell+1}, jj^{\ell+1})}\, K_{ii^{\ell+1}}^{\ell}$$

where in every case (1,2,...ℓ+1...) solid $S_{ii^{\ell+1}}$, $C_{ii^{\ell+1}}$ and basis element $Y_{jj^{\ell+1}}$ are considered in defining $a^{\ell+1}$, $K^{\ell+1}$, $T^{\ell+1}$ in terms of a^{ℓ}, K^{ℓ}, and T^{ℓ}.

and

$$\begin{bmatrix} S_{ii^2} = -\left(\sum_{\substack{i=1 \\ i \neq ii^1, ii^2}}^{m} a^1(i,jj^2)\, C_i - T'_{jj^2} \right) / a^1(ii^2, jj^2) \\ \\ S_{ii^1} = \left(\sum_{\substack{i=1 \\ i \neq ii^1}}^{m} a^\circ(i,jj^1)\, C_i - T^\circ_{jj^1} \right) / a^\circ(ii^1, jj^1) \end{bmatrix} \quad (2 \times 2)$$

Note that in the expression for S_{ii^1}, the summation over i does not exclude ii^2; thus C_{ii^2} in the summation is equivalent to S_{ii^2}, or,

$$S_{ii^1} = -\left(\sum_{\substack{i=1 \\ i \neq ii^1, ii^2}}^{m} a^\circ(i,jj^1)\, C_i + a^\circ(ii^2, jj^2) S_{ii^2} - T^\circ_{jj^1} \right) / a^\circ(ii^1, jj^1)$$

3.3.6 General Expression for Solids

It is now possible to write the general expression for an arbitrary number of solids ns.

$$\begin{bmatrix} Y_{j \neq jj(1,\ldots ns)} = \sum_{\substack{i=1 \\ i \neq ii(1,\ldots ns)}}^{m} a^{ns}(i,j) C_i - T_j^{ns} \\ \\ \text{where} \\ \\ C_{i \neq ii(,\ldots ns)} = K_i^{ns} \prod_{\substack{j=1 \\ j \neq jj(1,\ldots ns)}}^{n} X_j^{a^{ns}(i,j)} \end{bmatrix}$$

$(n\text{-}ns \times n\text{-}ns)$

and

$$\left[S_{ii^{\ell}} = -\left(\sum_{i=1,\, i \neq ii^{(\ell \ldots 1)}}^{n} a^{\ell-1}(i, jj^{\ell}) C_i - T_{jj^{\ell}}^{\ell-1} \right) / a^{\ell-1}(ii^{\ell}, jj^{\ell}) \right]_{\ell = ns, \ldots 1}$$

(ns × ns)

Appendix 2

Example of a Chemical Equilibrium Problem

A very simple problem will be solved in order to demonstrate the principles on which MINEQL functions. The chemical approach to the problem is emphasized.

The equilibrium composition of a solution to which 10^{-3} moles/liter of $CaCO_3$ has been added is to be determined. The aqueous phase and two possible solid phases will be considered, but the gas phase will not. The species to be considered in this computation and their respective stability constants are given in Table I. A schematic presentation of these data, similar to their representation in MINEQL, is given in Figure 1. The entities Ca^{2+}, H^+, and CO_3^{2-} have been chosen as components; every species can be written as a linear combination of these components. It is assumed initially that no solid phases are present. The solubility of each of the possible solids will be tested after the equilibrium speciation for the soluble species has been computed.

An initial uneducated guess for $[Ca^{2+}]$, $[H]$, and $[CO_3^{2-}]$ is made:

$$[Ca^{2+}]^\circ = 10^{-3}$$
$$[H^+]^\circ = 10^{-10}$$
$$[CO_3^{2-}]^\circ = 10^{-4}$$

and the concentration of each of the complexes is calculated:

$$[Ca^{2+}] = [Ca^{2+}] \cdot 10^{0.0} = 10^{-3.0}$$
$$[H^+] = [H^+] \cdot 10^{0.0} = 10^{-10.0}$$
$$[CO_3^{2-}] = [CO_3^{2-}] \cdot 10^{0.0} = 10^{-4.0}$$

TABLE I Species Considered in Carbonate Problem

1.	Ca^{2+}		
2.	H^{+}		
3.	CO_3^{2-}		
4.	$CaCO_3$	$[Ca^{2+}]\ [CO_3^{2-}]K = [CaCO_3]$	log K = 3.0
5.	$(CaHCO_3)^{+}$	$[Ca^{2+}]\ [H^{+}]\ [CO_3^{2-}]K = [CaHCO_3^{+}]$	log K = 11.6
6.	$(CaOH)^{+}$	$[Ca^{2+}]\ [H^{+}]^{-1}K = [CaOH^{+}]$	log K = -12.2
7.	HCO_3^{-}	$[H^{+}]\ [CO_3^{2-}]K = [HCO_3^{-}]$	log K = 10.2
8.	H_2CO_3	$[H^{+}]^2\ [CO_3^{2+}]K = [H_2CO_3^{*}]$	log K = 16.5
9.	OH^{-}	$[H^{+}]^{-1}\ K = [OH^{-}]$	log K = -14.0
10.	$Ca(OH)_2(s)$	$[Ca^{2+}]\ [H^{+}]^{-2}K = [Ca(OH)_2(s)]$	log K = -21.9
11.	$CaCO_3(s)$	$[Ca^{2+}]\ [CO_3^{2-}]K = [CaCO_3(s)]$	log K = 8.3

The following conditions are assumed: temperature = 25°C, ionic strength $\mu = 0$, concentration $\equiv$ activity

Figure 1. Schematic Representation of Data

Calcium Carbonate Problem

		Ca^{2+}	H^+	CO_3^{2-}	log K
1.	Ca^{2+}	1	0	0	0.0
2.	H^+	0	1	0	0.0
3.	CO_3^{2-}	0	0	1	0.0
4.	$CaCO_3(aq)$	1	0	1	3.0
5.	$CaHCO_3^+$	1	1	1	11.6
6.	$CaOH^+$	1	-1	0	-12.2
7.	HCO_3^-	0	1	1	10.2
8.	H_2CO_3	0	2	1	16.5
9.	OH^-	0	-1	0	-14.0
10.	$Ca(OH)_2(s)$	1	-2	0	-21.9
11.	$CaCO_3(s)$	1	0	1	8.3
		$[Ca^{2+}]_T$	$[H^+]_T$	$[CO_3^{2-}]_T$	
		10^{-3}	0	10^{-3}	

The analytical concentrations according to this representation are

$$[Ca^{2+}]_T = 10^{-3},\ [H^+]_T = 0,\ [CO_3^{2-}]_T = 10^{-3}$$

$$[CaCO_3] = [Ca^{2+}]\,[CO_3^{2-}2]\cdot 10^{3.0} = 10^{-4.0}$$

$$[CaHCO_3^{+}] = [Ca^{2+}]\,[H^{+}]\,[CO_3^{2-}]\cdot 10^{11.6} = 10^{-5.4}$$

$$[CaOH^{+}] = [Ca^{2+}]\,[H^{+}]^{-1}\cdot 10^{-12.2} = 10^{-5.2}$$

$$[HCO_3^{-}] = [H^{+}]\,[CO_3^{2-}]\cdot 10^{10.2} = 10^{-3.8}$$

$$[H_2CO_3^{*}] = [H^{+}]^2\,[CO_3^{2-}]\cdot 10^{16.5} = 10^{-6.5}$$

$$[OH^{-}] = [H^{+}]^{-1}\cdot 10^{-14.0} = 10^{-4.0}$$

The "calculated analytical concentrations" of Ca^{2+}, H^{+}, and CO_3^{2-} as determined from the concentration of complexes is then computed:

$$[Ca^{2+}]_T^{calc} = [Ca^{2+}] + [CaCO_3] + [CaHCO_3] + [CaOH^{+}] = 1.11 \times 10^{-3}$$

$$[H^{+}]_T^{calc} = [H^{+}] + [CaHCO_3^{+}] - [CaOH^{+}] + [HCO_3^{-}] + 2[H_2CO_3^{*}] - [OH^{-}]$$
$$= 5.62 \times 10^{5}$$

$$[CO_3^{2-}]_T^{calc} = [CO_3^{2-}] + [CaCO_3] + [CaHCO_3^{+}] + [HCO_3^{-}] + [H_2CO_3^{*}]$$
$$= 3.62 \times 10^{-4}$$

Then the difference function between the imposed analytical concentration $[x]_T$ and the calculated $[x]_T^{calc}$ is evaluated:

$$Y_{Ca^{2+}} = [Ca^{2+}]_T^{calc} - [Ca^{2+}]_T = 1.10 \times 10^{-4}$$

$$Y_{H^{+}} = [H^{+}]_T^{calc} - [H^{+}]_T = 5.62 \times 10^{-5}$$

$$Y_{CO_3^{2-}} = [CO_3^{2-}]_T^{calc} - [CO_3]_T = 6.37 \times 10^{-4}$$

According to the Newton-Raphson Method, the difference functions (Y's) and their derivatives may be used to find improved values for $[Ca^{2+}]$, $[H^+]$, and $[CO_3^{2-}]$, such that the calculated analytical concentrations, $[x]_T^{calc}$, more closely approach the imposed analytical concentrations, $[x]_T$. If the initial guesses for Ca^{2+}, H^+, $CO_3 2-$ are denoted by $[x]^\circ$ and the improved values by $[x]^{calc}$, the Newton-Raphson Method for this case yields the three simultaneous equations:

$$1.\quad \frac{\partial Y_{Ca^{2+}}}{\partial [Ca^{2+}]} ([Ca^{2+}]^\circ - [Ca^{2+}]^{calc}) + \frac{\partial Y_{Ca^{2+}}}{\partial [H^+]} ([H^+]^\circ - [H^+]^{calc})$$

$$+ \frac{\partial Y_{Ca^{2+}}}{\partial [CO_3^{2-}]} ([CO_3^{2-}]^\circ - [CO_3^{2-}]^{calc}) = Y_{Ca^{2+}}$$

$$2.\quad \frac{\partial Y_{H^+}}{\partial [Ca^{2+}]} ([Ca^{2+}]^\circ - [Ca^{2+}]^{calc}) + \frac{\partial Y_{H^+}}{\partial [H^+]} ([H^+]^\circ - [H^+]^{calc})$$

$$+ \frac{\partial Y_{H^+}}{\partial [CO_3^{2-}]} ([CO_3^{2-}]^\circ - [CO_3^{2-}]^{calc}) = Y_{H^+}$$

$$3.\quad \frac{\partial Y_{CO_3^{2-}}}{\partial [Ca^{2+}]} ([Ca^{2+}]^\circ - [Ca^{2+}]^{calc}) + \frac{\partial Y_{CO_3^{2-}}}{\partial [H^+]} ([H^+]^\circ - [H^+]^{calc})$$

$$+ \frac{\partial Y_{CO_3^{2-}}}{\partial [CO_3^{2-}]} ([CO_3^{2-}]^\circ - [CO_3^{2-}]^{calc}) = Y_{CO_3^{2-}}$$

The derivatives are readily calculated:

$$\frac{\partial Y_{Ca^{2+}}}{\partial [Ca^{2+}]} = 1 + \frac{[CaCO_3]}{[Ca^{2+}]} + \frac{[CaHCO_3^+]}{[Ca^{2+}]} + \frac{[CaOH^+]}{[Ca^{2+}]} = 1.11$$

$$\frac{\partial Y_{Ca^{2+}}}{\partial [H^+]} = \frac{[CaHCO_3]}{[H^+]} - \frac{[CaOH^+]}{[H^+]} = -2.33 \times 10^4$$

$$\frac{\partial Y_{Ca^{2+}}}{\partial [CO_3^{2-}]} = \frac{[CaCO_3]}{[CO_3^{2-}]} + \frac{[CaHCO_3^+]}{[CO_3^{2-}]} = 1.04$$

$$\frac{\partial Y_{H^+}}{\partial [Ca^{2+}]} = \frac{[CaHCO_3^+]}{[Ca^+]} - \frac{[CaOH^+]}{[Ca^+]} = -2.33 \times 10^{-3}$$

$$\frac{\partial Y_{H^+}}{\partial [H^+]} = 1 + \frac{[CaHCO_3^+]}{[H^+]} + \frac{[CaOH^+]}{[H^+]} + \frac{[HCO_3^-]}{[H^+]} + \frac{4[H_2CO_3^*]}{[H^+]} + \frac{[OH^-]}{[H^+]}$$

$$= 2.67 \times 10^6$$

$$\frac{\partial Y_{H^+}}{\partial [CO_3^{2-}]} = \frac{[CaHCO_3^+]}{[CO_3^{2-}]} + \frac{[HCO_3^-]}{[CO_3^{2-}]} + \frac{2[H_2CO_3^*]}{[CO_3^{2-}]} = 1.63$$

$$\frac{\partial Y_{CO_3^{2-}}}{\partial [Ca^{2+}]} = \frac{[CaCO_2]}{[Ca^{2+}]} + \frac{[CaHCO_3^+]}{[Ca^{2+}]} = 0.104$$

$$\frac{\partial Y_{CO_3^{2-}}}{\partial [H^+]} = \frac{[CaHCO_3^+]}{[H^+]} + \frac{[HCO_3^-]}{[H^+]} + \frac{2[H_2CO_3^*]}{[H^+]} = 1.63 \times 10^6$$

$$\frac{\partial Y_{CO_3^{2-}}}{\partial [CO_3^{2-}]} = 1 + \frac{[CaCO_3]}{[CO_3^{2-}]} + \frac{[CaHCO_3^+]}{[CO_3^{2-}]} + \frac{[HCO_3^-]}{[CO_3^{2-}]} + \frac{[H_2CO_3^*]}{[CO_3^{2-}]} = 3.62$$

Then the system of simultaneous equations

$$1.11 \quad x_1 - 2.33 \times 10^4 \quad x_2 + 10.4 \quad x_3 = 1.10 \times 10^{-4}$$

$$-2.33 \times 10^3 \quad x_1 + 2.67 \times 10^6 \quad x_2 + 1.62 \quad x_3 = 5.62 \times 10^{-5}$$

$$0.104 \quad x_1 + 1.63 \times 10^6 \quad x_2 + 3.62 \quad x_3 = 6.37 \times 10^{-4}$$

is solved for x_1, x_2, and x_3 where

$$x_1 \rightarrow [Ca^{2+}]^\circ - [Ca^{2+}]^{calc} = 3.54 \times 10^{-4}$$

$$x_2 \rightarrow [H^+]^\circ - [H^+]^{calc} = 1.83 \times 10^{-10}$$

$$x_3 \rightarrow [CO_3^{2-}]^\circ - [CO_3^{2-}]^{calc} = 2.68 \times 10^{-4}$$

Then the known values for $[Ca^{2+}]^\circ$, $[H^+]^\circ$, and $[CO_3^{2-}]^\circ$ are substituted in the equations above and

$$[Ca^{2+}]^{calc} = 6.45 \times 10^{-4}$$

$$[H^+]^{calc} = -8.33 \times 10^{-4}$$

$$[CO_3^{2-}]^{calc} = 3.68 \times 10^{-4}$$

It is seen that the new value for $[H^+]^{calc} < 0$; this is physically meaningless. Therefore, by use of an empirical algorithm, $[H^+]^{calc}$ is set to $[H^+]^{calc} = [H^+]^\circ/10$, and the iterative process is resumed with the values $[Ca^{2+}] = 6.45 \times 10^{-4}$

$$[H^+] = 10^{-11}$$

$$[CO_3^{2-}] = 3.68 \times 10^{-4}$$

This iterative process is carried out six times until the value of each difference function is small compared to the terms in the difference function. (The actual convergence criterion is that Y divided by the maximum of individual terms in Y is less than ε; here $\varepsilon = 10^{-4}$).

$$Y_{Ca^{2+}} = [Ca^{2+}]_T^{calc} - [Ca^{2+}]_T$$

$$= [Ca^{2+}] + [CaCO_3] + [CaHCO_3^+] + [CaOH^+] - [Ca^{2+}]_T$$

$$= 6.87 \times 10^{-4} + 2.97 \times 10^{-4} + 4.58 \times 10^{-6} + 1.12 \times 10^{-5} - 10^{-3}$$

$$= -2.29 \times 10^{-10}$$

$$Y_{H^+} = [H^+]_T^{calc} - [H^+]_T$$

$$= [H^+] + [CaHCO_3^+] - [CaOH^+] + [HCO_3^-] + 2[H_2CO_3^*] - [OH^-] - [H^+]_T$$

$$= 3.87 \times 10^{-4} + 4.58 \times 10^{-6} - 1.12 \times 10^{-5} + 2.65 \times 10^{-4} + 4.09 \times 10^{-8}$$

$$- 2.59 \times 10^{-4} - 0$$

$$= 4.07 \times 10^{-10}$$

$$Y_{CO_3^{2-}} = [CO_3^{2-}]_T^{calc} - [CO_3^{2-}]_T$$

$$= [CO_3^{2-}] + [CaCO_3] + [CaHCO_3^+] + [HCO_3^-] + [H_2CO_3^*] - [CO_3^{2-}]_T$$

$$= 4.33 \times 10^{-4} + 2.97 \times 10^{-4} + 4.58 \times 10^{-6} + 2.65 \times 10^{-4}$$

$$+ 2.05 \times 10^{-8} - 10^{-3}$$

$$= -2.02 \times 10^{-10}$$

The full solution to this problem is given in the first column of Table II.

Now it is necessary to compute the solubility of the solid phases:

$$Ca(OH)_2 \quad (s) \quad [Ca^{2+}]\,[H]^{-2} \cdot 10^{-21.9} = 10^{-4.24}$$

$$CaCO_3 \quad (s) \quad [Ca^{2+}]\,[CO_3] \cdot 10^{8.3} = 10^{+1.78}$$

TABLE II Solution of the Calcium Carbonate Problem

Species	Solution 1 no solid phases -log[x]	Solution 2 $CaCO_3$(s) present -log[x]
Ca^{2+}	3.16	3.91
H^+	10.41	9.91
CO_3^{2-}	3.36	4.38
$CaCO_3$	3.53	5.30
$CaHCO_3^+$	5.34	6.61
$CaOH^+$	4.95	6.21
HCO_3^-	3.58	4.09
H_2CO_3*	7.69	7.70
OH^-	3.59	4.09
$CaCO_3$(s)	-	3.13
$Ca(OH)_2$(s)	-	-

It is seen that the solubility product of $CaCO_3$ (s) is exceeded, i.e. $[Ca^{2+}]\ [CO_3^{2-}]\ K > 1$; the problem must be modified according to the description given in Appendix I to include the solid phase $CaCO_3$ (s). The schematic representation of the "modified" problem is given in Figure 2. Note that by modification the basis element CO_3^{2-} has effectively been eliminated from the computation; now the computation includes only Ca^{2+} and H^+.

The previously calculated values for $[Ca^{2+}]$ and $[H^+]$ are used as initial guesses, and the modified problem is solved as before for the soluble species. The computation converges after five iterations, and the results are given in Table II. The amount of $CaCO_3$ (s) is calculated from

$$[CO_3^{2-}]_T = [CO_3^{2-}] + [CaCO_3] + [CaHCO_3^+] + [HCO_3^-] + [H_2CO_3^*] + CaCO_3\ (s).$$

$CaCO_3$ (s) is found to be 8.73×10^{-4}; this is checked to insure that it is greater than 0.

The solubility of $Ca(OH)_2$ (s) is then checked:

$$[Ca^{2+}]\ [H^+]^{-1} \cdot 10^{-6.00} < 1$$

and found to be soluble.

The problem is solved.

Figure 2 Schematic Representation of Data for Calcium Carbonate Problem: Modified for $CaCO_3(s)$

		Ca^{2+}	H^+	log K	CO_3^{2-}
1.	Ca^{2+}	1	0	0.0	0
2.	H^+	0	1	0.0	0
3.	CO_3^{2-}	-1	0	-8.3	1
4.	$CaCO_3$	0	0	-5.3	1
5.	$CaHCO_3^+$	0	1	3.3	1
6.	$CaOH^+$	1	-1	-12.2	0
7.	HCO_3^-	-1	1	1.9	1
8.	H_2CO_3	-1	2	8.2	1
9.	OH^-	0	-1	-14.0	0
10.	$Ca(OH)_2(s)$	1	-2	-21.9	0
11.	$CaCO_3(s)$	1	0	8.3	1
		$[Ca^{2+}]_T$	$[H^+]_T$		
		0.0	0.0		

Appendix 3

Evaluation of Y and $(\partial Y/\partial K)$

The computation of Y_p^m and $(\partial Y_p^m/\partial K_u)$ from which S_u and $D_{u,v}$ can be computed is discussed. The computation of Y_m^p is straightforward: the chemical equilibrium problems posed by $T_{\bar{i}}^m$ ($\bar{i} \in \bar{I}$) and X_i^m ($i \in I$) and K_u (guess) ($u \in U$) are solved, and the value for each T_p^m ($p \in P$) calculated. The difference between the calculated and the experimentally observed value of T_p^m is the residual Y_p^m:

$$Y_p^m = T_p^m \text{ (calculated)} - T_p^m \text{ (experimental)}$$

The computation of the derivatives

$$\left(\frac{\partial Y_p^m}{\partial K_u}\right)_{T_{\bar{i}}\ X_i\ K_u\ T_p} \qquad \begin{matrix} i \in \bar{I} \\ i \in I \\ u \in U \\ p \in P \end{matrix} \qquad (1)$$

is somewhat more complicated. The superscript m is dropped hereafter since the equations below are equivalent for all $m \in M$; also for the remainder of this appendix the following subscripts are used exclusively to denote components belonging to the following sets (Table I):

$\bar{i},\bar{h} \in \bar{I}$

$i \in I$

$n \in N$

$u \in U$

$p \in P$

$r \in R$

We begin by writing Y_p as a function of the free concentration of each component, the K's to be determined, and T_p:

$$Y_p = Y_p(X_n, K_u, T_p) \qquad (2)$$

We may find the numerical value of the derivative of Y_p with respect to X_n or K_u as described earlier (Appendix 1):

$$\left(\frac{\partial Y_p}{\partial X_n}\right)_{X_n\ K_u\ T_p} = \sum_r \left(a_{r,p} \cdot a_{r,n} \cdot C_r/X_n \right)$$

$$\left(\frac{\partial T_{\bar{i}}}{\partial X_n}\right)_{X_n\ K_u} = \sum_r \left(a_{r,\bar{i}} \cdot a_{r,n} \cdot C_r/X_n \right)$$

$$\left(\frac{\partial Y_p}{\partial K_u}\right)_{X_n\ K_u\ T_p} = \sum_r \left(a_{r,p} \cdot a_{r,u} \cdot C_r/K_u \right) \qquad (3)$$

$$\left(\frac{\partial T_{\bar{i}}}{\partial K_u}\right)_{X_n\ K_u} = \sum_r \left(a_{r,\bar{i}} \cdot a_{r,u} \cdot C_r/K_u \right)$$

C_r is the concentration of the product of reaction r, and $a_{r,n}$ is the stoichiometric coefficient of component n in complex r. The values of C_r and X_n are available from the solved equilibrium problem: thus it is possible to compute directly the derivatives in equation 3.

It is necessary to transform the partial derivatives based on the function

$$Y_p = Y_p (X_n, K_u, T_p) \qquad (4)$$

which we can compute, to partial derivatives based on the function

$$Y_p = Y_p(T_{\bar{i}}, X_i, K_u, T_p) \qquad (5)$$

which we wish to find. We rewrite equation 4 in terms of the independent

variables of equation 5.

$$Y_p = Y_p\,(X_{\bar{I}}\ (T_{\bar{I}}, X_i, K_u), X_i, K_u, T_p) \qquad (6)$$

that is, $X_{\bar{i}}$ has been re-expressed in terms of $T_{\bar{I}}$, X_i, and K_u.

Then the derivative which we seek (expression 1) is given by the derivative of the composite function 6:

$$\left(\frac{\partial Y_p}{\partial K_u}\right)_{T_{\bar{I}}\ X_i\ K_u\ T_p} = \sum_{\bar{i}}\left[\left(\frac{\partial Y_p}{\partial X_{\bar{i}}}\right)_{X_{\bar{i}} X_i K_u}\left(\frac{\partial X_{\bar{i}}}{\partial K_u}\right)_{T_{\bar{I}} X_i K_u}\right] + \left(\frac{\partial Y_p}{\partial K_u}\right)_{X_{\bar{i}} X_i K_u T_p} \qquad (7)$$

The first factor of the first term and the second term in equation 7 may be computed directly (equation 3); the second factor in the first term in equation 7 may be found through the set of $\bar{I} \times \bar{I}$ linear equations:

$$\sum_{\bar{i}}\left[\left(\frac{\partial T_{\bar{h}}}{\partial X_{\bar{i}}}\right)_{X_n, K_u} \cdot \left(\frac{\partial X_{\bar{i}}}{\partial K_u}\right)_{T_{\bar{I}}\ X_i\ K_u}\right] = -\left(\frac{\partial T_{\bar{h}}}{\partial K_u}\right)_{X_n\ K_u} \qquad (8)$$

The coeffiecients for equation 8 may be computed from equation 3. The root of equation 8:

$$\left(\frac{\partial X_{\bar{i}}}{\partial K_u}\right)_{T_{\bar{I}}\ X_i\ K_u}$$

may be substituted into equation 7 to yield the derivative of expression 1, q.e.d.

Appendix 4

Evaluation of $(\partial Y/\partial T)$ and $(\partial Y/\partial X)$

The derivatives of

$$Y_p^m = Y_p^m(T_{\bar{i}}^m,\ X_i^m,\ K_u,\ T_p^m)$$

with respect to (A) $T_{\bar{i}}^m$, (B) X_i^m and (C) T_p^m which appear in the expression for the variance of Y_p are discussed. As in Appendix 3, the superscript m is dropped hereafter since the derivatives are equivalent for all $m \epsilon M$, and the following subscripts are used exclusively to denote components which are members of the following sets (Table I):

$$\bar{i},\ \bar{h} \in \bar{I}$$

$$i \in I$$

$$n \in N$$

$$u \in U$$

$$p \in P$$

$$r \in R$$

A. The derivative

$$\left(\frac{\partial Y_p}{\partial T_{\bar{i}}}\right) T_{\bar{i}},\ X_i,\ K_u,\ T_p$$

may be found by a method similar to the one used in Appendix 3:

Y_p is expressed as a composite function:

$$Y_p = Y_p(X_{\bar{i}}\ (T_{\bar{i}},\ X_i,\ K_u),\ X_i,\ K_u,\ T_p) \tag{1}$$

and the derivative with respect to $T_{\bar{i}}$ is taken:

$$\left(\frac{\partial Y_p}{\partial T_{\bar{i}}}\right) T_{\bar{i}},\ X_i,\ K_u,\ T_p = \sum_{\bar{h}}\left[\left(\frac{\partial Y_p}{\partial X_{\bar{h}}}\right) X_{\bar{i}},\ X_i,\ K_u \left(\frac{\partial X_{\bar{h}}}{\partial T_{\bar{i}}}\right) T_{\bar{i}},\ X_i,\ K_u\right] \tag{2}$$

The first term on the right hand side of Equation 2 may be found from Appendix 3, Equation 3, and the second term is an element of the inverse of the Jacobian matrix formed by the derivatives

$$\left(\frac{\partial T_{\bar{h}}}{\partial X_{\bar{i}}}\right) X_{\bar{i}},\ X_i,\ K_u$$

which are also found from Equation 3.

B. The evaluation of the derivative

$$\left(\frac{\partial Y_p}{\partial X_i}\right) T_{\bar{i}},\ X_i,\ K_u,\ T_p$$

follows exactly the method for evaluation of

$$\left(\frac{\partial Y_p}{\partial K_u}\right) T_{\bar{i}},\ X_i,\ K_u,\ T_p$$

in Appendix 3; substitute "∂X_i" for "∂K_u" in the equations of Appendix 3.

C. $$\left(\frac{\partial Y_p}{\partial T_p}\right) T_{\bar{i}},\ X_i,\ K_u,\ T_p = -1$$

Part III

Interactions among

Trace Metals, Organic Ligands, and Phytoplankton

PART III
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Introduction

The interactions among phytoplankton, trace metals, and organic chelating materials in natural waters are a complex phenomenon (Figure 1). That these interactions do occur to at least some degree has been established through a variety of laboratory and *in situ* experiments (1-4); however, the exact magnitude of these interactions and the magnitude of their effect on the aquatic ecosystem are largely unknown. It is the purpose of this paper to discuss one of the sub-systems within the network of interactions; namely, the effect of phytoplankton generated exudates on trace metal speciation.

Before examination of this particular exudate-trace metal speciation problem, it is useful to discuss Figure 1 in very general terms to ascertain the framework within which the exudate - metal speciation problem is couched.

Arrow I in Figure 1 shows that trace metals and organics are introduced into the aquatic environment from human and natural sources. The group of metals which are considered - sometimes referred to as "trace" or "heavy" metals - is somewhat ill-defined. A common trait among these metals is their coordination chemistry with certain organic ligands and their biological activity. This group of metals is generally taken to include the transition and rare earth metals, and to exclude the alkalai and alkaline earth metals. Since the transition and rare earth metals do generally occur at "trace" concentrations in natural environments, the descriptor "trace" may be appropriate; however, the term "heavy metals" is less appropriate since many of the metals always included in the

Figure 1. Interactions among Trace Metals, Organic Complexing Agents, and Phytoplankton

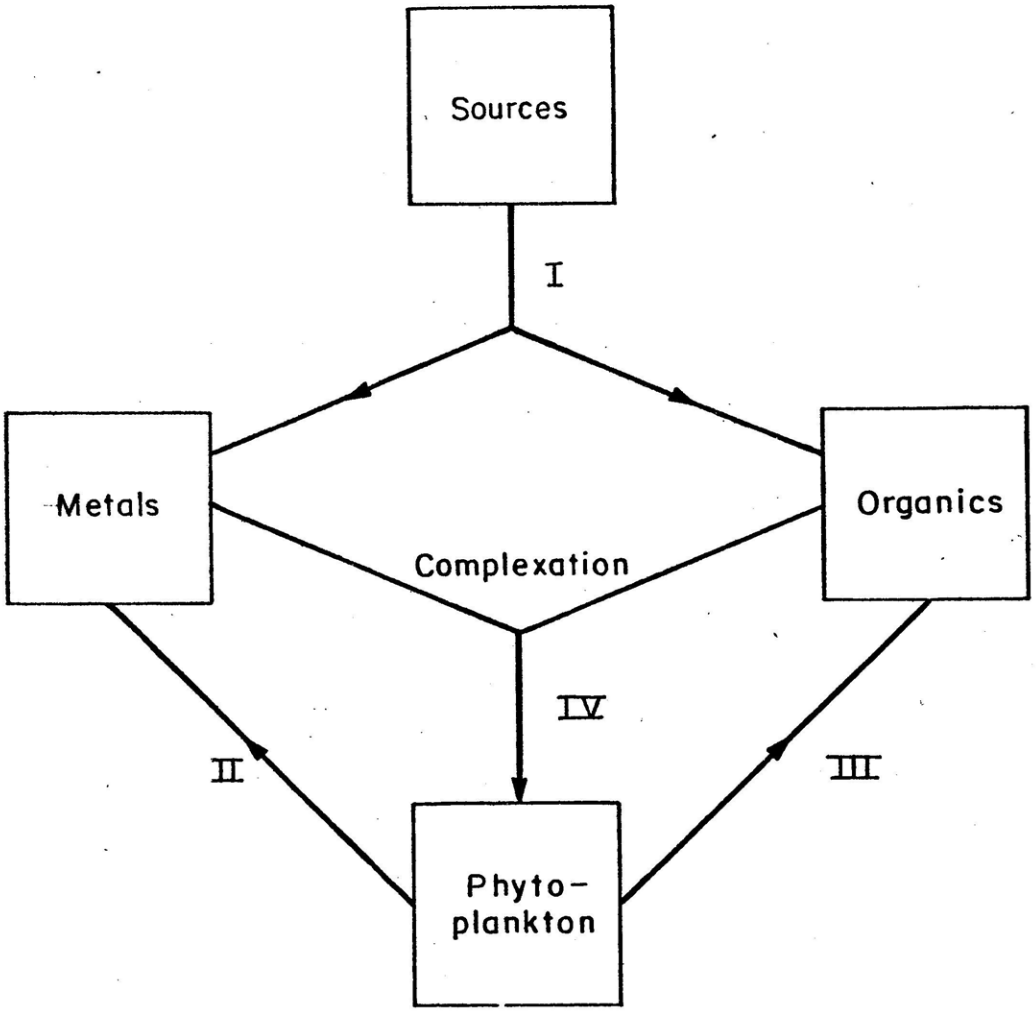

Sources
I
Metals
Organics
Complexation
IV
II
III
Phyto-
plankton

Figure 1

group simply are not "heavy", e.g., chromium and manganese.

The organics of interest in this discussion are those which form stable complexes with the trace metals, that is, primarily organic materials which have oxygen, nitrogen, or sulfur electron donating functional groups. The stability of the trace metal complexes increases according to the electron donor, generally in the order: $O < N < S$, but the abundance of the elements in the biosphere increases in the opposite order: $S < N < O$. The net effect is that, other than in very specific cases, the organic compounds in the aquatic environment most likely to bind trace metals are nitrogen and oxygen containing compounds, primarily those with amine, carbonyl and carboxyl functional groups.

Integrated over the entire globe, the flux of trace metals and organics through the aquatic environment due to human activities is small compared to the natural flux. But in areas of intense human activity, the human component of the trace metal-organic flux may completely outweigh the natural component.

Arrows II and III show the effect of phytoplankton on trace metal and organic concentration. The primary effect of the phytoplankton population on the concentration of trace metals is negative: metals are removed by uptake or adsorption. The primary effect of phytoplankton on the organic concentration is positive: organics are added by cell lysis or exudation from healthy cells. The magnitude of the phytoplankton contribution to the trace metal and organic cycles with respect to the anthropogenic contribution is again related to the

prevailing degree of civilization. In intensely civilized areas the natural component of the cycles is less likely to be significant.

Arrow IV represents the influence of trace metals and organics on phytoplankton. The effect of trace metals on phytoplankton does not necessarily depend on the total amount of metal present, but rather on the availability of the metal to the cell; the availability of the metal to the cell depends on the metal's speciation, and the speciation of the metal may be highly dependent on the presence of organic chelators. Thus the concentration of metal and organic together determines the metal's effect on the phytoplankton. This effect may be limitation of growth if too little of an essential metal is available or inhibition of growth if too much of a potentially toxic metal is available (Figure 2).

In this work, we wish to determine if there is evidence for an interaction of the type: (1) trace metal toxic to phytoplankton - phytoplankton exudes organic material - organic material chelates trace metal and renders it non-toxic; or (2) trace metal unavailable to phytoplankton - phytoplankton exudes organic material - organic material renders trace metal available to phytoplankton. Specifically we attempt to find evidence for metal chelators in pure phytoplankton cultures.

The lack of such evidence would in no way infirm the validity of Figure 1. The necessity of organic chelators, from some source, for phytoplankton cultures is well established (3 - 6). The question is whether the organisms can <u>themselves</u> produce the necessary chelators, or must they rely on an external source of chelators.

Figure 2. Effect of Metal Availability on Phytoplankton Growth

A. Limitation by Lack of an Essential Metal
B. Optimal Growth
C. Inhibition by Toxicity of Metal

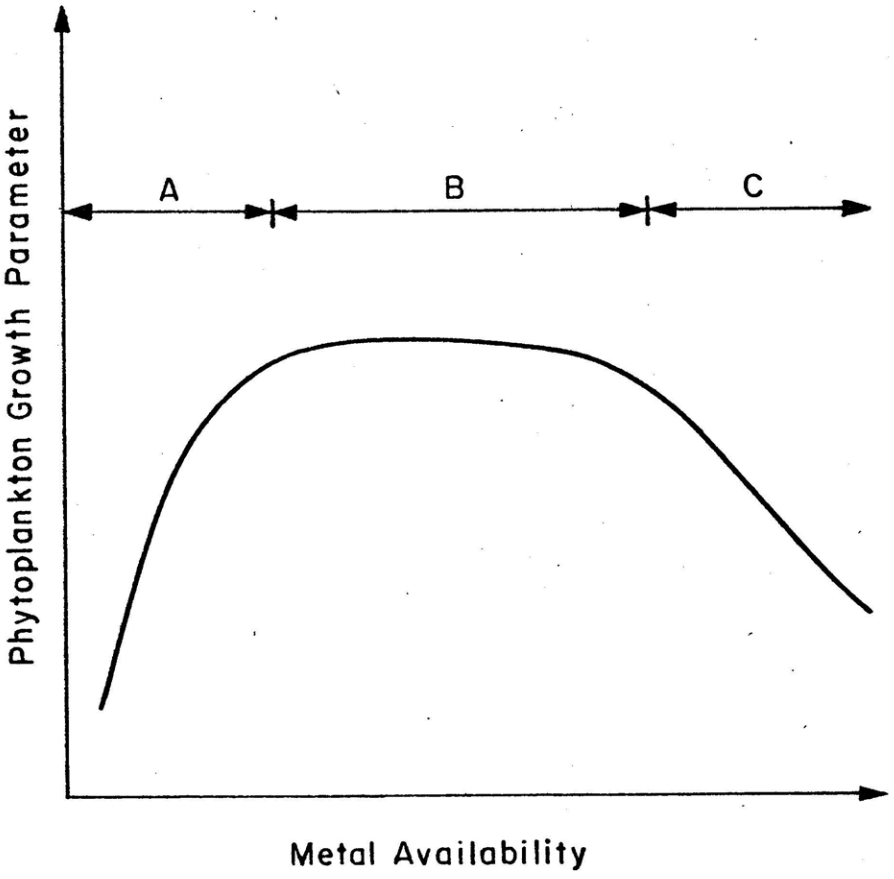


Figure 2

In environments where there is already an abundance of organic chelators from human or other terrestial sources, the question is of little importance; but in environments where there are naturally few organics, the release of organic chelators by phytoplankton may be a key factor in the determination of phytoplankton activity.

Summary of Existing Knowledge on Algal Exudates, With Particular Reference to Metal Complexing Compounds

Before we attempt to discuss the presence of metal complexing compounds exuded into phytoplankton culture media, it is useful to summarize some of the studies on the general problem of algal exudates.

Fogg (7) has defined an extracellular product as "a soluble substance liberated from intact living cells and excluding substances set free on injury or by decomposition of dead cells although it is realized that the distinction is not always easy to maintain either when considering mode of origin or ecological significance". Since, in the framework of Figure 1, it is immaterial whether a complexing material came from an intact cell or an injured cell, Fogg's last qualification will be dropped, and the term "algal exudate" will be used to denote any soluble substance liberated from algal cells. Indeed it is quite conceivable that the primary source of complexing materials in a culture medium stressed by trace metals in toxic concentrations would be exudation from lysed cells.

There are several recent review articles on algal exudates which reference studies on specific compounds (7 - 12). Hellebust (10) has divided extracellular products into different categories:

carbohydrates, nitrogenous substances, organic acids, lipids, phenolic substances, organic phosphates, volatile substance, enzymes, vitamins, sex factors, growth inhibitors and stimulators, toxins. Among these groups, the nitrogenous substances and organic acids are likely to include the strongest trace metal complexers, for reasons discussed previously. Simple substances such as organic acids and amino acids are generally released by diffusion through the cell membrane (8), and the rate and amount of release of these substances depend on their intracellular concentration, the membrane, and its permeability (8, 13). Inbatch cultures, cells in lag phase and stationary growth phase generally release more organic substances than do exponentially growing cells (10).

A list of amino acids and organic acids which have been found as "extracellular products" in various phycological studies is presented in Table I. The conditions of the studies and the organisms used varied, but the list and the accompanying compilation of thermodynamic data make it possible to determine conditions of pH and total ligand concentration which are necessary if a significant fraction of a trace metal (in this example, copper) is to be bound in organic chelates.

The concentration of the exuded substances given in the various studies have been expressed as per cent of photoassimilated carbon, percent of extracellular carbon or directly as concentration of the particular compound. In any case, for a dense culture, a generous estimate for the concentration of one of the relatively abundant extracellular products (such as glycollic acid) is 10^{-5} moles $\cdot$ liter^{-1} (15),

TABLE I

ORGANIC CHELATORS DETECTED IN PHYTOPLANKTON CULTURES

		H^+	Cu^{2+}
Malic acid	$= H_2L$	$K_1 = 5.05$	$K_1 = 3.4$
		$K_{12} = 3.46$	
Oxalic acid	$= H_2L$	$K_1 = 4.14$	$K_1 = 6.19$
		$K_{12} = 1.25$	$K_2 = 4.04$
Glycollic acid	$= HL$	$K_1 = 3.88$	$K_1 = 2.81$
			$K_2 = 1.77$
Lactic acid	$= HL$	$K_1 = 3.88$	$K_1 = 3.02$
			$K_2 = 1.82$
Glycine	$= HL$	$K_1 = 9.61$	$K_1 = 8.62$
		$K_{12} = 2.35$	$K_2 = 6.97$
Serine	$= HL$	$K_1 = 9.24$	$\beta_2 = 14.2$
		$K_{12} = 2.20$	
Aspartic acid	$= H_2L$	$K_1 = 9.47$	$K_1 = 8.57$
		$K_{12} = 3.63$	$K_2 = 6.78$
		$K_{13} = 1.91$	

Glutamic acid	= H_2L	K_1 = 9.67	K_1 = 7.85
		K_{12} = 4.25	K_2 = 6.55
		K_{13} = 2.19	
Proline	= HL	K_1 = 10.68	β_2 = 16.8
		K_{12} = 1.93	
Histidine	= HL	K_1 = 9.20	K_1 = 10.60
		K_{12} = 6.00	β_2 = 18.6
		K_{13} = 1.82	
Arginine	= HL	K_1 = 9.05	K_1 = 7.34
		K_{12} = 1.98	K_2 = 6.42

Table I gives the name, structure, and complex formation constants of the H^+ and Cu^{2+} complexes for several organic chelators detected in phytoplankton cultures. The equilibrium constants were taken from Sillén and Martell (14).

while for one of the less abundant products (for example aspartic or glutamic acid) is 10^{-6} moles · liter^{-1} (11). Using the value of 5×10^{-6} moles · liter^{-1} for total ligand concentration, and assuming that the ligand is in excess of copper, we have taken the data from Table I and computed the ratio of free (uncomplexed) copper to organically bound copper for each of the organic ligands at pH 6, 7, and 8 (Table II). It is seen that for only a few ligands under these conditions is a significant portion of copper bound with the ligand.

This result suggests that no extensive binding of trace metals by nonspecific chelators exuded from phytoplankton may take place. However in light of circumstantial bio-assay evidence to the contrary (e.g.,3,6), a direct analysis of phytoplankton culture media for exuded chelators is presented in this study.

Characterization of Metal Complexing Compounds

There are several ways to "characterize" the chelating properties of algal exudates; the most basic information is the quantity of chelator present, and its affinity for hydrogen ion and a particular trace metal. A second category of information relates to the identity of the chelator: what are the functional groups and the approximate molecular weight. One could also consider the phycological origin of the chelator: during which phase of growth of the phytoplankton was the chelator produced, and what is the influence of stress on the cells.

To answer these questions requires various methods of analysis, but in every case it is first necessary to determine whether a substance capable of trace metal complexation is present in a significant

TABLE II

CHELATION OF COPPER BY SELECTED ORGANIC LIGANDS

	pH 6	pH 7	pH 8
Arginine	+1.1	+0.1	-0.9
Histine	-1.5	-3.6	-5.6
Proline	+3.2	+1.2	-0.8
Glutamic Acid	+1.1	+0.1	-0.9
Aspartic Acid	+0.2	-0.8	-1.8
Serine	+2.8	+0.8	-1.2
Glycine	+0.3	-0.7	-1.8
Lactic Acid	+2.3	+2.3	+2.3
Glycollic Acid	+3.1	+3.1	+3.1
Oxalic Acid	-0.9	-0.9	-0.9
Malic Acid	+2.1	+2.1	+2.1

The values in the table are the logarithms of the ratios of free copper to bound copper for $L_T = 5 \times 10^{-6}$M and ligand in excess of copper. These values were calculated considering only the ligand and copper, i.e., hydroxy and carbonato copper species were ignored.

concentration. A direct method for this determination is the comparison of the cupric ion activity (as measured with a cupric ion selective electrode) in the exudate containing medium with that of a similar exudate-free medium. Copper is an ideal metal for use in this study because of the high stability of many copper-organic ligand complexes, the fact that it may be an essential nutrient as well as a toxicant to algae, and the ease with which analysis for cupric ion activity may be carried out. In the following section of this report the mathematical relationships for a hypothetical complexation reaction will be developed to illustrate limits to observable complexation phenomena.

Model System

We shall assume that our model system consists of the chemical components H^+ Cu^{2+} HL (HL is a protonated organic ligand) and their reaction products: L^-, and CuL. One may write the material balance equations for each of the components (see Appendix 1 of Part II for explanation of material balance equations.)

$$H_T^+ = [H^+] - [L^-] - [OH^-]$$

$$Cu_T^{2+} = [Cu^{2+}] + [CuL^+]$$

$$HL_T = [HL] + [L^-] + [CuL^+]$$

The symbol H_T^+ represents total hydrogen ion concentration and the symbol $[H^+]$ represents free hydrogen ion concentration. It is assumed that equilibrium constants are conditional constants, i.e. that they are

valid for concentrations in the given medium, not activity. For the sake of simplicity, the soluble hydrolysis species of copper and copper hydroxide solid have not been included in the equations above; instead the conditions under which these species are the predominant forms of copper are taken to be limiting conditions to the applicability of this discussion of copper-organic complexes. Those limiting conditions are shown in the figures which accompany the following discussion.

We choose L to be similar to one of the amino acids in Table I. Then if the equilibrium constant K_{HL} for the reaction

$$H^+ + L^- = HL \qquad K_{HL} \qquad \text{(I)}$$

is of the order 10^9, the ligand will be primarily in its protonized form HL in the pH range which we shall consider, pH = 4-8. However, since K_{HL} for the reaction is not readily obtainable experimentally, we shall not assume a value for it, but simply accept that the ligand species HL predominates in the range pH 4-8 and the species L^- is insignificant.

Then to investigate a metal complexation reaction of the type

$$Cu^{2+} + HL = CuL^+ + H^+ \qquad {}^*K_{CuL} \qquad \text{(II)}$$

we may vary either Cu_T^{2+} or H_T^+, and measure the cupric ion activity and the hydrogen ion activity with a cupric ion-selective electrode and a glass electrode. (It is reasonable that the primary copper ligand species might be CuL^+, and not CuL_2 or CuL_3^-, since the algal exudate

ligand concentration cannot be in great excess over the copper concentration, i.e., the greatest possible concentration for exuded complexing agents is of the order 10^{-5} equivalents · $liter^{-1}$ and the lowest possible copper concentration for proper electrode response is of the order 10^{-6}M. Thus the greatest possible ligand to metal ratio is of the order 10 and we might expect the predominance of the species CuL.)

Titration: pCu vs. pH

First we consider the titration in which H_T^+ is varied (Figure 3). If the ligand is in excess ($HL_T > Cu_T^{2+}$ and $[HL] \approx HL_T$), equilibrium in reaction (II) can be written:

$$[Cu^{2+}] \frac{HL_T}{[H^+]} \; {}^*K_{CuL} = [CuL^+] \qquad (1)$$

If virtually no complexation takes place, i.e., the factor

$$\frac{HL_T}{[H^+]} \; {}^*K_{CuL}$$ in Equation 1 is much less than 1,

then the material balance equation for copper is

$$[Cu^{2+}] \simeq Cu_T^{2+} \qquad (2)$$

On the other hand, if a very stable complex is formed, i.e., the factor

Figure 3. Theoretical pCu vs. pH Curve:

$Cu_T = 10^{-6}$ M

$HL_T = 10^{-5}$ M

$Cu^{2+} + HL = CuL^{+} + H^{+}$ $\quad *K = 10^{-1}$

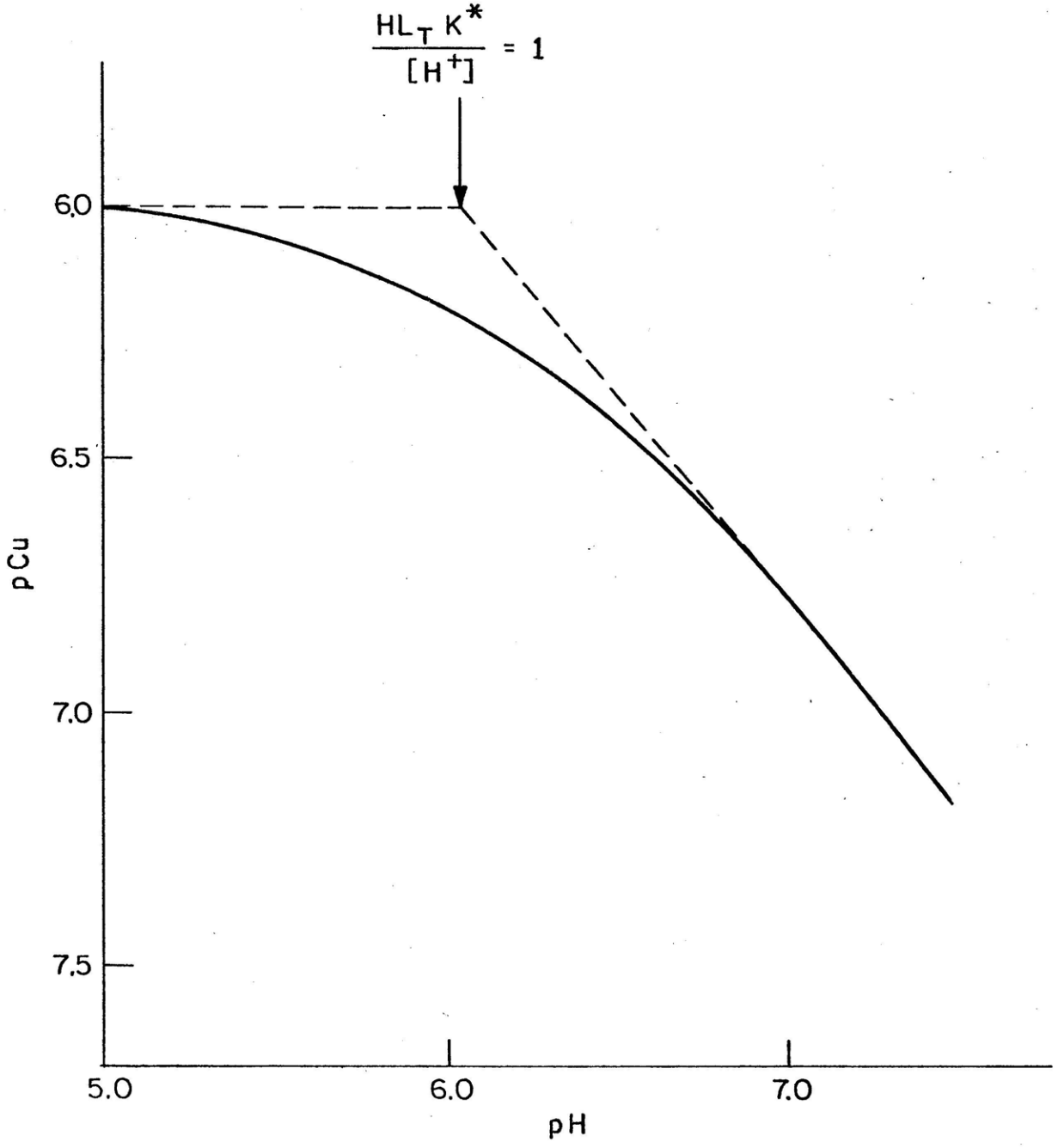


Figure 3

$\frac{HL_T}{[H^+]}\ {}^*K_{CuL}$ in Equation 1 is much greater than 1,

then the material balance equation for copper is

$$[CuL^+] \simeq Cu_T^{2+}$$

and the concentration of free copper is

$$[Cu^{2+}] \simeq \frac{Cu_T^{2+}[H^+]}{HL_T\ {}^*K_{CuL}} \qquad (3)$$

Then in the first case $[Cu^{2+}]$ as measured by the ion selective electrode is constant (Equation 2) while in the second, $[Cu^{2+}]$ is directly proportional to $[H^+]$ (Equation 3), which will vary as H_T^+ is varied. At the point of intersection of these two limiting cases

$$\frac{HL_T}{[H^+]}\ {}^*K_{CuL} = 1$$

This point can be used to evaluate the product $HL_T \cdot {}^*K_{CuL}$ (Figure 3).

Also, had the reaction involved two protons,

$$Cu^{2+} + H_2L = CuL + 2H^+ \qquad (III)$$

it would have been possible to observe the stoichiometric coefficient of $H^+ = 2$ from the pCu vs pH curve. But it is impossible to obtain from

this sort of titration curve HL_T or $^*K_{CuL}$ alone; only their product $HL_T \cdot {}^*K_{CuL}$ can be determined.

Titration: pCu vs Cu_T

In the second type of titration the pH is fixed and the total copper is varied (Figure 4). For this titration in an unbuffered system, the pH is likely to change to such a degree that it would be necessary to correct the copper activity to a reference pH value. This could be done if the pCu vs pH curve had already been determined.

Again we express equilibrim in reaction (I) by the equation

$$[Cu^{2+}] \frac{[HL]}{[H^+]} \quad {}^*K_{CuL} = [CuL^+] \tag{4}$$

Before the titration point the total ligand is in excess of total copper,

$$Cu_T < HL_T, \quad [CuL^+] \simeq Cu_T$$

and free cupric ion concentration is approximated

$$[Cu^{2+}] \simeq \frac{Cu_T}{\frac{HL_T \; {}^*K_{CuL}}{[H^+]}} \tag{5}$$

i.e., free cupric ion concentration is equal to the total copper concentration multiplied by the factor $\frac{[H^+]}{HL_T \; {}^*K_{CuL}}$

Figure 4. Theoretical pCu vs. Cu_T Curves for Several Values of *K

$HL_T = 5 \times 10^{-6}$ M

pH = 6.0

$Cu^{2+} + HL = CuL^{+} + H^{+}$ *K

$$F = \log \frac{{}^*K \cdot HL_T}{[H^+]}$$

A. log *K = 3 F = 3.7

B. log *K = 2 F = 2.7

C. log *K = 1 F = 1.7

D. log *K = 0 F = 0.7

E. log *K = -1 F = -0.3

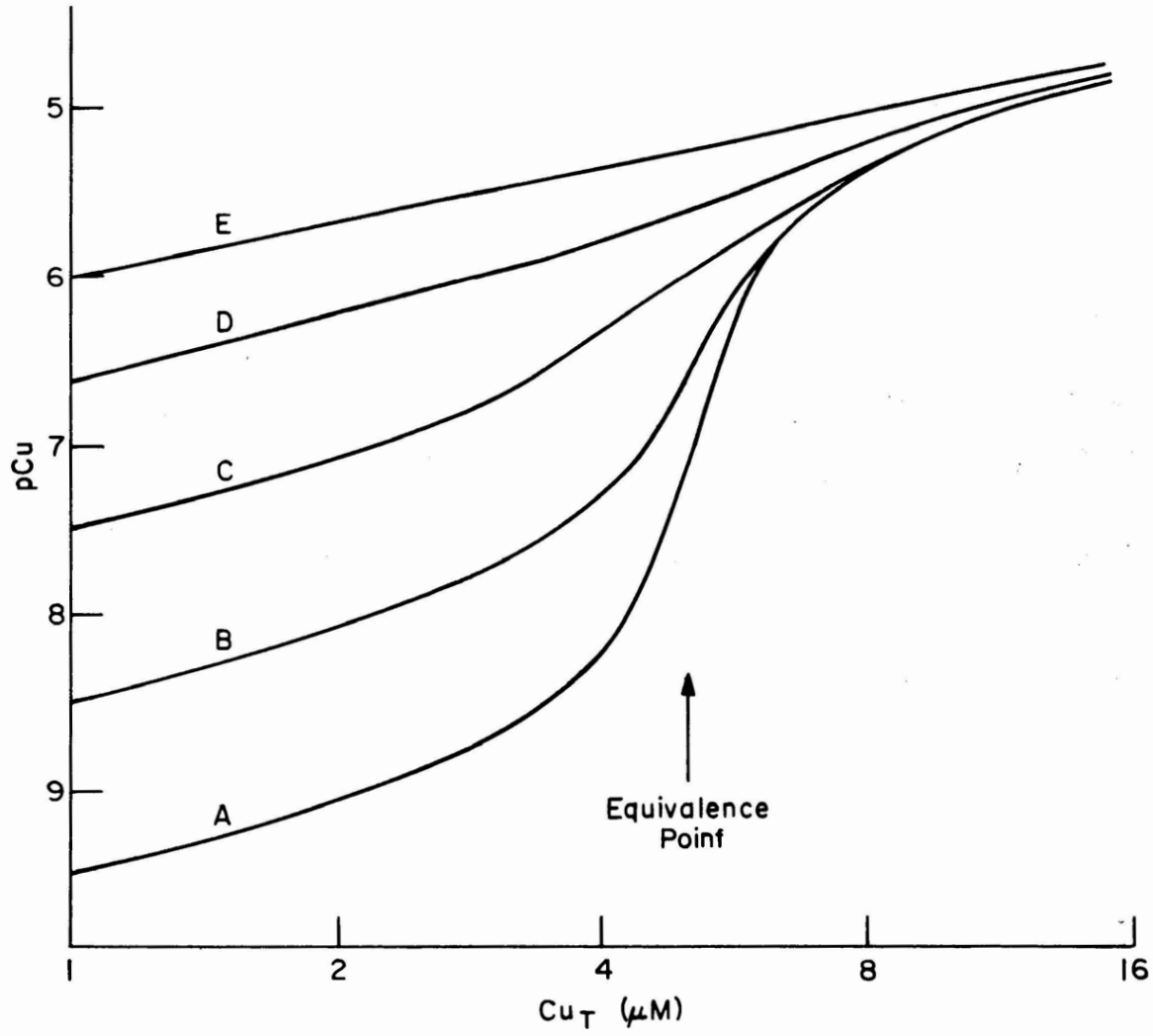


Figure 4

After the titration point, when a large excess of copper has been added,

$$Cu_T^{2+} > HL_T \text{ and } [CuL] \simeq HL_T$$

and the free cupric ion concentration is approximately equal to the total copper concentration

$$[Cu^{2+}] \simeq Cu_T^{2+} \tag{6}$$

At the inflection point in the titration curve $Cu_T = HL_T$; thus HL_T can be determined (Figure 4). And with HL_T known $^*K_{CuL}$ can be computed from the pre-titration point relationship, when total ligand is in excess over total copper;

$$[Cu^{2+}] = \frac{Cu_T}{HL_T \frac{^*K_{CuL}}{[H^+]}} \tag{7}$$

Limits of Titration Methods

The ability to detect a copper complexing agent with either of these titration methods depends on two factors: the amount of ligand present (HL_T), and the stability of the complex (i.e., the product $^*K_{CuL}\ HL_T[H]^{-1}$).

In both the pCu vs pH and the pCu vs Cu_T titration it is necessary that the total ligand concentration be in excess of the initial total copper concentration. It is also necessary that the total copper

concentration be within the domain of linear response of the cupric ion selective electrode. We may take $Cu_T^{2+} = 10^{-6}M$ as being the lower limit of linear response, and $HL_T = 4 \times 10^{-6}M$ as the lower limit for total ligand concentration. A theoretical pCu vs pH titration curve for total ligand concentration of $10^{-5}M$, $5 \times 10^{-6}M$, $10^{-6}M$ shows that the complexation of copper is hardly detectable at $HL_T = 10^{-6}M$ (Figure 5).

The other condition necessary for an observable complexation effect is that the metal ligand complex be stable enough to alter the free copper concentration significantly. In media in which no other inorganic ligands compete for complexation of copper, this requires that the factor $F = {}^{*}K_{CuL}\ HL_T[H^+]^{-1} > 1$. A theoretical pCu vs Cu_T titration curve shows little observable titration effect for values of $F < 1$ (Figure 4). The factor F can be made larger by increasing the pH (Figure 6), but it can be increased only to the point at which the hydrolysis species of Cu^{2+} and the solid $Cu(OH)_2$ limit the observable titration effect. The optimal pH for the titration may be found by comparing the pCu vs pH curves for an exudate containing medium and a similar exudate free medium and picking the pH at which the difference in the pCu values read from the two curves is maximum.

Buffering of Sample Solutions

Thus within the limits discussed, the pCu vs pH and the pCu vs Cu_T^{2+} curves make it possible to characterize the chelating properties of algal exudates. However, the crucial question of pH control in the pCu vs Cu_T^{2+} titration was overlooked. The pH will certainly vary during such a titration, and extrapolation of the pCu measured at a given pH, to pCu

Figure 5. Theoretical pCu vs. pH Curves for Several Values of HL_T

$Cu_T = 10^{-6}$ M

$Cu^{2+} + HL = CuL^{+} + H^{+}$ log*K = -1

$Cu^{2+} + 2OH^{-} = Cu(OH)_2(S)$ log K = 20

A. $HL_T = 10^{-5}$ M

B. $HL_T = 5 \times 10^{-6}$ M

C. $HL_T = 10^{-6}$ M

D. HL_T = 0 M (Formation of $Cu(OH)^{+}$)

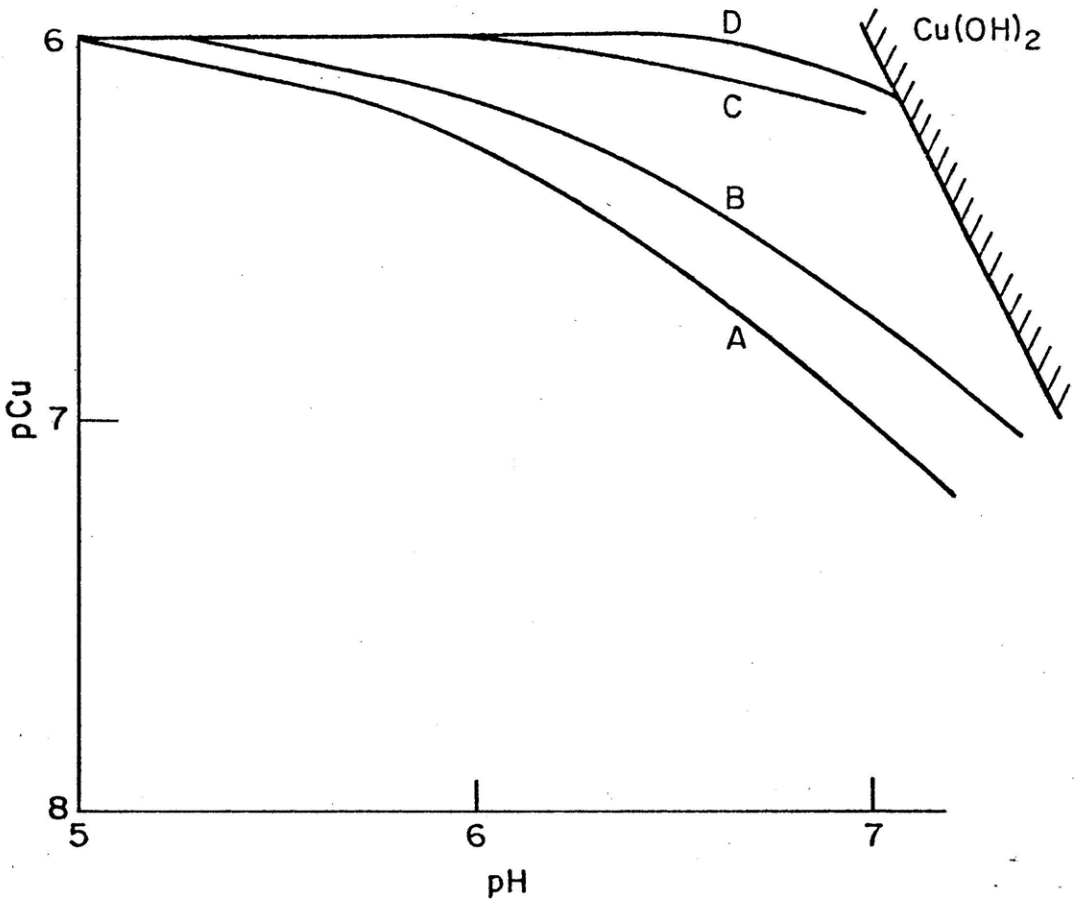


Figure 5

Figure 6. Theoretical pCu vs. Cu_T Curves for Several Values of pH

$HL_T = 5 \times 10^{-6}$ M

$Cu^{2+} + HL = CuL + H^+$ log*K = 1

$Cu^{2+} + 2OH^- = Cu(OH)_2(S)$ logK = 20

A. pH = 8
B. pH = 7
C. pH = 6
D. pH = 5

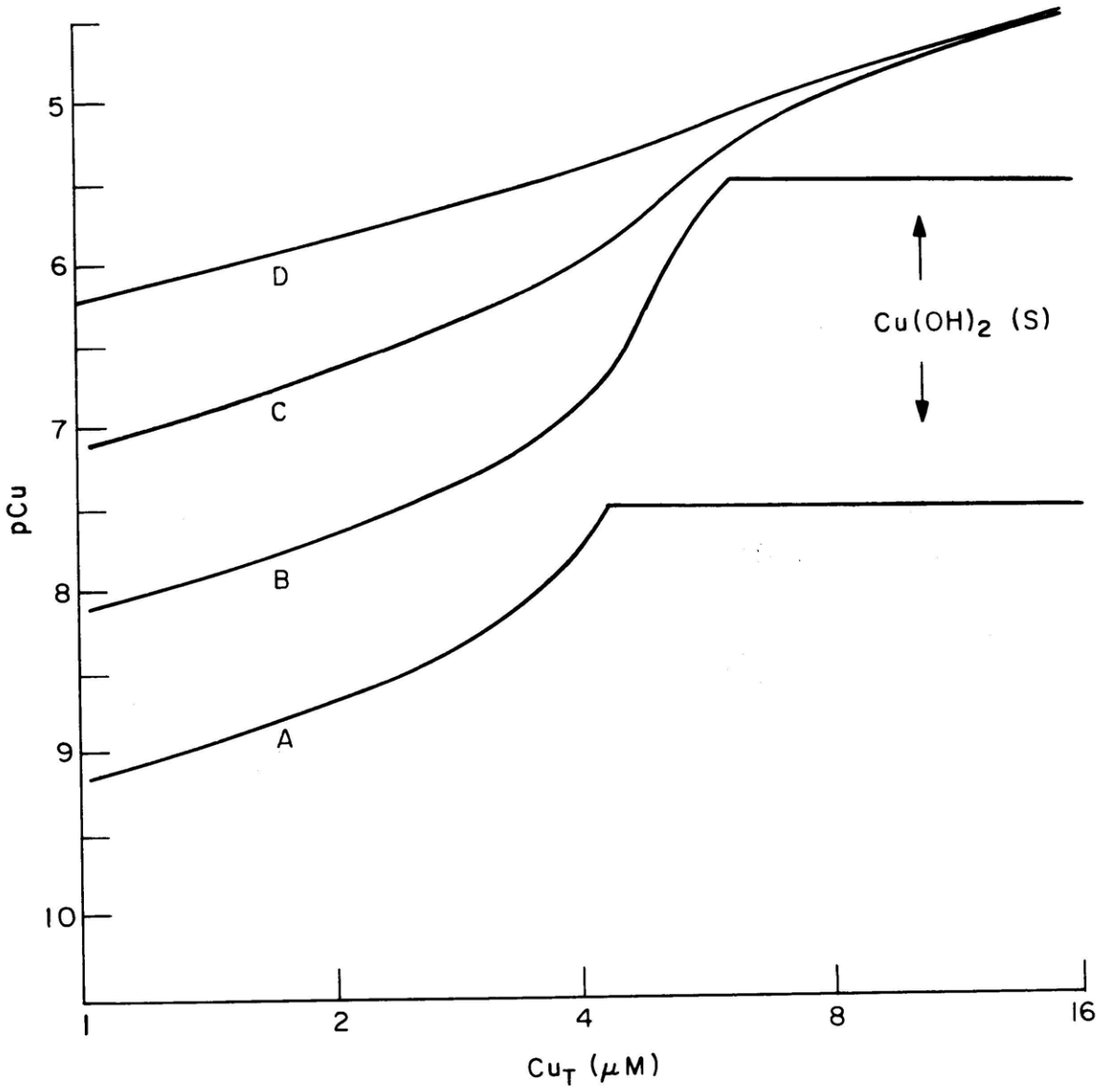


Figure 6

at a reference pH is undesirable. It would be possible to add strong base or a strong acid after each addition of copper to bring the pH back to its initial value, but this pH-stat method is inconvenient because of the long equilibration times that are necessary.

This situation presents the need for a non-complexing pH 6-8 buffer. Among inorganic buffers, phosphate is undesirable because of insoluble copper phosphate species, and carbonate is inconvenient because of the time necessary for equilibration of CO_2 (aq) and CO_2(g). Organic compounds which are good buffers in the range pH 6-8 are generally good complexing agents as well, and cannot be used.

Given the possibilities above, carbonate is probably the most desirable buffer. Carbonate does complex copper to some degree, but carbonate is present in phytoplankton culture media anyway; if a chelator were too weak to be detected in the presence of carbonate, it would also be too weak to be of importance in a natural system. If a carbonate buffer system were used, pH could conveniently be altered by a change in the partial pressure of CO_2(g). The major drawback is that attainment of equilibrium between CO_2(aq) and CO_2(g) is slow. Despite this disadvantage the use of the carbonate buffer will be discussed.

The theoretical pCu vs pH curves for a system in which the pH is changed by variation of the partial pressure of CO_2(g)are shown in Figure 7. The carbonate alkalinity is 10^{-3} equivalents $\cdot$ liter^{-1}, $HL_T = 10^{-5}$M and $*K_{CuL}$ is varied from 1 to .01 as in Figure 4. The carbonate reactions considered in these computations are:

Figure 7. Theoretical pCu vs. pH Curve in Carbonate Medium for Several Values of HL_T

$Cu_T = 10^{-6}$ M

$HCO_{3_T}^- = 10^{-3}$ M

$Cu^{2+} + HL = CuL^+ + H^+$ $\quad \log^*K = -1$

$Cu^{2+} + 2OH^- = Cu(OH)_2(S)$ $\quad \log K = 20$

A. $HL_T = 10^{-5}$ M

B. $HL_T = 5 \times 10^{-6}$ M

C. $HL_T = 0$ (Formation of $CuCO_3(aq)$)

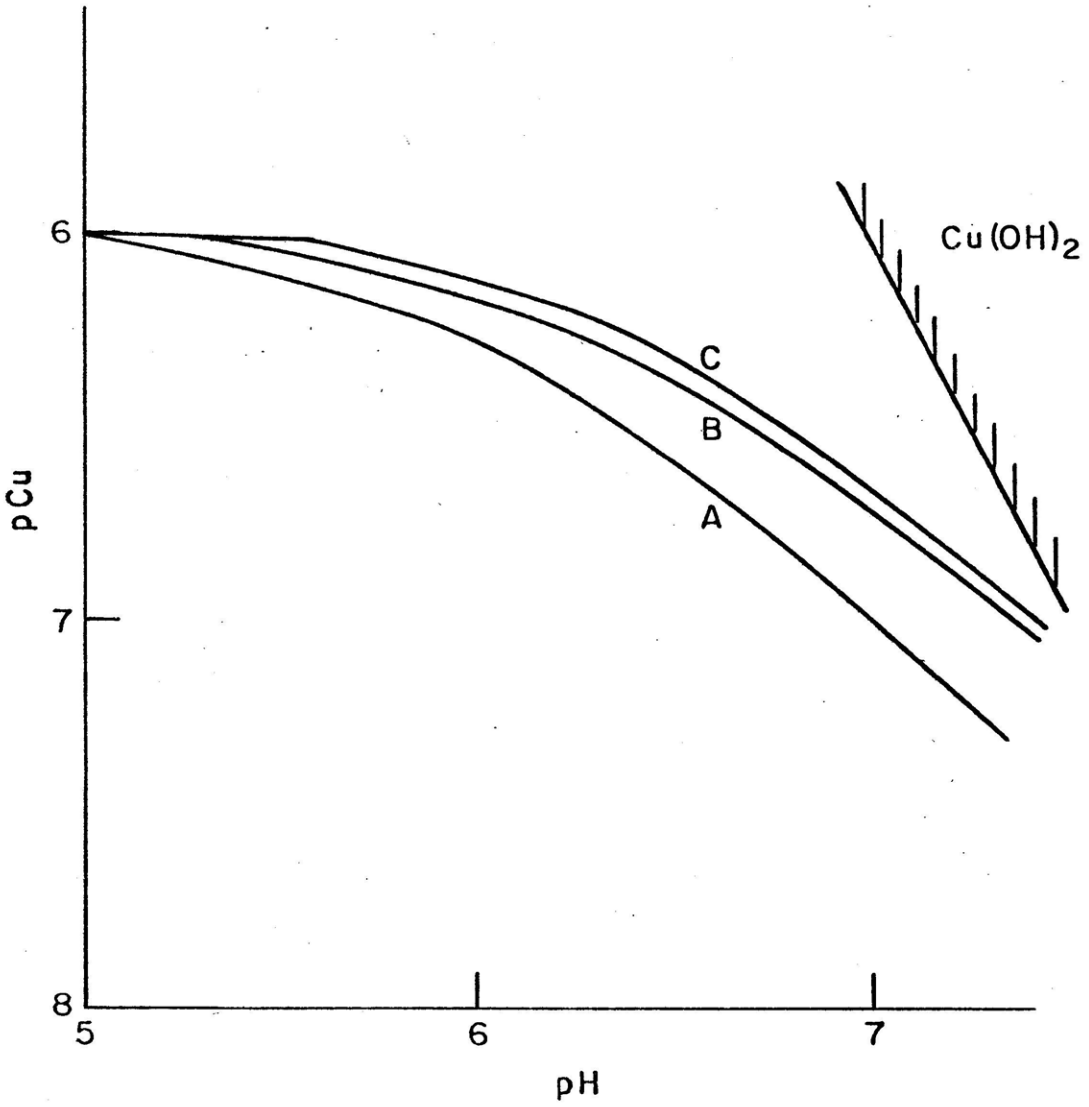


Figure 7

$$H^+ + CO_3^- = HCO_3^-$$

$$2H^+ + CO_3^- = H_2CO_{3*}$$

$$H_2CO_{3*} = CO_2(g)$$

$$Cu^{2+} + CO_3^{2-} = CuCO_3(aq)$$

No other carbonate copper species are formed in significant amounts under the conditions specified.

The limitof detection of organic complexation in the pCu vs pH curves in Figure 7 where carbonate is considered are not significantly different from those in Figure 5, where carbonate is absent. Similarly the pCu vs Cu^{2+}_{TOT} curves for the system containing carbonate (not shown) are similar to Figures 4 and 6 for the carbonate free system. That is to say, the detection limits of the method described for characterizing cupric ion chelating properties of algal exudates are not significantly impaired by the inclusion of milli-equivalent alkalinity in the system.

Summary

Thus there are four methods of titration to be considered: I. pCu vs. pH for fixed Cu^{2+}_T, carbonate free; II. pCu vs Cu_T unbuffered, carbonate free; III. pCu vs. pH for fixed Cu^{2+}_T, where pH is varied by varying P_{CO_2}; IV. pCu vs Cu_T buffered by fixed P_{CO_2}. These titrations will be discussed in the experimental work.

This method for the detection of cupric ion complexing substances is not applicable to seawater samples because of the interference of chloride with the cupric ion selective electrode.

Experimental

Biological. Because of the existing study on the effect of copper speciation in cultures of Thalassiosira pseudonana (6), this estuarine diatom was chosen for the initial study of copper complexing algal exudates.

The basic culture medium (Table III) for T. pseudonana contained a relatively high concentration of EDTA. Since a high level EDTA would mask the effect of a natural complexing agent, it was necessary to reduce the EDTA concentration in the culture medium and to modify the culture medium in some other way to satisfy the organism's requirement for a complexing agent in the culture medium. Three procedures were attempted: reduction of the EDTA concentration below the limit of detection of the cupric ion selective electrode complexometric titration method; addition of freshly prepared ferric chloride solution at the time of innoculation (this could increase iron availability or remove toxic metals by adsorption and coprecipitation); and dilution of an EDTA containing continuous culture medium with an EDTA free culture medium - then presumably natural complexing agents would take over the function of the artificial chelators.

Of these three procedures, the addition of iron to artificial chelator free medium yielded the most productive cultures over an extended period of time. Cultures in a no artificial chelator medium or the low artificial chelator media expired after successive transfers from the original chelator containing medium. The continuous culture method produced viable cultures for several weeks, but was abandoned because

TABLE III

COMPOSITION OF CULTURE MEDIUM*

SALTS	
$CaCl_2$	2.5×10^{-4} M
$MgSO_4$	1.5×10^{-4} M
$NaHCO_3$	1.5×10^{-4} M
NUTRIENTS	
K_2HPO_4	5.0×10^{-5} M
$NaNO_3$	1.0×10^{-3} M
Na_2SiO_3	1.0×10^{-5} M
TRACE METALS	
Cu^{2+}	1.0×10^{-9} M
Zn^{2+}	4.0×10^{-9} M
Co^{2+}	2.5×10^{-9} M
MoO_4^{2-}	1.5×10^{-9} M
Mn^{2+}	2.3×10^{-8} M
Fe^{3+}	4.1×10^{-7} M
EDTA	5.0×10^{-6} M
VITAMINS	
B-12	5.5×10^{-7} g·l^{-1}

*Salts and Nutrients from W.C. medium (16) and Trace Metals from FRAQUIL medium (17).

of the work necessary to maintain the continuous cultures relative to the work necessary for the iron addition method.

Batch cultures of 100 ml were grown in 250 ml Erlenmyer Flasks at 17°C under constant illumination of 3000-5000 lux. Cell counts were made with a Model Z Coulter Counter; culture samples were diluted with 0.05M KCl, the lowest electrolyte concentration possible for satisfactory Coulter Counter operation. There was no microscopically visible effect on T. Pseudonana when transferred from the culture medium (of ionic strength $\sim$0.005 $eq \cdot l^{-1}$) to the electrolyte (0.05 M KCl).

Prior to potentiometric analysis, all biological samples were filtered successively through 0.8 μm and 0.22 μm Millipore filters at a pressure differential 120 mm of mercury.

Apparatus: Titrations were performed on a stirred 50 ml sample in a 100 ml cell thermostated at 25°C $\pm$ 0.1°C, shielded from light to minimize photosensitivity of the electrodes, and isolated from the atmosphere. CO_2 and N_2 were metered through Matheson No. 601 and 801 rotometers, mixed in proportions from 0 to 100 percent, passed through a granular charcoal column and a water filled sparger tube. According to the experiment, the gases were bubbled through the sample solution or passed over the top of the sample solution.

The potential of an Orion Model 91-01-00 Glass Electrode, Orion Model 94-29 or Radiometer Model F-3000 Cupric ion-selective were measured against an Orion 90-02 double junction reference electrode with an Orion Model 801A or 701 Digital pH millivolt meter. The glass electrode was calibrated with Fisher pH 7.00 and pH 4.00 buffers. Samples were

U.V. irradiated with an Ultra Violet Products Co., San Gabriel, Calif., UV lamp.

Procedure: While the exact composition of a particular blank or sample depends on the titration procedure, there are six general categories of samples:

1) A solution of one millimolar KNO_3 referred to as a "salt blank";
2) A solution of the algal culture medium, sterile, never innoculated, referred to as "medium blank",
3) A solution of one millimolar KNO_3 to which glycine had been added, referred to as "glycine sample".
4) A solution of the algal culture medium, innoculated with phytoplankton and handled with sterile techniques. Two or three days after the culture had reached stationary phase ($\sim 2 \times 10^6$ cells/ml) the cells were removed from the solution by gentle filtration as described; the solution is referred to as "culture samples"
5) A solution of the algal culture medium in which phytoplankton had reached stationary phase, but subsequently not maintained under sterile conditions. The solution was maintained for approximately 1 month and a large population of bacteria was allowed to develop. This solution is referred to as "contaminated sample".
6) A solution of one millimolar KNO_3 to which cell lysate had been added. The cells from five 100 ml stationary phase cultures ($\sim 5 \times 10^8$ cells) were ground in a ground glass tissue grinder, and resuspended in 100 ml of millimolar KNO_3; this solution was filtered to yield the "lysate sample".

Results

The similarity of the titrations of the salt blank and the medium blank (Figure 8) confirm that there are no detectable interactions of cupric ion with other constituents of the culture medium (e.g. phosphate, vitamins).

The titration of the glycine sample with copper confirms that it is possible to detect complexation of copper by small amounts of glycine, and sets the limit of detection for copper complexation by glycine at approximately 5×10^{-6}M for glycine (Figure 9). In this titration the pH was held constant at pH 6.35 ± 0.08 by addition of strong acid or strong base.

The titration curves for culture samples of T. Pseudonana (Figures 10 and 11) do not exclude the possibility that a complexing agent is present at the detection limit, but the result is by no means conclusive. The depression of the copper activity in the pCu vs pH curve (Figure 10) at low values of Cu_T can be attributed to the change in pH over the course of the titration. The system was initially buffered by 1% CO_2 and one millimolar alkalinity; but as copper was added, time was not allowed for re-equilibration of the 1% CO_2 gas with the solution. The change in cupric ion activity was presumably caused by pH dependent carbonate complexation of copper. The pCu vs pH curve for a similar culture sample (Figure 11) shows no evidence of cupric ion complexation by an organic complexing agent. More extensive work by Swallow (18) using both titration methods indicated that there are no naturally produced copper complexing agents above the detection limit in cultures

Figure 8. pCu vs pH Curve: Salt Blank and Medium Blank

+ Salt Blank:

$HCO_{3_T}^- = 10^{-3}M$

$Cu_T = 10^{-6}M$

° Medium Blank

$HCO_{3_T}^- = 10^{-3}M$

$Cu_T = 10^{-6}M$

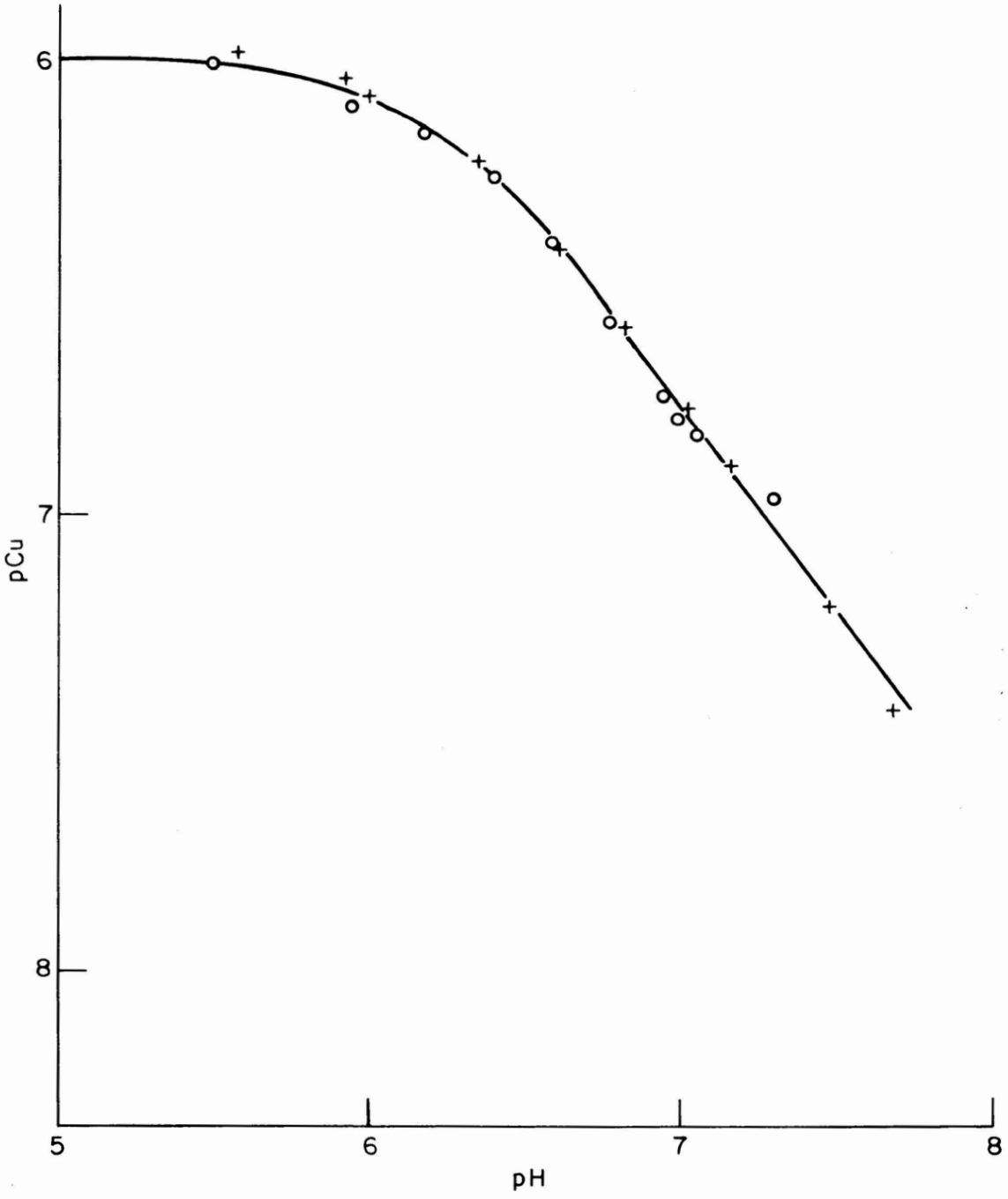


Figure 8

Figure 9. pCu vs Cu_T Curve for Glycine

pH = 6.35 $\pm$ 0.8

$GLY_T = 5 \times 10^{-6}$ M

Figure 9

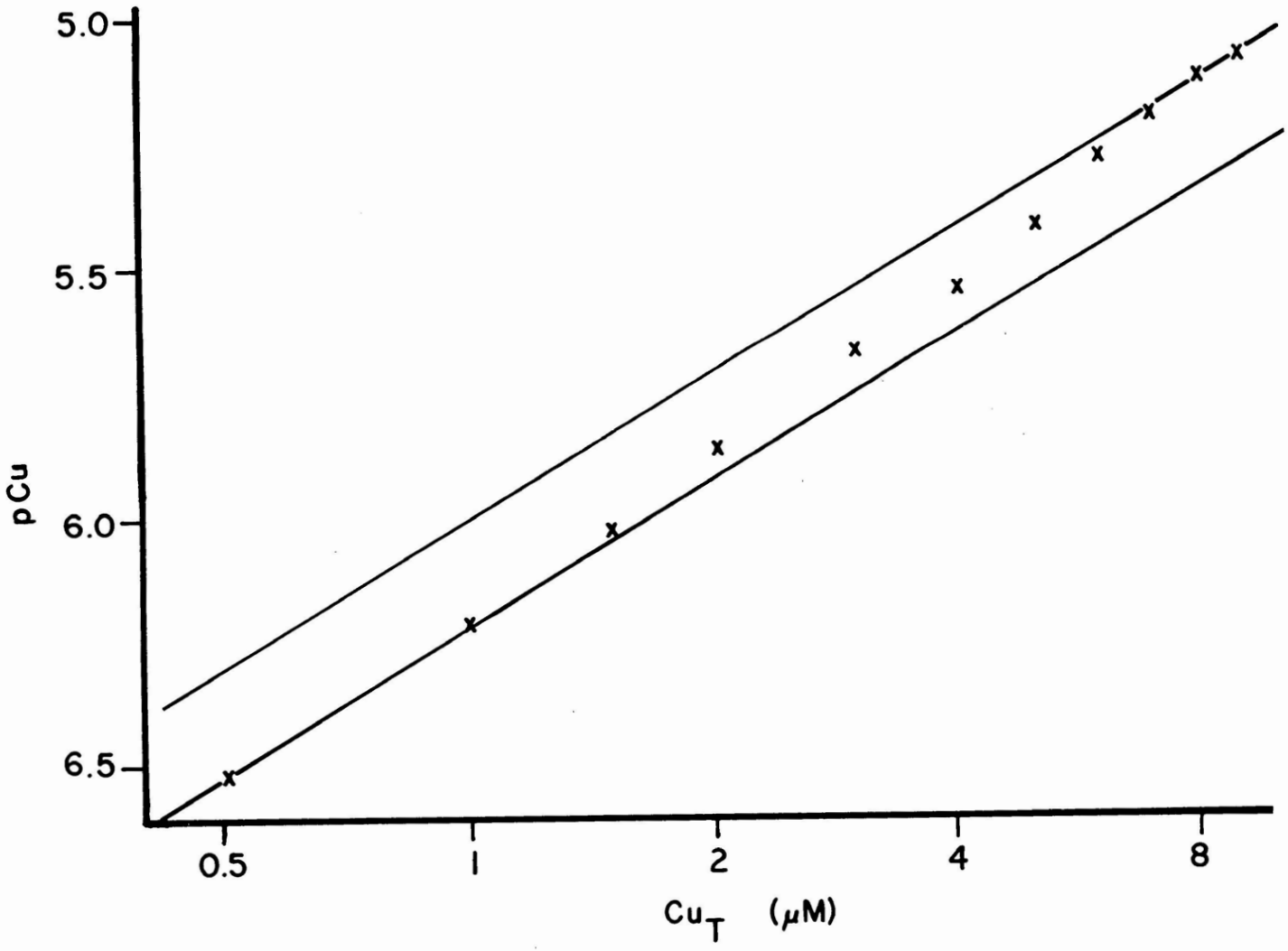


Figure 10. pCu vs Cu_T Curve for Culture Sample

° Culture Sample 9J pH 7.0-7.3

• Culture Sample 11J pH 7.0-6.7

✕ Culture Sample 12J pH 7.2-6.9

Figure 10

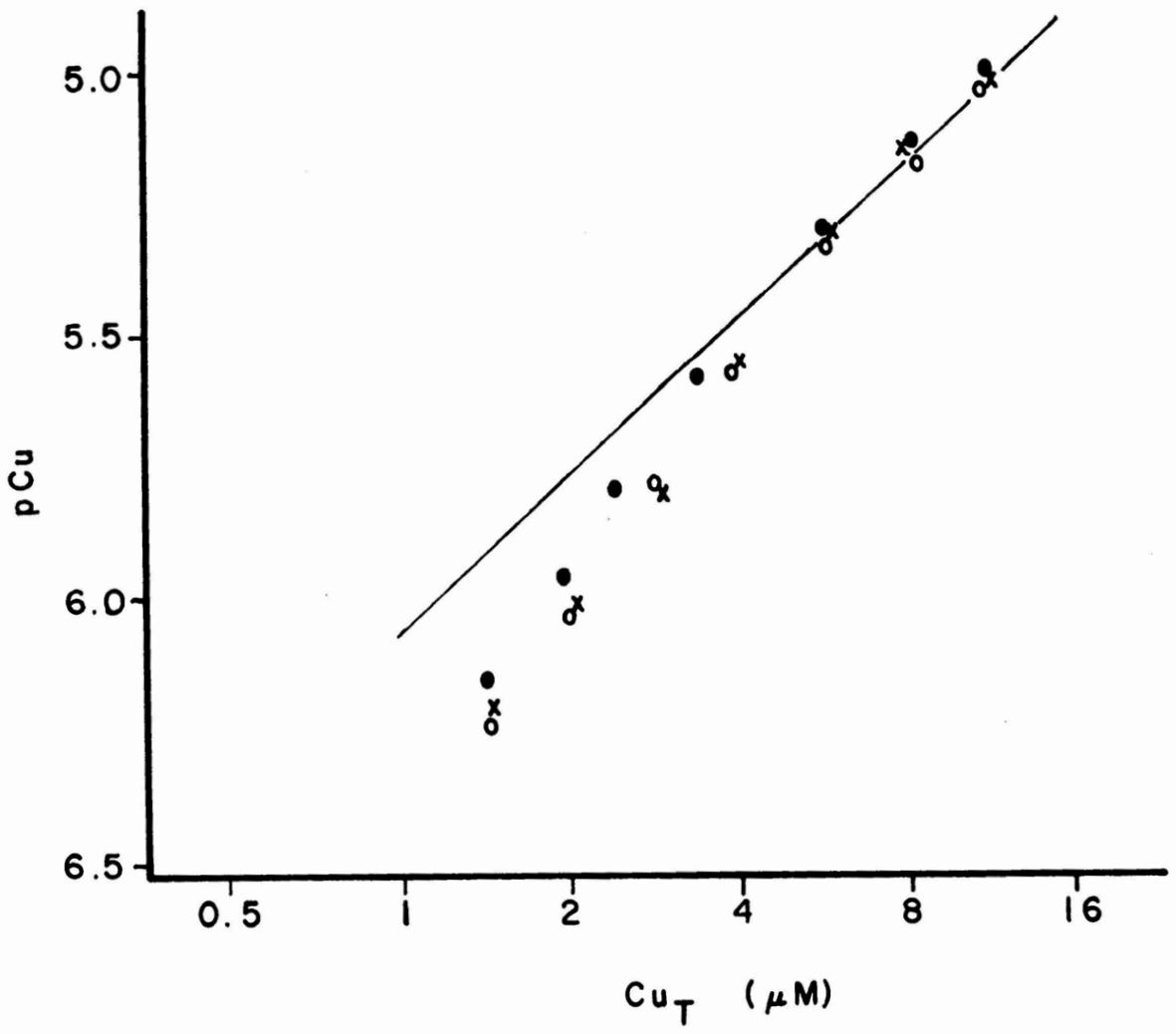
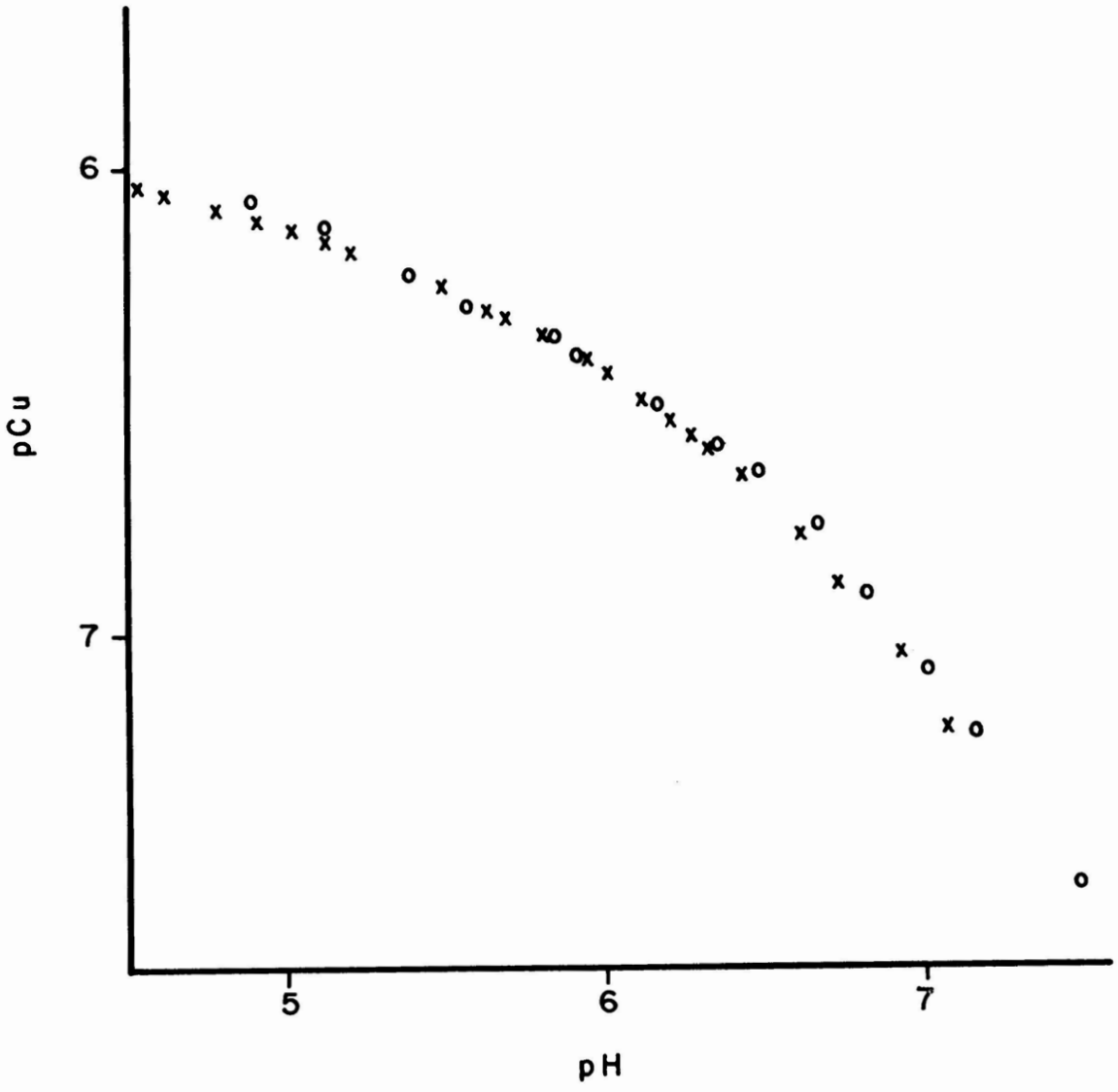


Figure 11. pCu vs pH Curve for Culture Sample and Medium Blank

x Culture Sample $Cu_T = 10^{-6}M$

° Medium Blank $Cu_T = 10^{-6}M$

Figure II



of T. Pseudonana grown under similar conditions.

A contaminated sample did show evidence of a copper complexing material. A value of $*K_{CuL} = 10^{0.0}$ and $HL_T = 10^{-5}$ was found (Figure 12). Another contaminated sample was titrated and then subjected to ultraviolet radiation for a period of two hours in order to destroy the organic chelator (Figure 13). The sample was retitrated and a reduced value of $*K_{CuL}HL_T$ was found, indicating that some of the chelator had been destroyed. Further ultraviolet treatment of the sample indicated further reduction in the total ligand concentration. Medium blanks were titrated during the intervals when the sample was being irradiated. The several titration curves for the medium blanks coincided with each other, indicating that the electrodes were stable during the period of the experiment.

The lysate sample was titrated at pH 6.88 $\pm$ 0.05 in a 1% $CO_2/10^{-3}$ Alkalinity buffer and yielded a very pronounced titration effect (Figure 14).

The question which motivated this research was whether phytoplankton could produce substances in sufficient quantity and of sufficient complexing strength to alter the trace metal speciation in the culture medium. For the case of the diatom T. Pseudonana and the trace metal copper, the answer is no, that the speciation of copper is not altered at the level of 10^{-6} moles·liter^{-1}. The fact that copper complexing compounds were not found in the culture sample of T. pseudonana, but were found in the contaminated sample and in the lysate is not too surprising in view of the high concentration of organics in the latter cases. Further interpretation of these results at this point is not warranted.

Figure 12. pCu vs Cu_T Curve for Contaminated Sample

° Contaminated Sample

x Computed for

$HL_T = 10^{-5}$ M

$Cu^{2+} + HL = CuL^{+} + H^{+}$ $\log^*K = 0.0$

Figure 12

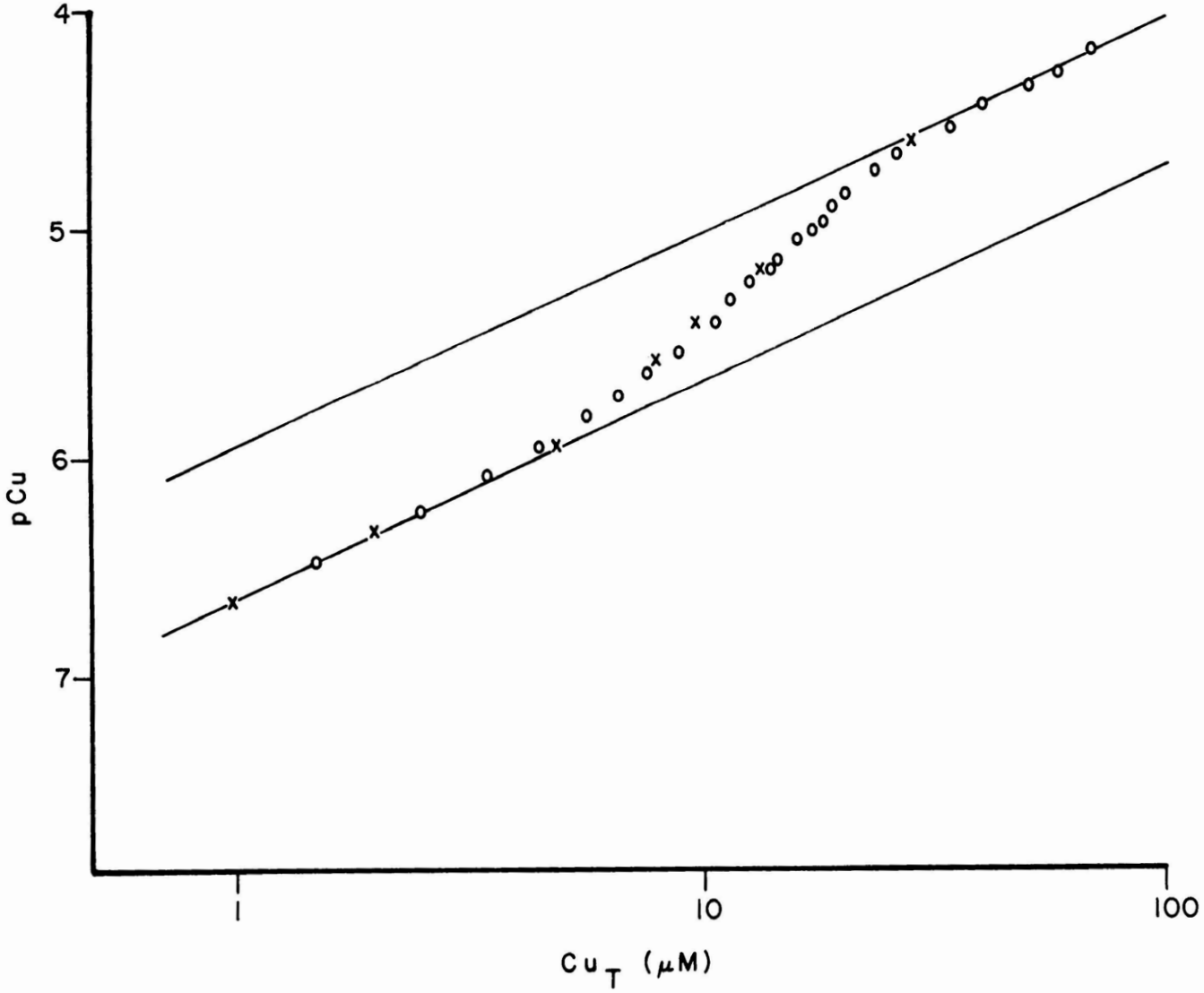


Figure 13. pCu vs pH Curve for Contaminated Sample

• contaminated sample

x contaminated sample + 1 hr. U.V.

o contaminated sample + 2 hr. U.V.

- medium blank

Figure 13

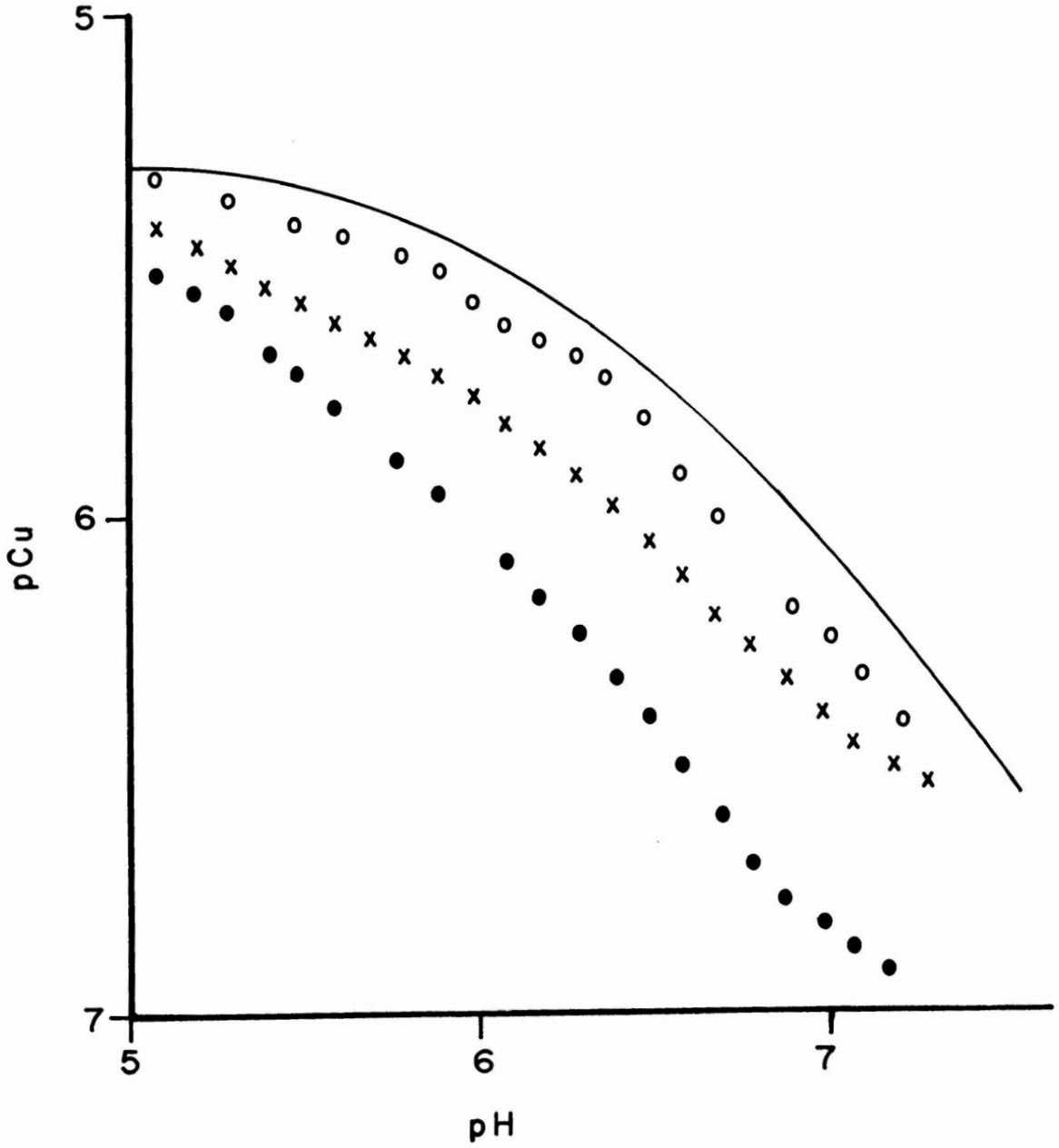
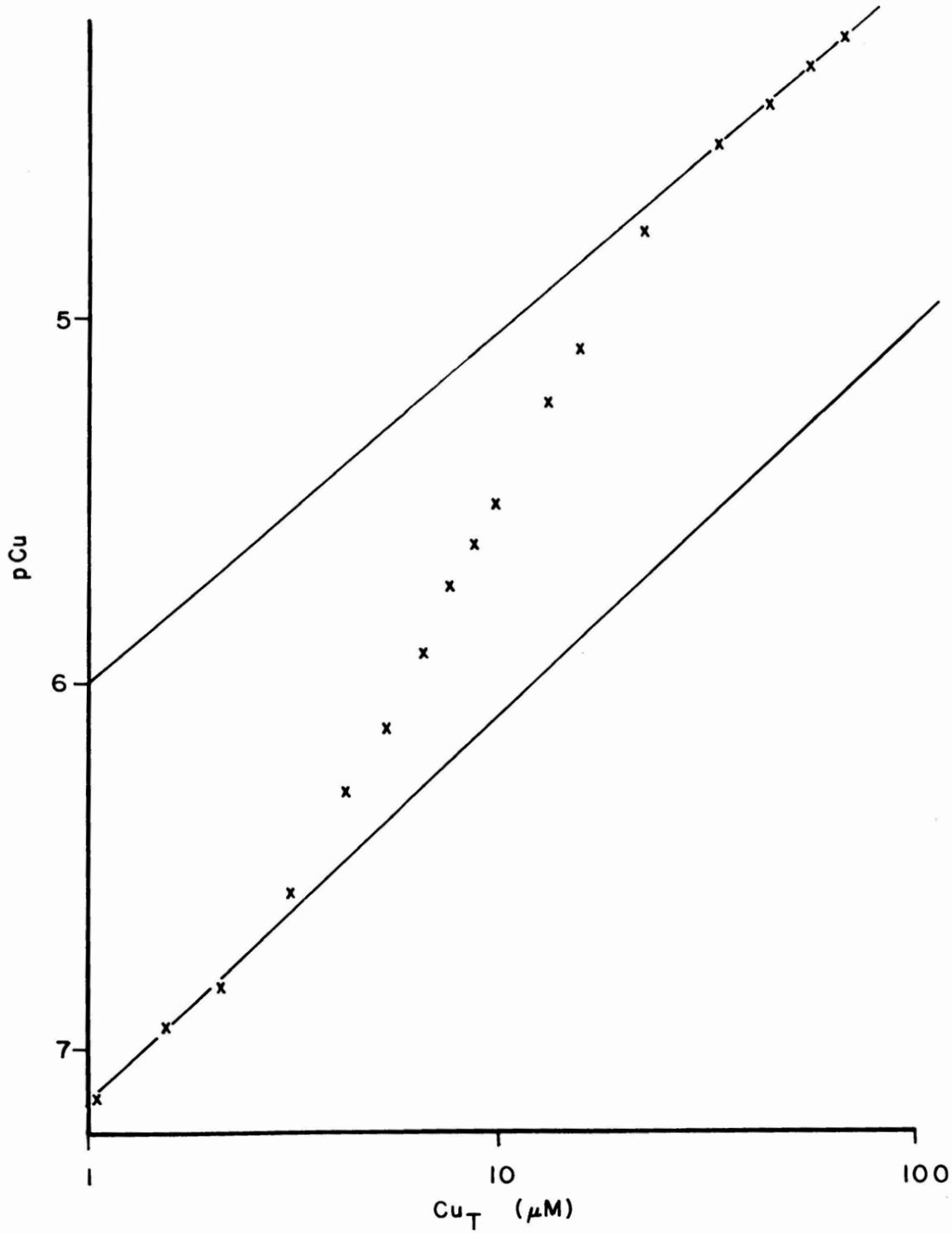


Figure 14. pCu vs Cu_T Curve for Lysate Sample

pH = 6.88

Figure 14



Discussion

The experimental results presented in this paper demonstrate that sound methods have been established for further investigation of the complexing characteristics of algal exudates; but the result for one metal and one species of algae should not be interpreted to apply in general. Since we do not present exhaustive evidence for or against extensive trace metal complexation by algal exudates--involving response to toxic concentrations of metals, samples taken from different phases of algal growth, and cultures of different species of algae--the discussion that follows may seem somewhat speculative, but it does address important questions which should motivate further research.

What is the interpretation of this work in terms of the cycles in Figure 1? One may consider two types of complexing agents produced by phytoplankton: non-specific complexing agents, by-products of algal metabolism, which are produced in relatively large quantities; and specific chelators, eg. ferrichrome, which form highly stable chelates, with specific transition metals, chelates so stable that they persist even at great dilutions or at extreme pH. Because of the intricacy of the specific chelating molecules, it is presumed that they appear in less abundance than the non-specific chelators.

If the non-specific chelators were not detected at concentrations of the order 10^{-6} M, the possibility that they could significantly alter the trace metal speciation can substantially be ruled out. As is evident from Table II, the formation of complexes formed from trace metals and non-specific complexing agents depends on a relatively high concentration of complexing agent (concentration greater than micromolar).

Submicromolar concentrations of complexing agents simply will not bind a significant fraction of trace metals.

Specific chelators will on the other hand bind trace metals quantitatively at any concentration; the question becomes how much of a specific chelator can algal cells produce? If one considers that the concentration of chelatable trace metals is of the order 1-100 nanomolar, (as is typical of natural waters) one could imagine that it would be difficult for algal cells to produce enough of a specific chelator to raise the bulk concentration to such a level in view of the amount of energy and material which would have to go into that specific function.

Then to return to the original question posed in the introduction, are there interactions of the type (1) phytoplankton produce chelator to reduce toxic availability of trace metals, or (2) phytoplankton produce chelator to render essential trace metals available: it is tempting to interpret the results of this work to suggest that if copper complexation for an algal culture was not detected at the micromolar level, that it is unlikely that phytoplankton exudates will significantly alter the overall speciation of trace metals (and render a metal which is toxic to the cell, non-toxic), but that it is still possible that a fraction of some trace metal which is essential to the phytoplankton could be chelated by a specific chelator and rendered available to the cell.

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