INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON EQUILIBRIUM DATA*

SPECIFIC PROBLEMS IN THE MEASUREMENT AND INTREPRETATION OF COMPLEXATION PHENOMENA IN SEAWATER

(Technical Report)

Prepared for publication by

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Abstract. Characterization of metal complexation phenomena in seawater is a formidable challenge. Adequate characterization of inorganic complexation schemes is currently limited by a paucity of well-defined hydrolysis constant data for chemical species which are important in the weakly alkaline conditions of normal seawater. Other important problems include the neglect of equilibria involving ligands and metals which form highly insoluble solids, and a poor understanding of the influence of pressure on stability constant behavior. Characterization of metal complexation by organic ligands in seawater is especially problematic. There is presently a large gulf between laboratory investigations of metal complexation by structurally characterized organic ligands, and investigations of complexation by natural organics whose molecular architecture is essentially unknown. In view of the wide variety of techniques used to investigate the complexation behavior of natural marine organics, and the diverse and occasionally conflicting attributes reported for metal complexation by natural organics in seawater, it would be most valuable if some level of effort were devoted to analysis of shared samples. In view of the multi-faceted strengths of the diverse procedures available for characterization of natural organo-metal interactions, conjugate analysis using a variety of techniques on identical samples seems essential for bringing a reasonable level of constraint to interpretation of complexation observations.

1. Complexation Phenomena in Seawater

The fertility of the oceans and the chemical composition of seawater are intricately interwoven (85MH, 91BD). Many elements in seawater exhibit widely varying solution concentrations due to their participation in complex, biologically-mediated cycles: uptake in and on biogenic particles in the surface ocean, downward transport via biological debris, regeneration (return to solution) at depth, and return to the surface ocean via oceanic mixing. While the distribution of many elements in the oceans is thus strongly influenced by ocean biology, the growth characteristics of organisms in the surface ocean, in turn, bear the strong imprint of ocean chemistry. The role of ocean chemistry, and, in particular solution chemistry, in the oceans' biological productivity is especially evident in relationships which exist between marine planktonic organisms and a variety of bioactive cations found in seawater at nanomolar and lower concentrations. For a wide variety of metals including Co^{II}, Mn^{II}, Ni^{II}, Cu^{II}, Zn^{II}, and Fe^{III} it is generally observed that bioavailability is intimately related to each metal's distribution among various chemical forms (speciation) in solution (90HM, 91S, 93HM, 94S, 94PB). For these nutrient metals, and others, partitioning of total metal among free hydrated cations, M^{n+}, and forms, ML_i, complexed with solution ligands (L_i) exerts a major influence on the oceans' biogeochemical cycles.

While the acknowledged importance of chemical speciation in marine biogeochemistry (85MH, 91BD, 91S) has made chemical speciation an area of intense interest, the experimental difficulties typically encountered in identifying and investigating species of suspected importance in seawater has made some aspects of speciation assessments very controversial. In the discussion which follows, specific problems and limitations in the description of complexation phenomena in seawater will be addressed. This account will be divided into inorganic and organic complexation assessments. In keeping with the general practice of saying most about that which is least understood, the greater part of the following discussion will be devoted to the very important phenomenon of metal association with marine organic matter.

2. Inorganic Complexation

Inorganic complexation assessments directly involving natural seawater as the experimental medium (85BM, 89KB) are uncommon. Instead, assessments of the extent of metal complexation by inorganic ligands in seawater are generally constructed through measurements obtained in simple synthetic solutions. Through the use of stability constants ($_x\beta_n = [MX_n][M]^{-1}[X]^{-n}$) derived from measurements in simple solutions at the ionic strength ($\mu = 0.7$ mol kg^{-1}) of typical seawater, the total dissolved concentration ($[M_T]$) of each metal in seawater can be numerically partitioned into concentrations of free hydrated cations ($[M]$) and inorganic complex species ($[MX_n]$) with equations of the form

$$[M_T]/[M] = 1 + \sum_x (_x\beta_1[X] + _x\beta_2[X]^2 + _x\beta_3[X]^3 + ...) \tag{1}$$

where $[X]$ denotes inorganic ligand (anion) concentrations including OH^-, HCO_3^-, CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, F^-, Cl^-, and Br^-. The seemingly straightforward summations in equation 1 foster a false sense of simplicity. Since the anion concentrations in the equation are generally expressed as free concentrations, these concentrations ($[X]$) must themselves be calculated in a manner which accounts for interactions (62GT, 79P, 82MSc, 91P, 92M) with the major cations (Na^+, Mg^{2+}, Ca^{2+}, K^+) in seawater. Furthermore, through equilibria involving H^+ (e.g. $H_2O \rightleftharpoons H^+ + OH^-$, $HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$) important ligands such as OH^- and CO_3^{2-} have sea water concentrations that range over approximately an order of magnitude in response to near-surface variations in ocean pH. Thus, the use of equation 1 in marine metal speciation calculations requires a detailed understanding of many equilibria which are not explicitly represented in the equation. The systematics of metal complexation by inorganic ligands in seawater at 25 °C have been summarized by Turner et al. (81TW), and this work was extended to include the influence of temperature on complexation by Byrne et al. (88BK). Further updating of these models is in progress (93L). Complexation assessments based upon data obtained in simple electrolyte solutions indicate that the dominant inorganic ligands in seawater are Cl^-, CO_3^{2-} and OH^-. Metals having speciation schemes dominated by Cl^- include Ag^I, Cd^{II}, Hg^{II}, Pt^{II} and Pd^{II} (81TW, 88BK). Metals with inorganic complexation schemes dominated by CO_3^{2-} include Cu^{II}, U^{VI}, Y^{III} and trivalent lanthanides and actinides (88BK). A large number of trivalent and tetravalent metals, including Al^{III}, Fe^{III} and Th^{IV}, have seawater complexation schemes dominated by hydrolyzed species ($M(OH)_n$) (81TW).

Problems occur in calculating $[M_T]/[M]$ (eq. 1) and partitioning total metal among various complex species when formation constant data for species of possible significance are not available or are inappropriate to natural seawater conditions. Such problems arise from a number of sources. Among the difficulties encountered in the use of equation 1 to describe the equilibrium behavior of cations in seawater, first and foremost is the paucity of well-defined hydrolysis constant data appropriate within the normal pH range ($7.4 \leq pH \leq 8.3$) of natural seawater at 25 °C. The inorganic complexation of many important trivalent and tetravalent metals in seawater (e.g. Fe^{III}, Al^{III}, Cr^{III} and Th^{IV}) is dominated by equilibria among the forms $M(OH)_2$, $M(OH)_3$ and $M(OH)_4$. Hydrolysis constants for these species are of poor accuracy relative to constants for simple species, MOH, which are of only marginal importance for most strongly hydrolyzed cations in seawater. Difficulties in the determination of hydrolysis constants for species important in neutral to mildly alkaline solutions are predominantly attributable (76BM) to the problems imposed by hydrolytic precipitation and polynuclear complex formation at the elevated metal concentrations prerequisite to laboratory equilibrium investigations. Polynuclear species formation greatly increases the complexity of data interpretation and modeling. Problems imposed by hydrolytic precipitation can be particularly severe since it has generally proved difficult to distinguish between dissolved and colloidal metal at total metal molalities lower than 10^{-7} mol kg^{-1}. Thus, even in the case of Fe^{III}, a metal of critical importance in marine biogeochemistry, the relative significance of the dissolved forms $Fe(OH)_2^+$ and $Fe(OH)_3^\circ$, and even dissolved vs. colloidal metal in seawater, is controversial (91BD, 95WP).

A second important problem encountered in assessments of inorganic speciation in seawater is the neglect of potentially important equilibria. Very few experimental data exist for metal

complexation involving ligands such as PO_4^{3-}, S^{2-} and $H_2SiO_4^{2-}$. The nearly complete absence of data for complexation of trace metals by such ligands is attributable to formidable analytical problems. Phosphate forms very insoluble salts with trivalent metals and, additionally, phosphate complexation must be studied at relatively high pH ($6.5 \leq pH \leq 8.5$) where hydrolytic equilibria may render data interpretation difficult. The latter problem is also likely to be quite important in assessments of complexation by $H_2SiO_4^{2-}$ since appreciable concentrations of this ligand are only generated at high pH. Sulfide, found even in oxic ocean environments (88CK, 89LT), potentially forms strong complexes (87CO, 88E, 88D) with soft metal ions (68A, 68P, 75A) such as Cu^I, Ag^I, Cd^{II}, Pd^{II} and Hg^{II}. However, investigation of the complexation of such metals by S^{2-} and HS^- is challenging because even at very low S^{2-} concentrations these metals may be either precipitated or reduced. The work of Zhong and Millero (94ZM) demonstrates that problems with sulfide precipitation can be mitigated by working in media which evidence strong chloride complexation (eg. seawater), and subsequently accounting for competitive equilibria among metal bisulfide and chloride complexes.

The neglect of potentially important equilibria in seawater includes a problem inherent in the formulation of eq. 1. Equation 1 does not explicitly provide for the possibility of mixed ligand complexation. Statistical considerations (60WD, 68DJ, 82BK) indicate that species such as $MCl_n(OH)_m$, $M(OH)_n(CO_3)_m$ and $MCl_n(HS)_m$ etc. are of occasional significance in seawater. Recent work in natural seawater indicates that $PdCl_3OH^{2-}$ is a significant solution species (89KB), and evidence from experiments in synthetic solutions indicates that $Th(OH)_3CO_3^-$ is an important species in seawater (94OB). Due to the neglect of mixed ligand complexation and the neglect of species for which no stability constant data exist, equation 1 can generally be expected to underestimate the extent of a metal's complexation in solution.

A third substantial problem encountered in representations of oceanic metal complexation based on equation 1 is the nearly complete absence of complexation data appropriate to the high pressures encountered in the deep ocean. Recent work (93LB) with Gd in synthetic seawater at pressures up to 600 bar indicates that, due to the influence of pressure on lanthanide carbonate complexation, the ratio $[M_T]/[M]$ decreases by an order of magnitude between the surface ocean and 5,000 m. Other than this characterization for a lanthanide, there are apparently no investigations which would allow assessment of the influence of pressure on the formation of complex species which are expected to dominate a trace metal's speciation in seawater.

Some of the most vexing shortcomings of current inorganic metal speciation models for seawater are not amenable to simple remedies. However, it is reasonable to expect that some progress in the areas identified above is possible. For environmentally important metals such as Cu^{II} and Fe^{III}, which have strong absorption bands in the ultraviolet, significant insights might be gained through the use of rapid mixing techniques in conjunction with rapid scan spectral analysis. The formation rates of monomeric complexes and, in particular, the formation rates of hydrolyzed monomers are rapid compared to rates of dimerization, polymerization, coagulation and precipitation. Through direct observations of a metal's uv-visible (UV/VIS) absorbance characteristics and/or observations involving UV/VIS observations of complexation by competitive ligands (83O, 88TB), resolution of complex mixtures of chemical species might be based on spectrophotometric observations and differences in the formation and dissociation rates between monomeric species (which are dominant in seawater) and species which form at the high metal concentrations requisite for laboratory analysis. If such potential remedies do not provide a means of circumventing, as a minimum, the solubility limitations confronting experimentalists, the path to an adequate understanding of metal speciation in sea water may be long indeed.

3. Organic Complexation

Over the past decade intensive investigation of marine organo-metal complexation phenomena has considerably altered our perspectives on the potential significance of such phenomena. An investigation of metal complexation by Mantoura et al. (78MD) concluded that ~10% of the total

copper in open ocean sea water was organically complexed. In a 1981 review of organo-metal interactions in natural waters, Mantoura (81M) indicated that, on average, approximately 20% of the total copper in seawater was present in the form of organic complexes. Note that organo-metal should not be identified with organo metallic which involves a direct metal-carbon bond. Due to procedural/analytical advances, it is now commonly reported (84V, 87SH, 87MZ, 90CB, 94CV) that 99% or more of the total copper in seawater exists as organic complexes. While the significance of metal ion-organic ligand interactions in seawater was once questioned (70SM, 77P) it is now generally acknowledged that the aqueous chemistries of a variety of metals are dominated or very strongly influenced by their interactions with marine organic matter.

A wide variety of techniques has been used to investigate complexation of trace metals by organics in seawater. Many, if not most, techniques used prior to 1980 did not provide modern measures of organic ligand complexation systematics. The earliest assessments of metal complexation by organic substances were used to provide estimates of total metal binding capacity and are now perceived (87MD) as being of limited interest in aquatic chemistry. Measurements of total binding capacity were eventually superseded by metal fractionation assessments in which total metal was partitioned into a variety of organic, inorganic, and particulate classes (76BF, 78BG, 83HQ). In recognition of the importance of free metal ion concentrations and activities in regulating biogeochemical processes, nearly all modern assessments of organo-metal interactions seek to define parameters which will allow calculation of free metal ion concentration, [M].

3.1 Complexation Systematics

It is generally appreciated that metal coordination with natural organics involves a wide variety of coordinative site types and metal-ligand affinities (90BA). The nature and distribution of sites is, of course, not known *a priori* but is deduced, typically, from observations of the changing extent of metal-ligand association as the total metal concentration is increased incrementally. Various mathematical devices have been used to interpret titration data. Fully satisfactory data analysis has proved problematic because a highly heterogeneous population of site types can give rise to titration data sufficiently similar in character that it is difficult to distinguish the efficacy of physically sound models from models which are undoubtedly simplistic. Turner et al. (86TV) compared, on statistical grounds, five models applicable to analysis of copper complexation by fulvic acid: a) the multi-site model in which metal and organic matter is assumed to be associated at a small number of site-types, each site having an associated stability constant (K_i) and total site concentration (L_i); b) the multi-dentate model (77BG) in which the ligand (L) of a single site-type can complex metal as either ML or ML_2 type complexes; c) the electrostatic model (77WK, 84CS) which involves a single binding site with a stability constant functionally dependent on the extent of metal occupation at the binding site; d) the normal distribution model (83PL) which assumes a continuous (smooth) variation in metal-site binding affinities that are normally distributed as a function of $\log K$; and e) the affinity spectrum model (80TM) which assumes a continuous (smooth) variation in binding site affinities with an unknown distribution. While Turner et al. (86TV) noted that the absence of appropriate structural information on fulvic acid precluded comparisons of the physical and chemical significance of the various models, based on practical considerations (conceptual and mathematical simplicity) and statistical considerations, the multi-site model was the most satisfactory model for describing their Pb and Cu titration data. Models appropriate to the interactions of metal ions with polydisperse organics in natural systems have undergone considerable development (90BA, 92BC, 95MR) since the introduction (73R, 75H) of multi-site models. However, quantitative use of complexation models in marine systems has, to date, been essentially confined to relatively simple multi-site models which make no explicit distinction between the fundamental thermodynamic character of inorganic and organic complexation. Due in large part to conceptual and mathematical simplicity and parallelism with existing inorganic speciation models, current characterizations of organo-metal interactions in seawater are generally provided in the form of models with strong and weak binding site constants (K_1' and K_2') and strong and weak binding site concentrations ([L_1] and [L_2]). With conditional stability constants written

in the form

$$K_i^{'} = [ML_i][M]^{-1}[L_i]^{-1} \qquad (2)$$

equation (1) is easily extended to encompass both inorganic and organic complexation

$$[M_T]/[M] = 1 + \sum_x ({}_x\beta_1[X] + {}_x\beta_2[X]^2 + {}_x\beta_3[X]^3 + ...) + K_1^{'}[L_1] + K_2^{'}[L_2] + ... \qquad (3)$$

It should be noted that the ligand concentrations, $[L_1]$ and $[L_2]$, in equation 3 denote the concentrations of free ligands plus the concentrations of ligands present as labile complexes with the major cations in seawater (Na^+, Mg^{2+}, Ca^{2+}, etc.). Since the concentrations of Ca^{2+} and Mg^{2+} are quite constant in seawater, free ligands and ligands complexed with major cations are present, at equilibrium, in nearly constant relative proportions.

3.2   Complexation Kinetics

Depictions of the equilibrium characteristics of metal ion interactions with organics in seawater are complicated by the role of coordination kinetics in complexation observations. It is generally expected (89HM) that the kinetics of coordination of trace metals with dissolved organics in seawater is slow. Reported equilibration times for investigations involving EDTA additions to seawater are on the order of 24 hours (82HD, 91SH). Investigations of ^{64}Cu equilibration with dissolved organic matter indicated that 70% of the exchange sites in an estuarine environment underwent relatively rapid exchange while 20% of the sites did not exchange in a 24-hour time period (82MH). Approximately 20% of the total zinc in an Atlantic Ocean seawater sample did not equilibrate with added ionic zinc in a two hour time period (91MK). Equilibration of lead with organic ligands in seawater appears significantly slower than is the case for copper (90CC). It was shown by Raspor et al. (80RN) that the slow kinetics of trace metal coordination by EDTA in seawater may be attributable, in large part, to the role of Ca^{2+} in organic complexation phenomena. Organic chelates are coordinated with Ca^{2+} (80RN), Mg^{2+} and trace metals (89HM) in seawater. Consequently, it is expected that trace metal complexation in seawater should generally be written as a replacement reaction.

$$M_1 + M_2L \rightleftharpoons M_1L + M_2 \qquad (4)$$

where M_1 is a trace metal and M_2 may be Ca^{2+}, Mg^{2+}, H^+, or another trace metal. Since it is generally observed that metal coordination with organics is strongly influenced by pH (84AL, 90ST, 91MK, 91SH), special consideration should be given to the role of H^+ in equation 4. Reaction rates for the above replacement reaction are generally slow when M_2 is an alkaline earth metal ion. It is likely (89HM) that the approach to equilibrium in such reactions will be particularly slow when M_2 is a transition metal ion. These observations demonstrate the importance of acknowledging, in terms of both kinetics (89HM) and equilibria (88H), that seawater is a multimetal medium. The very slow kinetics of Ni^{II} reactions with humate (87LL) provides a good example. Over a time scale of hours, some transition metal complexes, such as those of Ni^{II}, may be effectively inert (87LL). The analysis of Hering and Morel (89HM) indicated that, in the course of the metal titrations used for conditional stability constant determinations, added metal will first occupy higher lability sites, and any strong binding sites with exceptionally low lability might not participate in the equilibration process within the time frame of experimental observations. In this case, the approach to an equilibrium occupation of high affinity, low lability sites may be so slow that some strong binding sites might be undetectable. Measurements of metal complexation involving metal additions would therefore underestimate the concentration of strong binding sites in sea water. Consistent with this expectation, the complexation analysis of Campos and Van den Berg (94CV) detected a factor of two increase in the molality of strong Cu^{II} binding sites over a period of six hours.

It should be noted that, while many observations support the conclusion that metal-organic complexation in seawater is a slow process, there are also observations (89B) which indicate that complexation processes in some cases are relatively rapid. Coale and Bruland's (88CB)

investigation of Cu^{II} complexation in the northeast Pacific exhibited substantial constancy in titration results when copper additions were allowed to equilibrate with natural organic ligands over periods as short as 5.5 minutes and as long as 24 hours. Bruland's observations of Zn^{II} complexation (89B) and Cd^{II} complexation (92B) evidenced no significant changes in titration characteristics using equilibration periods between 10 minutes and 24 hours. Such observations argue for the existence of some types of organic binding sites which are highly specific for a single metal.

3.3 Analytical Methods

In order to explore the multi-faceted equilibrium and kinetic characteristics of metal ion-organic ligand associations in seawater, a wide variety of techniques has been developed which are capable of examining organo-metal interactions at very low concentrations. Some of these techniques are summarized below. The following descriptions exclude techniques, such as ion selective electrode measurements, which do not perform well at total metal molalities on the order of one nanomol kg^{-1} and lower.

Differential Pulse Anodic Stripping Voltammetry (DPASV): The use of voltammetric procedures, including DPASV, for examination of metal complexation in seawater has been reviewed by Nürnberg (82N, 84N) and van den Berg (88V). DPASV was one of the first and most widely-used techniques for modern analyses of metal-organic complexation in seawater. DPASV is used with either a hanging mercury drop electrode or a rotating glassy carbon electrode coated with a thin film of mercury. Copper and/or other dissolved metals are electrochemically reduced and amalgamated with the mercury electrode, followed by measurements of the oxidative current as copper is electrochemically stripped from the amalgam. Measurements of peak current are followed as a function of total copper concentration. Deconvolution of this titration data provides both conditional stability constants and binding site concentrations. Measurements and data interpretation are sensitive to the kinetics of dissociation of the various forms of complexed copper (87VL), hydrodynamical conditions (e.g. diffusion layer thickness) at the electrode/solution interface (78D), and deposition potential (92V). While the sensitivity of ASV to the kinetic characteristics of organo-metal complexes is generally perceived as a constraint on the interpretation of ASV titrations, Muller and Kester (91MK) have pointed out that this sensitivity also can provide for a richer description of complexation phenomena than can be gained solely through equilibrium analyses. Through the use of variable rotation rates of glassy carbon electrodes the sensitivity of the method to organo-metallic complexes with different dissociation kinetics can be systematically varied. DPASV has been used extensively to study organic complexation of Cu, Pb, Cd and Zn in marine systems.

Fixed Potential Amperometry: This direct measurement technique (83WM, 87HS) involves amperometric observations of the reduction of Cu^{II} to Cu^{I} at relatively positive voltages compared to DPASV analysis. Electrode response is calibrated using an organic-free electrolyte solution. Fixed potential amperometric titrations are dependent (as in the case of DPASV) on the method's discrimination between reducible and non-reducible organically complexed copper. For measurements of copper complexation, fixed potential amperometry offers some advantages relative to DPASV because the relatively positive voltage employed obviates or at least minimizes the reduction and dissociation of the most labile organo-metallic complexes. Reduction of organo-metal complexes results in underestimation of conditional stability constants and is perceived as a potentially important limitation (92V) in both fixed potential amperometry and DPASV analysis. Fixed potential amperometry is not suitable for all reducible metals since it is dependent on the stabilization of Cu^{I} in solution by extensive chloride complexation. Essentially, all previous work employing fixed potential amperometry to investigate organic complexation in seawater has solely involved complexation of Cu^{II}.

Bioassay Determinations: Estimation of formation constants through bioassay measurements is based on the toxicity of free Cu^{II} ions to aquatic microorganisms (76SG, 78AM, 79SG). Bioassay

measurements, which can assume a variety of forms, are exemplified by the investigation of Hering et al. (87HS) in which the relationship between Cu^{2+} activities and incorporation rates of ^3H-labeled amino acids was calibrated using nitrilotriacetic acid to generate Cu^{II} buffers. It is notable that a comparison of this technique (87HL) and fixed potential amperometry provided fundamentally consistent results. Bioassay techniques are potentially useful for examination of a variety of trace metals, including Cu, Pb, Cd, Zn, Co, Cr and Ni, provided the absence of hydrophobic metal complexes can be assumed. However, since trace metal toxicity can involve competitive interactions among a number of metals, experimental conditions must be carefully defined (91S).

Competitive Ligand Equilibration: The use of competitive ligand equilibrations in assessments of metal complexation by natural organics has assumed a variety of forms. A procedural feature common to all methods is the addition to solution of chelating agents or ion exchangers. In contrast to direct methods, this feature provides the investigator with the flexibility to systematically vary the overall extent of metal complexation in solution and to examine, thereby, the complexation characteristics of different fractions of the natural ligand pool. With increasing concentration of added ligand, a metal is progressively withdrawn from association with natural ligands. That which remains bound with natural ligands is increasingly associated with the most strongly binding chelation sites. It should generally be appreciated that competitive ligand equilibrations involve examination of a more complex system than is the case for direct methods. One particularly relevant concern here could be the formation of mixed complexes involving both natural and added ligands. This problem is potentially most significant when added ligands are bidentate. The significance of mixed complexes is likely to be minimal when a metal's coordinative capacity is saturated by a single ligand. One important form of competitive ligand equilibration is that which is used in conjunction with cathodic stripping voltammetry (CSV) (81PV, 87VN, 90VN, 90DB, 92DV). CSV is particularly versatile and has been used to investigate Al, Cd, Cr, Co, Cu, Fe, Ni, Pb, Pt, Sb, Se, Sn, Ti, V and Zn in seawater (93DB). Metal complexes of the added ligand are adsorbed, typically, on a hanging mercury drop electrode held at a fixed potential followed by observation of the peak current during a subsequent reductive stripping. The method is extremely sensitive (sub-nanomolar to picomolar metal concentrations) and is applicable to a wide variety of metals (93DB). In another form of competitive ligand equilibration, copper associations with organics in a variety of environments have been examined through liquid-liquid phase distribution analysis (87MZ, 90MZ, 94MB). Acetylacetone is added to seawater and the diacetylacetonato copper(II) complex is partitioned between seawater and toluene. Copper in the toluene is then back extracted with dilute nitric acid and copper concentrations are measured with graphite furnace atomic absorption spectrometry (94MB). The competitive ligand equilibrations of Sunda and Huntsman (87SH) involved liquid-solid phase distribution analysis in which copper was partitioned between adsorbed natural organic and inorganic species on C_{18} (Sep Pak) cartridges (82MH) and a variety of dissolved forms of copper including EDTA, natural organics, and inorganic species. The method is calibrated using metal ion buffers containing excess concentrations of copper and EDTA. Sorption characteristics are examined as a function of added copper. It is notable in this case that, in contrast to the procedure used in CSV analysis, the identity of the sorbed organic species is unknown. The competitive equilibrium techniques of Sunda and Huntsman (91SH) involved the use of EDTA in seawater in conjunction with chemiluminescence observations of Cu^{II}-phenanthroline chelates in the presence of H_2O_2 at alkaline pH. The method is based on the lack of chemiluminescent detectability of CuEDTA chelates. One of the earliest procedures to successfully employ competitive ligand equilibrations involved observations of the distribution of copper between MnO_2 and dissolved natural organic and inorganic ligands (82V, 84VB). The total copper concentrations in these titrations were determined by anodic stripping voltammetry. This ion exchange procedure is generally applicable to a variety of metals.

A selection of stability constants obtained using the various procedures outlined above is shown in Table 3.3. Conditional constants (K_2') for the weakly complexing class of ligands generally range between $10^{8.3}$ and $10^{10.6}$ kg mol^{-1}. Conditional constants (K_1') for the strongly complexing class of ligands are typically on the order of 10^{12} kg mol^{-1} or larger. The largest conditional constants

TABLE 3.3. Representative Stability Constants, $K_i'(Cu)$, and Ligand Concentrations, $[L_i]$, for Oceanic, Coastal and Estuarine Seawater Samples (Ligand molalities are given in nanomol kg^{-1}). $K_1'/[L_1]$ and $K_2'/[L_2]$ refer to strong-site and weak-site stability constants and ligand concentrations.

lg K_1'	$[L_1]$	lg K_2'	$[L_2]$	Technique[a]	Sample Location	Source
>12	5	9.8	15	Bioassay	SW Gulf of Mexico	83SF
10.3	31	9.4	87	Ion exchange MnO$_2$	Irish Sea	84VB
12.6	11	10.6	33	CLE/Catechol/CSV	South Atlantic	84V
12.3	3.8	9.2	75	CLE/EDTA/C$_{18}$	Coastal Peru	87SH
12.0	5.1	10.5	110	CLE/acetylacetone Solvent Exchange	SE Coastal USA	87MZ
11.7	20-50	9.1	50-70	Fixed Potential Amperometry	NE Coastal USA	87HS
12.9	1.8	9.5-10.6	7.6	DPASV	N. Pacific	88CB
13.0	2	10.0	6	DPASV	N. Pacific	90CB
11.4-12.7	13-200	-	-	CLE/Catechol/CSV	Severn Estuary, UK	90AG
13.2	3.3	10.0	26	CLE/EDTA/ Chemiluminescence	NC Shelf USA	91SH
12.4	16.2	-	-	CLE/Tropolone/CSV	North Sea	92DV
15.6	7.8	-	-	CLE/Catechol/CSV	North Sea	92DV
10.5	44	-	-	CLE/Tropolone/CSV	Sargasso Sea	92VD
12.4	3.5	-	-	CLE/Tropolone/CSV	Sargasso Sea	92VD
15.9	2.0	-	-	CLE/Oxine/CSV	Sargasso Sea	92VD
13.5	13	9.0-9.6	20-30	CLE/8-hydroxy-quinoline/CSV	San Francisco Bay	94DL
>14.2	0.8	-	-	CLE/acetylacetone Solvent Exchange	Sargasso Sea	94MB

[a]CLE: Competitive Ligand Equilibration

TABLE 3.4. Representative Stability Constants, $K_i'(M)$, for the Complexation of Ni, Zn, Co, Cd and Pb by Natural Organic Ligands (Ligand molalities are given in nanomol kg^{-1}).

M	lg K_1'	$[L_1]$	Technique[a]	Sample Location	Source
Ni	17.3	3.7	CLE/DMG/CSV	English Channel	87VN
Ni	17.8	5.3	CLE/DMG/CSV	Menai Strait UK	87VN
Ni	17.7-18.7	0.3-6.4	CLE/DMG/CSV	Liverpool Bay UK	89NV
Ni	>17	17-28	CLE/DMG/CSV	San Francisco Bay USA	94DL
Zn	11.0	1.2	DPASV	North Pacific	89B
Zn	11.2	1.76	DPASV	North Pacific	90DB
Zn	10.3	1.60	CLE/PDC/CSV	North Pacific	90DB
Co	15.6-17.5	0.3-1.1	CLE/DMG/CSV	Scheldt Estuary UK	90ZV
Cd	12.0	0.1	DPASV	North Pacific	92B
Pb	11.3	0.2-0.5	DPASV	North Pacific	90CC

[a]CLE: Competitive Ligand Equilibration; DPASV: Differential Pulse Anodic Stripping Voltammetry; CSV: Cathodic Stripping Voltammetry; DMG: Dimethylglyoxime; PDC: Pyrrolidin-1-yldithioformate.

shown in Table 3.3 are those obtained in a subset of the competitive ligand equilibrations wherein a substantial fraction of the total metal is associated with the added ligand. Through the use of varying concentrations and types of added ligands (90VN, 92DV, 92VD) different fractions of the natural ligand pool are brought into exchange equilibrium with the metal complexes of well-characterized synthetic ligands. In this manner a variety of natural ligand site-types is systematically examined. A given set of experimental conditions can be conceptualized as constituting a detection window (90VN) within which only a fraction of the total metal exchange sites is observed. Differences in detection windows can be created through the systematic use of competitive ligands, and also implicitly through an investigator's choice of analytical technique. The detection window analyses of van den Berg et al. (90VN) and van den Berg and Donat (92VD) produced (Table 3.3) conditional Cu^{II}-organic stability constant estimates (K_1') for the most strongly complexing class of sites in a single sea water sample which range over approximately five orders of magnitude. In instances where no competitive ligands are added in the course of an analysis, variations in K_1' and K_2' may be attributed, in part, to detection window differences inherent in different analytical techniques. The profound variations in K_1' in competitive ligand equilibrations is consistent with the polyfunctional nature of natural complexing material (80GU, 90BA) and will undoubtedly fuel ongoing consideration (88CS, 86TV, 90BA) of complexation model correctness and practicality.

3.4   Complexation Specificities

Although the strong-site conditional stability constants, $K_1'(Cu)$, obtained for copper through competitive ligand equilibrations (Table 3.3) have ranged to values as large as $10^{15.7}$, conditional stability constants obtained for Ni (Table 3.4) are approximately one to two orders of magnitude larger. In the context of a variety of stability constant comparisons in which $K_1'(Cu)$ is very commonly larger than $K_1'(Ni)$ (75SM, 76SM, 89SM, 74MS, 77MS, 82MS), comparative observations of $K_1'(Ni)$ and $K_1'(Cu)$ in seawater appear anomalous. To the extent that such observations ($K_1'(Ni)/K_1'(Cu) > 1$) are not attributable in some manner to disparate detection windows between Cu and Ni competitive ligand equilibrations, comparative $K_1'(Cu)$ and $K_1'(Ni)$ results strongly argue for the existence of ligands which are highly specific for complexation of Ni. Similar evidence is seen in Table 3.4 for the existence of cobalt-specific organic ligands. Evidence for copper- and zinc-specific ligands is provided by the studies of Coale and Bruland (88CB) and Bruland (89B). Coale and Bruland (88CB) observed that the addition of Zn, Pb, and Fe in molar excess of Cu did not measurably influence their DPASV analyses of organo-copper(II) complexation. While comparative Zn^{II} and Cu^{II} complexation constants (Tables 3.3 and 3.4) do not appear unusual, the Zn complexation analyses of Bruland (89B) indicated that even when Cu concentration levels were elevated to the point that total copper approximately equaled the total concentration of Zn binding ligands, zinc titrations were not measurably affected. The Pb^{II} complexation analyses of Capodaglio et al. (90CC) evidenced constancy in titration characteristics even in the presence of a large excess (20 nmol kg^{-1}) of added copper. These observations, in conjunction with the short equilibration times required in the Cu^{II} analyses of Coale and Bruland (88CB) and in the Zn^{II} and Cd^{II} analyses of Bruland (89B, 92B), are strongly suggestive of remarkable metal-ligand specificities for some organic ligands in seawater.

3.5   Complexation at Surfaces

The foregoing descriptions of complexation phenomena in sea water have been directed toward characterization of metal interactions in solution. However, it has become increasingly evident that investigations of metal complexation by natural organic ligands in seawater do not, in many cases, distinguish metals in the dissolved phase from metals associated with colloids and even bacteria (88HA). Solution and surface complexation phenomena in seawater are, to some degree, indistinct. While the size spectra for particles $\geq 0.5 \mu m$ are reasonably well known, the nature and abundance of colloids in seawater is poorly understood (92HS). Since organic material is a predominant component of marine particles (85WW), it is reasonable to presume that ocean colloids are also organic-rich and may be responsible for some portion of the very strong metal binding properties of natural seawater.

In addition to the indistinction between solution and surface complexation phenomena for particles $\leq 0.5\mu$m, solution complexation phenomena and the surface complexation phenomena for particles $\geq 0.5\mu$m are likely to have strong similarities. A variety of observations indicate that natural organic films are ubiquitous on particles immersed in seawater (72NL, 74NL, 77LN, 79HL, 80H, 82HL). Adsorbed natural organic films appear to be capable of dramatically transforming the surface chemistry of particles in natural waters (84D, 79HL, 90BK) including seawater. The investigation of Davis (84D) indicated that complexation of metal ions by adsorbed organic films can be described by apparent association constants similar in magnitude to the constants which describe solution phase equilibria. The work of Balistrieri et al. (81BB) indicated that metal-particulate matter interactions in seawater closely resemble the interactions between organic compounds and metals. However, in view of recent, rapid advances in the procedures (e.g. 90VN, 92VD) used to examine metal complexation by low concentration, high affinity binding sites in seawater, it is apparent that direct observations of particle surface chemistries utilizing modern competitive-ligand procedures are required in order to understand solution-particle metal exchange equilibria in the oceans.

Observations of metal sorption rates on marine particles do not provide evidence of a simple uptake mechanism. The work of Jannasch et al. (88JH) showed very fast sorption of Zn^{II}, Sc^{III}, Th^{IV} and Sn^{IV} on natural marine particles followed by much slower and extended uptake over a period between 1 minute and 100 days. The work of Li (81L) indicated that natural distribution coefficients are approximately an order of magnitude larger than any measured laboratory values. Explanations (88HA) for the observed slow sorption kinetics include (a) models involving rapid metal uptake by colloidal particles followed by aggregation with particles of filterable size (78TM, 85BH, 89HS) and (b) models involving rapid (millisecond) uptake by filterable particles followed by secondary processes including polymerization and/or diffusion into a lattice or pores (87BR). In view of the potentially central role of colloids in both chemical equilibria and chemical kinetics in the oceans, it is apparent that advances in our current understanding of ocean colloid chemistry is of critical importance to a general understanding of ocean chemistry.

A growing body of evidence indicates that the sorption of metals under natural conditions involves direct associations between complex solution species and particle surface sites. The work of Hunter et al. (88HH) indicated that, in natural seawater at pH 7.9-8.2, Th^{IV} bound to particle surface sites will dominantly be in the form of hydrolyzed thorium species ($ThOH^{3+}$, $Th(OH)_2^{2+}$, $Th(OH)_3^{+}$ and $Th(OH)_4^{\circ}$) rather than Th^{4+}. At high degrees of lanthanide complexation by CO_3^{2-}, sorption of $LnCO_3^{+}$ becomes important relative to Ln^{3+} (90SB). These observations and others (94GL) indicate that realistic models of the behavior of marine metals must involve experimental observations of metal sorption under natural solution complexation conditions. As such, investigations of marine surface complexation phenomena should be increasingly directed toward work at very low metal concentrations and accurately defined inorganic and organic solution complexation conditions. If very strong binding by dissolved organic binding sites is an important aspect of a marine metal's solution chemistry, then the surface complexation behavior of the metal should be studied with this phenomenon in view.

4.   Future Directions in Metal-Organic Complexation Analysis

Although the importance of organo-metal interactions in seawater is much better appreciated than a decade ago, in molecular terms organo-metal interactions in seawater remain poorly understood. The current indefinite understanding of organic complexation phenomena in seawater is attributable to formidable analytical difficulties and the substantial complexity of the natural system under investigation. The organic ligands of interest to marine chemists have total concentrations generally less than 100 nanomolar, and the most interactive class of organic ligands, which strongly influence the chemistry of copper in seawater, have concentrations commonly much lower than 10 nanomolar (Tables 3.3 and 3.4). A variety of reactive trace elements (Al, Cr, Mn, Ni, Cu, Zn) have concentrations in surface seawater (93DB) which are comparable to the total concentrations of this highly reactive class of organic ligands. Observations to date indicate that highly metal-specific

coordination may be a significant aspect of metal complexation phenomena in seawater. Metal complexation by organic ligands in most regions of the ocean, however, appears to also involve relatively non-specific ligands, with a wide spectrum of site characteristics. Slow and variable equilibrium rates have been observed and are attributable in part to the importance of replacement reactions in trace metal coordination. The equilibrium characteristics of this interactive system of metals and ligands are typically deduced through titrations with a single dissolved metal, occasionally in conjunction with addition of a non-specific competitive ligand. In view of the complexity of this system (multi-metal, multi-ligand, variable specificities, variable kinetics) what experimental designs might prove useful in elucidating the nature of organo-metal interactions in seawater?

One of the most fundamental problems underlying efforts to characterize organic complexation phenomena in seawater is the heterogeneity of the investigations. Different investigations involve analysis of different chemical systems. Both the character and concentrations of ligands in different seawater samples are variable, and the extent to which ligand sites are naturally occupied by a variety of metals is variable. Although variability in the spatial and temporal distributions of both organic ligands and complexed metals is an important and interesting question, toward the end of achieving a general understanding of the phenomenon of metal coordination with natural organics, it would be most valuable if some level of effort were devoted to analysis of shared (i.e. identical) samples. It is not uncommon that organo-metal complexation investigations are conducted in the laboratory subsequent to a brief period of storage. A variety of reports indicate that organic ligands are stable over a period of days if not weeks. It appears reasonable then to envisage experiments in which large seawater samples are collected, and using modern clean techniques the samples are subdivided for prompt shipment to a variety of laboratories equipped for this type of investigation. Through the concerted efforts of different laboratories using a diverse set of procedures, each sample could be much more fully characterized than is possible for any single laboratory. The suite of potential analyses which could be performed on such samples is very large and, in view of the multi-faceted strengths and limitations of the wide variety of methods applicable to analyses of natural organo-metal interactions, conjugate analyses involving the widest possible variety of methods would constitute an extremely powerful analytical system. Coupling the results obtained for analyses of shared samples seems essential for bringing increasing constraint to data interpretation and system characterization. It is, at present, too easy a matter to attribute large variations in organo-metal complexation phenomena to differences in the character of natural samples rather than differences attributable to analytical procedure.

Some of the most significant approaches to understanding marine organo-metal interactions may be found among the procedures and products of ligand design. Ligand architecture exerts profound effects on metal coordination properties, and molecular mechanics (84BJ, 89H, 89HMa) has proven to be a powerful quantitative tool in ligand design studies of metal-ligand coordination efficiency. Insights provided by molecular mechanics could be an essential aid in interpreting observations of metal complexation phenomena in natural systems. Observations of well-characterized ligands demonstrate that remarkable specificities can be obtained in the complexation of metal ions by organics. Such specificities are quantitatively explicable in terms of molecular mechanics (89HMa). The most "anomalous" specificity in marine systems is that embodied in the very large complexation constants reported for Ni^{II} relative to Cu^{II}. The specificity of certain classes of natural organics for Ni relative to Cu is quite reasonable in the context of the properties of TACNTA (1,4,7-triazacyclononane-N,N$^{/}$,N$^{//}$-triacetic acid), a hexadentate ligand with a preference for small metal ions with M-L bond lengths on the order of 0.200nm (89HMa). Whereas the TACNTA stability constant with Cu^{II} is on the order of $10^{19.5}$ at 25°C and ionic strength 0.1M, the TACNTA stability constant with Ni^{II} under the same conditions is $10^{28.3}$. In view of such observations and the role of trace elements in many essential functions of marine microorganisms, it is inherently reasonable that ligands with strong specificities should exist in seawater. Molecular mechanics may provide a useful interpretative link between comparative metal complexation observations and assessments of organic molecular architecture. In view of the insights offered to marine organic complexation phenomena by the field of ligand design and molecular mechanics, it is reasonable to argue for an

increasing consideration of relationships between metal reactivity and the ligand structure of natural marine organics such as those begun by Piotrowicz et al. (84PH).

There is presently a large gulf between laboratory investigations of structurally characterized organic ligands and investigations of the complexation behavior of natural organics in seawater. Whereas in the former case a variety of well-developed and well-understood analytical procedures can be brought to bear on any equilibrium system, in the latter case technique development and complexation phenomenology are proceeding hand in hand; uncertainties surrounding measurement technique and uncertainties in the characterization of organic complexation phenomena are interwoven. Investigations of the complexation behavior of some ligands (eg. EDTA, DTPA) have, in some instances, been undertaken as a means of assessing the efficacy of techniques and verifying the quality of model interpretations. In order to better understand the very complex phenomenon of organo-metal associations in seawater, it seems important that additional emphasis be given to systems intermediate in complexity between natural systems and systems involving single, non-specific ligands. Metal titrations conducted in well-defined synthetic systems containing multiple metals and multiple ligands could considerably advance our understanding of the performance of analytical procedures used in more complex systems. A wide variety of ligands with widely varying metal affinities and metal specificities is available for such studies (87B, 89HMa).

Among the many and diverse investigations being directed toward an improved understanding of ocean biogeochemistry, those which may most forcefully alter current views on marine metal speciation may well be studies involving characterization of marine colloids. The existence and potential importance of colloids in marine chemical processes has been recognized for some time (73S, 76M, 77O, 85CB, 89HS). Colloids are ubiquitous components of seawater (90KH, 91WG, 92WG), and, in response to theoretical arguments and laboratory studies suggesting that colloid aggregation may be the principal mechanism for removing metal ions from natural solutions (86FM, 89HS, 91HS), investigation of the nature of colloids in the marine environment is proceeding at a greatly accelerated pace (eg. 92BP, 92BS, 92HS, 94BO, 94GC, 94WG, 95DM, 95HP, 95MD). Evidence for the importance of marine metals in colloidal form (94BO, 95DM, 95HP, 95MD) alters the perspective of metal speciation studies from distributions among chemical forms to distributions among physical forms. Indeed, it has recently been proposed (94MZ) that "trace metals in seawater are mainly in the colloidal state with coordination bonds holding the colloid particles together." According to the conceptual model of Mackey and Zirino (94MZ), metal-organic associations in seawater occur principally in the form of onionlike structures, with layers of organic molecules bound together by hydrogen bonds and coordination bonds between trace metals and ligand donor atoms. This model is particularly appealing as a means of explaining the unusually slow kinetics and inertness of metal-organic interactions in seawater. Although the organic ligands involved in the layering process are considered to be humic and fulvic acids, Mackey and Zirino (94MZ) conclude that the "composition of the organic component of the matrix could be described by any of the proposed structures for these compounds (83HB, 84B, 88Z, 90B)." Thus, in both the model of Mackey and Zirino (94MZ) and in current marine speciation models which are dominated by very strong metal-specific ligands (L_1), the coordination structures involved in metal-humic interactions with non-specific ligands (L_2) are not a central issue. Mackey and Zirino (94MZ) conclude that the apparent presence of strong ligands with high specificities for a given metal in natural seawater is probably an artifact. Consequently, the onion model (94ZM) predicts a Cu^{II} speciation scheme in seawater wherein only 10-20% of the total Cu^{II} is in true solution. The dominant portions of Cu^{II}, Zn^{II}, Co^{II}, Ni^{II} and Pb^{II} are considered to be incorporated in particles having a size range between ten and one hundred nanometers.

The colloid model and the strong ligand model of marine metals should not be viewed as mutually exclusive. Although the colloid model is certainly appealing as a means of addressing many of the unusual observations (94MZ) associated with measurements of marine metal speciation, culture studies with cyanobacteria (90MB, 94WT) have produced an extracellular ligand with the same stability constant as the strong Cu^{II} binding ligand in seawater (90MB), and have shown (94WT) that multiple siderophore production is a common response to iron-limited growth conditions.

Thus, the appearance in seawater of Cu^{II} complexation constants on the order of 10^{13} kg mol^{-1} (Table 3.3) and Fe^{III} complexation constants on the order of 10^{20} to 10^{23} kg mol^{-1} (94GV, 95RB) certainly has a biogeochemical basis.

Resolution of speciation observations into dissolved ligand contributions and colloid contributions should be considerably facilitated by application of modern physical separation techniques including ultracentrifugation (94WG), cross-flow ultrafiltration (95DM, 95MD) and dialysis (94MT). While physical separations are an obvious and essential means of identifying the role of colloids in marine metal speciation, the use of such procedures combined with other types of measurements can constitute a particularly incisive analytical system. In the work of Midorikawa and Tanoue (94MT), for example, concentrated natural organic ligands were studied after demetallization. This potentially mitigates a problem common to some direct electrochemical analyses wherein low concentrations of natural ligands are completely associated with natural levels of Cu^{II} and cannot be observed. Another useful consequence of physical separation procedures is the ability to examine metal-organic associations in a variety of solutions other than seawater. The Cu^{II} complexation work of Midorikawa and Tanoue included analyses at an ionic strength near zero, and involved the use of a Cu^{II} ion-selective electrode, an analytical procedure inapplicable in seawater (94MZ) due to sensitivity limitations and interferences.

The foregoing descriptions of world-wide efforts to elucidate the chemical associations of marine metals suggest that trace metal speciation models appropriate to seawater could remain controversial for some time. In view of the growing spectrum of analytical procedures which are being brought to bear on metal speciation investigations at trace concentrations, it is appropriate to emphasize again the probable benefits of multi-investigator analyses involving shared seawater samples.

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ACKNOWLEDGEMENT