



The Effect of Metal Concentration on the Parameters Derived from Complexometric Titrations of Trace Elements in Seawater—A Model Study

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In this study we examine the impact of dissolved metal concentrations on the parameters that are commonly determined from complexometric titrations in seawater. We use the non-ideal competitive adsorption (NICA) model within the framework of the chemical speciation program visual MINTEQ with iron as a model metal. We demonstrate that dissolved iron concentrations effect the determined parameters for a heterogeneous binding site distribution with a fixed concentration of dissolved organic carbon. The commonly used terms “ligand concentration” and “binding constant” are therefore dependent on metal concentration, so we adopt the terminology suggested by Town and Filella (2000) and use the terms ligand quotient and stability quotient here. The systematic increase in the ligand quotient with dissolved iron concentration likely contributes toward the trend of increasing ligand quotient with dissolved iron concentration observed in field studies, and makes it hard to assign an objective meaning to the parameter. We suggest that calculation of the side reaction coefficient, a parameter that describes the probability that any added metal will be complexed, could be less prone to bias and misinterpretation than calculation of conditional stability and ligand quotients. We explore the impact of experimental design on side reaction coefficients by applying different detection windows, and multiwindow and reverse titration approaches. We identify the method that results in the best estimates of side reaction coefficients over a range of iron concentrations between 0.1 and 1.5 nmol L⁻¹. We find that single window titrations can only reliably estimate side reaction coefficients over a limited range of iron concentrations. Multiwindow titrations provided estimates of side reaction coefficients within the 99% confidence interval of the values calculated directly from the NICA model at all iron concentrations examined here. We recommend that future reports of speciation measurements consider the potential influence of metal concentrations on the determined parameters and future studies focus on developing and applying experimental designs that improve the robustness and rigor of chemical speciation analysis in the marine environment.

Keywords: trace element speciation, trace element biogeochemistry, iron, complexometric determination, organic complexation

INTRODUCTION

Metals that are present at concentrations lower than $1 \mu\text{mol L}^{-1}$ in seawater are described as trace metals. They are important as they act as nutrients (e.g., iron, copper, manganese, zinc, cobalt) and/or toxic agents to marine biota (e.g., copper, cadmium, silver). Trace metals influence marine productivity and phytoplankton community composition and therefore ultimately have an impact on the carbon fluxes into the deep ocean and the drawdown of CO_2 from the atmosphere. In particular iron, manganese, zinc and cobalt have been shown to be trace nutrients with strong influences on open ocean phytoplankton productivity and community composition (Moore et al., 2013). In coastal environments, elevated inputs of metals result in perturbations of seawater chemistry that can result in ecosystem impacts (Achterberg et al., 1999; Gledhill et al., 1999; Mayer-Pinto et al., 2010). Trace metal availability is directly related to the abundance and lability of chemical species rather than to dissolved metal concentration (Sunda and Guillard, 1976; Buffle et al., 2009; Shaked and Lis, 2012). Furthermore the distribution in the ocean of trace elements like iron cannot be explained without a mechanism that maintains iron in solution at concentrations far beyond its solubility product (Tagliabue et al., 2016; Fitzsimmons et al., 2017).

The inorganic chemistry of metals in seawater is calculated from basic thermodynamic approaches (Turner et al., 1981). Over the last few decades steady improvements have been made to the constants and approaches describing inorganic metal speciation in seawater through incorporation of Pitzer equations to account for the high ionic strength in seawater (e.g., Byrne and Miller, 1985; Pitzer, 1991; Hudson et al., 1992; Millero et al., 1995; Turner et al., 2016). In contrast to our framework for modeling inorganic chemical speciation of trace metals in seawater, we have a simplistic and operational framework for understanding metal organic complexation (Gledhill and Buck, 2012; Pižeta et al., 2015) that limits our ability to identify distinct biogeochemical trends in metal speciation (Hassler et al., 2017). The complexity of DOM in the natural marine environment, coupled with the challenges of analyzing samples of high ionic strength has meant that few specific marine organic compounds have been fully identified (Benner, 2002; Hertkorn et al., 2006), and still fewer specific metal chelators quantified (Gledhill et al., 2004; Mawji et al., 2008, 2011; Velasquez et al., 2011; Boiteau et al., 2016a,b). Total organic carbon concentrations in the open ocean are of the order of $40\text{--}100 \mu\text{mol C L}^{-1}$, of which the major portion ($\sim 90\%$) is considered to be in the dissolved fraction (DOC, $<0.7 \mu\text{m}$). In coastal waters, total organic carbon concentrations are enhanced, especially within and close to estuaries (Hoikkala et al., 2014; Avendaño et al., 2016). Despite the challenges associated with the analysis of such a complex mix of components in a highly saline matrix, much progress has been made in recent years in characterizing organic matter. Improvements in extraction techniques (Green et al., 2014) and the application of high resolution mass spectrometry (Ultra high resolution Fourier transform ion cyclotron mass spectrometry, HR-FTICR-MS) has identified thousands of molecules (Koch et al., 2005; Medeiros et al., 2015) and shown that significant differences in

molecular composition exist between surface and deep waters that likely relate to DOC aging processes (Hansell et al., 2012; Hansell, 2013; Medeiros et al., 2015). Mass spectrometry coupled to chromatography has also been utilized for the investigation of specific metal complexes in seawater. Specific iron binding complexes produced by bacteria (siderophores) were first determined in the Baltic Sea (Mucha et al., 1999) by capillary electrophoresis with detection by spectrophotometry. However it is becoming increasingly apparent that low concentrations of siderophores can be observed in many regions of the open ocean (Mawji et al., 2008; Velasquez et al., 2011, 2016; Boiteau et al., 2016a). Improved techniques have also recently shown that picomolar concentrations of chromatographically resolvable complexes with copper and nickel can be observed in surface waters of the Equatorial Pacific Ocean (Boiteau et al., 2016b). Furthermore broad unresolved chromatographic humps were identified for Fe, Cu, and Ni supporting the hypothesis that metals also bind with chromatographically unresolvable organic compounds (Boiteau et al., 2016a,b). In tandem with these advances, evidence has emerged that humic like materials and exopolysaccharides play an important role in metal speciation (Laglera et al., 2007; Yang and van den Berg, 2009; Hassler et al., 2011; Whitby and van den Berg, 2015). Thus it is increasingly evident that metal binding to organic matter occurs through complex interactions with many binding sites. Furthermore, such variability in DOM and thus binding site character is highly likely to result in a diverse range of binding site strengths and variable stoichiometry. For example, siderophores alone exhibit denticities of between 2 and 6 and formation constants that vary over 25 orders of magnitude (Hider and Kong, 2010).

Despite this complexity, the binding of metals to organic matter in the marine environment is currently interpreted using an adsorption isotherm that assumes a simple 1:1 stoichiometry and a limited number of binding sites (e.g., Pižeta et al., 2015). Typically, a Langmuir type isotherm is applied to metal titration curves and a conditional stability constant ($K_{\text{MeL}_i}^{\text{cond}}$) and the apparent ligand concentration of a group of binding sites, i , (L_i) derived. The Langmuir type isotherm approach has been useful in showing that organic matter plays an important role in metal biogeochemistry (see e.g., Gledhill and Buck, 2012 and references therein). The conditional approach means results cannot be extrapolated beyond the conditions of analysis (Town and Filella, 2000). The conditions influencing determined $K_{\text{MeL}_i}^{\text{cond}}$ and L_i are normally considered to be pH, temperature and salinity. However, binding site heterogeneity means that the ratio between the metal and the available binding sites is also a condition that influences the determined “constants,” a factor first pointed out over 15 years ago (Town and Filella, 2000). In practice, this means that if binding site concentrations remain the same but metal concentrations change, then the apparent concentration of L_i will vary. Naturally, such behavior makes it challenging to establish links between $K_{\text{MeL}_i}^{\text{cond}}$, L_i and the biogeochemical cycle of carbon and this limits our ability to incorporate organic complexation into seawater chemical speciation models (Turner et al., 2016). The dependence of $K_{\text{MeL}_i}^{\text{cond}}$ and L_i on the ambient conditions of the sample has led to a suggestion that the term “quotient” be

used to describe $K_{MeL_i}^{cond}$ and L_i (Town and Filella, 2000), a practice that we adopt in this study. Iron is used as the example metal because an extensive oceanic data set has recently been made available (Caprara et al., 2016). Furthermore, the principles and challenges described here have previously been demonstrated for other trace metals that bind with organic matter in seawater (Town and Filella, 2000). The consequences of variable metal to binding site ratios for $K_{MeL_i}^{cond}$ and L_i quotients for a heterogeneous mix of binding sites are demonstrated through calculations in the speciation program visual MINTEQ (Gustafsson, 2012) using the non-ideal competitive adsorption (NICA) model of fulvic acid (FA). Since trace element concentrations in the open ocean can vary by more than an order of magnitude (De Baar and De Jong, 2001; Morel and Price, 2003), while DOC concentrations only vary by a factor of two (Hansell, 2013), we illustrate the impact of changing metal to binding site ratios by keeping the DOC concentration constant and varying iron concentrations. We simulate titrations employing different experimental designs in an effort to find a strategy capable of reliable determination of metal speciation given heterogeneous binding sites, and thus provide the best estimate of the ratio of bound to inorganic species in a sample. Our overall aim is to improve understanding of how parameters describing metal speciation can be linked to other biogeochemical properties in the ocean, such as pH and DOC concentrations.

METHODS AND APPROACH

The principles behind complexometric titration experiments have been described extensively elsewhere (Gerringa et al., 2014; Laglera and Filella, 2015; Pižeta et al., 2015). Conditional stability quotients (K_{i, MeL_i}^{cond}) describe the apparent strength of a single or group of binding sites (L_i), assumed to complex with 1:1 stoichiometry (Town and Filella, 2000). They are related to the concentration of complexed metal ($[MeL_i]$), the concentration of unbound metal ($[Me']$) and the concentration of binding sites that are available for complexation by the metal ($[L'_i]$).

$$K_{i, MeL_i(Me')}^{cond} = \frac{[MeL_i]}{[Me'] \cdot [L'_i]} \quad (1)$$

In addition to conditional stability quotients, side reaction coefficients are also utilized for describing complex equilibrium processes (Ringbom and Still, 1972). The side reaction coefficient describes the likelihood a particular complex will form given the presence of competing ions within a complex mixture. For the formation of metal complexes with organic binding sites, it can be derived from two expressions

$$\alpha_{MeL(Me')} = K_{MeL_i(Me')}^{cond} \cdot L'_i \quad (2)$$

$$\alpha_{MeL(Me')} = \frac{[MeL]}{[Me']} \quad (3)$$

The side reaction coefficient will thus describe the ratio of bound to inorganic metal for a particular sample. An inorganic side reaction coefficient or side reaction coefficient for the unbound metal ($\alpha_{Me'}$) likewise describes of the sum of all the

concentrations of inorganic ion pairs divided by the free metal concentration.

Adsorptive cathodic stripping voltammetry (AdCSV) is most commonly utilized for complexometric titrations in the marine environment. AdCSV requires the addition of a ligand (the added ligand, AL) that reacts with the metal and allows the metal complex to be adsorbed onto the mercury drop and undergo electrochemical reduction. The electroactive complex redistributes metal species within the sample, lowering the concentration of Me' and MeL_i in the analyzed sample in proportion to the known strength of the $MeAL_x$ complex, and thus allowing for the calculation of Me' and MeL_i . The relationship between the concentration of MeL_i and Me' is interpreted as a Langmuir adsorption isotherm, and $K_{i, MeL_i(Me')}^{cond}$ and L_i derived. An important assumption of the Langmuir isotherm is that all sites are equal, an assumption known to be incorrect in practice. Application of “multiwindow” methods, that vary the amount of metal bound by AL through varying the AL concentration, with interpretation of data as composite isotherms (i.e., consideration of two or more L_i) have been proposed as ways of accounting for the presence of more than one type of binding site, whilst still allowing for the application of the Langmuir isotherm (van den Berg et al., 1990; Buck et al., 2007; Sander et al., 2011). Further improvement to the range of ligands that can be detected has been achieved through unification of multi-window datasets based on mass balance equations (Hudson et al., 2003; Sander et al., 2011; Wells et al., 2013; Pižeta et al., 2015), but this approach has yet to be applied widely in the field. The likelihood that the metal-added ligand complex will form is described by its own side reaction coefficient $\alpha_{MeALx(Me')}$, which is also thought to define the “detection window” encompassing the spectrum of binding sites that can be detected under the applied conditions. During a titration, $\alpha_{MeALx(Me')}$ is assumed to remain constant, while $\alpha_{MeL(Me')}$ decreases as a result of adding the metal (Equation 3). Furthermore consideration of Equation (2) makes it clear that when multiple binding sites are present, the initial metal concentration will also determine whether a binding site of a given strength will be detected, since, as any given $L'_i \rightarrow L_i$, $\alpha_{MeL} \rightarrow 0$.

In order to illustrate the potential importance of initial metal concentration for $K_{i, MeL_i(Me')}^{cond}$ and L_i the speciation program visual MINTEQ v. 3.0 (Gustafsson, 2012) was used to simulate titrations of diethylenetriaminepentaacetic acid (DTPA) and fulvic acid type organic matter. Binding site heterogeneity was represented using the NICA model. Thermodynamic stability constants for DTPA were from the NIST database (Martell and Smith, 2004). The NICA model is described extensively in Milne et al. (2003) and references therein. Briefly, the NICA model as applied in this study describes a heterogeneous bimodal distribution of binding sites. The two groups of binding sites are generally thought to encompass acidic (carboxylic like) and a basic (phenolic like) functional groups (Kinniburgh et al., 1996; Koopal et al., 2005). Although the NICA model is used in this study to describe heterogeneous binding, the observations are likely valid for any heterogeneous distribution of binding

sites as the issue highlighted here relate to the presence of a diverse mix of binding sites of different strengths and are not restricted to a particular binding site distribution (see e.g., Turoczy and Sherwood, 1997; Town and Filella, 2000; Pižeta et al., 2015). Major ions (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Cl^- , SO_4^{2-} , Br^- , CO_3^{2-} , H_3BO_3 , F^-) concentrations, adjusted for a salinity of 35 were used to calculate and correct for ionic strength in visual MINTEQ. Ionic strength corrections were made using the Davies and Debye-Hückel equations. pH was set to 8.05 and temperature 20°C. The solubility and equilibrium constants from Liu and Millero (1999) were used to calculate iron hydroxide concentrations. The NICA parameters derived for Fe^{3+} for shelf sea waters in Avendaño et al. (2016) and the generic NICA parameters for H^+ , Ca^{2+} , and Mg^{2+} described in Milne et al. (2003) were used in the model assuming dissolved organic carbon (DOC, $64 \mu\text{mol L}^{-1}$) was present as FA. Although the Donnan model was not excluded from the calculations, the Donnan volume is very small at the ionic strength of seawater and thus no iron was calculated as adsorbed in the Donnan layer. The added ligand, 1-nitroso-2-naphthol (NN), was included in visual MINTEQ for titration simulations (Gledhill and van den Berg, 1994; Avendaño et al., 2016). Three experimental designs for titrations were tested, each incorporating a total of 15–18 data points per titration. (1) single window “forward” titrations at three different detection windows ($\alpha_{\text{FeNN3}(\text{Fe}')} = 146$, 1,164 and 1.46×10^5 , $[\text{NN}] = 0.5$, 1, and $5 \mu\text{mol L}^{-1}$), employing iron additions of between 0.5 and 8 nmol L^{-1} . (2) A “reverse” titration, where the added ligand concentration was varied between 0.5 and $150 \mu\text{mol L}^{-1}$. (3) A “multiwindow” method, employing reduced resolution forward titrations at two different detection windows ($\alpha_{\text{FeNN3}(\text{Fe}')} = 146$ and 9,310, $[\text{NN}] = 0.5$ and $2 \mu\text{mol L}^{-1}$). A high detection window ($\alpha_{\text{FeNN3}(\text{Fe}')} = 9.3 \times 10^6$, $[\text{NN}] = 20 \mu\text{mol L}^{-1}$) was included in the simulation as an “overtitration” mechanism for validating the calibration of the slope (Pižeta et al., 2015). The added iron range was extended to cover the range $0.5\text{--}30 \text{ nmol L}^{-1}$ (Garnier et al., 2004). Eleven separate titrations were simulated for initial dissolved iron (DFe) concentrations of between 0.05 nmol L^{-1} and 1.5 nmol L^{-1} .

Simulated concentrations of iron bound to NN calculated in visual MINTEQ were used to calculate $K_{i, \text{MeLi}(\text{Me}')}^{\text{cond}}$ and L_i whilst making a one or two ligand assumption using the complete fitting approach of Garnier et al. (2004) within the speciation program ProMCC (Omanović et al., 2014). The conditional stability constant for NN was set at $\log K_{\text{FeNN3}(\text{Fe}')}^{\text{cond}} = 21$, as this was equivalent to the value calculated from the visual MINTEQ data. This is stronger than the range of values typically given in the literature (17–19) (Hawkes et al., 2013; Gledhill et al., 2015), a factor which is likely related to the ionic strength correction mechanism in visual MINTEQ. For the forward titrations the slope was fitted along with $K_{i, \text{MeLi}(\text{Me}')}^{\text{cond}}$ and L_i , as is usual for the complete fitting approach (Garnier et al., 2004). Even though the calculated FeNN_3 concentration was used to fit the data, the slope did not always equal 1 (especially at lower detection windows), since a small portion of the total metal concentration remained bound to very strong binding sites. For the unified multi-window approach, a slope of 1 was assumed as this approach incorporated

an overtitration at a high detection window, when all added iron is calculated to be present as FeNN_3 .

Simulated reverse titrations (Nuester and van den Berg, 2005; Hawkes et al., 2013) varied the concentration of NN between $0.1 \mu\text{mol L}^{-1}$ and $150 \mu\text{mol L}^{-1}$. The side reaction coefficient α_{MeL} was determined using non-linear least squares regression of the expression

$$\frac{[\text{FeNN}_3]}{[\text{FeNN}_3]_{\text{max}}} = \frac{\alpha_{\text{Fe}'} + \alpha_{\text{FeNN3}}}{\alpha_{\text{Fe}'} + \alpha_{\text{FeNN3}} + \alpha_{\text{FeLi}}} \quad (4)$$

where $[\text{FeNN}_3]_{\text{max}}$ is the maximum $[\text{FeNN}_3]$ concentration observed in the titration. The regression fit was carried out within the computer program R (R Development Core Team, 2016).

For the multiwindow method, the unified Sander-Wells method (Sander et al., 2011; Wells et al., 2013) in ProMCC (Omanović et al., 2014) was used to transform the data and derive $\log(K_{i, \text{FeLi}(\text{Fe}')}^{\text{cond}})$ and L_i .

Side reaction coefficients in the absence of the added ligand for DFe concentrations between 0.1 and 1.5 nmol L^{-1} were calculated directly from the visual MINTEQ output obtained when the concentration of $[\text{NN}] = 0$, using Equation (3). The sum of the dominant inorganic species ($\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})_3$, and $\text{Fe}(\text{OH})_4^-$) were taken to equal $[\text{Fe}']$ and the sum of the organic species ($\text{Fe}(\text{III})\text{FA1}$, $\text{Fe}(\text{III})\text{FA2}$, where FA1 is the acidic fraction and FA2 is the basic fraction) taken to equal $[\text{FeL}]$.

Side reaction coefficients from $K_{i, \text{MeLi}(\text{Me}')}^{\text{cond}}$ and L_i were calculated using Equation (2). The excess ligand ($[L'_i]$) can be obtained by subtracting DFe from $[L_i]$. However, as $[L_i]$ approaches DFe, $[L'_i]$ needs to be calculated by iteration in order to reduce error associated with subtracting two very large numbers in order to obtain a small number. We therefore used R to apply Newton's algorithm (Press et al., 1986) and iteratively resolved the following two equations

$$\begin{aligned} [\text{DFe}] &= [\text{Fe}^{3+}] \times (1 + \alpha_{\text{Fe}'} + K_{i, \text{FeLi}(\text{Fe}^{3+})}^{\text{cond}} \times L'_i) \\ [L_i] &= [\text{FeL}] + [L'_i] \end{aligned}$$

to obtain a solution for $[\text{Fe}']$ and $[L'_i]$, where $K_{i, \text{FeLi}(\text{Fe}^{3+})}^{\text{cond}}$ is the conditional stability quotient with respect to the free iron concentration.

A smaller set of multiwindow titrations were simulated at higher iron concentrations in order to investigate the impact of the existence of an inert colloidal DFe fraction that did not dissolve within the time frame of a typical titration experiment ($<24 \text{ h}$). The inert fraction was constrained by solubility—in other words the concentration of the ligand (in this case the hydroxide ion) was not limited, to allow for formation of a solid phase. The inert fraction was thus simulated by adding ferrihydrite as a possible solid in the visual MINTEQ environment and then calculating the speciation of the simulated sample in the absence of NN. The precipitated ferrihydrite concentration was then subtracted from DFe concentrations to give a soluble iron concentration (sFe) and the titration simulated in visual MINTEQ. However, as the inert fraction would still be determined as DFe if it is small enough

to be present as a colloid, it was re-added to the sFe concentration prior to transformations of titration data in ProMCC.

RESULTS AND DISCUSSION

Influence of the Dissolved Metal Concentration on the Determined Binding Site Concentration and Conditional Stability Constant

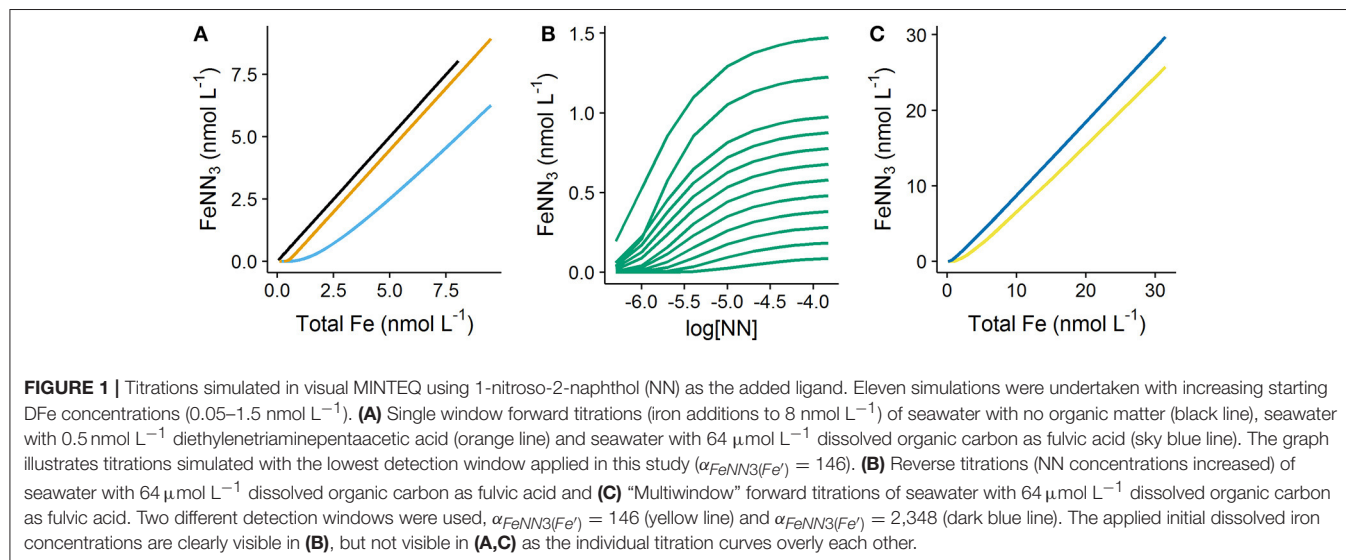
The simulated titration data obtained for DFe between 0.05 and 1.5 nmol L⁻¹ is shown in **Figure 1**. The difference between forward titrations undertaken with a simple single ligand (DTPA) and DOC characterized by a heterogeneous mix of binding sites (NICA-FA) is shown in **Figure 1A**. It is clear from this figure that the slope of the upper part of the titration curve for DTPA is the same as that observed in the absence of organic matter (slope of linear part (S) = 1), while that obtained for the NICA-FA is lower as a result of the presence of weak binding sites (S = 0.8 for $[NN] = 0.5 \mu\text{mol L}^{-1}$, $\alpha_{\text{FeNN3(Fe')}} = 146$). Thus the titration of NICA-FA does not reach a well-defined end point, a problem which is known to cause bias in transformed data (e.g., Turoczy and Sherwood, 1997; Garnier et al., 2004; Pižeta et al., 2015). Forward titration curves of DTPA and NICA-FA using different starting dissolved Fe concentrations all overly each other so that although all 11 simulated titrations are plotted on the graph, only one curve is observed. In **Figure 1B**, the reverse titration curves simulated at different iron concentrations are visible, but again in the multiwindow method (**Figure 1C**) data from the 11 different titrations are not distinct since all curves overlie each other. Titration data shown in **Figure 1C** shows how the slope can be expected to increase (and approach a value of 1) as the applied detection window increases.

Figure 2 plots $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$ and L_i obtained from transformations of the titrations obtained for DFe concentrations. The impact of complexity on $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$

and L_i can be observed by comparing results obtained for DTPA and NICA-FA. For DTPA, the expected $\log K_{\text{FeDTPA(Fe')}}^{\text{cond}}$ of 13.6 and the correct concentration of 0.5 nmol L⁻¹ was obtained irrespective of the NN concentration, although when DTPA was saturated with Fe, a small decrease in $\log K_{\text{FeDTPA(Fe')}}^{\text{cond}}$ with increasing DFe concentration was observed. Likewise, the fitting error increased at higher DFe concentrations as the number of datapoints (and thus the degrees of freedom) below the equivalence point of the titration decreased.

For NICA-FA, the determined parameters depended on applied conditions *including the dissolved iron concentration*. Calculation of $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$ and L_i assuming a one ligand model resulted in a trend of increasing L_i with metal concentration, coupled with a decrease in $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$. An assumption of two ligands resulted in a more constant L_i concentration across the range of metal concentrations for the weaker of the two assumed ligands, but a marked increase in L_i concentration for the stronger ligand, so that the sum of all ligand quotients (ΣL_i) still increased with dissolved metal. For both determined ligands $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$ decreased with increased DFe. Overall, it can clearly be seen that categorizing binding sites into “strong” and “weak” in this context becomes rather difficult, since the relative strength and concentration varies according to both the detection window and the dissolved metal concentration (Town and Filella, 2000).

The influence of detection windows on conditional stability constants has been discussed extensively since the concept was introduced by Apte et al. in 1988 (Apte et al., 1988; van den Berg et al., 1990; Town and Filella, 2000; Hudson et al., 2003; Pižeta et al., 2015). **Figure 2** also illustrates the impact of changing the detection window on our series of simulated single window iron titrations of NICA-FA. The three detection windows applied were $\alpha_{\text{FeNN3(Fe')}} = 146$, 1,164 and 1.46×10^5 . For a complex mixture of binding sites, $\log K_{i, \text{FeLi(Fe')}}^{\text{cond}}$ increased at higher detection windows and L_i decreased. For the lower detection window coupled with the one ligand model, which is more typical of



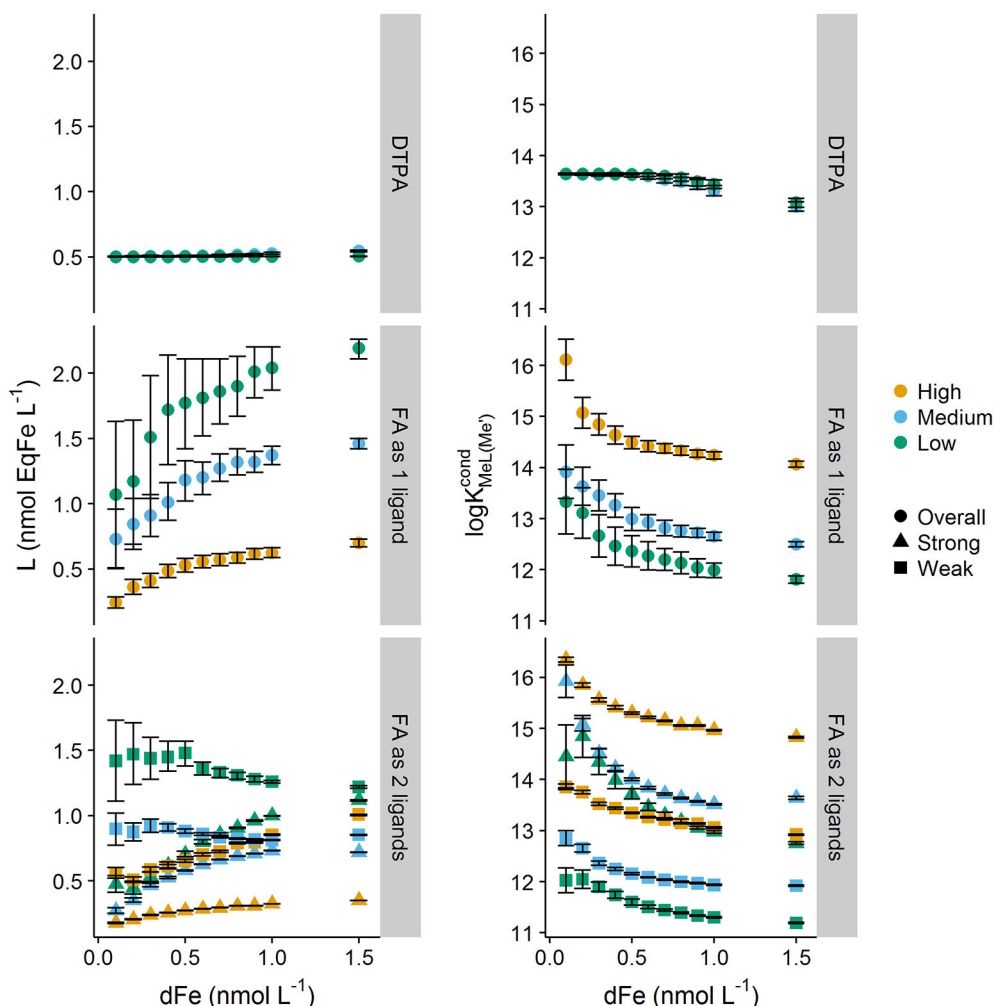


FIGURE 2 | Ligand and conditional stability quotients obtained from simulated forward titrations with different starting dissolved iron concentrations (0.1–1.5 nmol kg⁻¹) of diethylenetriaminepentaacetic acid (DTPA, [L] = 0.5 nM, $K_{FeDTPA(Fe')}^{cond} = 13.6$) and a heterogeneous mix of binding sites described by the NICA model of fulvic acid (DOC = 64 μmol L⁻¹) interpreted as only one ligand, and as two ligands. Three different added ligand concentrations were used to simulate titrations applying a high, medium and low detection window. Symbols indicate apparent class of ligand strength, where overall is the ligand class obtained when transformations only considered one ligand class to be present, and strong and weak differentiate the ligand classes for the two ligand transformations. Error bars represent 95% confidence intervals.

the approaches adopted for the analysis of field samples (e.g., Buck et al., 2012), $K_{i, FeL_i(Fe')}^{cond}$ varied by less than one order of magnitude over the modeled range of iron concentrations. The observed NICA-FA fitting error distributions were also contrary to that observed for DTPA titrations. For NICA-FA the largest errors for both $\log K_{i, FeL_i(Fe')}^{cond}$ and L_i were obtained at the lowest DFe concentrations. This most likely arises because it is at these concentrations that the most diverse and heterogeneous range of sites are available for binding, and thus the titration curve deviates most strongly from the Langmuir model.

Results from application of the multiwindow Sander-Wells method are shown in Figure 3. Similar trends are observed for the multiwindow experimental design and Sander-Wells fitting method as are observed for the single window methods, i.e.,

increasing metal concentrations results in increased L_i and decreases in $\log K_{i, FeL_i(Fe')}^{cond}$. Interestingly, for the one ligand assumption, the errors in L_i increased with increasing DFe (as for the Langmuir model and DTPA), which suggest that error pattern observed for the multiwindow fit is as would be expected from a reduction in degrees of freedom. The two ligand assumption resulted in a high estimate for the weak ligand quotient, associated with a high error.

Overall, the results obtained from applying a Langmuir type isotherm to a heterogeneous mix of binding sites across a range of metal concentrations confirm that it is inappropriate to assign an objective meaning to L_i and that the dependence of L_i on the dissolved metal concentration likely obfuscates meaningful biogeochemical interpretation of complexation quotients in the

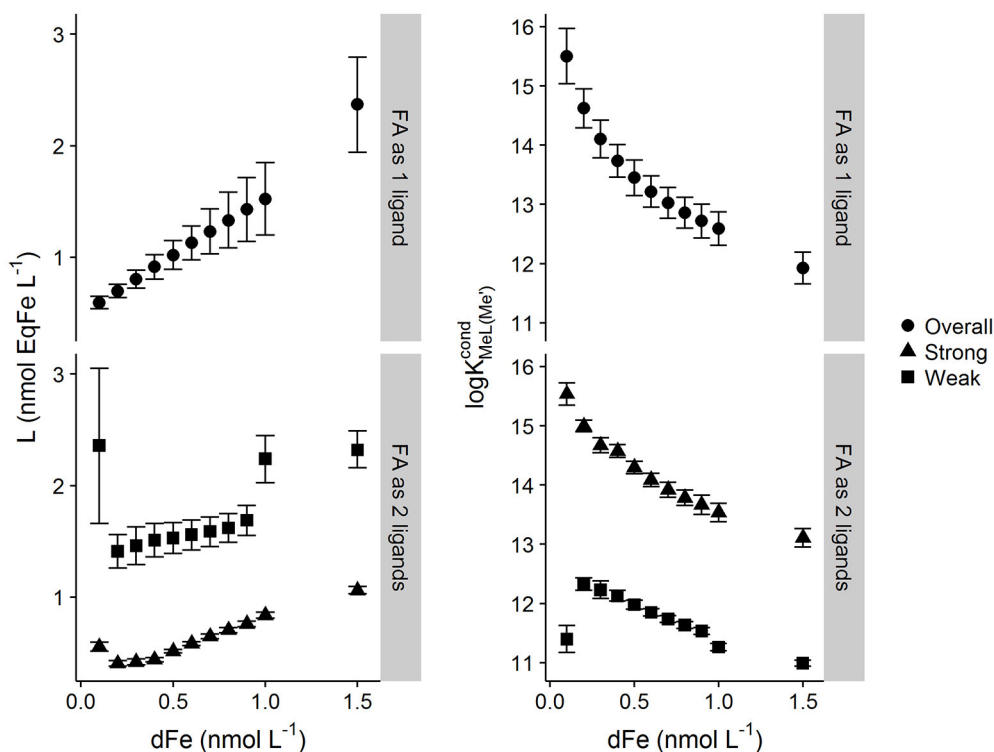


FIGURE 3 | Ligand and conditional stability quotients obtained from simulated multiwindow titrations of a heterogeneous mix of binding sites described by the NICA model of fulvic acid ($\text{DOC} = 64 \mu\text{mol L}^{-1}$) with different starting dissolved iron concentrations ($0.05\text{--}1.5 \text{ nmol kg}^{-1}$). $[L_i]$ and $\log K_{\text{MeL}_i}^{\text{cond}}$ were calculated using the unified Sander–Wells method within the computer program ProMCC. Titrations were interpreted as one overall ligand and as two ligands (“weak” and “strong”). Error bars represent 95% confidence intervals.

ocean (Town and Filella, 2000). The trend arising from changing metal concentrations (or more strictly speaking—changing metal to binding site ratio and thus changing $\alpha_{\text{MeL}(\text{Me}^')}$), is associated with the fact that stronger binding sites are filled first, thus at higher metal concentrations, only lower strength binding sites will be available for complexation by the metal added during the titration (Town and Filella, 2000). The side reaction coefficients of competing metals will also impact on the concentration of available sites. The bias is exacerbated by problems associated with calibrating the method, where *a priori* assumptions are made about the number of ligands present in the sample that influence the estimation of the slope in the titration curve and thus impact on the estimation of bound metal (Turoczy and Sherwood, 1997; Hudson et al., 2003; Gerringa et al., 2014; Pižeta et al., 2015). The determined L_i of natural organic binding sites with a heterogeneous distribution thus depends (a) on the number of ligands considered in the data treatment approach and (b) on the relative concentrations of binding sites and competing metals present in the sample. Increasing the detection window results in systematic shift of this bias to the determination of higher $\log K_{i, \text{FeL}_i(\text{Fe}')}^{\text{cond}}$ and lower L_i , since weaker binding sites cannot be filled by the metal as the added ligand is outcompeting these binding sites.

The result of this bias in for the determination of $\log K_{i, \text{FeL}_i(\text{Fe}')}^{\text{cond}}$ and L_i in real world samples should be a general trend of increasing L_i and decreasing $\log K_{i, \text{FeL}_i(\text{Fe}')}^{\text{cond}}$ with DFe as has been reported previously for other metals (Town and Filella, 2000). A trend of increasing L_i with iron has been shown in a recently published global ligand compilation (Caprara et al., 2016). The correlation between DFe and L_i increases in significance if only deep water samples are selected. DFe and ligand quotients are positively correlated in this data set ($>500 \text{ m}$, $r = 0.64$, $n = 669$, $p < 0.001$; **Figure 4A**), which is interesting as DOC concentrations and composition are known to be relatively constant at depth (Hansell, 2013). Furthermore, fitting errors for L_i were highest when L_i was high compared to DFe (i.e., high excess L_i), as would be expected for a heterogeneous distribution of binding sites (**Figure 4** and Supplementary Figure 1). In contrast, the fitting error for $\log K_{i, \text{FeL}_i(\text{Fe}')}^{\text{cond}}$ increased as the excess L_i decreased (**Figure 4** and Supplementary Figure 1), suggesting that the number of data points acquired below the equivalence point (and thus the degrees of freedom) also has an impact of the fitting error for $\log K_{i, \text{FeL}_i(\text{Fe}')}^{\text{cond}}$. Correlations between DFe and L_i can also be observed in individual studies such as the data obtained from the Western Atlantic GEOTRACES cruise ($r = 0.35$, $n = 162$, $p < 0.001$; **Figure 4B**; Gerringa et al., 2015). The potential that such a relationship may in fact result

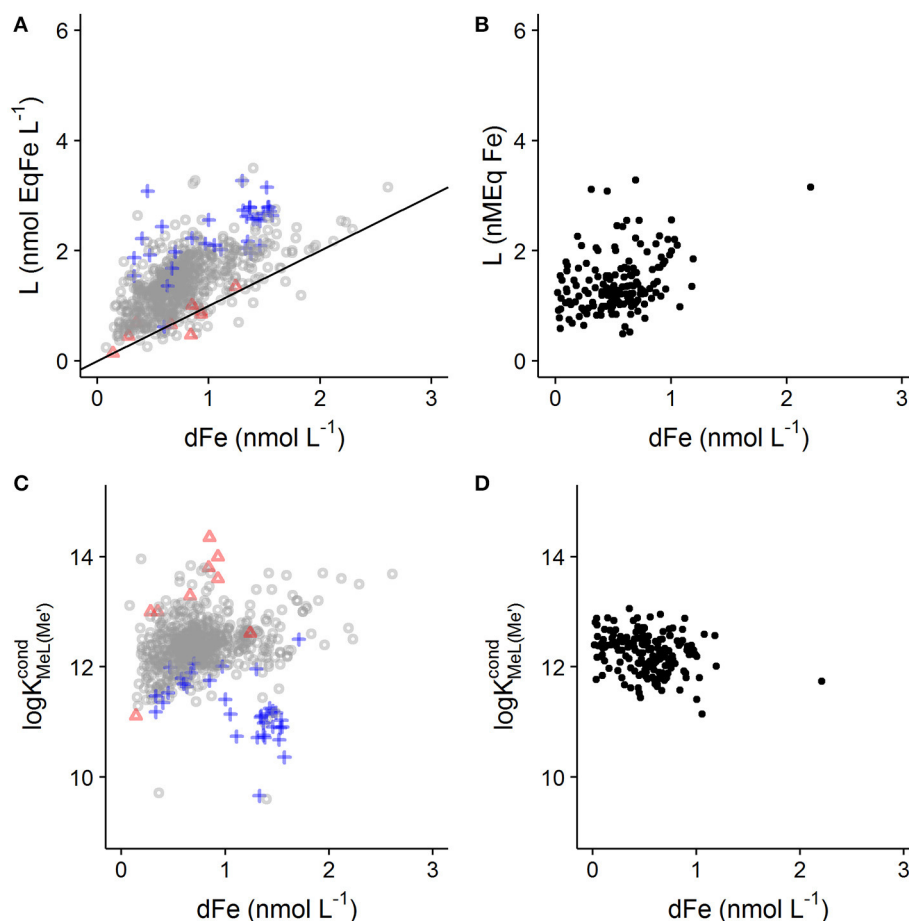


FIGURE 4 | Relationship observed between dissolved iron concentration, L_i and $\log K_{MeLi}^{cond}$. **(A,C)** Show L_i and $\log K_{MeLi}^{cond}$ for deep ocean samples (>500 m) from a global compilation (Caprara et al., 2016). Values of L_i with 95% confidence intervals $> 0.4 \text{ nmol EqFe L}^{-1}$ are illustrated with blue crosses, whilst values of $\log K_{MeLi}^{cond}$ with 95% confidence intervals > 1 log unit are displayed as a red triangle. It should be noted that not all values in the data set have associated confidence intervals. **(B,D)** Show data from the Western Atlantic GEOTRACES cruise (Gerringa et al., 2015). This data set was filtered to remove values of L_i with 95% confidence intervals $> 0.4 \text{ nmol EqFe L}^{-1}$. A 1:1 line is added to **(A)** show where concentrations of excess ligand are very low.

from a methodological bias in combination with variability in binding site concentration arising from changes in DOC concentrations or character is rarely addressed in the literature, but does bring into question the idea that metal concentrations and ligand quotients are somehow biogeochemically tightly coupled (e.g., Gledhill and Buck, 2012; Hassler et al., 2017). The interdependence of L_i on DFe almost certainly hinders our ability to link metal speciation to biogeochemical parameters commonly used to quantify changes in DOC concentration or composition (Hassler et al., 2017). This interdependence also means that iron concentrations in the titrated sample should ideally be determined and used in the calculations, since commonly applied sample storage methods (e.g., freezing) may change the dissolved iron concentration in the titrated sample. For example Gerringa et al. (2015) showed a loss of 13% of DFe when samples from the Western Atlantic were frozen as opposed to acidified. Furthermore the proportion of DFe lost could also be dependent on the dissolved iron and binding site concentrations.

Although a weak positive correlation between DFe and $\log K_{i, FeLi(Fe')}^{cond}$ is observed in the deep sea data ($r = 0.101$, $n = 667$, $p = 0.009$; **Figure 4C**), it is known that individual laboratory practices such as choice of added ligand can strongly influence determined $\log K_{i, FeLi(Fe')}^{cond}$ (Buck et al., 2016). Thus it could be that practices employed by different laboratories influence the global relationship between DFe and $\log K_{i, FeLi(Fe')}^{cond}$. In support of this, all of the data points with $\log K_{i, FeLi(Fe')}^{cond} < 11$ were obtained using NN as the added ligand, either at pH 6.9 (Witter and Luther, 1998), or using bromate as a catalyst (Boye et al., 2006), whilst weak but significant negative correlations can be apparent in datasets reported from individual laboratories ($r = -0.25$, $n = 162$, $p = 0.001$, **Figure 4D**, Gerringa et al., 2015).

It is also worth considering here detection limits for the determination of the iron-added ligand complexes and the constraints they impose on the determination of high $\log K_{i, FeLi(Fe')}^{cond}$ at low iron concentrations. Detection limits for

the most commonly applied voltammetric methods are given in **Table 1** and are typically of the order of 0.1 nmol L^{-1} . Our simulated titrations suggest that a concentration of 0.1 nmol L^{-1} FeNN_3 would not be observed until 0.5 nmol L^{-1} of total Fe at the higher detection window, and 1.2 nmol L^{-1} total Fe at the lower detection window. Thus an inability to confidently quantify low MeAL_x concentrations, will also have a detrimental effect on our ability to observe high $\log K_{i, \text{FeLi}}^{\text{cond}}(\text{Fe}')$. Any increases in $\log K_{i, \text{FeLi}}^{\text{cond}}(\text{Fe}')$ in the large data set that might arise from decreases in dissolved iron could thus be lost because of variability produced by e.g., use of different added ligand, changing salinity, temperature of titration and titration pH that also influence $\log K_{i, \text{FeLi}}^{\text{cond}}(\text{Fe}')$.

The Potential Influence of an Inert Fe Fraction

The discussion of influences on $K_{i, \text{FeLi}}^{\text{cond}}(\text{Fe}')$ and L_i in Section Influence of the Dissolved Metal Concentration on the Determined Binding site Concentration and Conditional Stability Constant above assumes that iron is present only in dissolved forms that are in equilibrium with the added ligand. However it was recently pointed out in Avendaño et al. (2016), that the interpretation of titration data can also be influenced by inert species that do not react with the added ligand on the timescale of the titration experiment. Such inert species could include e.g., aged oxyhydroxides or other colloidal minerals that exceed their solubility product in the sampled seawater and that take a long time ($>24 \text{ h}$) to equilibrate with the added ligand (Town and Van Leeuwen, 2005; Fitzsimmons et al., 2017). The kinetics of ligand assisted dissolution can vary considerably from ligand to ligand as a result of ligand solubility, hardness and denticity (Kraemer, 2004), so that although 15 min has been reported to be effective for dissolution of fresh oxyhydroxides by salicylaldoxime (Abualhaija and van den Berg, 2014), such dissolution rates may not be observed for all added ligands used in AdCSV. Inert DFe will not (by definition) be in equilibrium with the added ligand but in titration data such species will manifest as “strong” ligands. Furthermore, if this inert fraction is determined by solubility rather than complexation, then the concentration of the inert fraction will increase with increasing iron concentration (Avendaño et al., 2016). Theoretically, datasets where such behavior occurs can be identified by careful examination of titration curves. In particular examination of equivalent data points (i.e., same concentration of metal

added) from separate titration curves from an entire dataset can be illuminating. **Figure 5** shows the expected trend in the concentration of FeAL_x with increasing iron concentration for equivalent titration points (8 nmol L^{-1} added Fe) obtained for hypothetical samples with increasing DFe, but where binding site concentrations and organic matter composition do not change. In a system in equilibrium, increased iron concentrations will result in increases in both FeAL_x and FeLi . Importantly an increase is still observed even if the strength of FeLi is much greater than FeAL_x . However, in the presence of an inert fraction, then FeAL_x will not increase with total iron, and the equivalent points of the titration curves will simply be shifted to the right (along the blue line in **Figure 5**). Such a trend was observed for data in Avendaño et al. (2016), and datapoints from this study determined for pH 8, 8 nmol L^{-1} iron addition are presented along with the theoretical lines in **Figure 5**. Of course the interpretation of titration data in real data sets has added complexity because binding site concentrations and organic matter composition do vary with sample origin. Nevertheless, the fundamental principle that FeAL_x should increase in proportion to the total Fe concentration in a system in equilibrium should be kept in mind when considering titration data sets obtained for regions where variability in Fe concentrations far exceed changes in DOC concentration. Such systems include hydrothermal vent fields, or experiments investigating speciation in soluble vs. dissolved phases.

What Do We Need to Know?

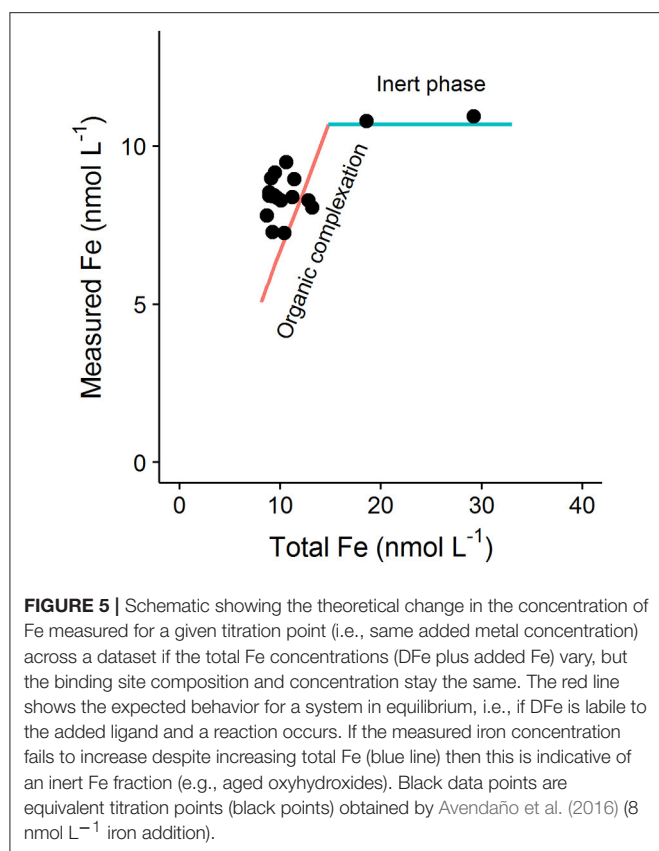
As with any analysis, it is important to consider the ultimate application of the generated data. In most cases the critical factors are understanding bioavailability and the balance between scavenging and solubility. For both these processes determination of the uncomplexed iron (Fe') concentration could be useful since this is the species that is thought to be most likely to be scavenged and also be the most bioavailable. Fortunately, it is possible to deduce this information from speciation measurements via calculation of side reaction coefficients using Equations (2) or (3).

Figure 6 shows side reaction coefficients obtained from applying Equation (1) to results obtained for discrete ligand approaches with varying detection windows (high, medium, low, multi) and transformation types (1 ligand assumption, 2 ligand assumption). Results are compared to side reaction coefficients obtained by applying equation 2 directly to the species concentrations calculated in vMINTEQ (shown on

TABLE 1 | Detection limits reported for the determination of iron by cathodic stripping voltammetry using different added ligands.

Added ligand	Ligand concentration ($\mu\text{mol L}^{-1}$)	Deposition time (s)	Detection limit (pmol L^{-1})	References	Detection limit normalized to 1 min deposition time (pmol L^{-1}) ^a
Salicylaldoxime	25	600	10	Buck et al., 2007	100
Dihydroxy naphthalene	10	60	10	Obata and van den Berg, 2001	10
2-(2-Thiazolylazo)-p-cresol	10	300	100	Groot and Johansson, 2000	500
1-nitroso-2-naphthol	20	60	160	Yokoi and van den Berg, 1992	160

^aDetection limits are also influenced by the mercury drop sizes of different electrodes, which is not accounted for here.



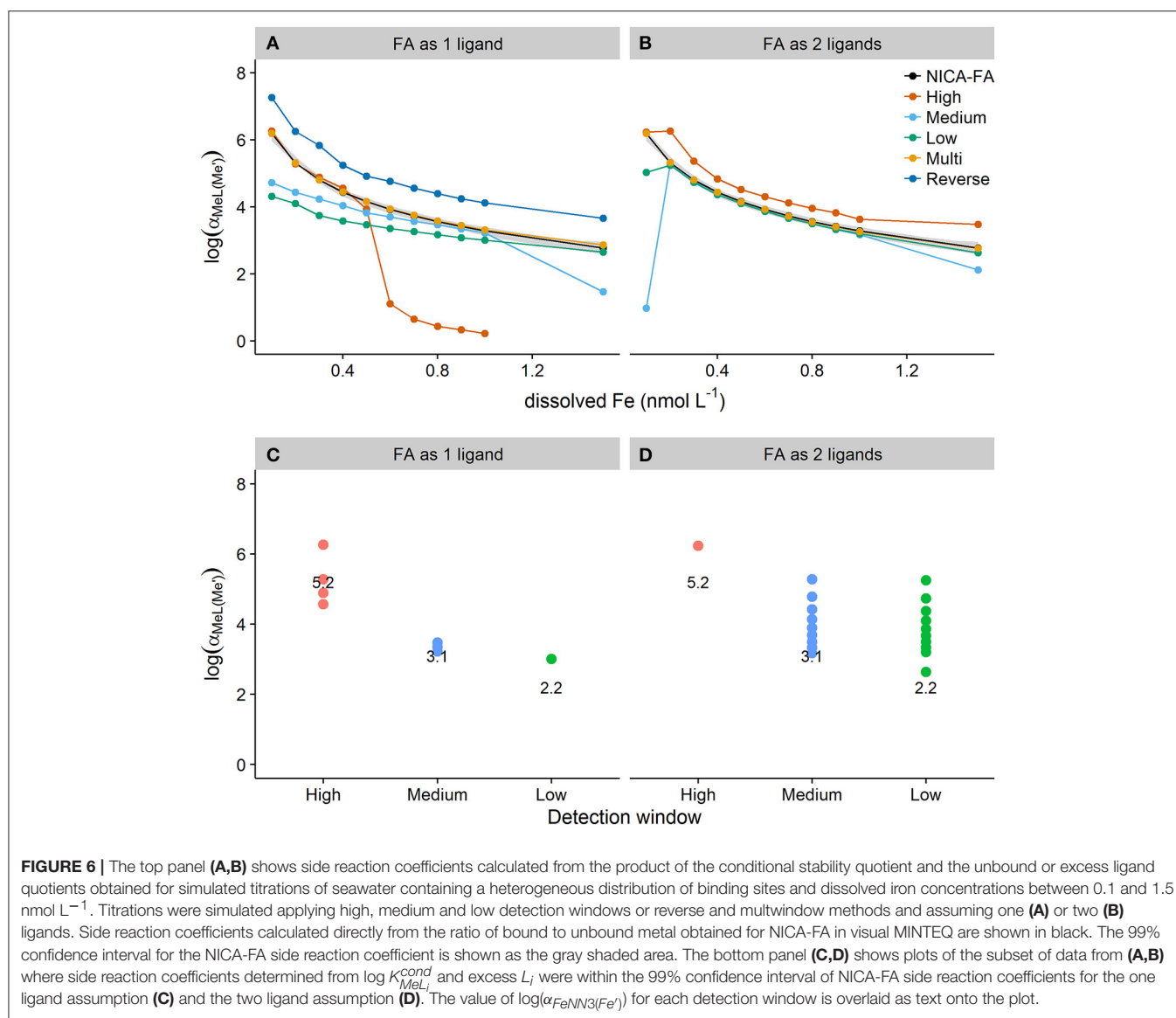
Figures 6A,B as NICA-FA values). For a one ligand assumption, the high detection window ($\alpha_{FeNN3(Fe')} = 1.46 \times 10^5$) resulted in estimates of $\alpha_{FeLi(Fe')}$ within the 99% confidence interval of the NICA-FA side reaction coefficient at DFe < 0.4 nmol L⁻¹ (**Figure 6**). At DFe concentrations over 0.4 nmol L⁻¹ [Fe'] is no longer determined by the stability quotient obtainable at this detection window and the calculated side reaction coefficient thus deviated strongly from the NICA-FA value. The medium and low detection windows underestimated α_{MeL} at low DFe but converged on the NICA-FA value of $\alpha_{FeLi(Fe')}$ as the ligand quotient approached the DFe concentration. These trends corresponded with trends observed for the fitting errors shown in **Figure 2**, which in turn relate to how much heterogeneity can be observed when applying the single detection window/one ligand assumption approach. The two ligand assumption improved the estimation of $\alpha_{FeLi(Fe')}$ for the low and medium windows, although obtaining a good estimate cannot be guaranteed as poor estimates were still obtained for medium and low detection windows at the lowest and highest DFe concentrations. A systematic bias toward an overestimate in $\alpha_{FeLi(Fe')}$ was observed for the high detection window at all but the lowest DFe concentration. This is most likely because the high detection window reduces the observable heterogeneity in the sample and this renders a two ligand fit inappropriate.

The relationship between the detection window (i.e., $\log(\alpha_{FeNN3(Fe')})$) and the range over which $\log(\alpha_{FeLi(Fe')})$ could be confidently estimated using the one ligand fit was not

linear or apparently straightforward (**Figures 6C,D**), making it difficult to recommend a particular protocol for any given sample. However, it can be seen that for a one ligand fit a higher detection window is required to estimate $\alpha_{FeLi(Fe')}$ at low iron concentrations, whilst lower detection windows are more suitable for estimation of $\alpha_{FeLi(Fe')}$ at higher DFe concentrations. Application of the two ligand model effectively widens the detection window (to three orders of magnitude above $\alpha_{FeALx(Fe')}$) and widens the range of DFe concentration that results in agreement (within the 99% confidence interval) with NICA-FA values calculated from vMINTEQ. However, it is the application of multiwindow titrations in combination with the unified fitting method that results in the best estimates of the side reaction coefficient at all DFe concentrations tested in this study—assuming both 1 and 2 binding sites. As the assumption of one ligand allows $\log(\alpha_{FeLi(Fe')})$ to be estimated to within the 99% confidence interval of $\log(\alpha_{FeLi(Fe')})$ for NICA-FA, the accompanying ligand and stability quotients can be thought of as “overall” quotients. Furthermore it appears that whilst a discrimination between two or more ligand classes is not strictly necessary for the confident calculation of $\alpha_{FeLi(Fe')}$ (Town and Filella, 2000), provided the multiwindow experimental design is utilized, it is necessary for a single window experimental design. Unfortunately, the two ligand model is often very difficult to apply, as analytical errors particularly at low concentrations of $FeAL_x$ often result in unsuccessful fits. We therefore suggest that the optimal and most robust experimental approach for metal titrations would be to estimate the side reaction coefficient using a multi-window experimental design with a one ligand model.

Side reaction coefficients can be directly determined via reverse titrations using Equation (4). However, simulation of reverse titrations with subsequent non-linear derivation of $\alpha_{FeLi(Fe')}$ shows that this approach results in overestimation of the side reaction coefficient across the range of iron concentrations examined. This is most likely because the method is biased toward the fraction of ligands that are complexed by the metal. This actually results in a significant disadvantage for reverse titrations in the case of iron since it is quite possible that the solubility product will be exceeded before full ligand saturation is achieved, resulting in further complications in interpretation (see Section The Potential Influence of an Inert Fe Fraction above). Furthermore, it is impractical to carry out reverse titrations at low iron concentrations, since the sensitivity of voltammetric methods is not high enough to register a sufficient response for confident fitting of non-linear models.

Determination of the side reaction coefficients and free metal concentrations does not provide information related to the biogeochemistry of the binding sites themselves (Hassler et al., 2017). However, in future it may be better to employ techniques that measure specific properties and concentrations of the ligand pool to investigate these compounds (Mawji et al., 2008; Velasquez et al., 2011, 2016; Boiteau et al., 2016a,b) rather than inferring properties and concentrations from measurements of the metal. It should also be noted here that as this is a simulated study, these results cannot



be used to identify the actual optimal conditions for sample analysis—multi-window analysis of real seawater samples are required to show this (Wells et al., 2013; Bundy et al., 2014). However, improved knowledge of how side reaction coefficients vary with metal concentrations, DOC and pH could provide basic information on how probable iron binding is within a sample, and thus allow us to improve our conceptual model of metal speciation in seawater. An understanding of the probability of complexation would allow for testing of the validity of the NICA model for marine DOM and also improve the NICA parametrization for trace metal complexation by marine DOC.

Considerations for Future Studies

The critical importance of determining metal speciation with improved accuracy and robustness is without doubt, yet our analysis suggests that current practices applied to the

investigation of this problem are not robust enough to account for the potential complexity of our samples. We have simulated the potential influence of the ambient metal concentration on determined complexation parameters. We have used variation in the concentration of one metal to show how the commonly determined parameters $K_{i, \text{MeL}_i}^{\text{cond}}$ and L_i can vary if metal to binding site ratio varies. It should also be noted that changes in the ratio of the metal of interest to other competing metals will also impact on $K_{i, \text{MeL}_i}^{\text{cond}}$ and L_i . In addition to these issues, there are other important factors that should be considered when studying metal speciation in natural waters. As already highlighted, a fundamental assumption of our conceptualization of metal complexation in seawater is that the system is at equilibrium. A recent review by Laglera and Filella (2015), has thoroughly covered the subject of ligand exchange processes and their (lack of) investigation in the field of marine metal speciation studies and so this topic is not covered in depth

here. Again, this issue is likely to have a particular impact on iron (Laglera and Filella, 2015) and relatively few studies have actually undertaken investigations into the kinetics of metal ligand formation and dissociation (Witter et al., 2000; Gerringa et al., 2007; Croot and Heller, 2012). Nevertheless recent field determinations of hydrothermal derived colloidal and soluble iron in the deep ocean suggest that there are processes, that could be phase changes or reactions, that likely take many years to reach equilibrium (Fitzsimmons et al., 2014, 2017). Thus, there is clearly a need to undertake more kinetic studies in order to improve our mechanistic understanding of the mode of formation and dissociation of metal complexes. As the time scale to reach true equilibrium may be very long, it is likely necessary to combine short term laboratory studies with isotopic observations in order to fully constrain the kinetics of marine trace metal chemistry. In addition, other titration approaches could be employed to improve our understanding of the importance of kinetic processes. Liu and Millero (2002) have shown that organic complexation in seawater only has an impact on solubility between pH 6 and 10. Thus it may be possible to investigate the proportion of DFe that is in short term equilibrium (<24 h) with binding sites by reversibly adjusting sample pH—i.e., undertaking pH titrations. pH titrations could also have the added bonus of allowing for increased certainty in predictions of changes in metal complexation over the pH range observed in contemporary and the future oceans.

Overall, the development of new, more sensitive methods with improved quantification of individual species would be highly beneficial to the determination of the speciation of iron and other metals in seawater. Recent developments (Mawji et al., 2008; Boiteau et al., 2016a,b; Velasquez et al., 2016) suggest we are on the cusp of a proliferation of novel techniques with exciting potential for analyzing specific metal complexes in seawater. Thus, we can look forward to a much improved knowledge base on specific metal complexes in the ocean in the coming years. In order to obtain a prognostic model of metal speciation in seawater however we also require kinetic and thermodynamic information. Electrochemistry is still the method most often employed for such studies in seawater. In this paper we have highlighted how complexation parameters change with changes in the relative concentrations dissolved metal concentration and DOC, and thus raised the question of how to assign objective meaning to the parameters $[L_i]$ and $\log K_{i, MeL_i(Me')}^{cond}$ which are commonly determined during titrations. The calculations undertaken as part of this study led to the hypothesize that determination of the side reaction coefficient for a given sample maybe informative as this overcomes potential bias resulting from *a priori* assumptions about the number of different types of binding sites. Furthermore, this study suggests that the multiwindow method coupled with the determination of overall ligand and conditional stability quotients is required for the determination of accurate side reaction coefficients at all metal concentrations, since use of one detection window will bias results obtained at either

high or low metal concentrations, depending on the detection window and data transformation approach adopted. Thus further experimental optimization of the multiwindow approach would be beneficial for application to large scale open ocean campaigns.

A further factor relates both to equilibrium and to the extrapolation of complexation data to *in-situ* conditions and in particular changes in seawater pH, which is important for modeling both present and future ocean metal biogeochemistry. pH titrations would offer potential improvements in knowledge of the competition of hydrogen ions for binding sites and also allow for the reversibility of the reactions to be assessed, and thus the question of equilibrium to be properly addressed, since pH can be manipulated in both directions.

The application of more sophisticated approaches to the conceptualization of binding with organic matter (e.g., the NICA model) would also greatly benefit our ability to model metal speciation in seawater. The complexity of these approaches poses potential limits for direct incorporation into biogeochemical models. Nevertheless, it is clear from modeling studies that improvements in our understanding of marine iron chemistry (and other metals) are required in order to develop and improve prognostic models predicting the impact of iron and other trace metals on productivity in the ocean under future climate change scenarios. Thus we must continue to seek robust solutions to these issues and continue to keep an open mind with respect to the development of new experimental designs and approaches to investigating metal speciation in seawater.

AUTHOR CONTRIBUTIONS

MG designed the study and worked on the data, LG worked on the data. Both authors interpreted the results, formulated the concepts and worked on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2017.00254/full#supplementary-material>

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