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Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work

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Abstract

A high-sensitivity cobalt speciation method was developed and applied to a profile in the North Atlantic. Method development included examining the redox chemistry of the analytical system and calibrating the electroactive cobalt ligand dimethylglyoxime (DMG) using EDTA as a model ligand. The method was applied to a depth profile at the Bermuda Atlantic Time Series Station (BATS) during a September 1999 cruise. Total dissolved cobalt, measured using adsorptive cathodic stripping voltammetry (ACSV) on ultraviolet light irradiated samples, revealed a nutrient-like profile for cobalt. Co speciation, measured using CLE–ACSV (competitive ligand exchange), showed a cobalt binding ligand concentration that was similar to that of total cobalt throughout the profile. An excess of ligand was observed in the chlorophyll maximum where *Prochlorococcus* and *Synechococcus* numbers were highest. A conditional stability constant for $CoHDMG_2$ was measured to be $\log K^{\mathrm{cond}}_{\mathrm{CoHDMG2}} = 11.5 \pm 0.3$ at pH 8.0. A pH dependence for $K^{\mathrm{cond}}_{\mathrm{CoHDMG2}}$ was observed and is consistent with model calculations based on the protonation constants for H_2DMG. The conditional stability constant for CoL was determined to be $\log K^{\mathrm{cond}}_{\mathrm{CoL}} = 16.3 \pm 0.9$ and total ligand concentrations varied from 9 to 83 pM as calculated by a one-ligand non-linear fit using the Levenberg–Marquardt algorithm. Alternate interpretations of the data are discussed, including the possibility for an underestimation of ligand concentrations and stability constants caused by the existence of Co(III) ligands, and kinetic and thermodynamic competition for natural ligands by Ni and Co(II).

Keywords: Cobalt; Electrochemistry; Sargasso Sea; Complexation; Bioavailability; Ligand

1. Introduction

Understanding the biogeochemistry of Co in seawater requires the ability to measure its oceanic chemical species. Zinc and cadmium have both been shown to be strongly complexed in seawater (Bruland, 1989; Bruland, 1992). However, due to the

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exceedingly low total dissolved cobalt concentrations in the picomolar range ($4–120 \times 10^{-12}$ M) (Jickells and Burton, 1988; Martin and Gordon, 1988; Martin et al., 1989), open ocean cobalt speciation has never been rigorously studied. Strong complexation of cobalt has been measured in the Scheldt estuary (Zhang et al., 1990) at nanomolar total cobalt concentrations, and Donat and Bruland (1988) have reported data suggestive of strong complexation of cobalt in open ocean samples. Since then, improvements in the analytical methodology for total cobalt measurements have lowered the detection limit by 1–2 orders of magnitude (Bobrowski, 1989; Bobrowski, 1990; Bobrowski and Bond, 1992; Herrera-Melian et al., 1994; Vega and van den Berg, 1997). With this added sensitivity, the 3 pM detection limit for electrochemical methods is below open ocean cobalt concentrations. However, significant method development has been required to adapt this total cobalt measurement for speciation work. This included verifying that the reagents were not oxidizing Co(II) to Co(III), and calibrating the conditional stability constant for $Co(HDMG)_2$ in seawater with this high-sensitivity method.

The use of cobalt and nickel dioxime complexes in analytical electrochemistry has been an area of active research for more than 50 years (Baxter et al., 1998). Dioxime complexes, such as those with dimethylglyoxime (DMG), are characterized by the presence of oxime groups (C=N–OH) and have provided significant enhancement of cobalt(II) reduction signal relative to the reduction of Co^{2+} cations or other cobalt organic ligand complexes. It is this enhancement of signal, coupled with other refinements in protocol such as computer assisted high speed scanning rates and nitrite catalytic activity (Bobrowski, 1989; Bobrowski and Bond, 1992) that have brought the detection limit and analysis time within the reach of the picomolar concentrations observed in oligotrophic marine environments. However, a mechanistic explanation for the enhancement in signal provided by the complexation of cobalt and nickel by dioxime ligands has eluded researchers until only very recently. Baxter et al. (1998) and Ma et al. (1997) make a convincing case that the unusual signal enhancement found when using dioxime ligands is caused by the reduction of the two dioxime ligands (partial reduction of the 2 C=N per dioxime molecule) in addition to the reduction of the metal center. This mechanism provides 10 electrons per Co molecule as opposed to the typical two or three electrons from the metal ion obtained during most high-sensitivity cathodic stripping voltammetry methods. It is a fortunate coincidence for environmental electrochemists that this cobalt dioxime reduction method is available since cobalt tends to be an order of magnitude lower in concentration than most other transition metals.

In order to make cobalt speciation measurements at oceanic concentrations, it is necessary to consider the redox state of cobalt. We were concerned that the use of nitrite as an additional signal enhancer could inadvertently oxidize Co(II) to Co(III), which was hypothesized by Vega and van den Berg (1997). The resulting system could be experimentally intractable for cobalt speciation due to the presence of two redox states each with its own set of thermodynamic constants. We used spectrophotometric methods to show that nitrite cannot oxidize Co(II) EDTA as a model for Co(II)$HDMG_2$ on time scales that are of experimental concern.

While the formation of Co(III) $HDMG_2^+$ complexes is possible (Costa et al., 1987), it is unknown if Co(III) $HDMG_2^+$ complexes are also electroactive at the low concentrations typical of seawater analyses. However, it is well known that Co(III) chelates have much higher stability constants than their corresponding Co(II) complexes. It is often stated that such Co(III) chelates once formed are chemically inert to further reaction (Ogino and Ogino, 1982). Moreover, the oxidation of inorganic and many organic Co(II) species is often kinetically slow, despite being thermodynamically possible at ambient oxygen concentrations. However, Co(II) oxidation does occur in natural waters. In coastal waters, Co(II) oxidation by manganese oxidizing bacteria appears to be the dominant removal pathway for dissolved Co (Moffett and Ho, 1996). In that study, Co(II) oxidation was associated with precipitation on a manganese oxide. However, there is no evidence for Co redox cycling in the dissolved phase in seawater. Moffett and Ho (1996) showed that in the open ocean, Co uptake is probably non-oxidative and dominated by phytoplankton. Although there is little experimental evidence, it is presumed that open ocean cobalt exists as Co(II) except for a small fraction as

cobalamin (vitamin B_{12}) (Menzel and Spaeth, 1962) which occurs as a Co(III) complex (Glusker, 1995).

The complexation of cobalt in the open ocean has been suggested in previous studies of open ocean total cobalt concentrations (Donat and Bruland, 1988; Vega and van den Berg, 1997). Cobalt is an essential micronutrient for many key phytoplankton species (Sunda and Huntsman, 1995; Saito, 2000). Given the exceedingly low total dissolved concentrations of cobalt in seawater, an understanding of the chemical species of cobalt present in seawater is crucial to improving our understanding of how phytoplankton acquire this necessary micronutrient.

2. Theory

A variety of electrochemical techniques have been used to achieve the sensitivity needed for the determination of trace elements found in seawater, including adsorptive cathodic stripping voltammetry (ACSV) and anodic stripping voltammetry (ASV). In this study, we have used ACSV for total cobalt measurements, using the method of standard additions, and ACSV coupled with competitive ligand exchange equilibria for cobalt speciation determinations (CLE–ACSV).

2.1. Competitive ligand exchange

Competitive ligand exchange methods determine the extent to which a metal is bound to natural organic complexes by competition between the electroactive synthetic reagent ligand and natural ligands (Eqs. (1) and (2)). Competitive ligand exchange refers to the equilibrium that is established between natural Co ligands (L) and the added electroactive ligand (DMG in this study). Inherent in this method is the assumption that no oxidation or reduction of cobalt occurs during the time needed to achieve equilibrium.

$$\mathrm{Co(II)L}^{n-} + 2\mathrm{HDMG}^{-} \xleftrightarrow{\mathrm{CLE}} \mathrm{Co(II)HDMG}_2 + \mathrm{L}^{n-2} \quad (1)$$

This competitive ligand equilibrium is then measured using a hanging mercury drop, which accumulates $CoHDMG_2$ on the surface of the drop by applying a negative potential for a programmed deposition time. The accumulated $CoHDMG_2$ is reduced by scanning from the deposition potential to a more negative potential (Eq. (3)). The resultant peak is proportional to the concentration of $CoHDMG_2$ in solution and to the deposition time. In addition to the reduction of the cobalt ion, the $HDMG^-$ groups are also irreversibly reduced to 2,3-bishydroxylaminebutane (DHAB, Baxter et al., 1998). The reaction at the electrode surface is:

$$\mathrm{Co(II)HDMG}_2 \xrightarrow{\mathrm{Hg-deposition}} \mathrm{Co(II)HDMG}_{2\ \mathrm{adsorbed}} \quad (2)$$

$$\mathrm{Co(II)HDMG}_{2\ \mathrm{adsorbed}} + 10\mathrm{e}^{-} \xrightarrow{\mathrm{CSV}} \mathrm{Co(O)} + 2\mathrm{DHAB} \quad (3)$$

The competition between the natural and synthetic ligands is influenced by their stability constants and the concentrations of each ligand. Due to the unique analytical matrix of seawater and a lack of sufficient literature data, conditional stability constants that have been calibrated at seawater pH and ionic strength are used in lieu of literature thermodynamic stability constants (Eqs. (4) and (5)).

$$K^{\mathrm{cond}}_{\mathrm{DMG}} = \frac{[\mathrm{CoHDMG}_2]}{[\mathrm{Co}^{2+}][\mathrm{DMG}']^2} \quad (4)$$

$$K^{\mathrm{cond}}_{\mathrm{CoL}} = \frac{[\mathrm{CoL}]}{[\mathrm{Co}^{2+}][\mathrm{L}^{2-}]} \quad (5)$$

In this study, $[\mathrm{DMG}'] = \mathrm{DMG}_{\mathrm{TOT}}$, since $CoHDMG_2$ and $NiHDMG_2 \ll \mathrm{DMG}_{\mathrm{TOT}}$. The thermodynamic stability constant for DMG with Co is not well characterized. The only reference we are aware of for $CoHDMG_2$ is a conditional stability constant ($\log K^{\mathrm{cond}}_{\mathrm{CoHDMG2}}$) by Zhang et al. (1990) of 12.85 ± 0.10. Moreover, this previous calibration was conducted in coastal waters with the traditional low speed scan protocol, much higher Co concentrations, and at a pH of 8.7. The recently developed high speed scan total cobalt protocols utilize high-speed scans ($10\ \mathrm{V\ s^{-1}}$) and a catalytic reaction. The relative concentrations and thermodynamic stability con-

stants of the natural ligands and the synthetic ligand (DMG) determine the partitioning of cobalt species in Eq. (1). With titration of a seawater sample with cobalt, the concentrations and strength of the natural ligands can be estimated.

2.2. Model ligands—calibration of speciation methodology

The non-electroactive synthetic ligand EDTA was used to determine a $K^{cond}_{CoHDMG2}$ for DMG. Constant cobalt and DMG concentrations were titrated with increasing concentrations of EDTA. Mass law equations for $CoEDTA^{2-}$, $CaEDTA^{2-}$ and $MgEDTA^{2-}$ complexes were taken from Martell and Smith (1993). The mass balance for cobalt in the EDTA calibration is:

$$[Co_{TOT}] = [Co'] + [CoHDMG_2] + [CoEDTA^{2-}] \quad (6)$$

Where Co′ indicates the summation of the important inorganic cobalt species in seawater:

$$[Co'] = [Co^{2+}] + [CoCl^{+}_{(aq)}] + [CoOH^{+}_{(aq)}] + [Co(OH)_{2(aq)}] + [Co(OH)^{-}_{3(aq)}] \quad (7)$$

X is then assigned to be the fraction of cobalt masked by binding to the non-electroactive EDTA, where i is the $CoHDMG_2$ reduction peak current, measured by peak height and in the presence $(i_{p,i})$ and absence $(i_{p,o})$ of EDTA.

$$X = \frac{i_{p,i}}{i_{p,o}} \quad (8)$$

Peak height can be related to the concentration of the electroactive $CoHDMG_2$ complex by the sensitivity (S), derived from the slope of the cobalt addition plot. Using the cobalt mass balance equation, $i_{p,i}$ (and $i_{p,o}$, where $[CoEDTA^{2-}] = 0$) is equal to:

$$i_{p,i} = S[CoHDMG_2] = S(Co_{TOT} - Co' - CoEDTA^{2-}) \quad (9)$$

$$\frac{i_{p,i}}{i_{p,o}} = \frac{S(CoHDMG_2)_{p,i}}{S(CoHDMG_2)_{p,o}} = \frac{S(Co_{TOT} - Co' - CoEDTA^{2-})}{S(Co_{TOT} - Co')} \quad (10)$$

Algebraic manipulations of Eq. (10) using mass law and mass balance equations yields:

$$X = \frac{K^{cond}_{DMG}[DMG']^2}{\left(K^{cond}_{DMG}[DMG']^2 + K^{cond}_{EDTA}[EDTA']\right)} \quad (11)$$

where $DMG' = DMG_{TOTAL}$ because $DMG' \gg Co_{TOT}$.

$$X = \frac{CoHDMG_2 - K^{cond}_{EDTA}Co'[EDTA']}{CoHDMG_2} \quad (12)$$

The conditional stability constant for $CoHDMG_2$ can then be calculated by fitting the equilibrated EDTA titration with:

$$K^{cond}_{DMG} = \frac{XK^{cond}_{EDTA}[EDTA']}{\left([DMG']^2 - X[DMG']^2\right)} \quad (13)$$

TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid hydrochloride hydrate) was used in this study as a model ligand for strong natural ligands. Unlike $CoHDMG_2$, TETA is not electroactive using the electrochemical parameters of our analytical system, so we calibrated CoTETA against DMG using the $CoHDMG_2$ conditional stability constant. This can be done using Eq. (13) after substituting K^{cond}_{TETA} for K^{cond}_{EDTA} and rearranging:

$$K^{cond}_{TETA} = \frac{K^{cond}_{DMG}\left([DMG']^2 - X[DMG']^2\right)}{(X[TETA'])} \quad (14)$$

2.3. Calculation of CoL, L_T, and K^{cond}_L

The calculations of the concentrations and conditional stability constants for natural ligands in previous work have utilized Langmuir isotherms (also described as van den Berg/Ruzic plots), Scatchard plots, and non-linear fits (Gerringa et al., 1995; Moffett, 1995). All of these algorithms begin with a calculation of CoL, which is a simple mass balance:

$$[CoL] = [Co_{TOT}] - [CoHDMG_2] - [Co'] \quad (15)$$

where Co_{TOT} and $CoHDMG_2$ are measured in separate analyses and Co′ is insignificant.

The equations for Langmuir isotherm calculations of L_T, and K^{cond}_{CoL} utilize a ligand mass balance Eq.

(16) substituted into a conditional stability constant equation to produce a linearized Eq. (17):

$$[L_{TOT}] = [L'] + [CoL] \quad (16)$$

$$\frac{[Co^{2+}]}{[CoL]} = \frac{[Co^{2+}]}{[L_{TOT}]} + \frac{1}{K^{cond}_{CoL}[L_{TOT}]} \quad (17)$$

where $x = Co^{2+}$, $y = Co^{2+}/CoL$, the slope is equal to the inverse of L_{TOT}, and the y-intercept is equal to $1/(K^{cond}_{CoL}\ L_{TOT})$.

The calculations of K^{cond}_{CoL} are sensitive to the value of S in calculating Co^{2+}. In work with Fe-salicylaldoxime (Rue and Bruland, 1995) and Cu-bzac (Moffett, 1995) the slope of equilibrated titrations approached that of titrations in ultraviolet light irradiated seawater. However, in this study we noticed that S was substantially lower in surface waters of the Sargasso than in UV-irradiated samples. Such a decrease can reflect matrix effects on S caused by surfactants; alternatively, an apparent decrease in S may be caused by the presence of additional chelators in excess of the highest Co concentration in the titration. The latter effect does not influence the actual value of S, so to distinguish the two, we determined S from a special set of titrations with no equilibration time. The assumption was that Co(II) complexes with natural ligands would not have time to form, but matrix effects would be unchanged. This approach has worked well for Cu in previous reports (Lucia et al., 1994; Moffett and Brand, 1997). S determined in this way is hereafter described as non-equilibrated S. Calculations of ligand concentration and stability constants were made using the non-equilibrated S, determined at each depth, in Eq. (9). Because Langmuir isotherm fits of titration data can lead to an increase in errors at the high concentration range of titrations, non-linear fits of titration data have been invoked as a means to reduce the error on estimates of K and L and have been shown to yield similar results to the traditional Langmuir isotherm algorithms (Gerringa et al., 1995). We utilized a single ligand non-linear fit in MATLAB using a Levenberg-Marquardt algorithm to solve for the parameters K and L using the titration data for CoL and Co^{2+} in the following equation:

$$\frac{[CoL]}{[Co^{2+}]} = \frac{[L]K^{cond}_{CoL}}{1 + K^{cond}_{CoL}[Co^{2+}]} \quad (18)$$

3. Materials and methods

3.1. Instrumentation and reagents

We used a high-speed scan protocol with a Metrohm 663 hanging mercury drop electrode stand with a teflon sample cup and an Eco-chemie μAutolab computer interface. Dimethylglyoxime (DMG) from Aldrich was recrystallized in Milli-Q (Millipore) water and 10^{-3} M EDTA (Sigma Ultra) to remove impurities, dried and redissolved in HPLC grade methanol to a concentration of 0.1 M. Final concentrations of DMG in the seawater samples were 0.000235 M. Solutions of 1.5 M sodium nitrite (Fluka Puriss) were equilibrated overnight with prepared Chelex-100 beads (Price et al., 1988/1989) to remove metal contaminants; 1.5 ml of this nitrite solution was added to 8.50 ml of seawater sample. A 0.5 M EPPS buffer solution (Fisher) was cleaned by running through a column with 3 ml of clean Chelex-100 beads (BioRad) and diluted in the sample to 0.0025 M. Cobalt additions for standard additions and speciation titrations prepared from Fisher certified $Co(NO_3)_2$ stock solution were freshly diluted in polymethylpentene volumetric flasks of Milli-Q water every few days.

The μAutolab analysis protocol involved a 3.25-min purge with filtered 99.999% N_2, a 90-s deposition time at -0.6 V, a 15-s equilibration period followed by a high-speed negative scan at 10 V s^{-1} from -0.6 to -1.4 V. The linear sweep wave form was used, and stirrer speed 5, and drop size of 0.52 mm^2. Cobalt signal was measured as the peak height from the baseline. Titration samples were analyzed in order of increasing concentration and rinsed with 10% HCl then pH 3 HCl (Baker Ultrex II) at the completion of each titration. The teflon sample cup was preconditioned with filtered seawater before adding equilibrated samples.

3.2. Sample collection and handling

Seawater samples were collected from the Sargasso Sea using acid-cleaned 10 L Go-Flo bottles, kevlar wire and PVC-messengers. Seawater was pumped into a positive pressure clean van with 0.2

μm filtered N_2 gas and teflon tubing, and was immediately filtered through acid-cleaned 0.2 μm Nuclepore polycarbonate filters into teflon bottles for speciation samples and polyethylene bottles for total dissolved samples. Samples were refrigerated in darkness until analysis. All labware was soaked with the acidic detergent Citranox and Milli-Q water solution overnight, rinsed with copious Milli-Q water, heated for 36 h with 1 M HCl (Baker Instra-Analyzed Grade) at 60°C, then rinsed three times and soaked with pH 3 HCl (Baker Ultrex II).

3.3. Total cobalt measurements: analytical standards, reagent blank and field samples

To measure the analytical blank, cobalt-free seawater was created by ultraviolet light irradiating Sargasso seawater (UV-SSW), followed by equilibration with clean chelex-100 resin beads (Price et al., 1988/1989). This seawater was then UV-irradiated again to degrade any iminodiacetate groups that were released from the chelex. This final irradiation step proved to be important at these picomolar levels, despite the rigorous protocol used to prepare the chelex.

The accuracy and precision of our analytical methodology was verified using CASS-3 Coastal Seawater Reference material and NASS-5 (National Research Council Canada-Institute for National Measurements). The CASS-3 seawater was diluted with Milli-Q water from a cobalt concentration of 700 ± 150 pM to 310 ± 69 pM, where the error is the 95% confidence interval of four different analytical techniques used by the National Research Council Canada. The NASS-5 was analyzed without dilution. The reference materials were UV-irradiated for 3.0 h in covered quartz vessels, then transferred to LDPE bottles and the pH was raised to between 7.5 and 9 using Gold Label NaOH (Johnson–Matthey).

Total samples were stored in darkness at 4°C after filtration until analysis immediately upon returning from sea. Total cobalt measurements on field samples were UV-irradiated at ambient pH for 3.0 ± 0.1 h in quartz vials covered with plastic caps, parafilm, and aluminum foil to prevent exposure to dust and losses by evaporation. Analysis was carried out 2–24 h after irradiation. This analytical protocol for total cobalt analysis with UV-irradiation and without strong reducing agents was shown to be effective by Vega and van den Berg (1997).

3.4. Calibration of DMG and TETA

Determination of the conditional stability constant of DMG relative to EDTA was performed using Na_2EDTA (Sigma Ultra) and 150 pM of $Co(NO_3)_2$ on three different occasions with separately prepared reagents. In all experiments the EDTA–DMG cobalt mixtures were allowed to equilibrate for between 12 and 24 h in teflon bottles and resulted in similar value for $K^{cond}_{CoHDMG2}$. The pH was measured using an Orion pH electrode calibrated using pH 7 and 10 buffers. In the first of three calibration experiments, the nitrite and EPPS were added prior to the equilibration period, while in the other two experiments these reagents were added immediately before analysis.

TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid tetrahydrochloride hydrate, Aldrich) was calibrated against 0.5 mM DMG by equilibration with 100 pM $CoCl_2$, EPPS, and nitrite in UV-irradiated seawater.

3.5. Co(II) oxidation experiments

Spectrophotometry experiments used a Hewlett Packard UV-VIS spectrophotometer and acid cleaned 1 cm pathlength quartz cuvettes. Solutions of Na_4EDTA (Fisher Biotech electrophoresis grade), $CoCl_2$, and Fluka Puriss $NaNO_2$ were prepared and allowed to equilibrate overnight before addition of 0.61 M Baker Ultrex H_2O_2 to cuvettes immediately before analysis.

3.6. CLE–ACSV Co speciation titrations

Equilibrations of seawater with cobalt and DMG were prepared in clean teflon bottles. 8.50 mL of filtered seawater was equilibrated with 0–510 pM of $Co(NO_3)_2$ for 1–2 h, followed by an equilibration with 20 μL of 0.1 M DMG (0.000235 M) for ≥ 12 h. The teflon sample cup was preconditioned with

filtered seawater from the same sample, and the EPPS and nitrite were added immediately before analysis (50 μl 0.5 M EPPS pH 8.0, and 1.50 mL NO_2^-). Titrations of irradiated Sargasso seawater (UV-SSW) were conducted with the same parameters and concentrations of reagents but without the equilibration periods. DMG kinetics in UV-SSW are extremely rapid due to the absence of natural ligands. Titrations were also conducted without equilibration time on filtered fresh Sargasso seawater samples by addition of reagents to the seawater sample in the electrochemical cup and direct additions of $Co(NO_3)_2$.

3.7. Flow cytometry analysis of Prochlorococcus and Synechococcus

Flow cytometry samples were collected by Niskin bottle and immediately analyzed at sea. Beads (0.474 μm) were added as an internal standard immediately prior to analysis on a modified FACScan (Dusenberry and Frankel, 1994). A syringe pump was used (Harvard Apparatus) with a flow rate of 10 μL/min, and 15 000 counts were collected on all samples.

4. Results

4.1. Method development: accuracy, precision, and blank

The accuracy of our total cobalt measurement protocol was determined using CASS-3 and NASS-5 coastal and open ocean reference seawaters, respectively, from the National Research Council Canada (NRCC). Standard additions analysis to diluted CASS-3 seawater with Fisher certified 1000 ppm $Co(NO_3)_2$, yielded a concentration of 249 ± 7 pM. The error was based on propagation of uncertainty associated with least-squares linear fit of the standard additions. Standard addition analysis of NASS-5 standards yielded a concentration of 169 ± 16 pM. The error here gives the standard deviation for three replicate analyses. These results showed excellent agreement with values reported by NRCC (Table 1): our results were within the NRCC error range and were slightly lower than their values, consistent with our low reagent blank measurements.

Table 1
The accuracy of total cobalt electrochemical measurements verified by standard additions of National Research Council of Canada reference seawater

Reference standard	NRCC value (pM)	Co by ACSV (pM)	Number of analyses
CASS-3 coastal seawater (diluted)	315 ± 75[a]	249 ± 7[a]	2
NASS-5 open ocean seawater	187 ± 51	169 ± 16	3

[a]NRCC error is calculated from different analytical techniques; our errors are from the standard deviation from triplicate samples or error propagation from the linear fit of titration data.

The analytical blank of our reagents and equipment determined using twice UV-irradiated chelexed SSW was close to detection limit (Fig. 1A). Raw spectra were analyzed by both peak height and 1 s scan baseline subtraction followed by peak height measurements; in most cases peak heights without baseline correction yielded better precision than those with baseline corrections due to slight baseline variations between samples (Fig. 1B and C). The Co blank was determined for each batch of reagents and subtracted from titration results.

4.2. Calibration and pH effects

There are few published data for the stability constant of dimethylglyoxime with cobalt(II). A calibration performed by Zhang et al. (1990) with EDTA yielded a conditional stability constant of log $K^{cond}_{CoHDMG2}$ of 12.85 ± 0.10 at pH 8.7 and 35‰ salinity. We conducted three calibration experiments with EDTA as a model ligand using UV-irradiated Sargasso seawater that showed a pH dependent conditional stability constant. Log $K^{cond}_{CoHDMG2}$ at pH 8.0 in our experimental system was determined to be 11.5 ± 0.3, where the error is calculated from the standard deviation of the three stability constants from these three separate calibration experiments each with freshly prepared reagents. Log $K^{cond}_{CoHDMG2}$ of 10.6 ± 0.01 at pH 7.6 was determined, representing a 1-log unit shift in conditional stability constant per half pH unit (Fig. 2A and Table 2). This pH dependence was

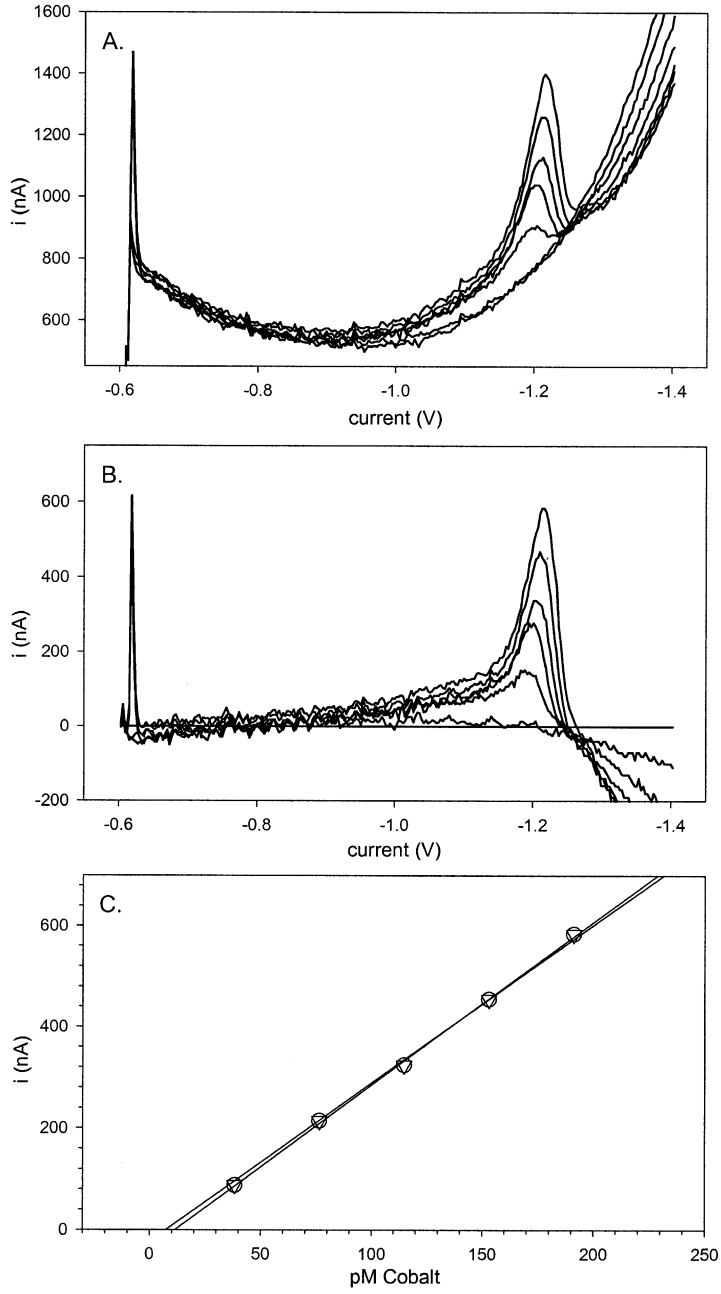


Fig. 1. (A) Blank determination: ACSV spectra of an UV-irradiated and chelexed Sargasso seawater sample with standard additions of 38.3 pM $Co(NO_3)_2$. Nickel peaks at −1.05 V are negligible, and cobalt peaks are at −1.17 V. (B) Baseline subtraction of the same data set using 1-s deposition time scan as a baseline. (C) Linear regression of baseline corrected peak height data with all points (triangles), and without the 0 pM point (circles), yielding a Co blank of 11 and 7 pM, respectively, for this batch of reagents.

predicted by calculations in which we modeled EDTA–DMG competition experiments as a function of pH (Fig. 2B). In these calculations we used protonation and binding constants for EDTA with

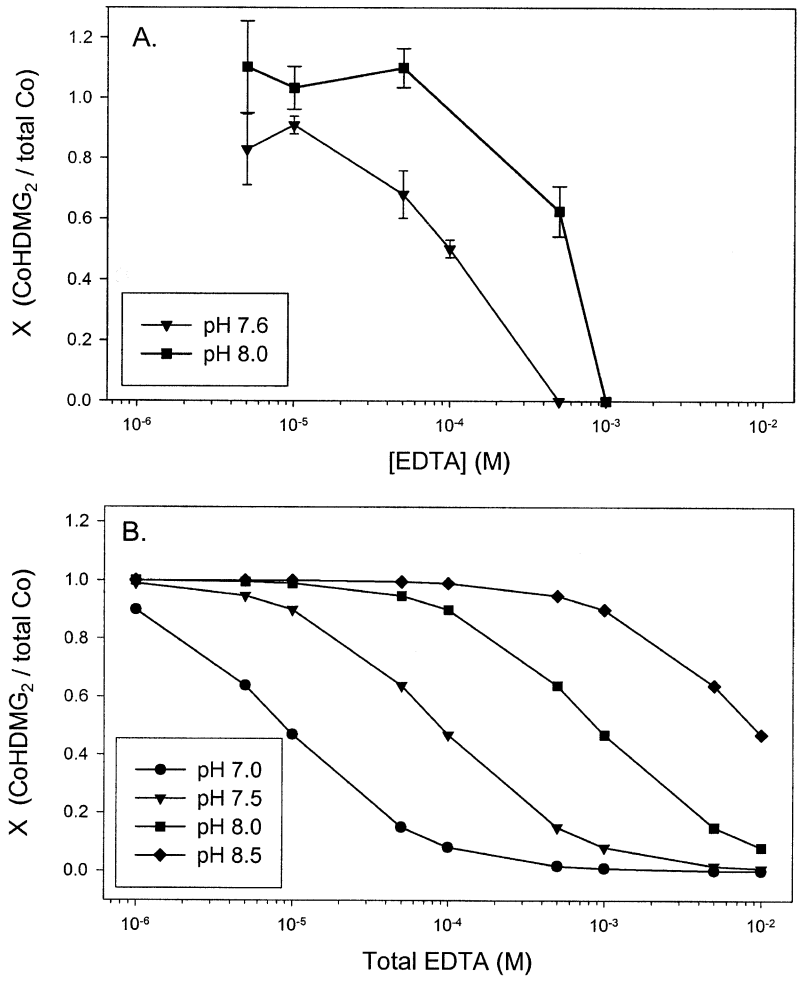


Fig. 2. (A) Calibration of the conditional stability constant of $CoHDMG_2$ using EDTA as a model ligand. [DMG] = 0.0005 M and Co_{TOT} = 191 pM. The higher pH causes an increase in $\log K_{CoHDMG2}$ and an increase in sensitivity. (B) Model cobalt speciation calculations showing the influence of protonation constants for H_2DMG on $K^{cond}_{CoHDMG2}$. K_{a1} and K_{a2} for H_2DMG are $10^{10.45}$ and $10^{11.9}$ from Martell and Smith (1993) were used with the calibrated $K^{cond}_{CoHDMG2}$ presented in Table 2. This use of protonation constants in these model calculations agrees well with our data in (A), and explains the discrepancy between our $K^{cond}_{CoHDMG2}$ constant and that of Zhang et al. (1990) as described in the text.

Co^{2+}, Mg^{2+}, and Ca^{2+} from the Martell and Smith (1993) database at $\mu = 0.5$, and protonation and a Co^{2+} conditional stability constant from Martell and Smith (1993) and this study, respectively. We were unable to find binding constants for DMG with major seawater divalent cations, calcium and magnesium. However, these ions tend to have a preferential affinity for carboxylic acid groups rather than the nitrogen ligands found in DMG. We assume that DMG does not participate in significant binding with Ca^{2+} and Mg^{2+}. Nevertheless, the conditional stability constants measured here would incorporate any effect of major ion binding.

The squared $HDMG^-$ term in the $CoHDMG_2$ stability constant equation (Eq. (4)) creates the strong pH dependence, where a one unit decrease pH change causes a two order of magnitude change in the conditional stability constant of $CoHDMG_2$

Table 2
Conditional stability constants for DMG and TETA calibrated using EDTA

Compound	Stoichiometry	$\log K^{cond}$
Co(II)Dimethylglyoxime (pH = 8.0)	[ML2]/[M][L]^2	11.5 ± 0.3[a]
Co(II)Dimethylglyoxime (pH = 7.6)	[ML2]/[M][L]^2	10.6 ± 0.01[b]
Co(II)TETA	[ML]/[M][L]	11.2 ± 0.1

[a] Error from standard deviation of mean results of three separate calibration curves.
[b] Error from standard deviation of individual points on titration curve $n \geq 3$.

($K^{cond}_{CoHDMG2}$). The difference in our conditional stability constant for $CoHDMG_2$ from that of Zhang et al. (1990) is explained by the differences in pH at which $CoHDMG_2$ was calibrated: our study was conducted at ambient seawater pH (8.0–8.1) with the addition of EPPS buffer immediately before analysis, while the Zhang et al. calibration was performed a pH 8.7 by equilibration with the triethanolamine (TEA)/NH_4Cl buffer. According to our calculations, this increase in pH would result in a conditional stability constant that is 1.4 log units higher, consistent with the difference between our value for $\log K^{cond}_{CoHDMG2}$ of 11.5 ± 0.3 and Zhang et al.'s value of 12.85 ± 0.10.

For Co(II)TETA, $\log K^{cond}_{CoTETA} = 11.2 \pm 0.1$ by calibration against DMG using the conditional stability constant measured in this study. CoTETA is a mono complex, while $CoHDMG_2$ is a bis complex;

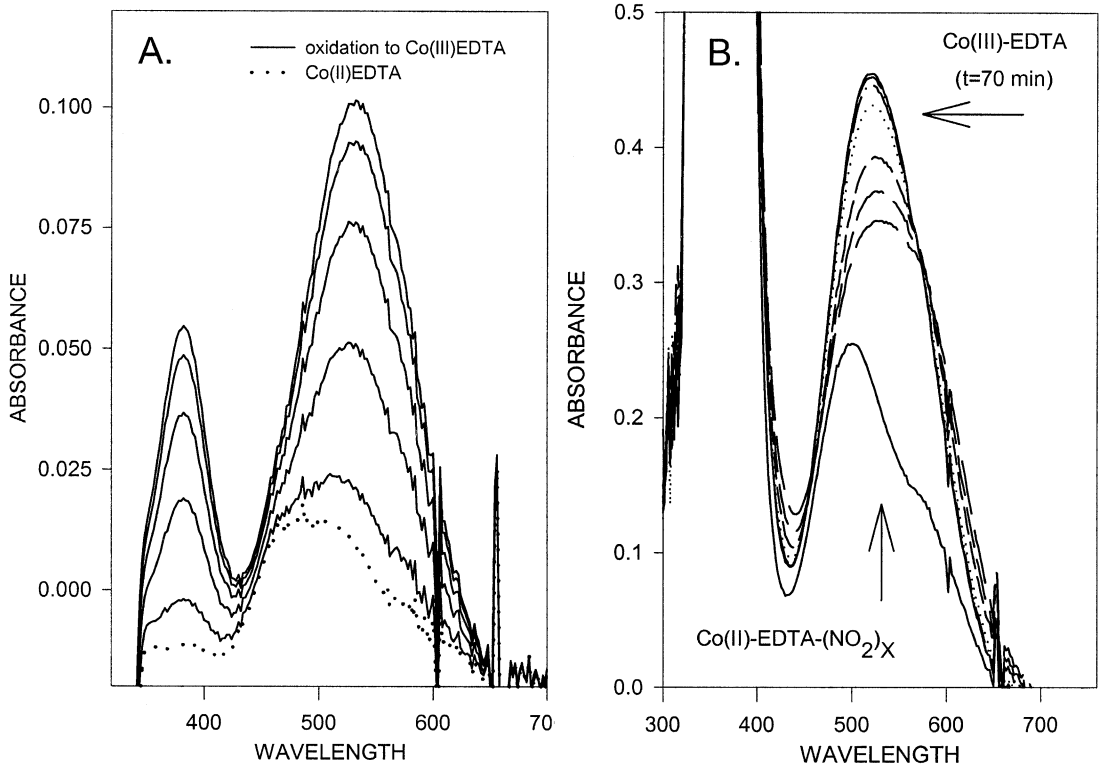


Fig. 3. (A) Oxidation of Co(II)EDTA to Co(III)EDTA by H_2O_2 can be observed on a UV-VIS spectrophotometer. Spectra are shown from before adding H_2O_2 until 48 min to illustrate the progression of the reaction. Co(II)EDTA has a peak at 490 nm and Co(III)EDTA has two distinct peaks at 380 and 535 nm. (B) Equilibration of nitrite with Co(II)EDTA produces a new spectra indicating a new complex has formed CoEDTA–$(NO_2)_x$, which can be oxidized to a Co(III)EDTA complex with the characteristic 535 nm maxima. Free nitrite has a broad absorption peak at 360 nM.

thus despite similar stability constants, the CoTETA complexes are significantly stronger than $CoHDMG_2$.

4.3. Method development: Co(II) and Co(III) redox chemistry

The high-speed scan/catalytic reaction utilizes nitrite to catalytically increase the reduction current. The reaction mechanism for this catalytic activity is unknown (Bobrowski and Bond, 1992; Vega and van den Berg, 1997). There have been several mechanisms proposed that account for the unusually high electrochemical signal of Co–dioxime complexes. Ma et al. (1997) argued convincingly that in addition to the reduction of the Co^{2+}, the dioxime ligand is itself also being reduced with $8e^-$ per two dioxime groups, for a total of $10e^-$ per $CoHDMG_2$ complex. However, the use of nitrite and its influence on the reaction mechanism have not been carefully explored yet. An important question that pertains to the development of a speciation method is whether the nitrite acts as a ligand for Co(II) or if it actually can oxidize cobalt(II) complexes to cobalt(III) complexes on experimental time scales.

A spectrophotometric study of Co redox chemistry was carried out using DMG and EDTA as ligands to explore the influence of nitrite on cobalt coordination and redox chemistry. At millimolar concentrations, $CoHDMG_2$, $Co(II)EDTA^{2-}$, $Co(III)EDTA^-$, and $CoEDTA(NO_2)_x$ have distinct absorbance spectra. Initially, we examined the oxidation of $Co(II)EDTA^{2-}$ to $Co(III)EDTA^-$ by H_2O_2, a ubiquitous oxidant in marine surface waters. EDTA was selected because the reaction can be monitored using a broad $Co(II)EDTA^{2-}$ peak at 472–520 nM and a Gaussian peak at 535 nM for $Co(III)EDTA^-$ (Fig. 3A) and because there is a strong thermodynamic driving force for this reaction caused by the much higher stability constant of the Co(III) chelate ($\log K_{Co(II)EDTA} = 16.45$, $\log K_{Co(III)EDTA} = 41.4$ from Martell and Smith, 1977; Xue and Traina, 1996, respectively). This reaction was very slow, requiring several hours for completion at millimolar H_2O_2 levels. A Co(II)EDTA complex was equilibrated overnight with NO_2^- yielding a unique spectra, suggesting coordination of nitrite had occurred (Fig. 3B). It is likely that the valence of Co in the new complex was still $+2$, since subsequent addition of H_2O_2 led to formation of the familiar Co(III)EDTA complex spectrum. Presumably, a mixed $CoEDTA(NO_2^-)_x$ complex was formed. These results suggest that nitrite probably does not induce a redox shift when added to a solution containing organically complexed Co(II), but may influence Co(II) speciation, even in the presence of a polydentate ligand like EDTA. It is possible that the addition of nitrite could influence the apparent stability constant of DMG or naturally occurring ligands through mixed complex formation. We think this is unlikely because conditional stability constants for $CoHDMG_2$ obtained here and by Zhang et al. (who did not use nitrite) are consistent when pH effects are accounted for (above), and our estimates of conditional stability constants for natural ligands are not substantially higher than those of Zhang et al.

4.4. Method development: wall effects

Given the low concentration of total cobalt and potential natural ligands we used a small addition of ^{57}Co radiotracer to our teflon equilibration bottles to determine if there were losses of Co-complexes to the surface area of the bottles during the equilibration period. An equilibration bottle with fresh filtered 1500 m Sargasso seawater was prepared in the same manner as those used for titrations. An aliquot of ^{57}Co ($^{57}CoCl_2$, Isotope Products) was added before adding DMG to make a final concentration of 100 pM ^{57}Co. 0.10 ml aliquots were withdrawn in triplicate at $t = 0$ and after 4 days. Samples were counted by germanium gamma counter (Canberra). We measured $97.98 \pm 2.1\%$ recovery of added ^{57}Co after 5 days of equilibration (28.39 ± 0.60 counts/s, $n = 3$, 27.81 ± 0.34 counts/s, $n = 4$, for t_0 and t_1, respectively), indicating no wall effects in our equilibrations.

4.5. Sargasso Sea field titrations

Titrations were conducted at sea and immediately upon returning to land. Filtered refrigerated samples were used instead of frozen samples to avoid any loss of ligands due to the freezing process and precipitation associated with the freeze/thaw cycle. Total cobalt measurements were conducted on a profile at BATS to 3000 m from September 1999 (Fig. 4A) and showed a nutrient-like profile with a

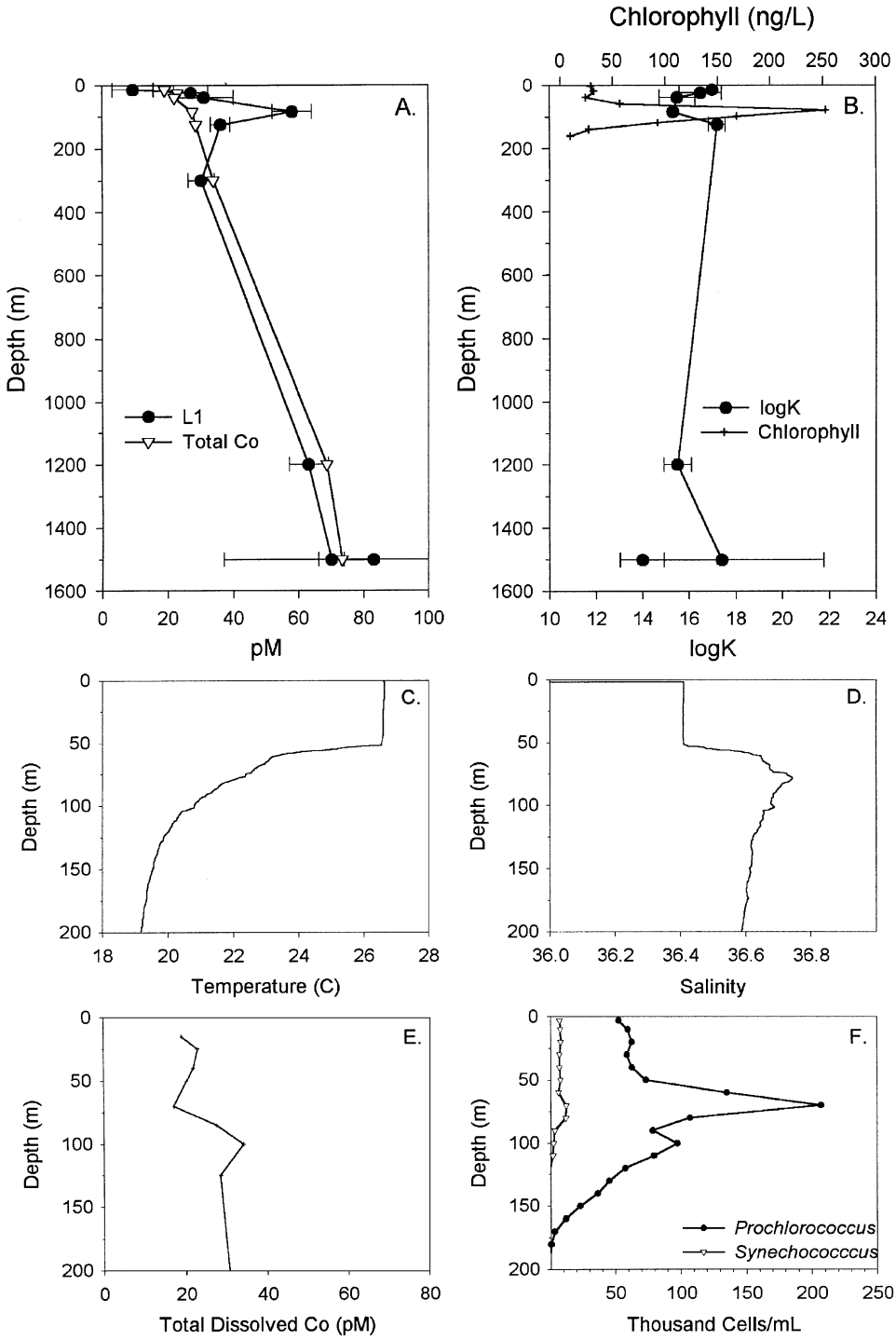


Fig. 4. (A) Total ligand concentrations at BATS, September 1999. Total L is calculated by non-linear fitting of titration data. (B) Calculated stability constants for natural cobalt ligands. $\mathrm{Log}\,K^{\mathrm{cond}}_{\mathrm{CoL}}$ is also calculated by non-linear fitting of titration data. Chlorophyll data has been overlaid for comparison (GF/F filter). (C–F) Hydrographic data and cell number abundances of *Synechococcus* and *Prochlorococcus* for BATS September 1999.

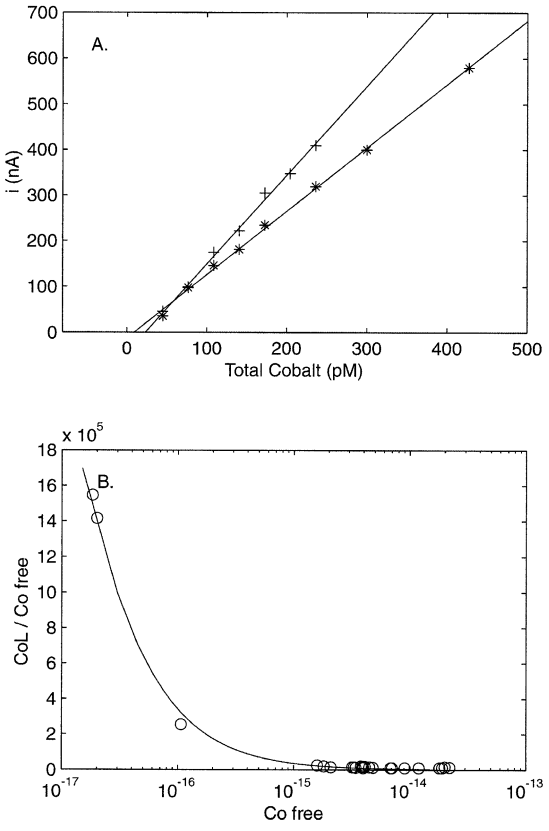


Fig. 5. (A) A typical titration (*), showing linear response to cobalt additions and a non-equilibrated titration (+) with a higher slope. (B) Titration data fit with a non-linear least regression analysis using the Levenberg–Marquardt algorithm. Co free refers to Co^{2+}.

maximum of 73 pM at 1500 m and approximately a three-fold increase in total cobalt concentration with depth. Only limited resolution was possible at greater depths due to difficult "chinese-finger" wire extensions used to acquire the deep samples.

Total L and K_{CoL}^{cond} were calculated using non-linear fits of titration data, incorporating the surface variability in slope as discussed in Section 2.3. Total L shows a peak in total ligand concentration near the *Prochlorococcus* and *Synechococcus* maximum and a decrease in the surface waters (Fig. 4). The calculation of K_{CoL}^{cond} is made relative to our calibration of $CoHDMG_2$ at ambient seawater pH. Log K_{CoL}^{cond} has a profile average of 16.3 ± 0.9 with the most variability occurring at or near the ligand maximum (Fig. 4B).

Each cobalt speciation measurement utilized two independent titrations to calculate final speciation results. In the first titration, subsamples were allowed to equilibrate for ≥ 12 h with added cobalt, natural ligands, and the synthetic DMG ligand (Fig. 5A). These titrations were remarkably linear and had consistently low *x*-axis intercepts, which when blank corrected resulted in labile cobalt values close to zero (Tables 3 and 4). A second titrations was run without equilibration on replicate samples to determine the sensitivity slope for the $Co(HDMG_2)$ chelate (S). These non-equilibrated cobalt titrations also resulted in linear responses but with a steeper slope than that of the equilibrated titrations (Fig. 5A). These non-equilibrated titrations were repeated for all titration depths and the ratios of non-equilibrated slopes to equilibrated slopes decreased with depth. In addition, standard addition analysis of UV-irradiated samples was conducted to measure total cobalt.

Table 3
Co-speciation incubations—BATS September and October 1999

Titration #	Date	Depth (m)	Slope/r^2	Non-equilibrated slope/r^2	Ratio	*x*-Intercept (pM)[a]
1	9/25	125	0.284/0.961	0.194/0.976	1.46	25.0 ± 1.2; 20 ± 0.9
2	9/27	40	0.195/0.997	0.139/0.998	1.40	25.7
3	9/29	85	0.217/0.969	0.149/0.994	1.46	21.4 ± 4.6
4	9/29	15	0.203/0.995	0.159/0.996	1.28	31.8 ± 6.5
5	9/29	25	0.270/0.976	0.160/0.992	1.69	25.4 ± 1.9
6	10/1	1500	0.185/0.998	0.153/0.993	1.21	20.4 ± 5.8
8	10/5	300	0.262/0.999	0.229/0.999	1.14	18.1
9	10/11	1200	0.224/0.983	0.215/0.994	1.04	31.9 ± 1.8
10	10/11	1500	0.191/0.999	0.188/0.976	1.02	81.1 ± 4.1 (cont.)

[a] Not dilution corrected—divide by 0.85 for reagent dilution correction.

Table 4
Comparison of totals of equilibrated bottles to UV-total cobalt

Depth (m)	Total equilibrated (pM)	Total equilibrated with diluted corrected (pM)	Minus blank (corrected total equilibrated) (pM)	Total cobalt (pM)	Error TotCo (pM)	[CoL] (totCo − TotEq) (pM)
15	31.8	37.4	21.1	19.0	4.6	−2.1[a]
25	25.4	29.9	13.7	23.0	0.0	9.3
40	25.7	30.2	13.9	21.9	–	8.0
85	21.4	25.1	8.9	27.4	0.5	18.4
125	25.0	29.4	13.1	28.5	–	15.3
300	18.1	21.2	5.0	33.7	1.1	28.6
1200	31.9	37.5	21.2	68.6	8.3	47.3
1500	81.1	95.4	79.1	73.3	2.1	−5.8[b]
1500	20.4	24.0	7.7	73.3	2.1	65.5

[a]This value is within the range of the error on the total cobalt measurement (4.7 pM).
[b]This equilibration sample was contaminated by comparison with a replicate measurement.

Estimates of CoL, total L and K_{CoL}^{cond} were calculated from these data. A representative non-linear fit is shown in Fig. 5B. Concentrations of CoL with respect to depth are calculated by simple mass balance subtraction shown in Eq. (15), and resemble the total cobalt nutrient-like profile.

5. Discussion

5.1. Total cobalt

The total dissolved cobalt profile at the Bermuda Atlantic Time Series Station (BATS, 31°79′N, 64°26′W) shows a depletion of cobalt in surface waters consistent with nutrient-like behavior (Fig. 4A). This is the first observation of a nutrient-like profile for cobalt at BATS. Jickells and Burton (1988) saw no trends with depth for Co at BATS, but were at their limit of detection. Our profile is similar to a profile reported by Martin et al. (1993) at an oligotrophic station in the North Atlantic at 59°30′N, 20°45′W, suggesting that the modest surface depletion we report may be a typical feature under oligotrophic conditions. Phytoplankton uptake is thought to be the dominant removal mechanism of cobalt in the euphotic zone of the Sargasso Sea (Moffett and Ho, 1996). The surface waters at BATS were dominated by *Prochlorococcus* and *Synechococcus* (Fig. 4F), both of which are known to have an absolute cobalt requirement (Saito et al., 2000; Sunda and Huntsman, 1995, respectively), and which may have contributed to the surface depletion of total Co.

5.2. Cobalt speciation

Results show that Co speciation is dominated by strong ligands at concentrations very similar to the total Co concentration (Fig. 6). Similar results have been reported for other metals, such as Cu and Fe, but Co titrations show two pronounced features. First, there is little or no DMG-exchangeable Co in

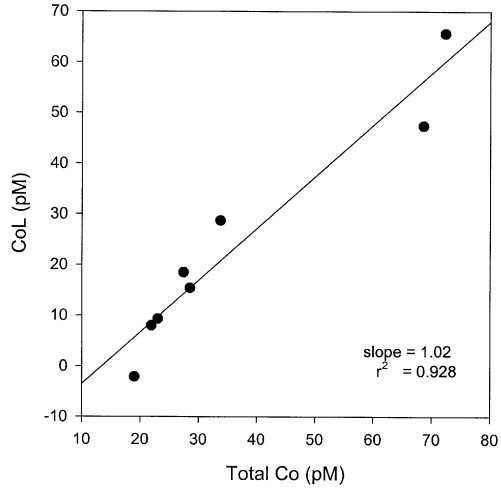


Fig. 6. The linear relationship between total dissolved cobalt and CoL. Cobalt appears to be tightly bound with a ligand concentration close to the total cobalt concentration.

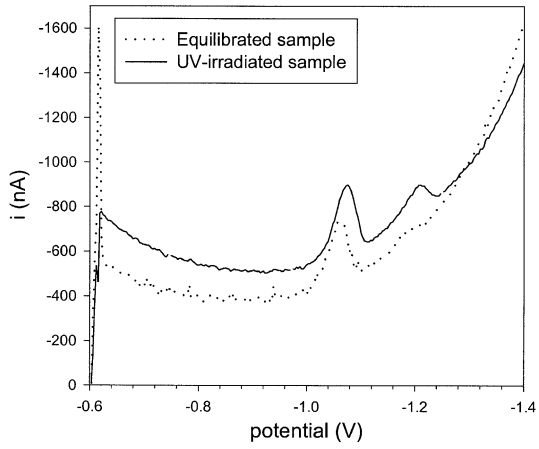


Fig. 7. A comparison of raw ACSV spectra showing the lack of cobalt signal in filtered samples after equilibration (dotted line), relative to the larger cobalt peak (−1.19 V) after UV-irradiation. By comparison, the nickel peak (−1.05 V) increases only slightly after UV-irradiation. In this figure, total cobalt is ~50 pM while total nickel is ~2 nM.

the initial sample. Secondly, there is no evidence for an excess of strong ligand that is titrated by additional Co. If there were, the slope of the titration plot would show a progressive increase with increasing Co (i.e. curvature). Instead, Co appears to be strongly complexed by a strong ligand in seawater, but there is no evidence for subsequent formation of this complex under the conditions of our titration. A total ligand concentration similar to the total cobalt concentration in equilibrated titrations, and a lack of curvature in those titrations were observed by John Donat in his thesis work; our findings over several years of work are consistent with this. Zhang et al. (1990) also did not observe any curvature in their titration plots of cobalt speciation work in estuarine waters.

The simplest explanation is provided by our calculations; a strong Co(II) ligand exists with concentrations very close to that of total Co. This explanation requires a mechanism by which metal and ligand concentrations should be similar. For instance, excess Co could be rapidly scavenged from the water column. However, there are alternative explanations that could account for the titration features even if ligand concentrations were higher.

First, it is possible that the natural Co complexes in the Sargasso are in fact Co(III) chelates. We are unable to remove significant amounts of cobalt from these natural ligands at the DMG concentration utilized, which is consistent with the properties of Co(III) complexes. Their stability constants tend to be much higher than the corresponding Co(II)-complexes (e.g. $\log K_{\mathrm{Co(II)EDTA}} = 16.45$, $\log K_{\mathrm{Co(III)EDTA}} = 41.4$ from Martell and Smith, 1977; Xue and Traina, 1996, respectively), indicating Co(III) complex dissociation should be slow. For instance, we found that Co(III)EDTA^-, prepared in Section 4.3 was exchangeable with DMG, but only fractionally (< 5%). Dissolved cobalt in seawater may be found in the form of a biogenic molecule such as cobalamin (vitamin B_{12}) or degradation products of cobalamin or other strong cobalt ligands. Cobalt in cyanocobalamin is known to be in the Co(III) redox state (Glusker, 1995) and, hence, would be kinetically inert to competitive ligand exchange reactions. Cobalamin is probably inaccessible to our speciation method, but the cobalt would be released in our total method by degradation of the corrin ring during UV-irradiation. Moreover, measurements of cobalamin in the Sargasso Sea by bioassay show subpicomolar concentrations, making them a small fraction of the total cobalt (Menzel and Spaeth, 1962).

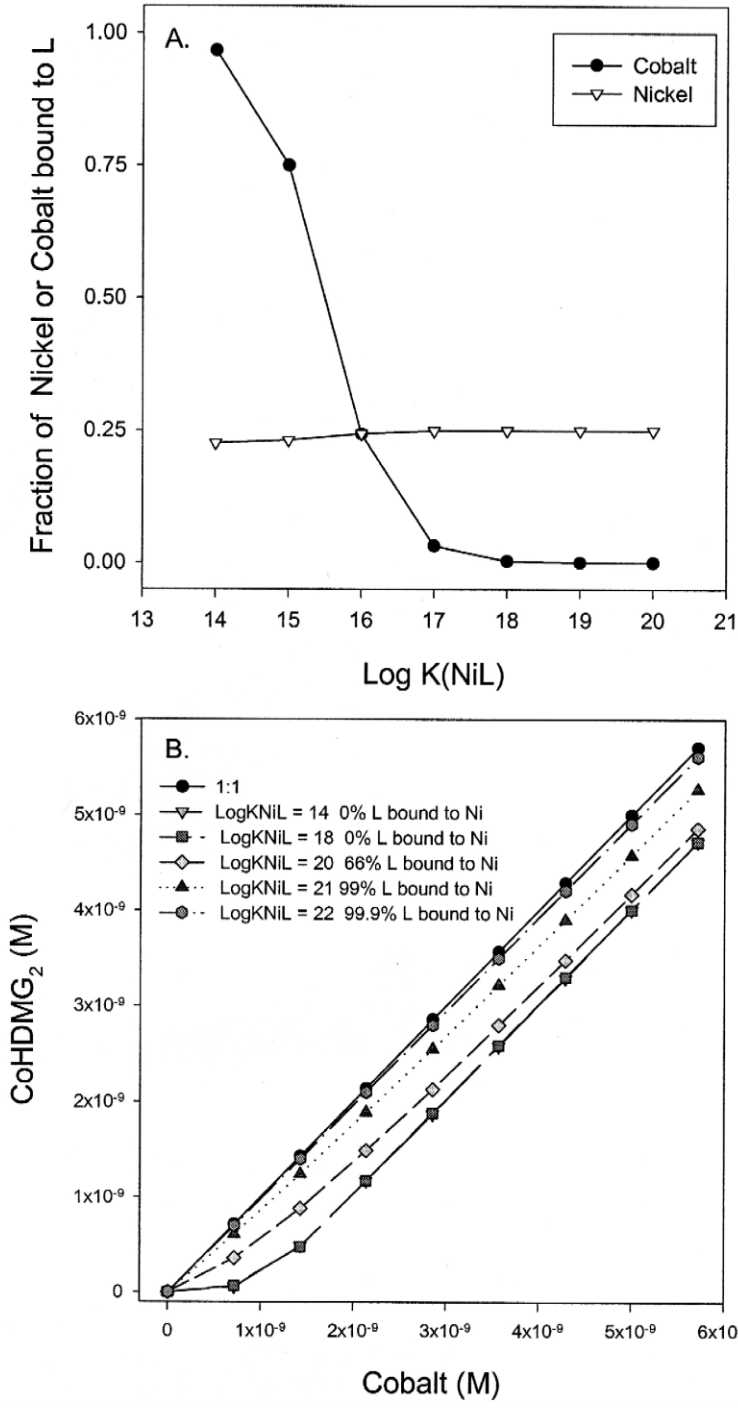
A second explanation for the similarity between CoL and total dissolved cobalt is that any ligand in excess of Co is bound to Ni, which is present at much greater abundance. While the Co peak is virtually absent from equilibrated samples, indicating lit-

Table 5
Stability constants of strong synthetic cobalt and nickel ligands

Compound	Stoichiometry	Log-K(Co(II))	Log-K(Ni)	Refs.
Dimethyl-glyoxime	$[ML2]/[M][L]^2$	12.85	17.2	a
Nioxime	$[ML2]/[M][L]^2$	15.62	21.5	a, b
TETA	$[ML]/[M][L]$	16.62	20.0	b
Cyclam	$[ML]/[M][L]$	12.71	22.2	b
Isocyclam	$[ML]/[M][L]$	10.9	–	b
Tetramethyl-cyclam	$[ML]/[M][L]$	7.58	8.65	c
Cyclen	$[ML]/[M][L]$	13.8	16.4	b, d
EDTA	$[ML]/[M][L]$	16.45	18.4	b
DTPA	$[ML]/[M][L]$	19.45	20.17	b

(a) Zhang et al., 1990. Conditional stability constants. (b) Martell and Smith, 1993. Thermodynamic stability constants. (c) Nakani et al., 1983. Thermodynamic stability constants. (d) Cyclen is 1,4,7,10-tetraazacyclododecance ([12]aneN4)cyclen.

tle or no free Co, the Ni peak is not saturated with strong natural ligands (Fig. 7). A database survey of strong synthetic Co ligands shown in Table 5 shows that most strong Co ligands have as strong an affin-



ity for Ni as for Co, supporting the idea of an equilibrium in seawater involving both metals. Moreover, Ni has not been reported to be saturated by natural ligands. In previous work by van den Berg and Nimmo (1986), Ni in coastal Britain was found to be partially complexed by strong Ni ligands with a $\log K_{\mathrm{NiL}}^{\mathrm{cond}}$ between 17.3 and 18.7 using cathodic stripping voltammetry and dimethylglyoxime as the electroactive synthetic Ni ligand. It is interesting to note that these investigators did not observe any curvature in their titrations of nickel, due to the excess of nickel over organic complexes in all of their titrations. We explored the possibility of competition for Co and Ni by a single ligand using model ligand calculations with a range of thermodynamic binding constants for nickel and cobalt (Fig. 8). The modeling work for the Co/Ni equilibria is derived from the mass law equations described above and the following mass balance equations.

$$\mathrm{L_{TOT}} = \mathrm{L_{Free}} + \mathrm{CoL} + \mathrm{NiL} + \Sigma\mathrm{ML} \quad (19)$$

$$\mathrm{TotCo} = \mathrm{Co}' + \mathrm{CoL} \quad (20)$$

$$\mathrm{TotNi} = \mathrm{Ni}' + \mathrm{NiL} \quad (21)$$

Given that many of the stronger cobalt and nickel ligands do not seem to have as much affinity for other metals and due to the lack experimental evidence, we assumed that ΣML was insignificant, allowing the simplified equation:

$$\frac{\mathrm{L_{Free}}}{\mathrm{L_{TOT}}} = \frac{1}{1 + K_{\mathrm{NiL}}^{\mathrm{cond}}[\mathrm{Ni}^{2+}] + K_{\mathrm{CoL}}^{\mathrm{cond}}[\mathrm{Co}^{2+}]} \quad (22)$$

Using $K_{\mathrm{NiL}}^{\mathrm{cond}}$ and $K_{\mathrm{CoL}}^{\mathrm{cond}}$ as parameters to study this system, we are left with an equation with $\mathrm{L_{Free}}$ as the only unknown:

$$\frac{\mathrm{L_{Free}}}{\mathrm{L_{TOT}}} = \frac{1}{1 + K_{\mathrm{NiL}}^{\mathrm{cond}} \dfrac{\mathrm{TotNi}}{\alpha'_{\mathrm{Ni}} + K_{\mathrm{NiL}}^{\mathrm{cond}}\mathrm{L_{Free}}} + K_{\mathrm{CoL}}^{\mathrm{cond}} \dfrac{\mathrm{TotCo}}{\alpha'_{\mathrm{Co}} + K_{\mathrm{CoL}}^{\mathrm{cond}}\mathrm{L_{Free}}}} \quad (23)$$

In addition to the potential competition of nickel for cobalt ligands, once bound, nickel ligands may appear recalcitrant to our speciation protocol due to kinetic factors. Nickel is known to have slow coordination kinetics because of its slow water-loss rate constants in the case of an disjunctive mechanism, or the stability of the partially dissociated complex for an adjunctive mechanism (Hudson et al., 1992). The water-loss rate constant for Ni^{2+} is two orders of magnitude slower than that of Co^{2+}, and adjunctive rate constants have been shown to correlate with water-loss rate constants in EDTA systems. The slow kinetics of nickel can be used to explain our speciation titration results: we would expect the metal exchange between NiL and CoL to be kinetically slow for both disjunctive and adjunctive mechanisms.

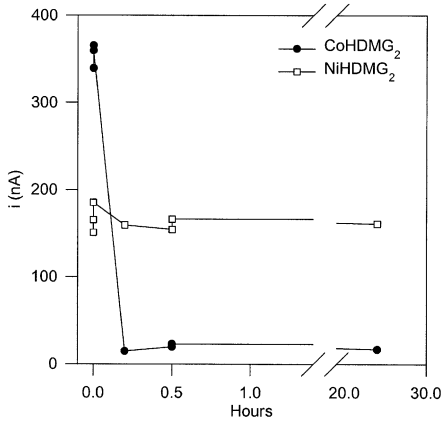


Fig. 9. A kinetic study of the formation of cobalt and nickel TETA complexes. $CoHDMG_2$ was pre-equilibrated prior to a TETA addition. The cobalt peak disappears rapidly while the nickel peak is not significantly affected even after 24 h.

Fig. 9 illustrates the effects of differing rates of Ni and Co exchange kinetics. An excess of TETA was added to a seawater solution containing $CoHDMG_2$ and $NiHDMG_2$ complexes. The Co peak decreases immediately reflecting formation of the non-electroactive TETA complex, but $NiHDMG_2$ is relatively unchanged. The relatively slow kinetics of Ni relative to Co are also apparent in the calibration

Fig. 8. (A) Competition of natural ligands for cobalt and nickel is modeled in the absence of DMG. In order for partial binding of nickel and cobalt, the conditional stability constant for CoL cannot be less than two orders of magnitude smaller than that of NiL. Since nickel is in excess of both natural ligands (the peaks are not saturated) and cobalt (2 nM versus 40 pM), there are no constraints on a conditional stability constant of CoL being greater than that of NiL. Total Co = 100 pM, total Ni = 4 nM, total L = 1 nM, $\log K_{\mathrm{CoL}}^{\mathrm{cond}} = 16$. (B) Model calculations of a natural ligand and dimethylglyoxime electrochemical system where affinity of natural ligands to nickel is included. A loss of curvature in the titrations is observed as the conditional stability constant for cobalt is increased. Total Ni = 2 nM, total L = 1 nM, $\log K_{\mathrm{CoL}}^{\mathrm{cond}} = 16$, $\log K_{\mathrm{CoHDMG2}}^{\mathrm{cond}} = 12.85$, $\log K_{\mathrm{NiHDMG2}}^{\mathrm{cond}} = 17.2$, total DMG = 0.0002 M.

of TETA where CoTETA complexes equilibrate with $CoHDMG_2$ in the 24 h equilibration period, but NiTETA complexes have only partially formed (Fig. 10).

Any of these explanations for the titration features must be considered in the context of the other important feature of the titrations—the difference in slope between equilibrated and non-equilibrated samples. We argued above that this reflects cobalt complexation by weaker ligands at concentrations substantially higher than our highest Co addition. How important are these weak ligands? The α coefficient for the weaker ligands is related to S as follows:

$$\frac{\alpha_{DMG} + \alpha_{WL}}{\alpha_{DMG}} = \frac{S_{non-equil}}{S_{equil}} \quad (24)$$

From this equation α_{WL} (where $\alpha_{WL} = K^{cond}_{CoL2} \cdot$ L2, L2 is the weak ligand and α_{DMG} = side reaction coefficient for $CoHDMG_2$ complexes) ranges from 1.7×10^3 to 1.2×10^4. By contrast the coefficient for the strong ligand, α_{SL}, calculated from K^{cond}_{CoL} and ligand concentrations, is 1.4×10^6. However, the strong ligand is saturated at most depths, so the relative importance of the weaker ligands increases. It is possible that the strong and weak ligands are the same species, but the former is a Co(III) complex and the latter a Co(II) complex, with oxidation kinetics that are too slow on the timescale of these titration experiments. The weak ligand class requires further investigation. Our efforts to fit the titration data using a two-ligand model resulted in large errors associated with K^{cond}_{CoL1}. It also removed the feature of the slight excess of strong cobalt ligands in the *Prochlorococcus* and *Synechococcus* maximum; this makes sense in the context of how these calculations have been performed, with the changes in S in surface waters forcing the increase in L at the *Prochlorococcus* and *Synechococcus* maximum. Although Co speciation is clearly dominated by the stronger ligand class, a small fraction complexed by the weaker ligands may constitute an important reactive and bioavailable class.

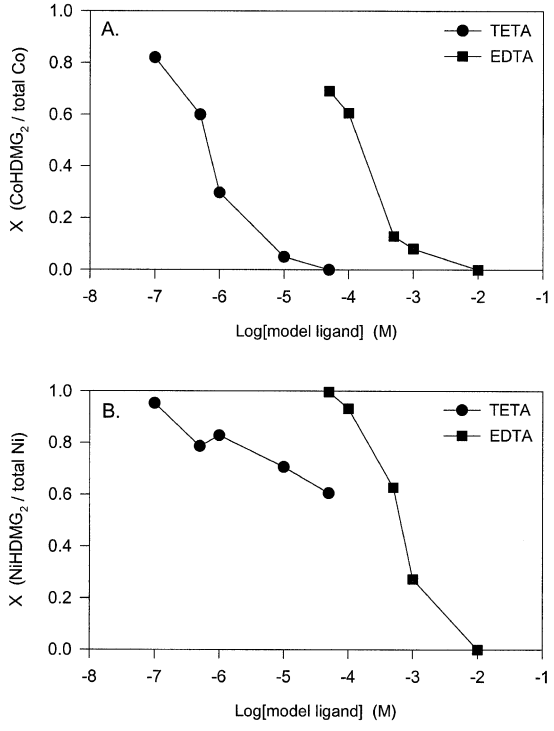


Fig. 10. Calibration of TETA and DMG against EDTA. (A) CoTETA forms significantly stronger complexes than CoEDTA. (B) Nickel appears to be kinetically slow in reacting with TETA with the incomplete formation of NiTETA complexes within the overnight equilibration period. This phenomenon of slow nickel kinetics could have implications for cobalt speciation in our Sargasso Sea samples.

The theoretical free Co^{2+} concentration is difficult to calculate from the data without large errors, since the strong ligand and Co concentrations are so similar. One approach is to simply divide the Co concentration that is in excess of the strong ligand by the α coefficient for the weak ligands. This would provide an upper limit on free Co^{2+} with concentrations that range from 10^{-14} to 10^{-17} M. The various interpretations of our cobalt speciation data presented here are not mutually exclusive: Co(II) and Ni ligands may exist in our samples, which also might bind Co(III) but with a much higher binding constant. This latter scenario could lead to an underestimate of ligand concentrations and conditional stability constants relative to the one ligand non-linear fit model that we have described.

6. Conclusions

In this study, we have begun to examine the difficult problem of cobalt speciation in oceanic environments. Our finding that cobalt is strongly bound by ligands of nearly equivalent concentration

is intriguing and warrants further study to determine if these ligands are inert Co(III) molecules (e.g. B_{12} degradation products) or if they are binding other metals like nickel. These findings have important implications for both the biological utility of cobalt and its geochemistry. With the predominant form of cobalt in the Sargasso as strongly bound CoL, the CoL is likely bioavailable since the resultant Co^{2+} concentrations would certainly limit growth of organisms with absolute cobalt requirements such as the photosynthetic cyanobacteria. Moreover, the strength of these ligands and their presence in deep Atlantic waters could result in a longer oceanic residence time than we would expect from a scavenged-type trace element as has been proposed for natural iron ligands (Johnson et al., 1997; Witter and Luther, 1998).

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References

Baxter, L., Bobrowski, A., Bond, A., Heath, G., Paul, R., Mrzljak, R., Zarebski, J., 1998. Electrochemical and spectroscopic investigation of the reduction of dimethylglyoxime at mercury electrodes in the presence of cobalt and nickel. Anal. Chem. 70, 1312–1323.

Bobrowski, A., 1989. Polarographic methods for ultratrace cobalt determination based on adsorption-catalytic effects in cobalt(II)–dioxime–nitrite systems. Anal. Chem. 61, 2178–2184.

Bobrowski, A., 1990. Determination of cobalt by adsorptive stripping voltammetry using Cobalt(II)-nioxime-nitrite catalytic system. Anal. Lett. 23, 1487–1503.

Bobrowski, A., Bond, A., 1992. Exploitation of the nitrite catalytic effect to enhance the sensitivity and selectivity of the adsorptive stripping voltammetric method for the determination of cobalt with dimethylglyoxime. Electroanalysis 4, 975–979.

Bruland, K.W., 1989. Complexation of zinc by natural organic ligands in the central North Pacific. Limnol. Oceanogr. 34 (2), 269–285.

Bruland, K.W., 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. Limnol. Oceanogr. 37 (5), 1008–1016.

Costa, G., Tavagnacco, C., Puxeddu, A., Balducci, G., Kumar, R., 1987. Electrochemistry of cobalt mixed Schiff base/oxime chelates. J. Organomet. Chem. 330, 185–199.

Donat, J.R., Bruland, K.W., 1988. Direct determination of dissolved cobalt and nickel in seawater by differential pulse cathodic stripping voltammetry preceded by adsorptive collection of cyclohexane-1,2,-dione dioxime complexes. Anal. Chem. 60, 240–244.

Dusenberry, J.A., Frankel, S.L., 1994. Increasing the sensitivity of a FACScan flow cytometer to study oceanic picoplankton. Limnol. Oceanogr. 39 (1), 206–209.

Gerringa, L.J.A., Herman, P.M.J., Poortvliet, T.C.W., 1995. Comparison of the linear van den Berg/Ruzic transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation in the estuarine environment. Mar. Chem. 48, 131–142.

Glusker, J.P., 1995. Vitamin B_{12} and the B_{12} coenzymes. Vitam. Horm. 50, 1–76.

Herrera-Melian, J., Hernandez-Brito, J., Gelado-Caballero, M., Perez-Pena, J., 1994. Direct determination of cobalt in unpurged oceanic seawater by high speed adsorptive cathodic stripping voltammetry. Anal. Chim. Acta 299, 59–67.

Hudson, R.J.M., Covault, D.T., Morel, F.M.M., 1992. Investigations of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes. Mar. Chem. 38, 209–335.

Jickells, T.D., Burton, J.D., 1988. Cobalt, copper, manganese and nickel in the Sargasso Sea. Mar. Chem. 23, 131–144.

Johnson, K.S., Gordon, R.M., Coale, K.H., 1997. What controls dissolved iron in the world ocean? Mar. Chem. 57, 137–161.

Lucia, M., Campos, A.M., van den Berg, C.M.G., 1994. Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime. Anal. Chim. Acta 284, 481–496.

Ma, F., Jagner, D., Renman, L., 1997. Mechanism for the electrochemical stripping reduction of the nickel and cobalt dimethylglyoxime complexes. Anal. Chem. 69, 1782–1784.

Martell, A.E., Smith, R.M., 1977. Critical Stability Constants, 3. Plenum Press, New York, 495 pp.

Martell, A.E., Smith, R.M., 1993. NIST Critical Stability Constants of Metal Complexes Database.

Martin, J.H., Gordon, R.M., 1988. Northeast Pacific iron distribution in relation to phytoplankton productivity. Deep-Sea Res. 35, 177–196.

Martin, J.H., Gordon, R.M., Fitzwater, S., Broenkow, W.W., 1989. VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res. 36 (5), 649–680.

Martin, J.F., Gordon, R.M., Hunter, C.N., Tanner, S.J., 1993. Iron, primary production and carbon-nitrogen flux studies during the JGOFS North Atlantic bloom experiment. Deep-Sea Res., Part II 40 (1–2), 115–134.

Menzel, D.W., Spaeth, J.P., 1962. Occurrence of vitamin B_{12} in the Sargasso Sea. Limnol. Oceanogr. 7, 151–154.

Moffett, J.W., 1995. Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. Deep-Sea Res., Part I 42 (8), 1273–1295.

Moffett, J.W., Brand, L.E., 1997. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnol. Oceanogr. 41 (3), 388–395.

Moffett, J.W., Ho, J., 1996. Oxidation of cobalt and manganese in seawater via a common microbially catalyzed pathway. Geochim. Cosmochim. Acta 60 (18), 3415–3424.

Nakani, B.S., Welsh, J.J.B., Hancock, R.D., 1983. Formation constants of some complexes of tetramethylcyclam. Inorg. Chem. 22, 2956–2958.

Ogino, H., Ogino, K., 1982. Redox potentials and related parameters of cobalt(III/II) complexes containing aminopolycarboxylates. Inorg. Chem. 22, 2208–2211.

Price, N.M. et al., 1988/1989. Preparation and chemistry of the artificial algal culture medium. Aquil. Biol. Oceanogr. 6, 443–461.

Rue, E.L., Bruland, K.W., 1995. Complexation of iron(III) by natural ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. 50, 117–138.

Saito, M.A., 2000. The Biogeochemistry of Cobalt in the Sargasso. PhD thesis, MIT–WHOI Joint Program in Chemical Oceanography, Massachusetts.

Sunda, W.G., Huntsman, S.A., 1995. Cobalt and zinc interreplacement in marine phytoplankton: biological and geochemical implications. Limnol. Oceanogr. 40, 1404–1417.

Vega, M., van den Berg, C.M.G., 1997. Determination of cobalt in seawater by catalytic adsorptive cathodic stripping voltammetry. Anal. Chem. 69, 874–881.

van den Berg, C.M.G., Nimmo, M., 1986. Determination of interactions of nickel with dissolved organic material in seawater using cathodic stripping voltammetry. Sci. Total Environ. 60, 185–195.

Witter, A.E., Luther, G.W., 1998. Variation in Fe-organic complexation with depth in the Northwestern Atlantic Ocean as determined using a kinetic approach. Mar. Chem. 62, 241–258.

Xue, Y., Traina, S., 1996. Oxidation kinetics of Co(II)-EDTA in aqueous and semi-aqueous goethite suspensions. Environ. Sci. and Technol. 30, 1975–1981.

Zhang, H., van den Berg, C.M.G., Wollast, R., 1990. The determination of interactions of cobalt (II) with organic compounds in seawater using cathodic stripping voltammetry. Mar. Chem. 28, 285–300.