

Determination of organic complexation of cobalt in seawater by cathodic stripping voltammetry

Michael J. Ellwood, Constant M.G. van den Berg*

Department of Earth Science, Oceanography Laboratories, University of Liverpool, P.O. Box 147, Bedford Street North, Liverpool L69 3BX, UK

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Abstract

A method was developed to determine the extent of cobalt complexation with organic ligands in seawater, and applied to samples from the North Eastern Atlantic Ocean. The cobalt speciation was determined using catalytic cathodic stripping voltammetry with ligand competition against the adsorptive ligand (nioxime). Optimised conditions include a nioxime concentration of 200 nM, 0.07 M ammonia/ammonium chloride pH buffer (pH 9.1), and 0.5 M nitrite. The stability of the mixed complex of cobalt, nioxime and ammonia was calibrated against EDTA. The cobalt speciation in the open-ocean surface waters was found to be dominated by complexation to natural organic ligands with conditional stability constants ($\log K'_{\text{CoL}}$) ranging between 15.6 and 16.1 and with ligand concentrations between 22 and 60 pM. The cobalt concentrations varied between 25 pM in the open-ocean waters and 103 pM in the English Channel, and were less than the ligand concentrations in many of the surface oceanic waters. The ligand concentration at depths between 30 and 115 m (average 22 pM) was lower than that of cobalt, whereas at other depths (shallower as well as deeper), the ligand concentrations were between 26 and 31 pM, sometimes greater than the cobalt concentrations. The cobalt and ligand concentrations in these waters are finely balanced, strongly binding all cobalt when the ligands are in excess.

Free Co^{2+} concentrations calculated for the open-ocean surface waters are extremely low (< 5 fM) which could cause cobalt limitation to certain phytoplankton such as coccolithophores and cyanobacteria if the organic complexes are unavailable. If this organic complexation of cobalt occurs also in other regions characterised by low cobalt concentrations, iron-limited waters in high-nitrate low-chlorophyll regions may be cobalt-limited too. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Organic complexation; Cobalt; Seawater; Cathodic stripping voltammetry

1. Introduction

The speciation of several trace metals in seawater is strongly influenced by complexation to natural

organic ligands, which has important implications for geochemical cycling, biological uptake and toxicity (Bruland et al., 1991; Hunter et al., 1997; Sunda and Huntsman, 1998). In open-ocean waters, cobalt has a relatively unique profile. Concentrations are generally low in surface waters (10–40 pM), which increase to a maximum in the upper thermocline (30–100 pM), and then decrease to values in deep waters of 10–30 pM (Knauer et al., 1982; Martin et al.,

* Corresponding author. Tel.: +44-151-794-4096; fax: +44-151-794-4099.

E-mail address: vandenbergliv@liv.ac.uk
(C.M.G. van den Berg).

1989, 1993). At present, comparatively little is known about complexation of cobalt in seawater; however, there is some evidence to suggest that it may be partially, but strongly complexed to natural organic ligands in estuarine waters (Zhang et al., 1990); the presence of a nonlabile voltammetric fraction indicates that there is at least partial complexation in the Mediterranean (Vega and van den Berg, 1997).

Cobalt is a biologically important metal although only few cobalt metalloproteins are known (Kobayashi and Shimizu, 1999). Likewise, even fewer studies have looked at cobalt–enzyme interactions in the marine environment. Cobalt is known to substitute for zinc in the enzyme carbonic anhydrase when marine phytoplankton is cultured under zinc-limiting conditions (Price and Morel, 1990; Morel et al., 1994; Yee and Morel, 1996).

More recently, laboratory culture experiments have shown that relative ratios of free Co^{2+} to free Zn^{2+} in seawater may be an important influence on the growth of coccolithophores, diatoms and cyanobacteria (Sunda and Huntsman, 1995). For example, higher Co^{2+} concentrations in culture experiments tended to promote coccolithophore growth compared to diatom growth when both phytoplankton groups were grown under zinc-limiting conditions (Sunda and Huntsman, 1995). The opposite tended to occur under cobalt-limiting conditions. Cyanobacteria, on the other hand, did not require zinc for growth; however, they do appear to have an absolute requirement for cobalt. Clearly, some microorganisms require free cobalt and its complexation by organic matter may make it unavailable.

We present here a method to determine the extent of cobalt complexation in open-ocean waters with low-cobalt concentrations, and include a preliminary study of the distribution of cobalt-binding ligands.

1.1. Theory

The underlying theory of determining cobalt speciation in seawater using cathodic stripping voltammetry (CSV) with ligand competition has been described in detail elsewhere (Zhang et al., 1990). Briefly, the complexation of cobalt in seawater by a natural ligand (L) can be defined as:



where K'_{CoL} is the conditional stability constant of the cobalt complex in seawater. $[L]$ is the concentration of L not complexed by cobalt, $[\text{CoL}]$ is the concentration of cobalt complexed with the ligand L , and $[\text{Co}^{2+}]$ is the free cobalt ion concentration. The total ligand concentration (C_L) is defined as:

$$C_L = [\text{CoL}] + [L]. \quad (2)$$

Substitution for $[L]$ in Eq. (1) using Eq. (2) and rearranging gives an equation similar to the Langmuir relationship (van den Berg, 1982; Ruzic, 1982):

$$[\text{Co}^{2+}]/[\text{CoL}] = [\text{Co}^{2+}]/C_L + 1/(K'_{\text{CoL}}C_L). \quad (3)$$

When values of $[\text{Co}^{2+}]/[\text{CoL}]$ vs. $[\text{Co}^{2+}]$ are plotted usually a linear relationship is obtained with a slope equal to $1/C_L$ and with the intercept yielding $1/(K'_{\text{CoL}}C_L)$. In solution, the Co^{2+} concentration is related to the current (i_p) measured at the mercury drop by:

$$[\text{Co}^{2+}] = i_p/(S\alpha'), \quad (4)$$

where S is the sensitivity (peak current/cobalt concentration, nA nM^{-1}) of the voltammetric analyses. S was determined from the linear portion of the titration curve after all of L had been titrated with cobalt. α' is the overall side-reaction coefficient for cobalt:

$$\alpha' = \alpha_{\text{Co}} + \alpha_{\text{Co}(\text{nioxime})_2}, \quad (5)$$

where α_{Co} is the inorganic side-reaction coefficient for cobalt in seawater, which was calculated to be 2.2 at $\text{pH} = 9.1$ using an ion-pairing model, with constants from Turner et al. (1981). $\alpha_{\text{Co}(\text{nioxime})_2}$ is the side-reaction coefficient for cobalt complexed with the added competing ligand, nioxime in this case. In solution $\alpha_{\text{Co}(\text{nioxime})_2}$ is fixed by the amount of nioxime added:

$$\alpha_{\text{Co}(\text{nioxime})_2} = \beta'_{\text{Co}(\text{nioxime})_2}[\text{nioxime}]^2, \quad (6)$$

where $\beta'_{\text{Co}(\text{nioxime})_2}$ is the conditional stability constant and $[\text{nioxime}]$ is the concentration of nioxime not complexed to cobalt. The concentration of nioxime added to solution is much greater than that of cobalt; hence, the total nioxime concentration (C_{nioxime}) added can be substituted for $[\text{nioxime}]$. It was assumed that cobalt forms a 1:2 stoichiometric

complex with nioxime. The Co–nioxime complex was calibrated against EDTA at the same nioxime concentration as used in the titration experiments (see below): thus, all the experimental results presented here are internally consistent, and the found complex stability is linked to that of cobalt with EDTA in seawater, even if the assumption of 1:2 stoichiometry were to be incorrect.

Finally, once C_L and K'_{CoL} had been determined the concentration of Co^{2+} in solution was calculated by solving the following quadratic equation:

$$[Co^{2+}]^2 \alpha_{Co} K'_{CoL} + [Co^{2+}] (K'_{CoL} C_L - K'_{CoL} C_{Co} + \alpha_{Co}) - C_{Co} = 0, \quad (7)$$

where C_{Co} is the total concentration of cobalt in solution.

2. Experimental

2.1. Instrumentation and reagents

The voltammetric system consisted of an Autolab PSTAT10 (Eco Chemie, The Netherlands) and a static mercury drop electrode (Metrohm, 663 VA), which were coupled to a Cardstar portable computer (486 PC). The reference electrode was double junction, Ag/AgCl, KCl (3 M), saturated AgCl, with a salt bridge filled with 3 M KCl, and the counter electrode was a glassy carbon rod. Water used to make up reagents was purified using a Millipore reverse-osmosis, ion-exchange water purification system (designated here as Milli-Q water). Acids and ammonia solutions were purified by subboiling evaporation in a quartz still (designated here as Q-acid or Q-NH₃).

All plasticware used in this study was acid-cleaned. Typically, 500 ml sample bottles were soaked for 1 week in a 50% HCl (AR grade) solution, rinsed with Milli-Q water, and then soaked for a further week in ~2 M HNO₃ (AR grade). Lastly, the bottles were rinsed with Milli-Q water, filled with 0.5% Q-HCl and then double bagged and stored until needed. Laboratory plasticware (except polystyrene “Sterilin” tubes) was acid-cleaned by heating for 3 days in ~25% *aqua regia* followed by

a 1 week soak in 0.5% Q-HNO₃. The plastic-ware was rinsed with Milli-Q water before being transferred between acid baths and before use.

A 5 M ammonia buffer was prepared by mixing subboiled hydrochloric acid and ammonia together. The buffer was cleaned by passing it through a Chelex-100 column followed by in-line UV-digestion (Achterberg and van den Berg, 1994). Additions of 140 μl of buffer to 10 ml seawater yielded a pH of 9.1 (NBS pH scale). A stock solution of 0.1 M nioxime (1,2-cyclohexanedione dioxime) was prepared in 0.2 M sodium hydroxide. A 40 μM solution of nioxime was prepared by serial dilution of the stock solution. Cobalt standards were prepared in 0.05% HCl by dilution of a 1000 ppm stock solution (BDH, Spectrosol grade).

A 5 M nitrite solution was made dissolving the sodium salt (BDH Analar grade) in Milli-Q water. The nitrite solution was cleaned electrochemically following the procedure described before (Vega and van den Berg, 1997). The blank due to the purified nitrite solution was determined accurately and was ~3 pM.

2.2. Total cobalt determinations

The procedure used to determine total dissolved cobalt was similar to that described before (Vega and van den Berg, 1997). Briefly, ~25 ml of sample was UV-digested at natural pH in preconditioned silica tubes for 45 min; to eliminate possible cobalt losses by adsorption the silica tubes were conditioned by UV-digestion of a seawater aliquot prior to the aliquot used for the analysis; this aliquot was poured out and replaced by a new aliquot of the same seawater. Experiments showed that cobalt losses were insignificant by this procedure, as shown before (Vega and van den Berg, 1997). Once cool, a 10 ml aliquot of UV-digested seawater was pipetted into a voltammetric cell, to which buffer (80 mM), nioxime (~800 nM) and nitrite (0.5 M) were added. The sample was purged with nitrogen for a period of 4 min. The cobalt–nioxime complex was then adsorbed onto a fresh mercury drop at an applied potential of –0.7 V for a period of 60 s. The potential was then jumped to –1.0 V for 1 s to reduce nickel–nioxime complexes, which also adsorbed onto the mercury drop. The electrode was

then equilibrated in quiescent solution for a period of 10 s at a potential of -0.8 V, after which the potential was scanned in a negative direction to -1.3 V. The differential-pulse scanning mode was used: pulse amplitude 50 mV, modulation time 10 ms, interval time 100 ms, and a step height of 2.4 mV giving a scan rate of 24 mV s^{-1} . The scans were repeated twice, and this procedure was repeated after additions of 75 and 150 pM cobalt.

2.3. Determination of $\alpha_{\text{Co(nioxime)}}$

The stability of the Co–nioxime complex was calibrated in UV-digested seawater by competition for Co^{2+} against EDTA (ethylenediaminetetraacetate). Thereto ten, 10 ml aliquots of seawater (salinity ~ 35) containing 200 nM of nioxime, 0.08 M of ammonium buffer (pH = 9.1) and about 1 nM of cobalt, were equilibrated overnight with EDTA at concentrations between 0 and 10 mM. Then the labile cobalt concentrations were determined by CSV after the addition of 1 ml of 5 M nitrite. A ratio (X) of the reduction current in the presence (i_p) and absence ($i_{p,0}$) of EDTA was calculated for each EDTA concentration:

$$X = i_p / i_{p,0} \quad (8)$$

From X , $\alpha_{\text{Co(nioxime)}}$ was calculated using the following equation (Zhang et al., 1990):

$$\alpha_{\text{Co(nioxime)}} = \left[(\alpha_{\text{Co}'} + \alpha_{\text{CoEDTA}}) X - \alpha_{\text{Co}'} \right] / (1 - X), \quad (9)$$

where $\alpha_{\text{CoEDTA}} = K'_{\text{CoEDTA}} [\text{EDTA}']$ (the product of the conditional stability constant of CoEDTA in seawater with the concentration of EDTA not complexed by Co^{2+}). The value for $\log K'_{\text{CoEDTA}}$ was calculated to be 7.66.

2.4. Cobalt titrations

Seawater samples, which had been stored deep-frozen, were used for this work. Previous work using iron (van den Berg, 1995; Wu and Luther, 1995) showed no change in the complexing ligands for that metal, suggesting that the same could be true for organic complexing ligands of other metals including

cobalt. Shipboard measurements of cobalt speciation in upwelling seawater from the eastern Pacific showed the presence of cobalt complexing ligands as in this work, but at much greater cobalt concentrations where the ligands were saturated (van den Berg, in preparation). The frozen samples were carefully thawed over ~ 8 h at room temperature, and then swirled to ensure they were well mixed prior to subsampling. A 142 ml aliquot was poured into a 250 ml Teflon bottle (marked to indicate the volume) to which 80 mM of ammonia buffer and 200 nM of nioxime were added. Eleven 10 ml aliquots were then pipetted from the Teflon bottle into 30 ml polystyrene vials (Bibby Sterilin), which were spiked with cobalt; two of these were without cobalt addition. Before first use each polystyrene vial was acid-cleaned in 10% HCl, rinsed with Milli-Q water, and conditioned twice overnight with seawater and cobalt at the same concentrations as used in the titration. Spiked samples were left to equilibrate overnight and at room temperature ($\sim 25^\circ\text{C}$). The following morning individual samples were measured by CSV after they were transferred from the polystyrene vial into the voltammetric cup and after 1 ml of nitrite had been added. The voltammetric cell was rinsed with seawater prior to the first measurement, and the first, zero-addition, aliquot was replicated to ensure, and check for, conditioning of the voltammetric cell. The voltammetric conditions used to determine labile cobalt were almost the same as for the total cobalt determination, except the adsorption time was increased from 1 to 2 min. Polystyrene vials were only rinsed with Milli-Q water between titrations and the same order of tubes was maintained to retain the conditioning. Comparative titrations in 25 ml Teflon (Nalgene) bottles left a mark on the bottle walls at the level of the solutions, suggesting that adsorption was occurring; no such evidence was apparent using the Sterilin tubes, suggesting that these were better for the cobalt complexation titrations.

2.5. Model ligands (vitamin B_{12} and coenzyme B_{12})

Stock solutions of vitamin B_{12} and coenzyme B_{12} were prepared in Milli-Q water from their bovine solids (Sigma). These compounds were added to seawater free of cobalt and organic matter (see be-

low) to 100 pM of either vitamin B₁₂ or coenzyme B₁₂. The B₁₂ containing seawater was then treated as sample and titrated with cobalt using the procedures described above.

2.6. Sample collection

Seawater used for the method development and preliminary experiments was from the North Atlantic (PRIME cruise with the RRS Discovery; 46°N, 19°W; pumped from a depth of 3 m) and had been filtered (0.2 μm filtration cartridge) and stored in a 50 l high-density polyethylene container. Before use the seawater was passed through a Chelex-100 column to obtain “cobalt-free” seawater, followed by in-line UV-digestion to remove any organic material (Achterberg and van den Berg, 1994). Transect samples from NE Atlantic were collected during a cruise in March 1998 with the RV *Pelagia* (Netherlands) as

part of the MERLIM project (EU) (Fig. 1), using a peristaltic pump (flow rate 4 l min⁻¹) from a depth of 3 m via a Teflon hose attached to a plastic “fish” positioned next to the research vessel. Seawater was continuously flushed through the tubing. Subsamples were collected periodically, filtered through a 0.2 μm filter cartridge (Sartobran), and then immediately frozen.

Samples from the water column of the NE Atlantic were collected during a cruise (76/91) with the RRV Challenger in March 1991 at 48 17.8'N, 12 28.8'W (station 13, Fig. 1). Samples were collected in precleaned 10 l Teflon coated Go-Flo bottles (General Oceanics) attached to a modified CTD-rosette (Morley et al., 1988) and collected on the upcast. The seawater was pressure filtered (0.5 Bar) through 0.4 μm acid-cleaned polycarbonate membrane filters using an in-line Teflon filtration unit into sample bottles. The sample bottles were re-

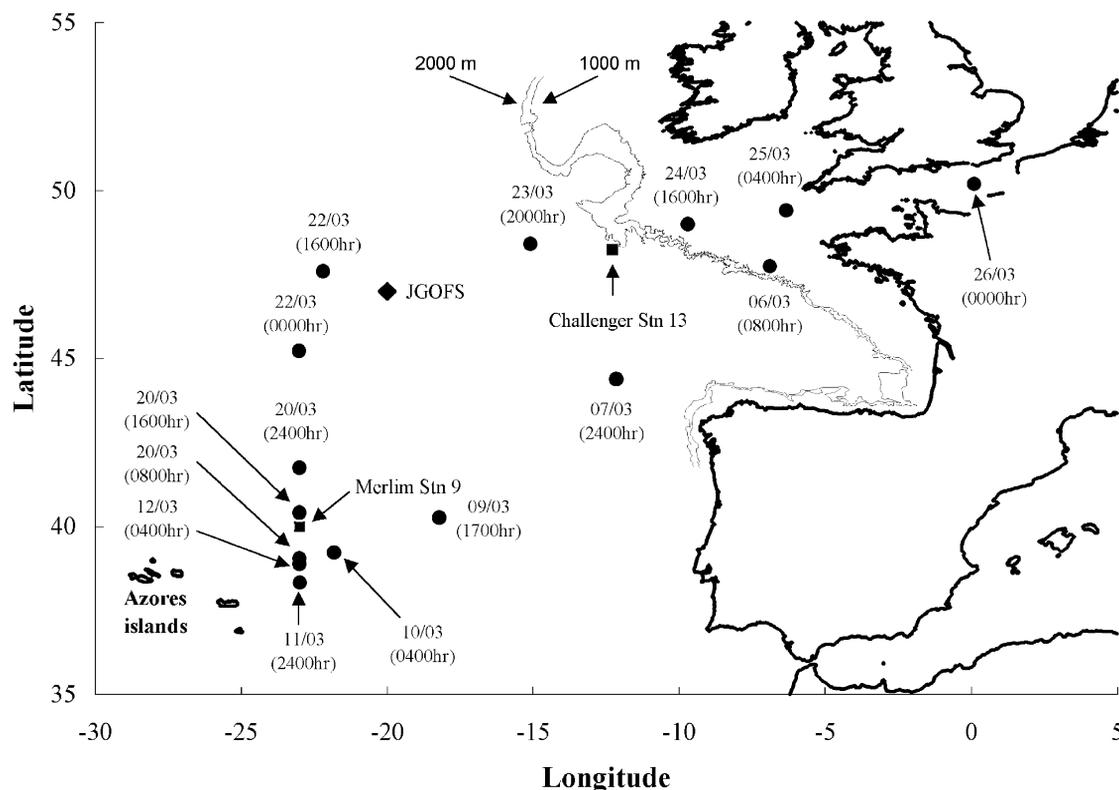


Fig. 1. Map showing sampling positions for Challenger station 13, MERLIM station 9, transect samples and the 47°N JGOFS station (Martin et al., 1993).

bagged and immediately frozen. These samples were used to obtain a comparative cobalt concentration profile for the region, not for speciation. Zinc analyses in the same samples showed that they had not been contaminated (Ellwood and van den Berg, 2000). Depth samples during the Pelagia (MERLIM) cruise were collected using 10 l Go-Flo bottles attached to a Kevlar line. Upon recovery Go-Flo bottles were transferred into a class 100 clean-container where 500 ml sample bottles were filled, rebagged and frozen. The Pelagia samples were used for cobalt speciation.

3. Results

3.1. Nioxime concentration optimisation and determination of $\alpha_{\text{Co}(\text{nioxime})}$

In preliminary experiments the nioxime concentration was varied to obtain the optimum nioxime concentration for the cobalt speciation. The dependence of the labile cobalt concentration on the concentration of nioxime was measured in two surface transect samples (Fig. 2). It can be seen that high concentrations of nioxime outcompete the natural complexing matter, making virtually all cobalt labile. However, at lower nioxime concentrations of 100–

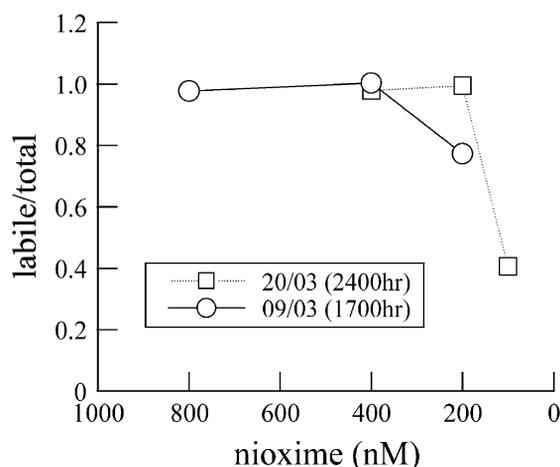


Fig. 2. Effect of varying the nioxime concentration on the lability of complexed cobalt in seawater; the data is for transect samples 20/03 (2400 h) and 09/03 (1700 h); each point on the graph calibrated by standard additions of cobalt.

200 nM, a significant fraction of the cobalt became nonlabile. To avoid possible effects on the sensitivity by variable levels of other trace metals competing for a limited amount of free nioxime, the high end of this range was selected (200 nM) for the cobalt speciation.

The stability of the cobalt complex with nioxime was calibrated against EDTA. The effect of varying the EDTA concentration on the cobalt reduction current in seawater containing ~ 1 nM cobalt and 200 nM of nioxime is shown in Fig. 3. As the EDTA concentration was increased, the labile cobalt peak decreased due to the EDTA complexation of cobalt in competition with nioxime. Values for X in Eq. (9) were generated from ratios of the peak current with and without EDTA (the last three points in the EDTA calibration (see Fig. 3) were not used because the concentrations of added EDTA approached the concentrations of Ca and Mg in seawater). An average value for $\alpha_{\text{Co}(\text{nioxime})}$ of 56126 was obtained from the single values of X .

A value for $\beta'_{\text{Co}(\text{nioxime})_2}$ was calculated from $\alpha_{\text{Co}(\text{nioxime})}$ using Eq. (6), giving $\log \beta'_{\text{Co}(\text{nioxime})_2} = 18.1$. As before (Zhang et al., 1990), it was assumed that cobalt forms a 1:2 complex with nioxime. Our value for $\beta'_{\text{Co}(\text{nioxime})_2}$ is approximately two and half log units higher than that found by Zhang et al. (1990). In view of a strong effect of ammonia on the CSV sensitivity (Vega and van den Berg, 1997), it is likely that the complex is a mixed (ternary) species which includes ammonia as well as nioxime; the stability constant is expressed on basis of nioxime only, which may explain the apparent difference with the previous value. The large effect on the apparent value for $\beta'_{\text{Co}(\text{nioxime})_2}$ could also indicate that 1:1 Co(nioxime) (rather than 1:2) complexes are formed as then the effect on the conditional stability constant is much smaller. When expressed on basis of the alpha-coefficient ($\alpha_{\text{Co}(\text{nioxime})}$), the effect is much smaller too: at the same nioxime concentration, the expected complex stability is about $13 \times$ greater in the presence of ammonia. However, because we use here a $25 \times$ lower nioxime concentration, the calibrated complex stability used here is about half that used previously for estuarine waters.

Our method utilizes catalysis to enhance the CSV sensitivity necessary for the low-cobalt concentrations in the oceanic samples, whereas the previous

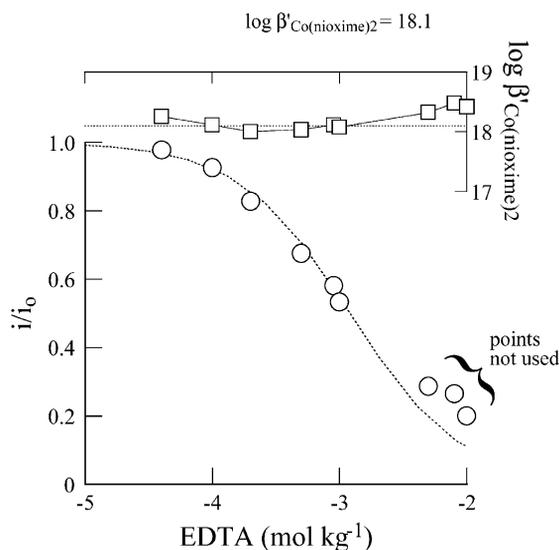


Fig. 3. The calibration of $\beta'_{\text{Co}(\text{nioxime})_2}$ by ligand competition with EDTA. The complex stability of cobalt with 200 nM nioxime is similar to that with approximately 1 mM EDTA.

work (Zhang et al., 1990) was at much greater cobalt concentrations in estuarine and coastal waters. Our titrations were carried out at pH 9.1 using ammonia buffer, whereas previously a pH 8.7 TEA buffer was used (Zhang et al., 1990). However, in spite of the possible disagreement regarding the value of the conditional stability constant, our results are internally consistent and are directly related to the complex stability of CoEDTA as $\alpha_{\text{Co}(\text{nioxime})_2}$ was calibrated against EDTA at the same nioxime concentration of 200 nM as used for the seawater titrations.

As it happens, the complex stabilities of the natural complexes with cobalt (the values for $\log K'_{\text{CoL}}$) obtained in this study (Tables 1 and 2) are very similar to those found previously in the estuarine waters (Zhang et al., 1990), suggesting that the differences between the two methods have little effect on the determined complex stability with the natural ligands present in seawater. The standard deviation of the stability constants (log values) varied between 0.1 and 0.4: it was high for the samples where the initial cobalt concentration was greater than the ligand concentration due to the scarcity of data points before the ligand was saturated, which

caused the intercept with the Y-axis to be low and error of its estimate to be relatively large.

3.2. Total cobalt concentrations

The results for the dissolved cobalt concentration in the NE Atlantic are presented in Figs. 4, 5 and Tables 1 and 2. The profiles for the cobalt concentration in the water column of the Atlantic appears to be oceanographically consistent for an element of this type. At Merlim station 9, a decrease in the cobalt concentration is apparent from the surface (33 pM) to a minimum at 115 m (21 pM). This is followed by an increase in concentration to a maximum at around 200 m (45 pM). In the deep waters below 500 m, the cobalt concentrations are relatively uniform with values ranging from 30 to 40 pM. The upper water column (above 1000 m) at Challenger station 13 shows clear enrichment compared to Merlim station 9, probably due to releases from the shelf sediments as the Challenger station is situated at the onset of the shelf. Cobalt data from Martin et al. (1993) at the JGOFS station are somewhat in between the two, although closer to those at the open-ocean Merlim station. The upper water column data are, therefore, consistent with Atlantic upper water column cobalt levels of 20–50 pM, with inputs from the atmosphere overshadowed by shelf inputs nearer the continent.

Table 1

Cobalt, ligand, $\log K'_{\text{CoL}}$ and free Co^{2+} data for station 9 from the MERLIM cruise

Position 40° 00N, 23° 00W				
Depth (m)	Co (pM)	Ligand (pM)	$\log K'_{\text{CoL}}$	Co^{2+} (pM)
15	33.1	35	15.8	0.003
30	36.9	21	15.7	7
40	30.8	25	16.2	3
65	25.2	23	16.0	1
115	20.5	21	15.2	0.04
165	27.8	30	15.6	0.003
200	45.3	29	16.0	7
250	43.0	29	15.6	6
500	31.4	31	16.2	0.2
1000	36.9	28	15.8	4
1250	40.5	26	15.7	6
2000	38.3	–	–	–

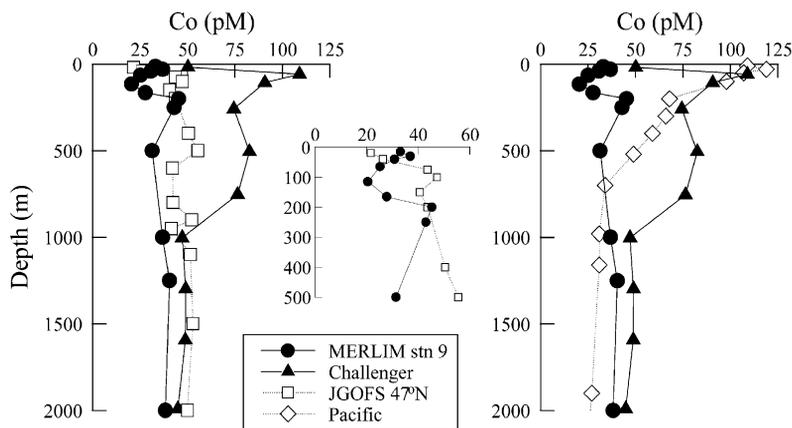


Fig. 4. Vertical profiles of cobalt for Challenger station 13, MERLIM station 9, the nearby JGOFS station (Martin et al., 1993), and for comparison a station from the Pacific Ocean (Knauer et al., 1982).

The deep-water cobalt concentrations tend to converge at the three stations: deep-water cobalt concentrations at station 9 are about 20% less than at station 13 and than those reported at the JGOFS 47°N station (Martin et al., 1993), whereas deep-water cobalt levels at station 13 and JGOFS show good agreement. The location of station 9 is about 7° (400 miles) further south than the JGOFS station, which could explain systematic differences. Atmospheric inputs at the latitude of station 9, followed by northward surface water transport and scavenging of cobalt to deeper waters, could explain the different shape of the cobalt profile in the upper water column at the two stations. Zinc concentrations were also found to be slightly elevated in the surface waters at station 9, thereby suggesting atmospheric input of both cobalt and zinc (Ellwood and van den Berg, 2000).

Cobalt concentrations for the surface transect samples were lowest in open-ocean waters at approximately 24 pM and increased coastward to reach a maximum of 103 pM in the English Channel (Fig. 5, Table 2).

3.3. Cobalt speciation

In most cobalt titrations, the CSV response was found to increase linearly with the cobalt concentration, thereby indicating that the natural ligands present were almost fully saturated with cobalt initially present in the sample. Curvature was only apparent when the initial cobalt concentration was less than

the ligand concentration. A titration curve of an open-ocean sample, showing clear curvature at low-added cobalt concentrations, is shown in Fig. 6a. The presence of cobalt–nioxime complexes at low-cobalt concentrations, before any cobalt additions to samples with excess natural ligand concentrations, is evidence for cobalt release from the natural complex (complex *dissociation*) when the competing ligand (nioxime) is added: without the dissociation the free cobalt would be undetectable as it occurs at femto-

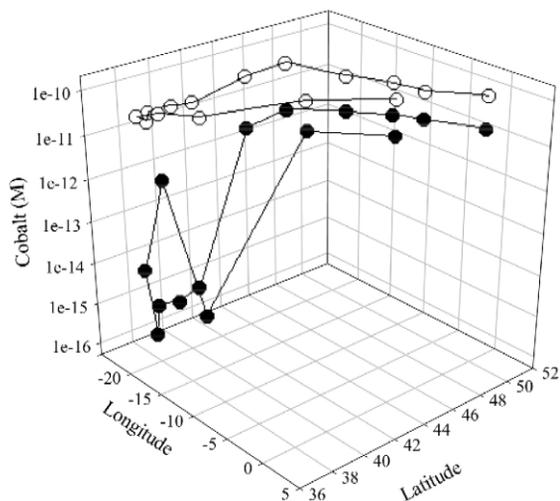


Fig. 5. Three dimensional plot of total cobalt and Co^{2+} concentration versus latitude and longitude. Open circles are total cobalt whilst closed circles are for free Co^{2+} .

Table 2

Cobalt, ligand, $\log K'_{\text{CoL}}$ and free Co^{2+} for transect samples collected at a depth of 3 m

Transect samples						
Sampling date and time	Longitude	Latitude North	Co (pM)	Ligand (pM)	$\log K'_{\text{CoL}}$	Co^{2+} (pM)
06/3/98 (0800 h)	06° 9W	47° 7	56.7	38	15.7	8
07/3/98 (2400 h)	12° 2W	44° 4	55.2	30	15.9	11
09/3/98 (1700 h)	18° 2W	40° 3	26.0	33	15.9	0.0005
10/3/98 (0400 h)	21° 8W	39° 2	24.0	22	15.6	0.7
11/3/98 (2400 h)	23° 0W	38° 3	21.6	22	15.9	0.005
12/3/98 (0400 h)	23° 0W	38° 9	14.4	32	16.1	0.0001
20/3/98 (0800 h)	23° 0W	39° 1	22.8	27	16.1	0.0005
20/3/98 (1600 h)	23° 0W	40° 4	22.3	27	16.1	0.0004
20/3/98 (2400 h)	23° 0W	41° 8	19.0	24	15.8	0.0006
22/3/98 (0000 h)	23° 0W	45° 2	33.5	29	16.2	2
22/3/98 (1600 h)	22° 2W	47° 6	46.7	39	15.8	3
23/3/98 (2000 h)	15° 1W	48° 4	51.1	34	15.7	8
24/3/98 (1600 h)	09° 7W	49° 0	65.7	38	15.7	12
25/3/98 (0400 h)	06° 3W	49° 4	61.3	29	15.8	15
26/3/98 (0000 h)	00° 1E	50° 2	103.0	60	15.6	19

molar levels. The curved CSV response with cobalt additions is evidence that cobalt is taken up (complex formation) until the ligand is saturated. The forward and backward reactions mean that these complexes are chemically reversible within the reaction time of these titrations (8 h). Saturation of the ligands causes the linear response seen at higher cobalt concentrations.

Plots of $[\text{Co}_{\text{labile}}]/[\text{CoL}]$ vs. $[\text{Co}_{\text{labile}}]$ were linear for all samples (Fig. 6b) indicating that a simple one-ligand one-metal model can be used to describe the data (van den Berg, 1982; Ruzic, 1982).

Cobalt binding ligand concentrations were between 21 and 35 pM in the water column (Fig. 7, Table 1), and between 22 and 39 pM on the transect, with a high value of 60 pM for the sample in the Channel. The ligand concentration profile shows a maximum (35 pM) right at the very surface, which decreases to a minimum (~ 22 pM) below 30 m. Below 115 m, ligands concentrations increased to a relatively constant concentration of about 28 pM (Fig. 7).

Although the ligand concentrations were similar to the cobalt concentrations, there were systematic

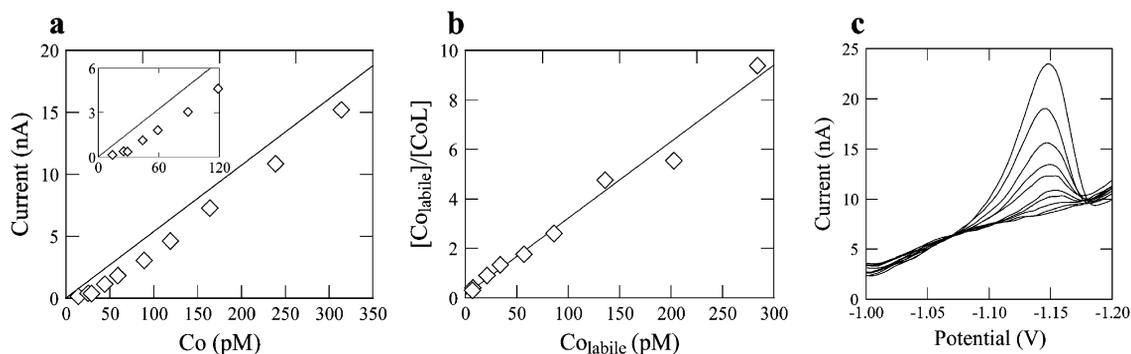


Fig. 6. Cobalt titration data for transect sample 12/03 (0400 h). (a) Peak current vs. added cobalt concentration. (b) A plot of $[\text{Co}_{\text{labile}}]/[\text{CoL}]$ as a function of $[\text{Co}_{\text{labile}}]$. (c) Individual voltammetric scans of cobalt additions.

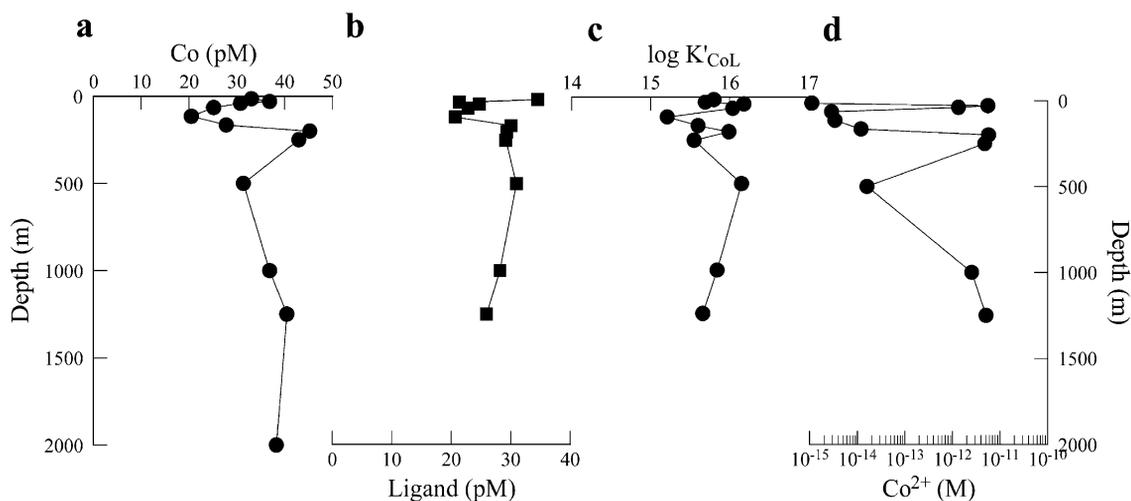


Fig. 7. Vertical profiles for station 9 of (a) dissolved cobalt concentration, (b) ligand concentration, (c) $\log K'_{CoL}$ and (d) free Co^{2+} concentration.

differences: cobalt concentrations tend to be greater than the ligand concentration in samples nearer the coast, whereas open-ocean waters tend to have ligand concentrations similar to, or greater than, the cobalt concentration.

$\log K'_{CoL}$ values ranged between 15.2 and 16.2 with no clear trend (Fig. 7). The high stability of these complexes, compared to the very low stability of inorganic complexation of cobalt ($\alpha_{Co} = 2.2$) means that virtually all ionic cobalt is complexed very strongly when the ligand concentration is greater than the cobalt concentration. It is, therefore, impor-

tant to have accurate data with a small relative standard deviation.

Replicate titrations were carried out to determine the reproducibility of the speciation method. The replicate titrations were in very good agreement giving ligand concentrations within 1 pM of each other (Table 3), equal to the standard deviation of the measurements. One replicate titration was carried out on a sample which was stored at room temperature for 36 days (Table 3): here the ligand concentration was found to be 17 pM less than expected. In light of this apparent room-temperature storage effect, most

Table 3

Results for replicate titrations for a sample from station 9 and for transect samples collected on the MERLIM cruise

	Co (pM)	Ligand (pM)	$\log K'_{CoL}$	Comments
<i>Station 9</i>				
Depth = 65 m	25.2	23 ± 1	16.0 ± 0.1	
		21 ± 1	16.0 ± 0.4	measured the same day
<i>Transect</i>				
11/3/98 (2400 h)	21.6	23 ± 1	15.7 ± 0.3	
		22 ± 1	15.9 ± 0.3	measured the same day
20/3/98 (1600 h)	22.3	27 ± 1	16.1 ± 0.4	
		10 ± 1	15.9 ± 0.1	measured 36 days later
20/3/98 (2400 h)	19.0	24 ± 1	15.8 ± 0.2	
		26 ± 1	15.8 ± 0.1	measured the same day

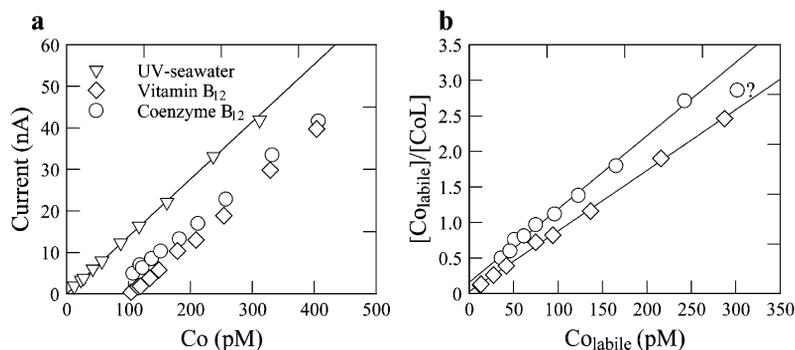


Fig. 8. (a) Titration data for vitamin B₁₂, coenzyme B₁₂ and UV-digested seawater. (b) Linearisation of the titration data for vitamin B₁₂ and coenzyme B₁₂.

of the titrations were set up and completed within 24 h of the sample being thawed.

3.4. Co²⁺ concentrations

The free Co²⁺ concentrations presented in Figs. 7, 8 and Tables 1 and 2 for transect and profile samples were calculated by solving Eq. (7). Surface Co²⁺ concentrations ranged from 10 to 20 pM in samples originating from over the continental shelf and for samples collected north of 45°. For samples collected near the Azores islands, free Co²⁺ concentrations ranged from 0.7 pM down to 0.4 fM, with the majority of the samples being subfemtomolar in concentration (Table 2).

Free Co²⁺ concentrations at station 9 were rather variable with values ranging between 3 fM and 7 pM down the water column (Fig. 7d, Table 1).

3.5. Vitamin B₁₂ and coenzyme B₁₂ ligand titrations

Cobalt complexation by vitamin B₁₂ and coenzyme B₁₂ was investigated to elucidate the possible nature of the organic ligands causing complexation of cobalt in seawater. These compounds were thereto

added to seawater and titrated with cobalt as before. The titrations of these compounds are shown in Fig. 8 along with a comparative titration of UV-digested seawater (to make it free of ligands) containing ~ 13 pM of cobalt, and the results are summarised in Table 4. The plots of CSV-labile vs. added cobalt were linear as both vitamin B₁₂ and coenzyme B₁₂ were almost fully saturated with cobalt (Fig. 8): our data, therefore, do not establish whether or not these compounds are reversible complexes like those in the seawater. The absence of curvature in these titrations could indicate that the complexes are irreversible; however, the shape of the titrations is consistent with the high stability of the complexes and is in fact inconclusive regarding the chemical reversibility.

Calculation of the binding strength for the Co–B₁₂ species from the linearisation titration data revealed that cobalt is complexed within vitamin B₁₂ with a log $K'_{\text{Co-B}_{12}}$ of 16.4 and coenzyme B₁₂ with a log $K'_{\text{Co-B}_{12}}$ of 15.5: these complex stabilities are similar to those of the seawater species.

4. Discussion

4.1. Co speciation in the Atlantic

To date no data has been published for cobalt speciation in open-ocean waters. The one study that has looked a cobalt speciation in seawater was a coastal study in the Scheldt Estuary (Zhang et al., 1990). Cobalt concentrations measured during that

Table 4

Cobalt, B₁₂ and log $K'_{\text{Co-B}_{12}}$ measured for vitamin B₁₂ and coenzyme B₁₂

B ₁₂ Complexes	Total Co found (pM)	B ₁₂ (pM)	log $K'_{\text{Co-B}_{12}}$
Vitamin B ₁₂	104	115	16.4
Coenzyme B ₁₂	107	97	15.5

study were high with concentrations ranging from about 0.6 to 1.5 nM. Likewise, the natural ligand concentrations measured were high with values ranging from 0.3 to 1.1 nM and with conditional stability constants ranging from 15.6 to 17.5 (log values), average = 16.2 ($n = 12$) (Zhang et al., 1990). Although the total cobalt and ligand concentrations measured in this study are much lower than those measured by Zhang et al. (1990), the conditional stability constants measured here covered a similar range, i.e. 15.2 to 16.2 (log values), average = 15.8 ($n = 26$).

The conditional stability constants measured for vitamin B₁₂ and coenzyme B₁₂ are similar to those measured for the cobalt–natural ligand complex. When these conditional stability constants are compared to that of EDTA, which is generally regarded as a strong metal chelator, it appears that cobalt is very strongly bound within the B₁₂ system and by natural ligands in seawater. In fact, the conditional stability constants measured for the natural ligand are more than eight orders of magnitude higher than that for cobalt–EDTA complexation in seawater (log $K'_{\text{CoEDTA}} = 7.66$, whereas log $K'_{\text{CoL}} = 15.8$).

This work was carried out at pH 9.1, one pH unit above the “normal” pH of seawater, as this gave optimal sensitivity of the catalytic detection of cobalt. In view of the competition by the major cations Mg²⁺ and Ca²⁺, which are present at 10 and 50 mM, at much greater level than the protons (10 nM), it is unlikely that the complex stability is altered by this change in pH. For comparison, the complex stability of EDTA with trace metals in seawater is not significantly affected until the pH is lowered to below 2. No change was apparent in the organic complexation of iron (on basis of Fe³⁺) when the pH was lowered from 8 to 7 (van den Berg, 1995); therefore, by analogy, there may have been no change by this pH change. Also, there is no significant difference from the complex stabilities at pH 8.7 in estuarine waters (Zhang et al., 1990) although this could be a coincidence. However, a pH effect cannot be discounted without experimental verification, which was not possible with the CSV method used here. If there were to be an overestimate of the conditional stability constants (contrary to expectation) of one or two log units due to the increased pH, the estimated concentrations of Co²⁺ for seawater

with excess ligand concentrations would be underestimated by a factor of 10–100. However, in that case, our general conclusions would still be unaffected due to the very great stability of the complexes.

A second potential artefact is introduced by the high ammonia concentration (0.08 M), which is used as pH buffer and to enhance the CSV sensitivity; its complexation with Co²⁺ is very weak (log $K \cong 2$); however, it is probably involved in a ternary complex with nioxime, along with NO₂⁻, as it increases the overall complex stability. However, the nioxime complex is special in that it is planar in view of its ability to adsorb on the mercury drop. If the natural ligand were to form a complex with cobalt similar to that with nioxime, or sufficiently small to form a bidentate species with cobalt, then the stability of that complex could be enhanced by the ammonia similar to its effect on nioxime complexation. For larger, more bulky, ligands, like for instance the coenzyme B₁₂ tested here, this effect is not likely to be significant.

4.2. Redox state of complexed cobalt: Co^I, Co^{II} or Co^{III}?

The most common and probably the most stable oxidation state for dissolved uncomplexed cobalt in water is thought to be Co^{II}; however, all three oxidation states are known to exist in solution (Cotton and Wilkinson, 1972). However, many Co^{II} complexes are readily oxidised to Co^{III} especially if the complex contains N groups. For instance, it is thought that the voltammetric detection step makes use of the reduction of Co^{III} (as a mixed complex with nioxime and ammonia) to Co^{II} (Vega and van den Berg, 1997). However, this is not certain, and other data suggests that any Co^{III} (produced during UV digestion) has to be reduced to Co^{II} (using borohydride) before it is bound by nioxime (Donat and Bruland, 1988). This effect was not confirmed in this work; however, it does suggest that there may still be questions about the redox state of cobalt.

On the other hand, there is little evidence for Co^I in seawater (Moffett and Ho, 1996). Work on B₁₂ models has demonstrated that all three cobalt oxidation states are possible when cobalt is bound within a *corrin* ring system although the normal oxidation

state in vitamin B₁₂ is Co^{III} (Cotton and Wilkinson, 1972; Gerli et al., 1992). Redox potentials for the reduction of Co^{III} to Co^{II} within model B₁₂ complexes range from -0.02 to -1.9 V, and for the reduction of Co^{II} to Co^I from -1.00 to -1.42 V (Gerli et al., 1992). The reduction potential for the Co-nioxime complex during the CSV scan was situated at about -1.14 V. The exact redox state of cobalt in seawater is still uncertain; however, it has been assumed here that the more likely one is Co^{II} whilst bound to the natural organic ligand. The stability constants have been calibrated on basis of Co^{II} complexation with EDTA, and the titrations have been carried out with additions of Co^{II}. However, this is no guarantee for cobalt remaining in this oxidation state as any complexation as Co^{III} would readily cause its oxidation although inorganic cobalt would mostly remain as Co²⁺. This problem is analogous to that for copper, which is known to be reduced from Cu^{II} to Cu^I in the presence of thiol-ligands in seawater (Leal and van den Berg, 1998). The calculated equilibrium concentrations of Co²⁺ are not affected by the oxidation state of the complexed cobalt as the complex stability has been calibrated against EDTA; however, it is possible that all organically complexed cobalt in seawater is actually Co^{III}.

4.3. Co and Fe colimitation of phytoplankton in HNLC regions?

The free Co²⁺ concentrations calculated in the open-ocean region of our study are extremely low, i.e. low femtomolar levels. Both bottle and large-scale iron enrichment experiments have shown that in certain parts the world oceans are iron limited. It has also been noted that diatoms tend to dominate following iron fertilisation of the iron-limited waters (Martin and Fitzwater, 1988; Martin et al., 1989, 1990; Coale et al., 1996). Why diatoms should benefit most is still unknown; however, here, we suggest that certain phytoplankton are also likely to be cobalt limited. Like iron, cobalt concentrations in high-nitrate, low-chlorophyll (HNLC) waters also tend to be low: typically concentrations range between 20 and 30 pM (Martin et al., 1989, 1990; Gordon et al., 1998).

Laboratory culture studies have shown that the marine cyanobacterium, *Synechococcus bacillaris*

and the coccolithophore *Emiliania huxleyi* have a strong requirement for cobalt, whereas the marine diatoms *Thalassiosira pseudonana* and *T. oceanica* require zinc rather than cobalt for maximum growth (Sunda and Huntsman, 1995). Decreases in the growth of *E. huxleyi* were observed when culture medium Co²⁺ concentrations were lowered below 1 pM. When Co²⁺ concentrations were lowered below 25 fM, both *E. huxleyi* and *S. bacillaris* were unable to grow. Even the addition of 74 pM of vitamin B₁₂ to the culture medium did not encourage *E. huxleyi* growth (Sunda and Huntsman, 1995). Speciation results for vitamin B₁₂ (Table 4) shows that the cobalt atom is very tightly bound within the B₁₂ system ($\log K'_{\text{CoL}} = 16.4$); hence, the lack of growth in *E. huxleyi* and *S. bacillaris* cultures even after vitamin B₁₂ was added was probably because they were unable liberate cobalt from the B₁₂ complex.

Our results show that in open-ocean waters cobalt also tends to be very strongly bound to a natural ligand, which is perhaps similar in nature to the B₁₂ complex. Furthermore, free Co²⁺ concentrations within these waters were extremely low (< 1 fM; Fig. 5 Table 2). If cobalt is also fully complexed in HNLC regions, such as the Southern Ocean and eastern equatorial Pacific, and if this complexed cobalt is unavailable, then some phytoplankton that are iron limited are also likely to be cobalt limited. The addition of pure ferrous iron to seawater, such as in the IronEx experiments, is unlikely to stimulate growth in cobalt-limited phytoplankton. The results from the two IronEx experiments showed that diatom growth was greatest when iron was added to iron-limited water, which may be because diatoms can substitute zinc for cobalt at low-cobalt concentrations (Sunda and Huntsman, 1995); for instance the marine diatom *T. oceanica* does not require cobalt at all. Plankton enumeration in IronEx II showed no evidence for iron limitation of the coccolithophores (Landry et al., 2000), suggesting that something else must have been limiting the growth of this group of algae. Cobalt perhaps?

5. Conclusions

A method for determining cobalt speciation in seawater was developed and applied to water sam-

ples collected from the NE Atlantic Ocean. Cobalt concentrations measured down the water column are low, but are consistent with other cobalt measurement made for the NE Atlantic Ocean. Cobalt concentrations in open-ocean surface waters were low with cobalt speciation being dominated by its complexation to natural organic ligands with concentrations that tend to be higher than that of cobalt. Cobalt concentrations increased landward from about 25 pM in open waters to 103 pM in the English Channel. Ligand concentrations also increased landward, but north of about 43° cobalt concentrations were always higher than ligand concentrations. Correspondingly, free Co^{2+} concentrations also increased landward to reach a few tens of picomoles per litre in the English Channel. Conditional stability constants for the CoL complex were extremely high with an average log value of 15.8.

The low free Co^{2+} concentrations determined in open-ocean waters, i.e. subfemtomolar, suggests that certain phytoplankton, such as coccolithophores and cyanobacteria, may well be cobalt limited in the NE Atlantic Ocean. Furthermore, HNLC regions which tend to be iron limited, are also characterised by low-cobalt concentrations, thereby suggesting that they could also be cobalt limited.

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