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Determination of total dissolved cobalt in UV-irradiated seawater using flow injection with chemiluminescence detection

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Abstract

A sensitive flow-injection method with chemiluminescence detection (FI-CL) for the determination of dissolved cobalt in open ocean samples, suitable for shipboard use has been developed. To date, FI methods for dissolved cobalt have been used only in coastal and estuarine waters. Therefore, significant modifications to existing methods were required, including (1) the use of a commercially available iminodiacetate (IDA) resin (Toyopearl AF-chelate 650M) in place of resin immobilized 8-hydroxyquinoline for online preconcentration and matrix removal, (2) the introduction of acidified ammonium acetate (pH 4) as a column-conditioning step before sample loading and rinse steps, and most importantly, (3) UV irradiation of acidified seawater samples to determine total dissolved cobalt, rather than an operationally defined fraction. This method had a detection limit of 4.5 pM (3σ of the blank). The accuracy of the method was evaluated by determining total dissolved cobalt in acidified North Pacific deep seawater (1000 m) samples from the Sampling and Analysis of Iron (SAFe) program and NASS-5. The method yields a mean (\pm SD) value of 40.9 ± 2.6 pM ($n = 9$), which is in excellent agreement with the SAFe consensus value of 43 ± 4 pM, and 208 ± 30 pM for NASS-5 (certified value 187 ± 51 pM). This study demonstrates that UV irradiation is an essential step for the determination of total dissolved cobalt in seawater by FI-CL. The method was applied to vertical profiles from the Sargasso Sea, indicating that total dissolved cobalt is influenced by both biological and physical processes.

Cobalt is an essential micronutrient for phytoplankton growth in the oceans. It is the central metal cofactor in the vitamin B₁₂ (cobalamin) complex. In addition, cobalt is a metal cofactor in the enzyme carbonic anhydrase (CA), which is required for inorganic carbon acquisition by marine phytoplankton (Lane and Morel 2000). Whereas certain groups of phytoplankton (e.g., the centric diatoms) are able to substitute zinc for cobalt or cadmium as the metal cofactor in CA, others (particularly *Prochlorochoccus* and *Synechococcus* spp.), hav-

ing an absolute requirement for cobalt, cannot (Sunda and Huntsman, 1995; Saito et al. 2002). Given that *Prochlorochoccus* may be the most abundant autotroph in the ocean (Partensky et al. 1999) and responsible for a significant proportion of global photosynthesis (Goerick and Welschmeyer 1993; Campbell et al. 1994), there is a need to understand the factors that control the growth and distribution of this organism.

A combination of extremely low concentrations in the open ocean (~ 4 – 120 pM, e.g., Knauer et al. 1982; Martin et al. 1989; Saito et al. 2004) and the complexity of the seawater matrix (which contains the major seawater ions at concentrations 10^9 greater than dissolved cobalt) makes the determination of dissolved cobalt in ocean waters a formidable challenge. Determination of dissolved cobalt therefore requires a preanalysis step that entails both the separation of the analyte from the bulk seawater matrix and preconcentration of the analyte to increase the effective sensitivity of the analytical method. These steps have been achieved in a variety of ways: (1) chelation/solvent extraction (Bruland et al. 1979; Martin and Gordon 1988), (2) voltammetric techniques (Saito and Moffett 2001), and (3) use of chelating ion-exchange resins

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(Sakamoto-Arnold and Johnson 1987; Cannizzaro et al. 2000; Sohrin et al. 2008; Milne et al. 2010). In recent years, the increased use of inductively coupled plasma mass spectrometry (ICP-MS) and flow injection (FI) techniques for trace metal analysis has resulted in widespread use of chelating ion exchange resins for separation and preconcentration.

Traditionally, the preconcentration resin of choice for the FI analysis of trace metals has contained 8-hydroxyquinoline (8-HQ) as the chelating group. Resin-immobilized 8-HQ is attractive, as it has a strong affinity for binding a number of trace metals of interest in seawater (Landing et al. 1986). Because resin-immobilized 8-HQ is not commercially available, however, it needs to be synthesized (Landing et al. 1986; Dierssen et al. 2001), which produces resins of varying quality. Commercially available chelating resins are arguably preferable since the quality of the resins is reproducible, and such resins have been successfully used in FI systems (e.g., Aguilar-Islas et al. 2006; Lohan et al. 2006) and combined with ICP-MS detection (Lohan et al. 2005; Sohrin et al. 2008; Milne et al. 2010). Commercially available resins that have been used for the analysis of trace metals in seawater include an NTA-type chelating resin for Fe and Cu (Lohan et al. 2005), an EDTA-type resin for Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn (Sohrin et al. 2008), and Toyopearl AF-Chelate 650M resin, which contains tridentate iminodiacetate functional groups, for Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn (Warnken et al. 2000; Ndung'u et al. 2003; Aguilar-Islas et al. 2006; Brown and Bruland 2008; Milne et al. 2010).

Flow injection systems are particularly well suited to oceanographic analyses, since they are portable and robust, contain relatively simple and inexpensive components, and offer analytical determinations over a dynamic range of as much as three orders of magnitude (e.g., Xu et al. 2005). Modern FI systems are capable of high sensitivity, although the limits of detection vary with the method of detection and the analyte. Flow injection systems can also be coupled with towed, trace metal-clean sampling devices, enabling the near-real-time determination of trace metals in surface ocean waters (e.g., Bowie et al. 2002; Bruland et al. 2005). In addition, FI methods are ideal for kinetic catalytic methods that use the analyte of interest as a reaction catalyst, resulting in highly sensitive analytical techniques (Resing and Mottl 1992; Measures et al. 1995; Aguilar-Islas et al. 2006). Previously developed FI methods for the determination of dissolved cobalt in coastal and estuarine waters have used the Trautz-Schorigen reaction (TSR), which involves the oxidation of either gallic acid (3,4,5-trihydroxybenzoic acid; Sakamoto-Arnold and Johnson, 1987) or pyrogallol (Cannizzaro et al. 2000) with hydrogen peroxide in the presence of cobalt as a catalyst to produce chemiluminescence emission in the visible region.

The aim of the work described in this article was to develop an FI method that can determine total dissolved cobalt in open-ocean waters and to highlight the need to UV-irradiate seawater samples before determination of dissolved cobalt.

The recent launch of the international GEOTRACES program, which aims to determine trace metal concentrations in diverse oceanic regions, requires accurate methods that are highly sensitive. The focus of the study described here was the analysis of dissolved cobalt in open-ocean seawater, for which no FI method has been developed to date. This method was then applied to water column samples collected from the oligotrophic Sargasso Sea in June 2008.

Materials and procedures

Apparatus—A schematic diagram of the flow-injection manifold used is shown in Fig. 1; it consists of an eight-channel peristaltic pump (Minipuls 3, Gilson); two micro-electronically actuated six-port, two-position injection valves (VICI, Valco Instruments); a photomultiplier tube (PMT, Hamamatsu H 6240-01) containing a quartz glass spiral flow cell (internal volume 130 μ L; Baumbach and Co.); and a thermostatic water bath (Grant). The peristaltic pump tubing used was two-stop accu-rated™ PVC (Elkay). All other manifold tubing was 0.8 mm i.d. PFA Teflon (Cole-Parmer). The FI manifold had one mixing coil (1.85 m) and one reaction coil (5 m). The reaction coil was constructed by French-knitting 5 m of 0.8 mm i.d. PFA Teflon tubing using a four-pronged knitting spool. Two acrylic columns (internal volume 70 μ L), with porous HDPE frits (BioVion F, 0.75 mm thick), were incorporated in-line, one on the sample-buffer line to remove trace-metal impurities from the buffer solution, and a second for the preconcentration of cobalt and removal of the cations from the seawater sample matrix. Both columns were filled with Toyopearl AF-Chelate-650M resin (Tosohaas) (hereafter referred to as "IDA-Toyopearl"). The direction of flow through the cleanup column was one-way, so it was necessary to reverse the column at the end of each day to prevent the resin from becoming compacted. This was not required for the sample preconcentration column, which was loaded and eluted in opposing flow directions. The T-piece before the 1.85-m mixing coil, the mixing coil itself, and the 5-m reaction coil were maintained at 60°C by placing them inside a thermostatic water bath (Grant). The data acquisition module (Ruthern Instruments) and valve control software (LabVIEW v. 7.1) were operated using a laptop computer (Dell). To minimize contamination, all sample handling was carried out in a Class-100 clean bench. The FI system was flushed daily and after any configuration changes (such as pump tube or reagent replacement) with 1 M HCl solution.

Reagents—Unless otherwise stated, all chemicals were obtained from Fisher Scientific, and used as received. All solutions were prepared inside a Class-100 clean bench using ultra-high-purity (UHP) water (≥ 18.2 M Ω cm, Elgastat Maxima). The eluent, 0.1 M hydrochloric acid (HCl), was prepared by diluting 8.8 mL of 10 M ultrapure subboiling distilled HCl (SpA, Romil) to 1 L in UHP water. The 0.05 M ammonium acetate column rinse and conditioning solution was prepared by dissolving 3.8 g of ammonium acetate crys-

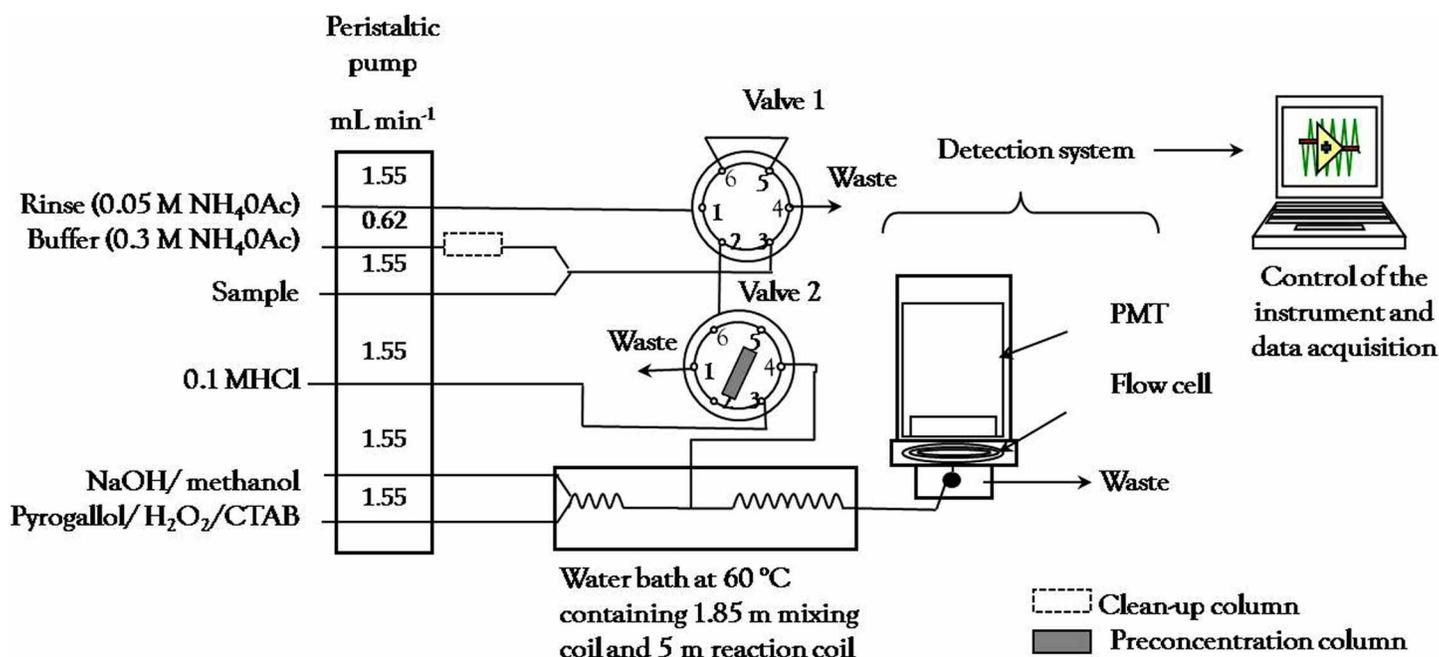


Fig. 1. FI-CL manifold configuration for the determination of total dissolved cobalt in seawater.

tals in 1 L UHP water and adjusting to pH 4 with 3.8 mL of 10 M ultrapure HCl (SpA, Romil). The 0.3-M ammonium acetate sample buffer solution was also prepared in UHP water from ammonium acetate crystals (23.11 g L⁻¹), and the acidified seawater samples were buffered online to between pH 5.2 and 5.5 by mixing with this solution. Because the detection limit of FI methods is often limited by the blank value rather than the sensitivity of the instrumental technique (Bowie and Lohan 2009), the potential contribution to the blank from the sample buffer solution was minimized by using a cleanup column (identical to the preconcentration column) on the sample buffer line. The 0.17-M sodium hydroxide (NaOH) reaction buffer solution was prepared by dissolving sodium hydroxide (NaOH) pellets (6.7 g L⁻¹) in 1 L solution (20% vol/vol methanol [HPLC grade], 80% vol/vol UHP water). The 50 mM pyrogallol reagent was prepared by sonicating 6.30 g pyrogallol and 9.12 g acetyltrimethylammonium bromide (CTAB) in UHP water; when the pyrogallol and CTAB were fully dissolved, 58.4 mL of 35% hydrogen peroxide (H₂O₂) was added, and the solution was diluted to 1 L with UHP water. As the pyrogallol solution is reportedly stable for only 48 hours (Cannizzaro 2001), this reagent was prepared daily as required.

Sample collection and pretreatment—Surface seawater (0–1 m depth) was collected using a trace metal-clean pole sampler: two 1-L widemouth low-density polyethylene (LDPE, Nalgene) bottles were secured in a Plexiglas frame at the end of a bamboo pole, which was extended from the ship's stern for sample collection while backing slowly into the wind. Water column samples were collected in modified 5-L Teflon-lined external closure Niskin-X samplers (General Oceanics) sus-

ended from a Kevlar line using a stainless-steel end weight and solid PVC messengers (Sedwick et al. 2005). Samples were immediately filtered at sea inside a shipboard Class-100 clean container laboratory, through a 0.4- μ m pore-size Supor Acropak filter capsule (Pall) that was prerinsed with 5 L UHP water followed by several hundred milliliters of sample. Filtered samples were acidified to 0.024 M with ultrapure HCl (SpA, Romil) before analysis.

Procedure—The peristaltic pump (Minipuls 3, Gilson) was set at 5.50 rpm to attain the flow rates shown in Fig. 1. After stabilization of the baseline (typically 30–45 min), with valve 1 and valve 2 in position B, acidified ammonium acetate was passed through the preconcentration column for 40 s. Valve 1 was then switched to position A, and a buffered sample was loaded onto the preconcentration column for 300 s. Valve 1 was then switched to position B, and the preconcentration column was rinsed for 40 s with the acidified ammonium acetate solution. After this rinse step, valve 2 was switched to position A for 90 s, and the eluent (0.1 M HCl) was passed over the chelating resin in the opposite direction to that of the loading phase, thus eluting the cobalt from the preconcentrating resin into the reagent stream that was then carried to the PMT detector. In total, one complete analytical cycle took 7.8 min. During the load and rinse phases, the eluting acid bypassed the column and mixed with the other reagents to produce the baseline signal.

Standardization—The analytical system was calibrated daily by simple linear regression of standard curves. Stock standard solutions were prepared in acidified UHP water by serial dilution of a 17-mM cobalt atomic absorption standard solution (Spectrosol). Working standards (additions of 12.5–75 pM) were

prepared in acidified low-trace-metal seawater (Atlantic Ocean surface seawater collected from 28°51'S, 4°41'W; total dissolved cobalt 13.7 ± 2.7 pM). Standards were run at the beginning and end of each program of analysis in triplicate, and concentrations were calculated from peak heights. Standard curves were linear ($r^2 > 0.99$) up to concentrations of 2 nM.

Assessment

In this section, an assessment of the commercially available chelating resin, Toyopearl AF-chelate 650M, in the preconcentration step of the FI-CL method for the determination of total dissolved cobalt in seawater is presented. The accuracy of the method was assessed using SAFe seawater reference samples, and the method was applied to the determination of total dissolved cobalt in water-column profiles from the Sargasso Sea. The key analytical parameters are shown in Table 1. Of particular interest is the improvement in detection limit of this FI-CL method compared with a previously published FI method (the average detection limit of this new method was 4.5 pM, c.f. ~8 pM for the method of Sakamoto-Arnold and Johnson 1987), and the wide dynamic range of this method (more than three orders of magnitude, ~4.5–2000 pM).

Online chelating resins—The commercially available IDA-Toyopearl chelating resin was compared with resin-immobilized 8-HQ, which has been used in previous FI-CL methods for cobalt determination (Sakamoto-Arnold and Johnson 1987; Cannizzaro et al. 2000). Figure 2 shows the FI elution profiles for resin-immobilized 8-HQ and IDA-Toyopearl resin. The same analytical conditions were used for both resins to allow a direct comparison. The use of IDA-Toyopearl provided consistently higher sensitivity and sharper elution profiles, e.g., for a 50-pM Co standard addition to seawater (Figure 2), use of IDA-Toyopearl gave a peak height of 1.28 mV, compared with 0.59 mV for resin-immobilized 8-HQ.

A comparison of the characteristics of the two resins is presented in Table 2. One of the advantages of IDA-Toyopearl is its greater binding capacity compared with resin-immobilized 8-HQ (25–45 $\mu\text{Eq mL}^{-1}$ versus 10 $\mu\text{Eq mL}^{-1}$, respectively). The breakthrough capacity (defined as the concentration of cobalt sorbed to the resin before 5% of the total concentration of the analyte in the eluant was detected) of the two resins (profiles not shown) was determined using inductively coupled plasma optical emission spectroscopy detection (ICP-OES, Varian 725-ES). An acidified 10- μM cobalt solution (pH 1.7) was buffered

to pH 5.2 and loaded onto a column containing a preconcentration resin (either IDA-Toyopearl or 8-HQ). Cobalt in the column eluent was continuously monitored by ICP-OES at an emission wavelength of 238.9 nm. The IDA-Toyopearl resin retained 25.3 nM Co before the 5% breakthrough limit was exceeded, compared with 13.4 nM Co for the resin-immobilized 8-HQ. Therefore, IDA-Toyopearl was selected as the preferred chelating resin for this method.

Reaction conditions—

Column-loading pH: Using ICP-MS as a detection system, Willie et al. (1998; 2001) demonstrated that Toyopearl AF-Chelate-650M quantitatively preconcentrated cobalt from seawater over the pH range 5.0–6.0. In the present study, the optimum pH range for the quantitative preconcentration of cobalt from seawater samples was found to be 5.2–5.5. Seawater samples were buffered online with 0.3 M ammonium acetate solution. Ammonium acetate buffers effectively within the pH range 3.8–5.8 ($pK_a = 4.8$) and is therefore suitable for this FI-CL method. A critical component of cobalt determination is characterization of the blank: even very low-level contamination (≤ 10 pM) of reagents can obscure the accuracy of a data set. Because the buffer is loaded on the column for the same period as the sample, any cobalt in the buffer solution could constitute a significant contribution to the blank (Willie et al. 1998). Therefore, a cleanup column, packed with IDA-Toyopearl resin, was placed in the sample buffer line before the sample preconcentration column, to remove any cobalt that might be present in the buffer solution.

Column-conditioning and rinse step: Previous FI-CL methods using resin-immobilized 8-HQ for cobalt determination (Sakamoto-Arnold and Johnson 1987; Cannizzaro et al. 2000) used UHP water as a column rinse solution and did not use a column-conditioning step. On the other hand, FI methods that have used IDA-Toyopearl as the chelating resin have included a column-conditioning step using a buffer with a pH similar to that of the in-line buffered seawater sample to ensure an optimum pH in the resin column before sample preconcentration (Aguilar-Islas et al. 2006; Brown and Bruland 2008). The column-conditioning step removes any residual acid that remains from the previous sample elution step and ensures that the resin is no longer in the protonated form, which might otherwise reduce retention of the analyte and thereby lessen the sensitivity of the method. Willie et al. (2001) demonstrated that a 0.1-M ammonium acetate rinse

Table 1. Key analytical parameters.

Parameter	This study	Literature values
Detection limit (seawater), pM	4.5	~8 (Sakamoto-Arnold and Johnson 1987)
Detection limit (UHP water), pM	3.8	5 (Cannizzaro et al. 2000)
Linear range, pM	3.8–2000	5–850 pM (Cannizzaro et al. 2000)
Blanks, pM	4.2 ± 1.5	
RSD ($n = 9$)	$\leq 4\%$	

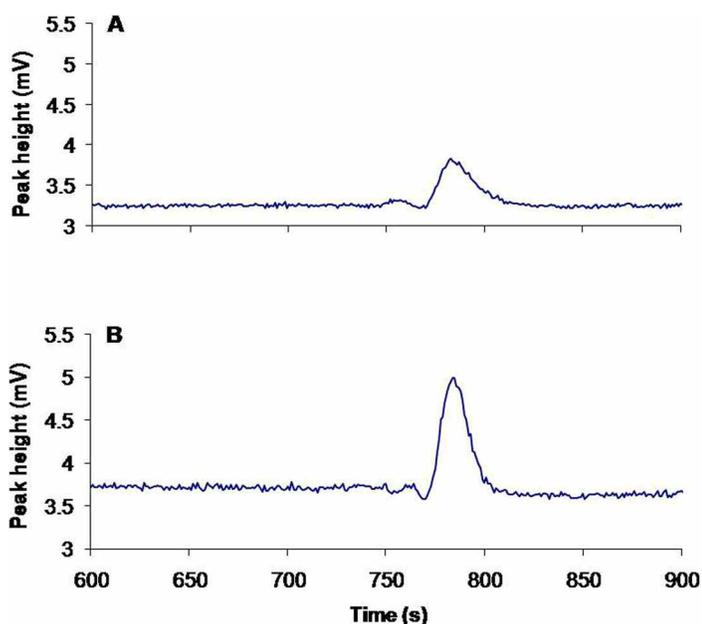


Fig. 2. Effect of two different chelating resins, resin-immobilized 8-hydroxyquinoline (A) and Toyopearl AF-Chelate 650M (B), on sensitivity and elution profile for a 50-pM standard in seawater using the same analytical conditions for both resins.

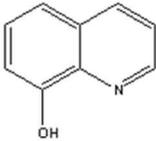
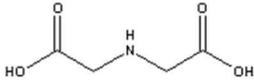
solution, buffered in the range of pH 3–5, was sufficient to eliminate seawater matrix components while still retaining the analyte on the IDA-Toyopearl chelating resin. Investigations during this study found that a further reduction in strength of this ammonium acetate rinse solution to 0.05 M did not result in reduced sensitivity, so the lower concentration was used. Thus the column-conditioning and rinse steps were incorporated in the analytical cycle to remove sea salt and excess acid from the preconcentration column before the sample loading and elution steps.

Eluting acid concentration: A range of ionic strengths of eluting acid are reported in the literature for FI systems using chelating resins for the preconcentration of dissolved cobalt from seawater. For example, Cannizzaro et al. (2000) used 0.05 M HCl to elute cobalt from an 8-HQ chelating resin. In this study, it was essential to maintain an optimum reaction pH of 10.35 ± 0.2 . A reaction pH outside this range resulted in changes to the baseline signal and significantly reduced sensitivity; e.g., at pH 10.6 the signal for a 25 pM cobalt addition was reduced by ~44%. To determine a suitable eluent strength while maintaining this reaction pH, eluents of 0.05, 0.1, and 0.5 M HCl were investigated, with standard additions of 25–75 pM cobalt in seawater. Increasing the acid concentration from 0.05 M (Cannizzaro et al. 2000) to 0.1 M HCl resulted in the rapid elution of cobalt from the IDA-Toyopearl resin and produced sharp sample peaks with CL detection, although there was no further improvement in peak shape between 0.1 and 0.5 M HCl. The increase in the eluting acid concentration (from 0.05 to 0.1 M HCl) required that the concentration of the NaOH solution be increased from 0.15 to 0.17 M to achieve the optimum reaction pH of 10.35.

Matrix effects and interferences: An investigation of the potential suppression of the cobalt CL signal by the major seawater cations was conducted by directly injecting solutions containing 9.9 nM cobalt in UHP water spiked with the chloride salts of Na, K, Ca, and Mg for 300 s (i.e., without a preconcentration step). These cobalt solutions were spiked with NaCl (10.7 g L⁻¹ Na), KCl (0.387 g L⁻¹ K), CaCl₂ (0.413 g L⁻¹ Ca), or MgCl₂ (1.29 g L⁻¹ Mg). A control solution contained cobalt and no added salts. Between injections, a solution of 0.05 M HCl (SpA, Romil) was injected for 120 s to rinse the previously injected solution.

With respect to the CL signal of the control cobalt solution, the alkaline earths produced the strongest reduction in chemiluminescence (51% and 55% reduction in signal for calcium

Table 2. Comparison of 8-HQ and IDA-Toyopearl resin characteristics.

	8-HQ ^a	Toyopearl AF-Chelate-650 M ^b
Bead	HW-75F	HW-65
Chelating group	8-hydroxyquinoline (8-HQ)	Iminodiacetic acid (IDA)
Chelating group structure		
Capacity	10 μEq mL ⁻¹	25–45 μEq mL ⁻¹
Particle size	32–63 μm	65 μm
Pore size	>1000 Å	1000 Å
Exclusion limit	50 MDa	5 MDa

^aLanding et al. (1986).

^bTosoh Bioscience (2008).

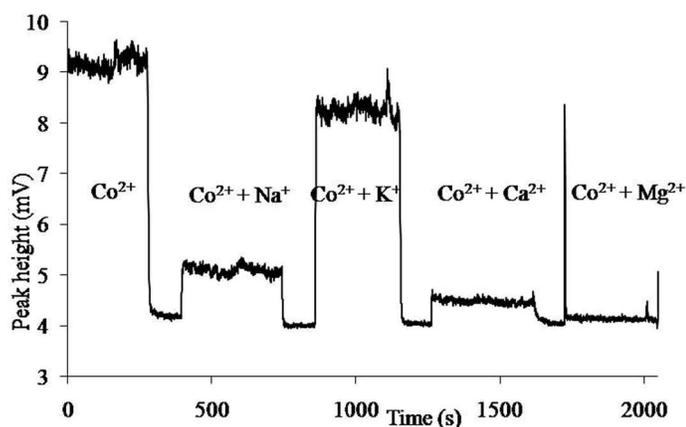


Fig. 3. Effect of the major seawater cations on the chemiluminescence signal (0.5 M Na⁺, 0.01 M K⁺, 0.01 M Ca²⁺, and 0.05 M Mg²⁺).

and magnesium, respectively; Fig. 3). This significant reduction in chemiluminescence, combined with a 44% signal reduction in the presence of sodium ions, illustrates the need for a matrix elimination step to remove the major seawater cations before the CL reaction and detection of the analyte.

For the analysis described here, the online preconcentration column must ideally have low affinity for the major ions in seawater, while quantitatively retaining the analyte on the resin. Elimination of the seawater ions can be achieved by rinsing the column with a dilute ammonium acetate solution before eluting the analyte with HCl. Even with the inclusion of this rinse step, however, some residual seawater matrix ions could still reduce the sensitivity of the method. Comparing standard additions of cobalt to seawater versus UHP water (12.5–75 pM), a 35% reduction in sensitivity was observed for standards prepared in seawater (data not shown). Consistent with this finding is the lower detection limit for standards prepared in UHP water compared with those prepared in seawater (Table 1). Therefore the sensitivity of this technique was monitored via calibration with standard additions (12.5–75 pM) before commencing analysis.

The potential for interference by the metal ions Ag⁺, Fe³⁺, Cu²⁺, Pb²⁺, Mn²⁺, and Cd²⁺ in the determination of Co²⁺ in seawater of the pyrogallol reaction was investigated by Cannizzaro et al. (2000). Of the ions tested, only Ag⁺ was found to cause a detectable interference, although it was concluded that this species was unlikely to interfere with cobalt analysis given the typical Ag concentrations in open ocean seawater (≤ 0.7 pM, Flegal et al. 1995).

Reaction temperature: Chemiluminescence reactions are sensitive to temperature, with the rate of reaction typically doubled for every 10°C increase in temperature. Cannizzaro et al. (2000) used a reaction temperature of 80°C; however, optimization experiments during this study showed that a reaction temperature of 60°C provided good sensitivity. The advantages of working at this lower temperature are twofold.

First, by working below the boiling point of methanol (64.7°C), the formation of bubbles in the NaOH/methanol reagent stream is avoided. Second, the use of this lower reaction temperature reduces evaporation losses from the thermostatic bath, thus avoiding the need to refill it (and potentially alter the reaction temperature) during the analysis.

Sample treatment for determination of total dissolved cobalt—

UV-irradiation: Similar to several other bioessential trace metals in seawater, a large fraction (>90%) of dissolved cobalt is complexed by uncharacterized organic ligands (Ellwood and van den Berg 2001). When using other analytical techniques, such as cathodic stripping voltammetry (CSV) and cation-exchange liquid chromatography with luminol chemiluminescence detection (LC-CL) as the analytical method, it is well established that it is necessary to break down these ligands to achieve full recovery of total dissolved cobalt (CSV, Donat and Bruland 1988, Vega and Van den Berg 1997, Saito and Moffett 2001, and Noble et al. 2008; LC-CL, Boyle et al. 1987). Because the degree of retention of organically bound cobalt by chelating resins is unknown, investigations were conducted to establish whether it was necessary to UV-irradiate seawater samples before determination of total dissolved cobalt by FI-CL. Previously acidified samples collected from 1000-m depth in the North Pacific subtropical gyre (30°N, 140°W) as part of the Sampling and Analysis of Iron (SAFe) program (Johnson et al. 2007) were analyzed to allow comparison between samples with and without UV pretreatment. Acidified SAFe samples were UV-irradiated in acid-washed quartz vials for 3 h using a 400-W medium-pressure Hg lamp (Photochemical Reactors). After UV irradiation, samples were decanted into acid-clean LDPE bottles and left for 48 h before analytical determination, to eliminate UV-generated free radicals that might otherwise interfere with the CL reaction. The concentration of total dissolved cobalt determined in UV-irradiated SAFe seawater samples was 40.9 ± 2.6 pM ($n = 9$), which was almost double the concentration measured in nonirradiated samples (25.4 ± 1.2 pM, $n = 8$). The results of this experiment demonstrate conclusively that UV irradiation is essential for the determination of total dissolved cobalt in acidified seawater by FI-CL.

There is evidence for the presence of strong, cobalt-binding organic ligands in seawater that have a stability constant (K_s) of $10^{16.3 \pm 0.9}$ (Saito and Moffett 2001; Saito et al. 2005) compared with the reported K_s value of $10^{13.08}$ for strong Fe(III)-binding ligands in seawater (Rue and Bruland 1997). However, in contrast to organic iron complexes, which appear to fully dissociate when seawater samples are acidified to pH 1.7 (Lohan et al. 2005), the results of this and earlier UV-irradiation experiments using other analytical techniques (e.g., Donat and Bruland 1988; Vega and van den Berg 1997; Noble et al. 2008; Milne et al. 2010) indicate that organic cobalt complexes do not completely dissociate at pH 1.7.

Although IDA-based resins such as Toyopearl AF-Chelate 650M are able to selectively complex free metal ions and other metal species that are thermodynamically and kinetically

labile, they may be unable to extract metals bound to strong organic ligands. Where it is necessary to increase pH to ≥ 5 before the preconcentration step, such as is the case for FI-CL analysis of acidified samples, some of the metal analyte may become recomplexed to form thermodynamically or kinetically inert complexes (in terms of chelation by the resin) with organic ligands present in the sample, and thus pass through the column without being retained on the resin. Unless the dissolved organic material (DOM) that contains these ligands is destroyed (e.g., by UV irradiation), such organic complexation may result in trace metal analytes not being fully retained by the preconcentrating resin (Ndung'u et al. 2003), and hence the determination of only the labile (rather than total) dissolved concentrations of trace metals such as cobalt. The recent GEOTRACES intercalibration exercise and program launch serves as a timely reminder that it is essential to UV-irradiate samples before determination of dissolved cobalt.

Addition of a reducing agent: The pyrogallol-FI-CL method described here determines dissolved cobalt by reducing the thermodynamically and kinetically labile cobalt fraction to Co(II). The SAFe samples were collected and acidified to pH 1.7 in 2004. Lohan et al. (2005) reported that the longer a sample was acidified, the greater the percentage of iron that exists as Fe(II). Noting the similarity between the redox speciation of iron and cobalt in seawater (both are present in the +2 and +3 oxidation states under typical seawater conditions), cobalt may similarly tend toward Co(II) in acidified seawater. However, to verify that the reduction of Co(III) to Co(II) was complete in this method, an experiment was conducted to investigate the effect of incorporating a strong reducing agent in the reaction scheme. A buffered 0.04-M solution of sodium sulfite (NaSO_3) was prepared by dissolving 0.202 g of NaSO_3 in 30 mL UHP water plus 10 mL of 0.4 M ammonium acetate buffer, to ensure no changes in the sample pH that could affect the sensitivity of the CL reaction. Before use, this solution was passed through two columns in series containing IDA-Toyopearl resin, to remove trace metal impurities; the first 10 mL of the purified NaSO_3 solution was discarded, and the next 30 mL was collected and left for at least 24 h to stabilize before the acidified SAFe seawater was spiked with 25 μL per 10 mL sample. Samples were then left at least 8 h, to allow time for the reduction of Co(III) to Co(II), before dissolved cobalt was determined by FI-CL. The samples used in this experiment were not UV-irradiated. Using a *t*-test, no significant difference ($P = 0.06$) was observed between samples with and without NaSO_3 addition (25.4 ± 1.2 and 30.5 ± 1.7 pM, respectively; $n = 8$), suggesting that the method effectively determines all dissolved cobalt in previously acidified seawater samples.

Saito et al. (2005) described natural cobalt-binding ligand complexes with stability constants greater than $10^{16.8}$ (c.f. $10^{11.48}$ – $10^{13.08}$ for iron-binding ligands in seawater; Rue and Bruland 1995) and reported that addition of excess Ni(II) ions did not liberate organically complexed cobalt. On this basis, it

was argued that organically bound cobalt was present as Co(III) complexes—a further parallel with iron biogeochemistry, since dissolved iron is thought to exist mainly as organically complexed Fe(III) in seawater (e.g., Rue and Bruland 1995). Acidification of samples may dissociate these organic complexes, releasing Co(III), which is then reduced to Co(II) over time, thus explaining the apparent lack of effect of the added reducing agent (*see* above). If this were the case, however, one would predict no increase in total dissolved cobalt determined after UV irradiation of acidified samples, which is inconsistent with the results presented here. This then implies that it may not be the redox chemistry of cobalt that is preventing complete recovery of total dissolved cobalt from acidified seawater samples on the IDA-Toyopearl resin, but rather that the acidified samples contain organic ligands that complex dissolved cobalt upon readjustment of the sample pH (Ndung'u et al. 2003; Saito et al. 2005).

The addition of a reducing agent resulting in no difference in cobalt concentrations has been previously reported by Vega and van den Berg (1997). However, their results contrast with results of a similar study by Donat and Bruland (1988), using sodium borohydride (NaBH_4) as the reducing agent, who found that it was still necessary to add a reducing agent even after samples had been UV-irradiated, to fully recover total dissolved cobalt. Vega and van den Berg (1997) argue that the reason for this contrast may result from the less extensive UV-irradiation step used by Donat and Bruland (1988). As no difference in cobalt concentration was observed between samples with and without addition of a reducing agent, it is suggested that the samples in this study contained Co(II).

Without UV irradiation, the dissolved cobalt concentration in the SAFe D2 reference samples was 38% lower. A similarly reduced concentration was observed by Donat and Bruland (1988) in their non-UV-irradiated samples, using CSV. However, the key differences between this study and Donat and Bruland's (1988) was the addition of reducing agent to samples after UV irradiation. It is therefore important to investigate the amount of time necessary to UV-irradiate open-ocean samples before analysis using SAFe or GEOTRACES reference samples. In addition, further insights might be gained by investigating the redox state of complexed cobalt during the analytical process.

Analytical figures of merit—

Blanks and detection limits: For this FI-CL method, the major contribution to the blank was from the addition of the ammonium acetate sample buffer. Although Cannizzaro et al. (2000) found no detectable signal from the added ammonium acetate buffer, this was not the case in the present work. The extremely low concentrations of cobalt in the open-ocean samples analyzed in this study required a fivefold increase in loading time compared with those used for estuarine samples (300 versus 60 s, respectively). Because the sample buffer and sample are loaded simultaneously, any cobalt present in the buffer potentially contributes to the analytical signal. There-

fore, a cleanup column (containing IDA-Toyopearl resin) was added to the sample buffer line to reduce the amount of cobalt loaded onto the preconcentration column. In common with other flow injection techniques, the manifold blank was determined by running an analytical cycle without the sample (e.g., loading the sample buffer only onto the preconcentration column) (Bowie and Lohan 2009), resulting in a concentration of 4.2 ± 1.5 pM ($n = 21$). The detection limit was calculated as the cobalt concentration corresponding to three times the SD of the blank, resulting in a detection limit of 4.5 pM ($n = 21$) in seawater.

Accuracy and precision: The accuracy of previous methods determining dissolved cobalt in seawater has been assessed using the North Atlantic Surface Seawater (NASS; National Research Council of Canada) certified reference material (CRM). In this study, a concentration of 208 ± 30 pM for total dissolved cobalt was determined in UV-irradiated NASS-5, which is within the certified range (187 ± 51 pM). This result is within error, and is consistent with the results of Vega and van den Berg's (1997) determination of total dissolved cobalt in UV-irradiated NASS-2. Like NASS-2, NASS-5 has a large standard deviation, and has a mean concentration that is up to 20 times greater than typical open-ocean surface concentrations of dissolved cobalt. Therefore, the combination of a large uncertainty and high cobalt concentration combined with the lack of a certified concentration of cobalt after UV irradiation renders this CRM unsuitable with respect to the determination of dissolved cobalt in open-ocean surface waters. Consequently, an alternative low-level cobalt reference sample was analyzed to assess the accuracy and precision of this FI-CL technique. The concentration of total dissolved cobalt was determined in acidified seawater samples (D2) collected from 1000-m depth in the North Pacific as part of the SAFe program (Johnson et al. 2007). Sample collection details were described by Johnson et al. (2007). The cobalt concentrations for the SAFe D2 reference samples after UV irradiation (40.9 ± 2.6 pM; $n = 9$) are in excellent agreement with results obtained for the same sample by other analytical techniques that also used a UV-pretreatment step (43 ± 3.2 pM, <http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html>). The analytical precision of the method was determined from repeat analyses of the SAFe D2 reference sample, yielding an uncertainty of $\pm 4\%$ expressed as relative SD of the mean ($n = 9$). Samples that were not UV-irradiated were also in excellent agreement with the consensus values for non-UV-treated SAFe D2 samples (this study, 25.4 ± 1.2 pM, $n = 8$; SAFe D2 non-UV, 26.9 ± 4.7 pM).

From the data already submitted to the GEOTRACES/SAFe intercalibration Web site (<http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html>) it has become apparent that a UV-irradiation step is necessary to determine total dissolved cobalt in the SAFe reference samples. At present, however, there is too much spread in the cobalt values for the SAFe reference samples, and further work needs to be conducted to investigate optimal UV-irradiation parameters (Bruland, pers. comm.). As

additional laboratories continue to submit data for SAFe and GEOTRACES reference samples, using UV pretreatment, confidence in the consensus values will increase.

Application to water column samples from the Sargasso Sea: This method was used to determine dissolved cobalt in samples from the FeAST-6 cruise in the Sargasso Sea, during which water column samples were collected in the region southeast of Bermuda. As an example, the vertical concentration profile of dissolved cobalt from the analysis of samples collected on June 6, 2008 from $31^{\circ}29'N$, $62^{\circ}45'W$, close to the Bermuda Atlantic Time Series (BATS) station, is shown in Fig. 4. The data shows a nutrientlike profile, but unlike a typical nutrientlike element, this profile exhibits surface enrichment (see Fig. 4). In contrast to Saito and Moffett (2001), who reported modest surface depletion (19 pM at 15 m), this study observed enrichment at a similar depth (40.2 pM at 10 m), followed by rapid depletion to a minimum of 25.3 ± 2.2 pM between 25 and 50 m, consistent with concentrations reported at the equivalent depths by Saito and Moffett (2001). The cobalt concentrations increased below 200 m, associated with remineralization below the base of the permanent pycnocline, to the maximum depth in this study (1000 m), where 70.1 ± 3.0 pM was determined, compared with 68.6 pM at the equivalent depth (1200 m) reported in Saito and Moffett (2001). The cobalt concentration at 300 m depth was higher in this study compared to Saito and Moffett (2001) (49.1 ± 0.9 pM, c.f. 33.7 pM), indicative of a shallower permanent pycnocline, possibly associated with the passing of a cyclonic eddy. The FeAST-6 cruise took place during a season of high eolian dust deposition, the effect of which was evident by the degree of enrichment of aluminum in surface waters (data not shown). In apparent contrast to conclusions drawn from analysis of time-series data of total dissolved cobalt from the MITESS mooring near the BATS station (Saito and Moffett 2002), these findings indicate that there may be a correlation

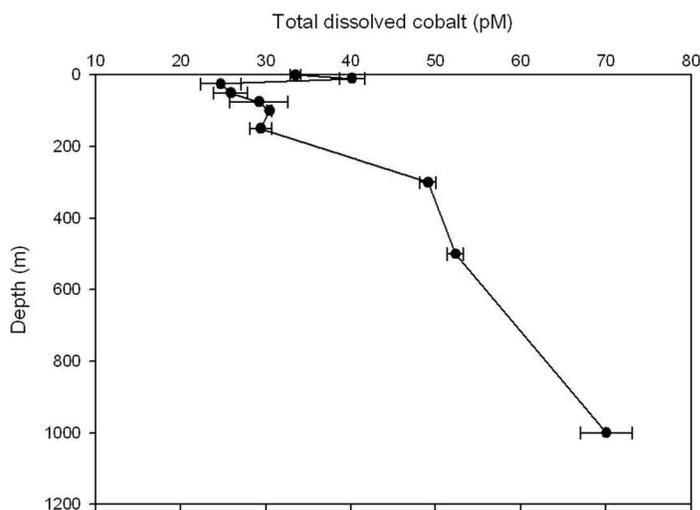


Fig. 4. Vertical concentration profile of total dissolved cobalt (dCo) in the subtropical North Atlantic Ocean ($31^{\circ} 29'N$, $62^{\circ} 45'W$) with error bars of ± 1 SD.

between eolian dust deposition events and surface concentrations, raising questions about the nature of the primary control of this micronutrient in the Sargasso Sea. However, the data reported by Saito and Moffett (2002) is from the MITESS sampler, which is moored at ≤ 40 m, below the seasonal pycnocline, and may therefore mask any atmospheric inputs that would be isolated in the shallow mixed layer, thus obscuring any potential relationship.

Discussion

The UV-irradiation experiment represents an important analytical development in the analysis of dissolved cobalt in seawater by FI-CL, in that it demonstrates the absolute requirement for UV irradiation to liberate organically complexed cobalt in acidified samples before FI-CL determination of dissolved cobalt. Previous FI-CL methods (and other analytical techniques) that did not include this step may have measured only dissolved labile cobalt (as defined in Noble et al. 2008). The use of the commercially available resin Toyopearl AF-Chelate 650M improves the reproducibility of the method and simplifies the preparation of the analytical system, as there is no need to synthesize the chelating resin. The introduction of a resin-conditioning step (acidified ammonium acetate rinse) also led to increased sensitivity. In addition to highlighting the need to UV-irradiate seawater samples and the advantages of using a column-conditioning step, this method also demonstrates the importance of effective sample buffering, both for the pH at which the sample is loaded onto the chelating resin column (pH 5.2–5.5), and the reaction pH of the combined reagent stream as it enters the detector (pH 10.35 ± 0.2). In this method, incorrect buffering results in a significant reduction of sensitivity.

The FI-CL method presented here is selective, has a low detection limit (4.5 pM), and is portable, making it an ideal method for determining total dissolved cobalt in open ocean regimes. The suitability of this method for shipboard use enables the mapping of total dissolved cobalt distributions while at sea, which make this technique an attractive analytical tool for examining high-resolution spatial and temporal trends in the oceanic distribution of cobalt. Although CSV can be used to determine total dissolved cobalt, FI methods are generally less time-consuming. Given that cobalt is an essential micronutrient for carbon acquisition for certain key groups of phytoplankton (e.g., *Prochlorochoccus*, *Synechococcus*), it is important to attain high-quality data to further our understanding of the biogeochemical cycling of this element in marine waters.

Comments and further recommendations

The relative simplicity and low risk of contamination make this method well suited for use in the laboratory and for shipboard analyses. The implementation of an automated sample selection valve could further reduce the risk of contamination associated with transferring the sample line from one sample

to the next. In this study, the preconcentration columns were fabricated according to Cannizzaro et al. (2000). Commercially available columns potentially improve the reliability and reproducibility of the separation/preconcentration step by minimizing leakage and contamination. Such columns have successfully been used in other FI applications (e.g., Aguilas-Islas et al. 2006; Lohan et al. 2006), and would likely be suitable for application in this method.

Owing to the absence of a suitably low-concentration certified reference material for cobalt, it is recommended that SAFE and GEOTRACES seawater reference samples be used for quality assurance when analyzing open-ocean samples. The consensus values of the SAFE reference seawater materials are now available at: <http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html> and www.geotraces.org/Intercalibration.html#standards_certifiedrefs. The GEOTRACES values are soon to be published on the same two Web sites. However, a cautionary note must be added at this point with regard to the cobalt numbers. A UV-irradiation step is necessary to determine total dissolved cobalt in the SAFE reference samples. However, at present there is still too much variation in the values, even after UV irradiation, to make a definitive conclusion. Consequently more research needs to be performed to evaluate the intensity and duration of the UV pretreatment required to release all the cobalt for the various analytical methods. Therefore, although there is a slight caveat attached to the accuracy of the cobalt values, the use of these low-metal, open-ocean reference materials is strongly advocated as they will likely benefit scientists determining dissolved cobalt and other trace elements in much the same way as they have done for the determination of total dissolved iron in seawater.

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