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Real-Time Monitoring of Picomolar Concentrations of Iron(II) in Marine Waters Using Automated Flow Injection-Chemiluminescence Instrumentation

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A shipboard-deployable, flow-injection (FI) based instrument for monitoring iron(II) in surface marine waters is described. It incorporates a miniature, low-power photon-counting head for measuring the light emitted from the iron-(II)-catalyzed chemiluminescence (CL) luminol reaction. System control, signal acquisition, and data processing are performed in a graphical programming environment. The limit of detection for iron(II) is in the range 8−12 pmol L^{-1} (based on 3s of the blank), and the precision over the range 8−1000 pmol L^{-1} varies between 0.9 and 7.6% (n = 4). Results from a day−night deployment during a north-to-south transect of the Atlantic Ocean and a daytime transect in the Sub-Antarctic Front are presented together with ancillary temperature, salinity, and irradiance data. The generic nature of the components used to assemble the instrument make the technology readily transferable to other laboratories and the modular construction makes it easy to adapt the system for use with other CL chemistries.

Introduction

In much of the open-ocean, the major nutrients (nitrate, phosphate, and silicate) control primary production by phytoplankton, but in vast areas of the Southern and Pacific Oceans, iron has been shown to be growth limiting (*1*−*3*). Accurate determination of iron in seawater is therefore fundamentally important to our understanding of the dynamics of marine ecosystems and their role in global climate change. However, despite its abundance in the Earth's crust, dissolved iron is present at extremely low levels (<1.0 nmol L^{-1}) in the open ocean (*4*), which is primarily the result of its low solubility in seawater.

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It is particularly important to determine the lower redox state, iron(II), because of its greater solubility and potential bioavailability (*5, 6*). However, iron(II) is a transient species in oxygenated waters, existing at low picomolar concentrations, and therefore making measurements that do not perturb the redox balance is extremely difficult. At seawater pH, iron(II) is rapidly oxidized by O_2 and H_2O_2 to the more thermodynamically favored iron(III) form (*7, 8*). Iron(II) persists due to a combination of photochemical (*9, 10*), thermal, enzymatic, and microbial cycling pathways (*11, 12*). Its oxidation rate is retarded at low temperatures (*7*), and although there is no direct evidence for the presence of iron-(II)-specific ligands in marine waters, organic complexation may promote iron(III) photoreduction and thus slow oxidation rates further (*10, 12*). Moreover, iron(II) may be supplied to the ocean through atmospheric deposition (*13*) and diffusion from reducing sediments (*14*). The ratio of iron(II) to "total" dissolved iron(II+III) in surface seawater is thus a balance between the strength of the sources of iron(II), rates of reduction and oxidation, and complexation of each redox state by (in)organic ligands.

Due to the short half-life (several minutes) of iron(II) species in seawater, measurements are best performed in situ or immediately after underway sampling, by delivering seawater directly from the ocean to the analyzer. Discrete vertical sampling for iron(II) using Go-Flo bottles is problematic because during collection, retrieval, and analysis, the redox speciation may change due to oxidation to iron-(III), hydrolysis, and precipitation to form colloidal and oxyhydroxide species. Alternatively, the analyte can be stabilized in situ using strong iron(II) chelators (e.g. ferrozine) (*15, 16*) for later analysis, but this may promote a redox shift toward the reduced form (*17, 18*). Ferrozine can also be used without preconcentration by incorporating long path length capillaries (2−5 m) to achieve moderate detection limits (100−200 pM) with absorbance spectroscopy (*19, 20*). It is important to appreciate that all iron speciation results are essentially operationally defined.

Existing techniques for iron determinations in seawater can be broadly categorized into "land-based" (graphite furnace atomic absorption spectrometry (GFAAS) (*21*) or inductively coupled plasma mass spectrometry (ICP-MS)) (*22*) and "ship-based" (flow injection (FI) with chemiluminescence (CL) *(23*) or spectrophotometric (*24*) detection or cathodic stripping voltammetry (CSV)) (*25*) methods. The advantages of shipboard systems are near real-time data with increased temporal and/or spatial resolution and the ability to modify research strategies in response to environmental change or inadvertent contamination.

In this paper, we describe a portable FI-CL instrument for the on-line monitoring of iron(II) in surface seawater, based on the catalytic effect of iron(II) on the luminol reaction in the absence of added oxidant (*26*−*29*). The method can also be used for dissolved iron(II+III) measurements, after minor modification to the manifold and software (*23*). FI-CL systems for dissolved iron determinations in discrete samples (*30*−*32*) and for investigating iron(II) oxidation rates in synthetic media (*33*) and eutrophic lakes (*34*) have been reported. The mechanism of the two step luminol oxidation chemiluminescence reaction has recently been described in detail (*35, 36*). An automated FI-spectrophotometric system has previously been described (*37*) for underway determination of dissolved iron in the surface ocean (5 min resolution), but the reaction, based upon the catalytic oxidation of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (DPD), cannot be used for iron redox speciation studies. The automated

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instrumentation reported here, however, can be used for the continuous underway measurement of ambient iron(II) in seawater. The system incorporates a miniature, low-power (5 V) photon-counting head detector and is controlled through graphical user software programmed in National Instruments LabVIEW. Performance characteristics and analytical figures of merit are presented, together with results of shipboard deployment and evaluation over several day−night cycles during a north−south transect through the subtropical Atlantic Ocean and a daytime transect in the Sub-Antarctic Front south of Australia.

Experimental Section

Instrumentation. The system was designed as a multipurpose instrument which facilitates computer control of mains-powered peristaltic pumps, low voltage microsolenoid pumps and microelectronic switching, injection and autosampler valves, while simultaneously acquiring measurement data from one or two photomultipliers (PMTs), and up to 14 other unspecified analogue units. The instrument consists of two enclosures: a main control unit houses the power supply and power control sections, and a separate smaller, isolated enclosure houses the photomultiplier interface and associated electronics.

Instrument control is performed using a type II PCMCIA DAQCard-DIO-24 input/output (I/O) card (National Instruments Corp., Newbury, UK) providing 24 digital transistor-transistor logic (TTL) lines. Signal acquisition is achieved through a multifunction NI DAQCard-700, incorporating a 16 channel, 12 bit analogue-to-digital converter. In addition, eight digital TTL outputs on this card provide supplementary lines used in switched gain amplification of the PMT signals (four modes). Both cards are operated through a Toshiba Satellite 310CDS Pentium laptop computer (Toshiba Information Systems Ltd., Weybridge, UK). Communications software was written in LabVIEW version 5.1 (National Instruments Corp.). A generic LabVIEW *VI* (virtual instrument subroutine), produced by Ruthern Instruments Ltd. (Bodmin, UK), provides basic control of the instrument acquisition and output functions. This *VI* was later integrated within a secondary routine for automation, display, and data processing functions. In combination, the two *VIs* provide a fully functional virtual instrument designed for a variety of trace metal analyses based on incorporation of CL chemistries into FI manifolds (*38*).

A LabVIEW *VI* consists of two component windows: the front panel and the wiring diagram. The front panel is where the program is designed and contains ready-to-use switches, buttons, controls, and graphical displays of detector readings. The connections between each element in the front panel appear in a wiring diagram, which also includes additional functions for mathematical operations, file management, and I/O of data and controls via acquisition cards.

Figure 1 shows the flow-through design used for the sampling and determination of iron(II) in surface seawater. The pumps, valves, and detector used for the online iron(II) FI manifold (shown on the right-hand side of Figure 1) were coupled to the automated instrument described above. The FI manifold is a modification of that described in Bowie et al. (*23*) for the determination of "total" dissolved iron(II+III). For shipboard use, where additional space was available, conventional peristaltic pumps (Gilson Minipuls 3, Anachem Ltd., Luton, UK) were used to minimize flow pulsing. These pumps could be replaced by solenoid operated self-priming micropumps to miniaturize the system further for remote field deployments, although in-line filtration is essential with such devices to remove particulate material and prevent blockages inside the pumps (*39*). Injection valves I1 and I2 are 6-port Cheminert low-pressure valves (model C22, Valco Instruments Co., Houston, U.S.A.) with microelectronic two position actuators and $^1/_4$-28 fittings. Electronic switching valves are 3-way, 2-position direct lift solenoid valves, containing PTFE wetted parts and zero dead volumes (model EW-01367-72, Cole-Parmer Instrument Co., Hanwell, UK). Switching valves are set by default to normally open-common (NO/COMM) when de-energized and to the normally closed-common (NC/COMM) position when power is supplied. Pumps and switches are operated at 5 V DC (TTL) and 12 V DC, respectively, supplied from the main control unit. A power saver relay was used to reduce the input voltage from 12 to 8 V DC when energizing the solenoids for extended (>2 min) periods, to prevent coil damage.

The detection system consists of a flow cell constructed from coiled transparent PVC tubing (1.0 mm i.d., Altec, Hants, UK) and mounted on the window of a side-on photon-counting head (model H6240-01, Hamamatsu Photonics, Welwyn Garden City, UK). This compact unit incorporated a low-noise PMT and internal high-voltage power supply. It is supplied with a low voltage (5 V DC) source from the main control unit. The photon counting circuitry produces a TTL output pulse train, modulated by the light intensity received at the PMT window. This pulse train is integrated in a resistor-capacitor network to produce a low-level voltage, which in turn is amplified and filtered, resulting in a clean signal suitable for collection at the analogue-to-digital converter of the DAQCard-700.

All manifold tubing is 0.75 mm i.d. PTFE tubing (Fisher Scientific, Loughborough, UK), except peristaltic pump tubing which is flow-rated PVC (Elkay, Basingstoke, UK). Preconcentration, matrix elimination, and sample buffer cleanup was performed using in-line microcolumns (*23*) containing 8-hydroxyquinoline (8HQ) immobilized on a vinyl copolymer resin, synthesized according to a modified version of Landing et al. (*40*).

Shipboard Field Trials and Sampling. Shipboard trials were conducted during expedition ANT XIII/1 (September 29−October 23, 2000) aboard PS *Polarstern*. A north−south transect of the Atlantic Ocean was undertaken during the cruise from Bremerhaven (Germany) to Cape Town (South Africa). The regions of the open Atlantic Ocean covered during this voyage receive trace metal inputs dominated in the tropics and subtropics by wet and dry atmospheric deposition, predominantly due to episodic, long-range transport of Saharan dust, and precipitation through the migrating Inter-Tropical Convergence Zone. Moreover, high daytime irradiance levels experienced through large sections of this transect meant that this was an interesting area in which to study the possible effects of photochemistry on iron redox speciation.

A further shipboard trial was conducted in a contrasting environment aboard RSV *Aurora Australis* as part of the CLIVAR SR3 expedition in the Southern Ocean south of Australia (October 28−December 12, 2001). Cold seawater temperatures and low H_2O_2 concentrations may lead to extended iron(II) oxidation half-lives in these waters (*12*). During the return voyage to Hobart (Australia) a north−south transect was undertaken across a filament of the Sub-Antarctic Front (SAF). In contrast to the open Atlantic Ocean, this is an area of extremely low atmospheric iron deposition and extended daylight periods, although irradiance levels are variable due to persistent cloudy skies, even during the austral summer.

Continuous underway sampling of surface (1−2 m) seawater was performed using a towed torpedo-shaped fish (left-hand side of Figure 1) deployed off the crane arm of a hydrographic winch at a distance of ∼5 m from the ship's starboard side (*32, 41*). For the Atlantic Ocean survey, seawater was pumped on-board using a variable speed high volume peristaltic pump (model 7591-00, Cole Palmer Instrument Co.), fitted with silicone pump tubing and filtered

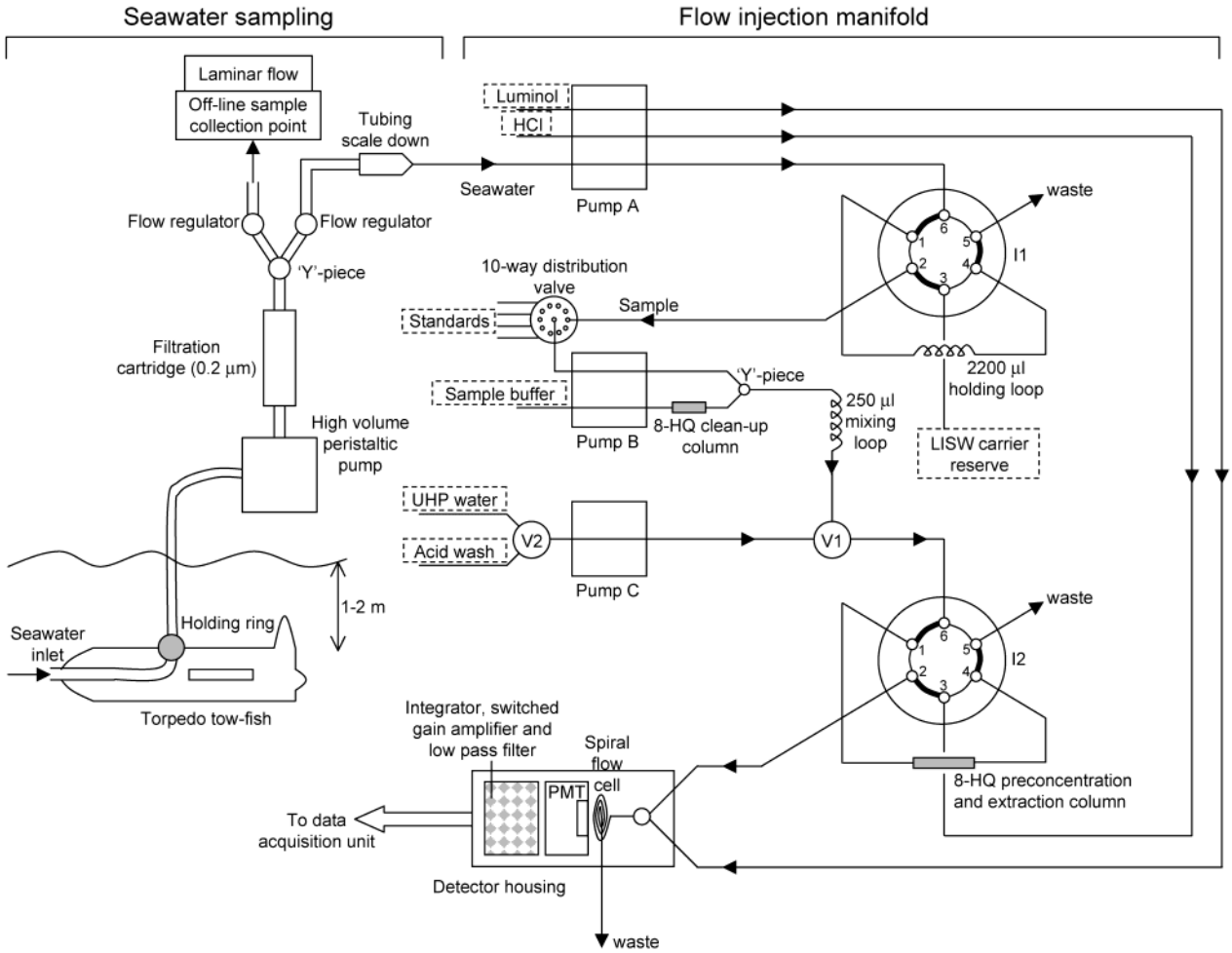

FIGURE 1. Schematic of the flow design used for the online determination of iron(II) in surface seawater. The left-hand side shows the in situ torpedo fish, high volume pumping and filtration system, while the right-hand side shows the components and tubing within the FI manifold. Pump A delivers a continuous stream of surface seawater and CL reagents, pump B delivers sample solution and sample buffer, and pump C delivers UHP water and acid wash solutions. Seawater from the LISW carrier reserve is not preconcentrated onto the 8HQ column and is simply used to prevent air entering the holding loop when sample solution is drawn up by pump B. The holding loop consists of 5 m of 0.75 mm i.d. PTFE tubing. V1 and V2 are solenoid switching valves, I1 and I2 are 6-port, 2-way injection valves. Boxes shown as dotted lines indicate the solution is sealed from the atmosphere within double zip-lock bags.

through a Sartobran-P polypropylene cartridge unit with a 0.2 μm cellulose acetate filter membrane (Sartorius Ltd., Epsom, UK). For the Southern Ocean survey, seawater was pumped on-board using a pneumatic PVDF double-diaphragm pump (model P.025, Wilden) and filtered through a Whatman Polycap (model 150TC) filter with 0.2 μm polysethersulfone filter membrane. Water from the sampling tubing entered a clean container laboratory positioned on the ship's aft deck, passed through a flow regulator, and was split into two channels. Seawater from one line was fed directly to the FI-CL iron(II) analyzer (Figure 1), while the other line provided a collection point for discrete samples which were later analyzed for dissolved iron(II+III) by FI-CL.

Elevated signals were observed for the first few (2−3) sample injections of each automated run of the iron(II) analyzer. This phenomenon was believed to be due to either low level contamination from the Cheminert injection valves or photoreduction of iron in seawater sitting in the transparent PTFE flow lines contained within the laboratory van. Thus data from the initial peaks for each run are not considered. The timing of each sample injection was determined by back calculation from the time (in UTC, Coordinated Universal Time) each data file was written to disk, and this time was adjusted for lag time delays due to surface water pumping and analysis (5.5 min on the Atlantic Ocean survey, 13.0 min on the Southern Ocean survey). The iron(II) concentration data were then merged with the ship's position and underway surface water parameters recorded on the ship's data logger system.

Reagents and Standards. Reagents were prepared as described previously (*23, 42*) for the determination of "total" dissolved iron (II+III). One liter volumes of buffered luminol reagent and HCl eluent were prepared in order to allow continuous determination of iron(II) species over a >10 h period. Low-level iron impurities in these reagents contributed to the detector baseline, which was above the background PMT noise level recorded in the absence of reagent flow. Blank correction was performed by subtracting the signal that was generated due to one load and elution cycle, without seawater sample present (i.e. sample buffer load plus ultrahigh purity (UHP) water rinse). To obtain this blank measurement, the sample line was stopped at the 'Y'-piece (to the right of pump B in Figure 1) and the associated pump tubing disconnected. A 1 s load can be used to differentiate the blank contribution from the 8HQ microcolumn and the switching of the injection valve, which were not negligible, from the reagent (sample buffer plus UHP water) contribution.

System calibration was performed as follows: low-iron seawater, which had been previously collected and allowed to age in the dark, was adjusted to pH 2.0 with triple quartz-distilled hydrochloric acid (Q-HCl) and 100 μM sodium sulfite added to ensure the iron in the sample was present in the reduced, ferrous form. Standards prepared at pH 2.0 in the presence of a reducing agent were necessary in order to prevent reoxidation of iron(II) which may have occurred at a higher pH. After a >8 h reduction period, standard additions of iron(II) in the range 0−1.0 nmol L^{-1} were made to this solution and immediately introduced into the FI-CL analyzer. The sensitivity of the system to iron(II) was ascertained from the slope of the standard curve.

System Operation. Prior to use, the PTFE flow lines, fittings, connectors and 8HQ microcolumns of the FI manifold were cleaned with 0.5 M Q-HCl and UHP water for >8 h. The system was calibrated at the start and end of each batch of reagents and also after any change in sensitivity (e.g. after change in temperature). For sample analysis, filtered (0.2 μm) ambient pH seawater was continually pumped from the towed torpedo fish into a 5 m (2.2 mL) holding loop (Figure 1) contained within injection valve I1. If air bubbles form in the holding loop (e.g. due to the degassing that may occur when very cold seawater enters a warm container laboratory) (*37*), the injection valve I1 can be replaced by a polyethylene bottle or PTFE debubbling vial that temporarily stores a small volume (e.g. 2 mL) of seawater pumped from the tow-fish for subsequent subsampling using a PTFE tube from the FI system. On switching I1 to the elute position, 1.6 mL of sample was drawn into the FI manifold where it was buffered in-line to pH 5.5 on passing through a 0.57 m (250 μL) mixing coil. The iron(II) in the buffered sample was preconcentrated and separated from the seawater matrix as it passed for 1 min over the 8HQ microcolumn contained within injection valve I2 (see *Method Chemistry* below). A distribution valve allows the system to be switched from sample analysis mode to calibration mode whereby up to 9 flow lines may be fed to spiked standard solutions. During calibration, seawater from the fish is continually pumped through the holding loop in injection valve I1 and to waste.

One complete analytical cycle was completed within 3 min. During this time, injection valve I1 was returned to the load position and seawater continually pumped through the holding loop ready for the next sample load. Using one batch of reagents, two blank measurements (in triplicate), two calibration curves (seawater standard plus two additions, in triplicate), and up to 8 h (160 peaks) of continuous online determination of 8HQ-reactive iron(II) can be performed. With a sampling and analysis sequence taking place every 3 min, a measurement is made every 0.9 km of the ship's track if the cruising speed is ~10 knots (18 km h^{-1}).

Results and Discussion

Analytical Performance. Instrumental drift was monitored during the Atlantic Ocean shipboard trials by regularly measuring the CL background emission and background peak-to-peak noise, blank signals, and calibration slopes. Sensitivity variations may result from temperature fluctuations (affecting both the PMT detector and CL chemistry), differences in reagent composition between batches, reagent aging, and degradation of pump tubing (affecting flow rates). The effect of temperature on the CL background noise and analyte signal generated using a 2.0 nmol L^{-1} iron(II) standard prepared in UHP water with the FI system in direct injection mode (i.e. 8HQ microcolumn removed; see *Method chemistry* below) was studied over a 24 h period. No significant changes in CL background noise (<2% drift, one measurement made each second, n > 80 000) or analyte signal (<6%, 86 ± 5 mV, n = 36) were observed, despite a 5.5 °C change in laboratory temperature. However, to minimize the risk of possible changes in sensitivity with temperature, the complete system was housed in an air conditioned clean air laboratory container.

A switched gain amplifier (gain on the PMT anode current) contained within the PMT interface provided four settings, ×100, ×1000, ×2000, and ×5000, which were selectable by the control *VI* software. Signal-to-noise ratio was proportional to gain, and therefore the highest gain setting (×5000) was used for most open-ocean applications. A lower PMT gain setting can be used for the higher concentration ranges expected in coastal and estuarine waters.

The sensitivity of the system was evaluated by comparing the slopes of calibration curves for standards prepared in low iron seawater (LISW), collected from the Southern Ocean Iron RElease Experiment (SOIREE) site (61°S 140°E) at a depth of approximately 500 m (*41*). Standard additions of 0.2, 0.4, 0.6, 0.8, and 1.0 nmol L^{-1} iron(II) were made. System sensitivity varied <5% on a single day (800 ± 40 mV per nmol L^{-1}, r^2 = 0.998) but varied up to 10% between days (typically 775 ± 73 mV per nmol L^{-1}, r^2 = 0.995). The precision for the standard addition solutions was in the range 0.9−6.2% RSD (n = 4). The iron(II) blank was typically 24 ± 4 pmol l^{-1} (n = 4), resulting in a limit of detection of 12 pmol L^{-1} (defined as three times the standard deviation of the blank).

Method Chemistry. In-line adjustment of sample pH was necessary to ensure optimum preconcentration of iron(II) species onto the 8HQ resin, while minimizing the effect of any interfering species which bind to 8HQ at higher pH (e.g. Mn) (*31*). Many other species are also known to catalyze the luminol reaction in the absence of selective analyte extraction and preconcentration (*43*). As such, these iron(II) measurements represent the operationally defined fraction that is extracted onto an 8HQ microcolumn after short (1 min) in-line buffering of sample to pH 5.5. These iron(II) measurements are hereafter referred to as 8HQ-reactive iron(II), recognizing that the iron(II) oxidation rate will be retarded and stability enhanced at lower pH (*7*, *9*). Ideally, iron(II) measurements should follow a sampling and pretreatment protocol that maintains speciation integrity as closely as possible.

The iron(II) detected using this approach is not an artefact of the analytical method. Since the residence time of the sample in the 8HQ microcolumn is short (<1.7 s), no significant reduction of iron(III) was observed in aged, filtered Southern Ocean seawater solutions spiked with up to 5.0 nmol L^{-1} iron(III) (<7% of analyte signal obtained for equimolar spiked iron(II) standards). Direct injection iron-(II) measurements without the preconcentration protocol (and hence not operationally defined) are also possible by removing the 8HQ microcolumn and sample buffer line. To achieve this, the 8HQ column within injection valve I2 is replaced with a sample loop (typically 100−150 μL), UHP water is used as carrier instead of an HCl eluent stream, and the column wash step (see Figure 1) is eliminated. In this manifold, injection valve I1 is not required, as seawater is fed directly to the loop within valve I2. The detection limit of this method is an order of magnitude higher than with the 8HQ column manifold (~0.2 nmol L^{-1}) and thus unsuitable for open-ocean iron(II) measurements where dissolved iron-(II+III) concentrations are extremely low (<0.1 nmol L^{-1}). However, this no-preconcentration version of the iron(II) system is particularly useful as a tracer for rapid underway mapping during iron fertilization experiments (e.g. SOIREE) (*3*), although it is important that the protocols described above are followed when determining the analytical blank. Any small positive matrix interference from Co(II) (*23*) can be removed by adding dimethylglyoxime (20 μM) to the luminol stream.

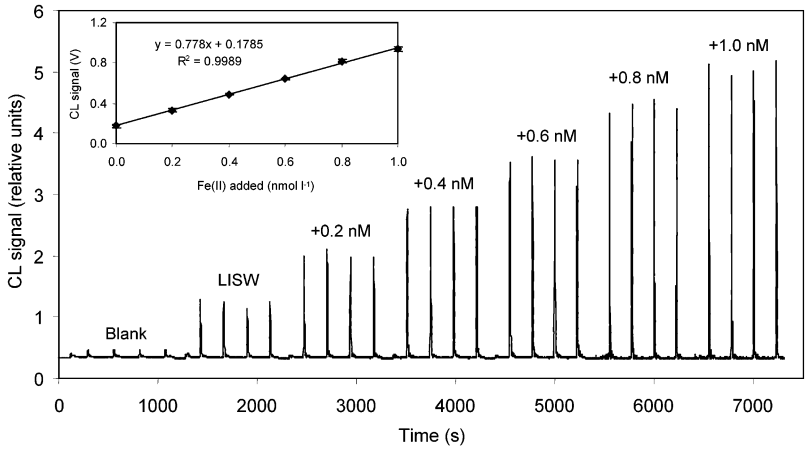

FIGURE 2. Shipboard calibration peaks and corresponding graph (inset) for iron(II) in the range 0−1.0 nmol L^{-1}.

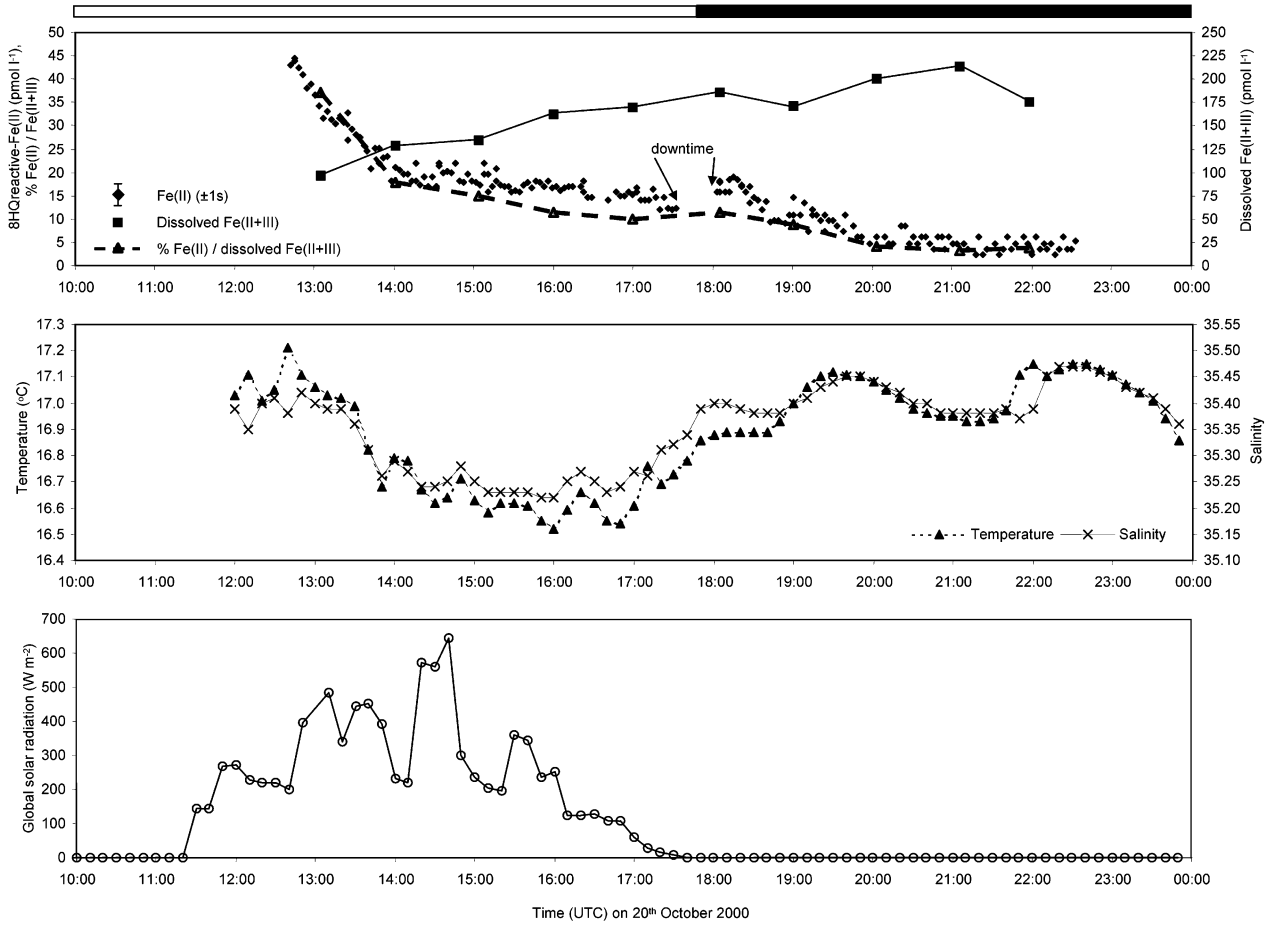

FIGURE 3. (a) Concentration of 8HQ-reactive iron(II), dissolved iron(II+III), and the iron(II)/iron(II+III) ratio during a 10 h shipboard deployment in the Atlantic Ocean, (b) surface temperature and salinity (10 min resolution), and (c) global solar radiation (10 min resolution) over the period 10:00−00:00 (UTC) on October 20, 2000. A typical error bar (1s) for the iron(II) concentrations is shown. The horizontal black bar represents the dark period during the survey.

Shipboard Trials. *Atlantic Ocean Survey.* Shipboard calibrations were performed at the beginning and end of each reagent batch (10 h periods). Figure 2 shows a typical set of peaks and corresponding standard curve for the blank solution and calibration standards prepared in LISW, with 0.2, 0.4, 0.6, 0.8, and 1.0 nmol L^{-1} additions of iron(II). Four replicates of each solution were made. The CL background noise showed good stability and reproducibility between replicate ($n = 4$) injections was typically <3%.

Figure 3 shows the results from the continuous determination of iron(II) in surface waters of the southeast Atlantic Ocean during one 10 h day−night period. Concurrent sea-surface temperature, salinity, and incident solar radiation are also shown. The online system was calibrated prior to switching to fully automated mode at 12:40 UTC on October 20, 2000. Continuous analyses were conducted until 22:34, except between 17:33 and 18:03 when a flow problem through the filtration cartridge needed to be rectified. The ship transited between 23°17′ S, 8°39′ E and 24°48′ S, 9°59′ E during the trial. During daylight hours, light cloud cover was present. Dusk occurred between 17:45−18:15 UTC. Subsamples were also taken hourly from the online system during the trial,

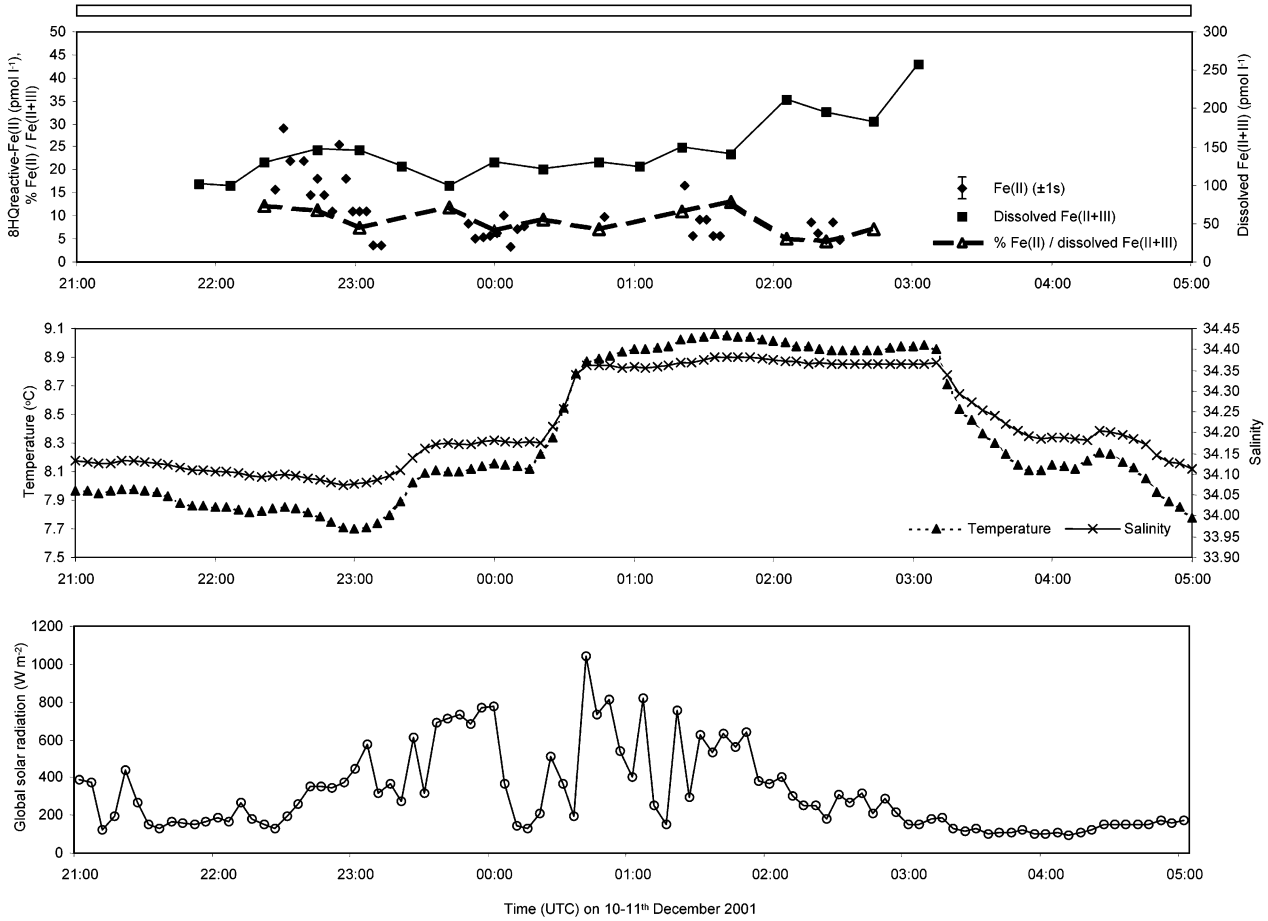

FIGURE 4. (a) Concentration of 8HQ-reactive iron(II), dissolved iron(II+III) and the iron(II)/iron(II+III) ratio during a 5 h shipboard deployment in the Southern Ocean, (b) surface temperature and salinity (5 min resolution), and (c) global solar radiation (portside, 5 min resolution) over the period 21.00 on December 10, 2001 to 05.00 (UTC) on December 11, 2001. A typical error bar (1s) for the iron(II) concentrations is shown. The horizontal white bar represents the daytime period during the survey.

acidified, reduced (0.01 M Q-HCl, 100 μM Na_2SO_3), and analyzed at sea by FI-CL for dissolved iron(II+III) (*23*).

The results (Figure 3) for 8HQ-reactive iron(II) showed concentrations in the subtropical, oligotrophic Atlantic Ocean ranging from below the detection limit (<12 pmol L^{-1}) during darkness up to 45 pmol L^{-1} at 12:50 UTC, at which time the solar intensity was close to the maximum experienced during the trial. The concentration of dissolved iron(II+III) in surface waters generally increased during the survey, but was low compared to other data (*4*) for the Atlantic Ocean (164 ± 35 pmol L^{-1}, $n = 10$). Dissolved iron(II) or iron(II+III) distributions are not correlated with subtle changes in temperature and salinity. Interestingly, the iron(II) to iron(II+III) ratio decreased steadily from 37% at 13:05 to 3% at 21:07, suggesting that the iron(II) concentration was independent of changes in water mass. These data are consistent with earlier work that suggests photochemical reduction of iron-(III) to be the dominant mechanism for iron(II) production in the Southern Ocean (*12*), offshore waters of Peru (*14*), and northern Australian shelf waters (*44*). Our data also support previous results demonstrating diurnal cycling between total "reducible" (dissolved/bioavailable) and "nondetectable" (colloidal/particulate) iron concentrations in natural seawater incubations spiked with added iron (*10*). The reduction of iron(III) to transient iron(II) species, either photochemically or involving marine microorganisms, may increase the solubility and bioavailability of iron in seawater.

Southern Ocean Survey. A short (5 h) trial was also undertaken in the Australian sector of the Southern Ocean between 50°92′ S, 143° 38′ E and 51°25′ S, 143°03′ E, in the Sub-Antarctic Front (SAF). The objective here was to demonstrate the generic capability of the instrumentation by deploying it in a contrasting marine environment. Iron(II) was measured from 21.00 on December 10, 2001 to 05.00 UTC on December 11, 2001 (local daytime) during austral summer. Conditions were partly overcast. Calibration standards were prepared as described above, and three replicates of each solution were made. The mean detection limit from daily shipboard calibrations was 8.7 pmol L^{-1}, and reproducibility between replicate ($n = 3$) blank injections was RSD <7.6%. A debubbler polyethylene subsampling bottle replaced injection valve I1 during this survey, as described in *System Operation*. Unfortunately, there are several short periods where no data were recorded. This was due to a problem with the continuous supply of surface water from the tow-fish, which was a deployed in increasingly rough seas.

The results (Figure 4) show concentrations ranging from below the detection limit up to 29 pmol L^{-1} for 8HQ-reactive iron(II). Dissolved iron(II+III) concentrations generally increased during the transect from 99 pmol L^{-1} at 22:06 to 257 pmol L^{-1} at 03:03 UTC, although total dissolvable iron (i.e. unfiltered and acidified, TDFe) levels (not shown) were fairly constant (399 ± 33 pmol L^{-1}, $n = 16$). These data are consistent with the low surface dissolved iron concentrations (0.1−0.2 nmol L^{-1}) observed in previous studies of this region (*45*, *46*). In the period 00:40 to 03:10, a significant shift in temperature (1.0 °C) and salinity (0.2 units) was observed, suggesting the intrusion of a different water mass, although this resulted in no clear trend in iron(II), dissolved iron-

(II+III) or TDFe concentrations. The iron(II) to iron(II+III) ratio was variable and ranged from 4 to 13%. The Southern Ocean 8HQ-reactive iron(II) concentrations are also in the same range as the southeast Atlantic data, but are closer to the detection limit, have greater temporal variation, and are out of phase with the variable solar irradiance profile, suggesting that photochemically mediated reduction of iron-(III) to iron(II) was not dominant. Earlier work by O'Sullivan et al. (*9*) showed that iron(II) concentrations may be highest in the early morning and lower in the late afternoon, due to the photochemical production of significant steady-state concentrations of transient oxidants of iron(II), such as H_2O_2. Unfortunately, we have no iron(II) data during the night-time period for comparison in this region.

Our contrasting Atlantic and Southern Ocean observations highlight the need for further shipboard studies on the cycling of iron(II) at ambient surface ocean concentrations. Such surveys should examine changes in iron(II) concentrations through complete 24 h periods in warm, temperate, and polar oceans and should address which processes (e.g. photoredox, thermal, microbial) are necessary for the transient production of iron(II). The FI-CL instrument reported here would facilitate the high resolution, ultratrace level investigations necessary to address these important questions.

Other Applications. The shipboard FI-CL instrument described here is a low cost, portable, rugged system suitable for online determination of iron(II) in surface marine waters. Moreover, the generic nature of system components and graphical programming software make it easily adaptable to other CL-detectable analytes (e.g. Co, Cu, Mn, H_2O_2). The performance and reliability of the instrument and the analytical figures of merit are sufficient to allow iron(II) to be determined at picomolar concentrations in real-time over long (>10 h) periods, without user intervention.

In open-ocean environments, iron(II) measurements and observations of iron redox cycling are inherently difficult due to the extremely low dissolved iron(II+III) concentrations, but our preliminary results show that the FI-CL approach can provide acceptable high resolution data in such settings. This instrument would also be particularly useful for examining subtle changes in iron redox chemistry during in situ iron fertilization experiments or in coastal and freshwater environments where dissolved iron(II+III) concentrations are significantly higher. The system could also be used for monitoring biologically mediated redox cycling and uptake of iron(II) in laboratory culture experiments, deckboard incubations, or chemostats. Further development is planned to adapt the manifold for concurrent online determination of "total" dissolved iron(II+III), after an in-line HCl acidification and Na_2SO_3 reduction step.

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