

Direct determination of iron in acidified (pH 1.7) seawater samples by flow injection analysis with catalytic spectrophotometric detection: Application and intercomparison

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Abstract

A sensitive flow injection method for determining iron in seawater developed by Measures et al. (1995) has been substantially modified to allow the direct preconcentration of dissolved iron in acidified seawater samples (pH 1.7) onto a nitrilotriacetic acid (NTA) chelating resin. This removes the need to adjust the pH and buffer samples before the preconcentration step, and the low pH eliminates potential interference from the presence of strong iron-binding organic ligands. As part of an international intercalibration exercise for the Sampling and Analysis of Fe (SAFe), we investigated at sea the precision and accuracy of this flow injection method with its preconcentration step plus catalytic spectrophotometric detection with *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (FI-NTA-DPD). Acidified seawater samples analyzed using FI-NTA-DPD were shown to be in excellent agreement with other ship- and lab-based methods. The acidification of seawater samples to pH 1.7 is an important protocol if total dissolved iron in seawater is to be determined within hours of collection. A ship- and lab-based analytical intercomparison of two flow injection methods (FI-NTA-DPD and FI-NTA-ICP-SFMS) for the determination of total dissolved iron in seawater was carried out on SAFe samples collected from surface waters and at 1000 m depth from the North Pacific Ocean. For the two methods, total dissolved iron concentrations in surface samples were 0.101 ± 0.009 and 0.098 ± 0.009 nM, respectively, and in samples from 1000 m, 0.93 ± 0.04 and 0.92 ± 0.08 nM. No statistical difference between the FI-NTA-DPD and FI-NTA-ICP-SFMS methods was observed ($P = 0.05$).

Introduction

Because of the extremely low concentrations of iron in seawater (pM to nM), the determination of total dissolved iron in seawater requires sensitive analytical methods to provide high-quality data (see Bruland and Rue 2001, review and references therein). In the past decade, shipboard methods for determining trace metals such as dissolved iron in seawater have proliferated, many of which are based on portable flow injection (FI) systems (Achterberg et al. 2001). One such FI

system is a catalytically enhanced spectrophotometric method that employs the catalytic oxidation of *N,N*-dimethyl-*p*-phenylenediamine (DPD) by iron cycled with hydrogen peroxide (Measures et al. 1995; Sedwick et al. 1997; Weeks and Bruland 2002). The use of FI systems coupled with a towed, trace metal-clean, continuous sampling device (e.g., Vink et al. 2000; Bruland et al. 2005) allows rapid, near real-time measurements of dissolved iron, thus allowing scientists to examine high-resolution spatial and temporal trends in the oceanic distribution and cycling of this important micronutrient and reducing the chance of sample contamination. Accurate shipboard determination of iron in seawater has become a high priority for marine chemists, as this important limiting nutrient exists at such low concentrations in seawater and is prone to contamination during sampling and analysis.

Flow injection systems for determining dissolved iron in seawater have typically used a preconcentration chelating resin to both concentrate and separate iron from the bulk seawater matrix. To date, analysts have primarily used 8-hydroxyquinoline (8-HQ) as the preconcentration chelating resin (Landing et al.

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1986). In organic-free seawater, the recovery of iron bound to 8-HQ is pH specific; Fe(III) is quantitatively recovered at pH values from 3 to 4 with no recovery of Fe(II), whereas Fe(II) is recovered along with Fe(III) at pH 5.2 to 6 (Obata et al. 1997). However, dissolved iron in seawater has been shown to exist primarily as Fe(III) strongly complexed with organic ligands (Gledhill and van den Berg, 1994; Rue and Bruland 1995; Nolting et al. 1998). Siderophores appear to make up some fraction of these Fe(III) chelating organic ligands (Macrellis et al. 2001; Barbeau et al. 2001). A study using the 8-HQ chelating resin at pH of 3 to 4 in the presence of an iron binding ligand, EDTA, showed poor recoveries of iron (Obata et al. 1997). Johnson et al. (2003) have shown that acidification to pH 3.2 for a short time period does not obtain quantitative recoveries in the presence of model siderophores. Therefore iron bound to organic ligands present in seawater may be partially inert with respect to such chelating resins.

Acidification of seawater samples to pH 1.7 to 1.8 has been demonstrated to be important for release of iron from two model strong iron-binding ligands, the commercially available siderophores desferrioxamine B (DFOB) and rhodotorulic acid (RTA) (Lohan et al. 2005). Recently Lohan et al. (2005) showed that Fe(III) in seawater samples acidified to pH 1.7 to 1.8 can be preconcentrated directly and quantitatively onto an NTA chelating resin even in the presence of these two strong iron-binding model ligands. Total dissolved iron can be determined in seawater by the addition of an oxidizing agent, hydrogen peroxide, to the acidified sample [to oxidize any Fe(II) to Fe(III)] prior to preconcentration. During the recent iron inter-comparison exercise (Sampling and Analysis of Fe [SAFe]), acidification of samples to pH 1.7 to 1.8 was found to be necessary to release iron bound to both colloidal forms and potentially soluble iron-binding ligands, and thus important for shipboard methods where samples are analyzed within hours of collection. Open-ocean samples that were first acidified to pH 1.7 during SAFe and buffered on-line to pH 3.2 for analyses allowed the complete recovery of dissolved iron. If, however, there was an excess of an exceptionally strong iron siderophore such as desferal, incomplete recovery of iron has been observed by adjusting the pH from 1.7 to 3.2 immediately before loading onto an NTA chelating resin (Lohan et al. 2005). Coastal samples that were acidified to pH 1.7 for longer than 6 months needed to be UV-oxidized before on-line adjustment to pH 5.5 using the Toyopearl chelating resin for determining total dissolved iron (Hurst and Bruland 2006). Therefore, for determination of total dissolved iron in seawater, the ability to preconcentrate seawater samples at pH 1.7 is useful.

A major advantage of spectrophotometric methods for shipboard use is that they only require simple, compact, low cost detectors (Achterberg et al. 2001). This article describes the modifications made to the flow injection catalytic spectrophotometric method developed by Measures et al. (1995) to accommodate the use of a new commercially available NTA chelating resin (Lohan et al. 2005) for iron determination in

seawater (hereafter referred to as FI-NTA-DPD). A recent inter-comparison study highlighted the need for a certified seawater reference material containing subnanomolar iron concentrations (Bowie et al. 2004). A major goal of the SAFe project was the introduction of low-Fe, reference seawater samples to be made available to the scientific community (Johnson et al., unpubl. data). The accuracy of the FI-NTA-DPD method was tested using this new seawater reference sample at sea with other shipboard methods, such as cathodic stripping voltammetry and FI with chemiluminescence detection, and with shore-based laboratory methods, such as isotope dilution with inductively coupled plasma-sector field mass spectrometry (ICP-NTA-SFMS) and chelation and solvent extraction with flameless graphite furnace atomic adsorption spectrometry (GFAAS) (Johnson et al., unpubl. data). This article also reports the results of a direct comparison of this method with a previously published flow injection method coupled to ICP-SFMS (Lohan et al. 2005). Total dissolved iron determinations were made on replicate subsamples of acidified (pH 1.7) SAFe open-ocean surface and 1000 m depth seawater with concentrations of ~0.1 and ~0.9 nM, respectively.

Materials and procedures

Apparatus—Total dissolved iron was determined using a custom-made flow injection system based on the catalytically enhanced spectrophotometric method of Measures et al. (1995). A schematic diagram of the flow injection manifold with flow rates is shown in Figure 1. The manifold consists of an 8-channel peristaltic pump (Dynamax, Rannin), two electronically actuated 6-port, 2-position injection valves (VICI; Valco Instruments Co.) which were controlled by a software program (FIALAB Instruments Inc.) on a laptop computer (Dell). The reaction manifold was constructed from TFE Teflon connectors and 0.25 mm i.d. by 0.5 mm o.d. PFA Teflon tubing (Upchurch Scientific). The pump tubing used was PharMed 2-stop tubing (Cole-Palmer), and the flow rates were set at a pump speed of 4.5 rpm (Figure 1). A quartz flow-through cell (Starna Cells) with a 10-mm path length was placed in a cuvette holder (CUV-UV; Ocean Optics) which has 2 SMA connectors for fiber optics (100 μ m; Ocean Optics). One optical fiber was connected to an LS-1 light source (Ocean Optics) and the other to a USB 2000 spectrometer (Ocean Optics). The spectrometer was set to measure absorbance at 514 nm, and absorbance was quantified using the FIALAB software. Temperature control was achieved by wrapping the knotted reaction coil around an aluminum heating block set to 27 °C. Mini-columns (1 cm; Global FIA) consisting of a tapered inner chamber (85 μ L) and sealed with nonmetal frits were used for both the preconcentration column and the clean-up columns. The column was packed with NTA Superflow (Qiagen) using a syringe and 20 μ L Milli-Q water. No additional preparation steps are required in using this resin—columns only need to be repacked with NTA resin when left unused for long periods. Unlike the preconcentration column, where the direction of

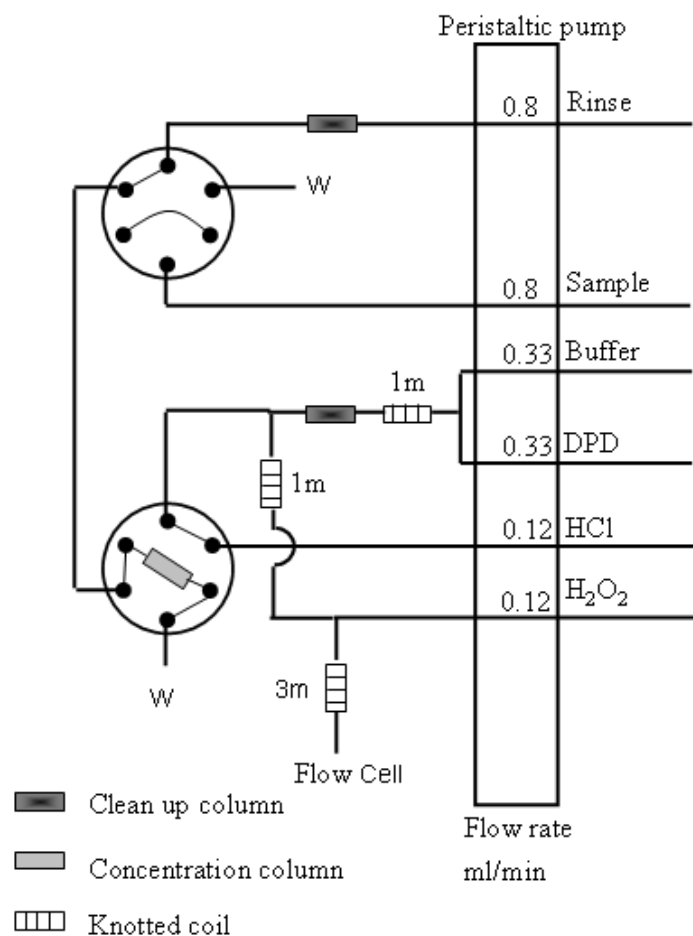


Fig. 1. Manifold configuration for rinse and preconcentration and determination of total dissolved iron in seawater.

flow is reversed between the load and elute phases, the direction of flow through the cleaning columns is always in the same direction. After several days of continuous use, the cleaning columns are reversed to ensure that the resin does not become tightly packed. To minimize contamination, sample and reagent bottles were contained within a Class-100 laminar flow bench. The system was flushed daily with 2 M HCl and after any changes such as changing reagents.

Reagents—All reagents were prepared in a Class-100 laminar flow hood and stored in acid-washed low-density polyethylene (LDPE) bottles. Subboiling quartz distilled hydrochloric acid (6 M) and glacial acetic acid were prepared by a single distillation of trace metal grade 6 M HCl and glacial acetic acid (Fisher Scientific) in a quartz-finger subboiling still (Q-HCl, Q-HAc). The eluting acid, 1.5 M Q-HCl, was made by diluting the 6 M Q-HCl with Milli-Q water with a resistance of $>18 \text{ M}\Omega \text{ cm}^{-1}$. A supersaturated ammonium acetate solution was prepared by bubbling anhydrous ammonia gas into Q-HAc. This solution was then slowly cooled, forming crystals of ammonium acetate. Milli-Q was added to the crystals to form a saturated ammonium acetate solution (19.2 M). Ammonium

hydroxide was prepared by bubbling anhydrous ammonia gas into Milli-Q water until saturated (Q-NH₄OH). A 3.5 M ammonium acetate buffer was prepared from the saturated ammonium acetate solution, and the pH was adjusted to 9 with Q-NH₄OH. A 1.5 M ammonium acetate/acetic acid rinse solution was prepared from saturated ammonium acetate and adjusted to pH 3.5 with Q-HCl (at this pH, the solution is primarily acetic acid with a smaller amount of ammonium acetate). A 0.05 M solution of *N,N*-dimethyl-*p*-phenylenediamine (4-amino-*N,N*-dimethylamine) dihydrochloride (DPD) (Fluka) was prepared daily by dissolving DPD in Milli-Q water. As this solution slowly oxidizes over time, 75 μL of 6 M HCl is added to 75 mL of 0.05 M DPD to slow the oxidation. The DPD reagent (solid) was kept in a refrigerator before dissolution, to prevent oxidation. A 5% hydrogen peroxide was prepared by diluting 30% H₂O₂ (Baker, Ultrex) with Milli-Q.

Sample collection and pretreatment—Surface seawater samples were collected using a trace metal-clean surface pump “sipper” system (Bruland et al. 2005), whereas samples from depth were collected using acid-cleaned and seawater-conditioned 30 L Teflon-coated Go-Flo samplers (General Oceanics) attached to a Kevlar line (Bruland et al. 1979). Seawater was prefiltered with a 0.45- μm polycarbonate track etched (PCTE) filter cartridge and then filtered through a 0.2- μm PCTE filter cartridge into large volume (500 L) acid-cleaned polyethylene tanks. In addition, a vertical profile of samples was collected using a series of Teflon-coated 10-L Go-Flo bottles mounted on a trace metal-clean rosette attached to a Kevlar conducting cable (C.I. Measures and W.M. Landing, unpublished data). Seawater samples from the vertical profile samples were filtered through acid-cleaned AcroPak 200 capsule filters with 0.8 μm /0.2 μm Supor membranes.

Filtered samples were acidified to pH 1.7 with 6 M Q-HCl (250 μL of 6 M Q-HCl added to 60 mL seawater sample). As the chelating resin used here to preconcentrate the sample binds only Fe(III) at pH 1.7, the addition of an oxidizing agent is necessary to ensure the oxidation of any Fe(II) in the sample. For this purpose, 50 μL of 12 mM H₂O₂ solution was added to a 60-mL sample for a final concentration of 10 μM H₂O₂, and the sample was allowed to sit for 10 min before analysis.

Procedure—The manifold is set up as shown in Figure 1. The addition of two 1-m mixing coils was to ensure complete mixing of the buffer with DPD and of the two reagents with the eluting acid before the final mixing coil. The NTA Superflow chelating resin is first conditioned with a 1.5 M ammonium acetate/acetic acid solution at pH 3.5 for 15 s. The seawater sample is then loaded onto the resin for 2 to 10 min, depending on the concentration of total dissolved iron in the sample. After a 15-s rinse with 1.5 M ammonium acetate/acetic acid (pH 3.5), the resin is then eluted for 3 min with 1.5 M HCl in the opposite direction to the flow of the sample during the load phase. During the condition, load, and rinse phases, the eluting acid bypasses the column and mixes with the other reagents, producing a baseline. Once the valve is switched to the elute mode,

the dissolved iron is eluted from the column and mixes into the reagent stream. This composite stream then passes through the 3-m reaction coil where the oxidation of DPD produces colored semiquinone derivatives, which are detected spectrophotometrically at 514 nm (Measures et al. 1995).

Total dissolved iron was also determined on replicate samples using an existing flow injection ICP-NTA-SFMS method (hereafter referred to as FI-NTA-ICP-MS) described by Lohan et al. (2005).

Assessment

In this section, an assessment of the FI-NTA-DPD method is followed by the results of an intercomparison study. This study was carried out to examine whether values for the acidified samples analyzed within 2 h onboard ship were comparable to those for acidified samples that were stored for a longer period (5 months) and analyzed in the laboratory.

Rinsing and conditioning the NTA resin—To ensure rapid desorption of iron from the NTA chelating resin, the analyte is eluted with 1.5 M HCl (Lohan et al. 2005). This is a significantly stronger eluting acid than the 0.15 M HCl used to elute iron from an 8-hydroxyquinoline (8-HQ) chelating resin in the previous reports of the FI-DPD method (Measures et al. 1995; Sedwick et al. 1997; Weeks and Bruland 2002). As the reaction chemistry downstream from the resin is identical to that developed by Measures et al. (1995), the concentration of the ammonium acetate buffer had to be increased to 3.5 M and made more basic (pH 9 compared to 6.3) to ensure the DPD-oxidation reaction occurs at pH 5.8. In the FI-NTA-DPD method presented here, the eluting acid is made up in Milli-Q and not in low-iron seawater as reported in Measures et al. (1995). Therefore, a rinse step is added before elution to remove any matrix interferences such as sea-salt concentrated on the resin. A similar step is carried out to condition the resin before loading with acidified seawater.

The optimal reaction pH is between 5.5 and 6.0 (Measures et al. 1995). Although the dead volume in the preconcentration column is only 85 μL , once the valve is switched from rinse to elute mode, the 85 μL of rinse solution present in the column will also be mixed into the reagent stream and produce an absorbance signal detected by the spectrometer (Figure 2). Owing to the pH sensitivity of the reaction, any change in pH will change the observed baseline. As shown in Figure 2, rinsing with Milli-Q causes the pH of the reaction stream to be pH 9, and a depression in the baseline is observed before the iron peak. Two considerations were important when choosing the rinse/conditioning solution. First, the pH of the rinse/conditioning solution combined with the reagent stream is similar to the pH of the elution acid in the reagent stream; second, the pH is not so acidic that iron could be removed from the NTA chelating resin. A 1.5 M ammonium acetate/acetic acid solution adjusted to pH 3.5 was chosen because its ionic strength matches that of the 1.5 M HCl, and at this pH any iron bound to the resin will not be removed during the rinse step. As the rinse/conditioning solution is a buffer and at a pH of 3.5, it will minimize any pH

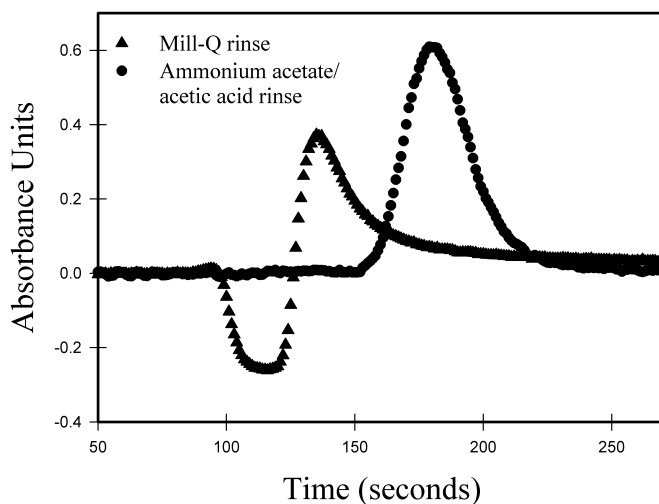


Fig. 2. Elution profile of total dissolved iron with a Milli-Q rinse step (\blacktriangle) and with a 1.5 M ammonium acetate/acetic acid rinse step (\bullet).

effects in the downstream solution after mixing with the pH 9 ammonium acetate buffer (3.5 M) used to neutralize the acid. The rinse/conditioning solution was made using trace metal-clean reagents and was then passed through NTA chelating resin (cleaning columns) to remove iron before rinsing or conditioning (Figure 1). The optimal time for both rinsing and conditioning was found to be 15 s. An example of the peak shape obtained after conditioning and rinsing for 1 s and 20 s is shown in Figure 3.

Interferences—In previous methods, triethylenetetramine (68.9 μM) was added to the reaction buffer to remove any copper interference (Measures et al. 1995; Sedwick et al. 1997; Weeks and Bruland 2002). Because the NTA chelating resin

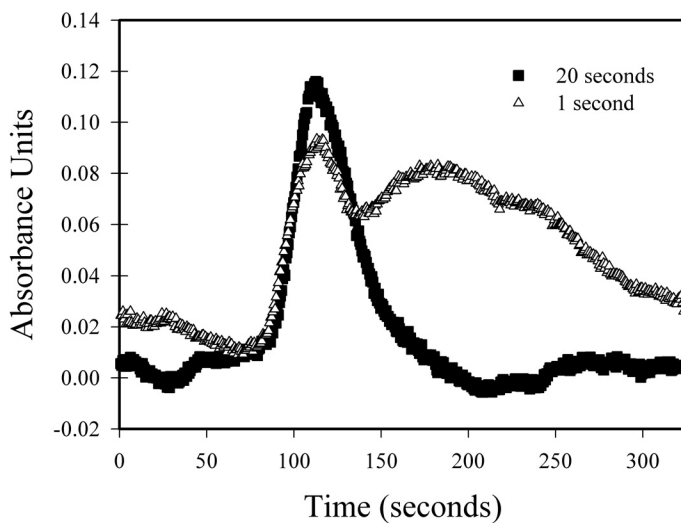


Fig. 3. Elution profile of total dissolved iron with a 1-s rinse step (\blacksquare) and with a 20-s rinse step (\triangle).

can bind both iron and copper at pH 1.7 (Lohan et al. 2005), the possible interference from copper was investigated. Copper standards were prepared by serial dilution of 1000 $\mu\text{g L}^{-1}$ stock solution (SPEX plasma standard) in acidified (pH 1.7) UV-oxidized seawater (UVSW) from which trace metals and metal chelating organic ligands were removed (see Donat and Bruland 1988). Concentrations of copper up to 70 nM (50 times that normally found in the ocean) were added to acidified seawater solutions containing 3 nM iron. No interferences on the iron peak were observed. Therefore, triethylenetetramine was not added to the reaction buffer.

Standardization—This method was calibrated daily using standard curves. Working standards ranging from 25 to 75 nM were prepared by serial dilution of 1000 $\mu\text{g L}^{-1}$ stock solutions (SPEX plasma standard) in acidified (pH 1.7) Milli-Q. Acidified surface Pacific Ocean seawater (pH 1.7) was used to prepare the standards, and 10 $\mu\text{M H}_2\text{O}_2$ was also added to the standards. Standard additions of the working standards to this low-iron Pacific Ocean seawater were used to determine the concentration of this background seawater, and the standard curve generated was applied to other samples. Standards were run at the start and end of each program (typically 20 samples), and concentrations were calculated from these standards based on the absorbance peak areas.

Blanks and detection limits—The major blank contribution to the FI-DPD-NTA method arises from the reagents that can be preconcentrated onto the NTA chelating resin. The contribution to the blank from the addition of H_2O_2 (50 μL of 12 mM H_2O_2 to a 60-mL sample) to acidified samples was investigated by spiking acidified seawater samples with 100 and 200 μL of 12 mM H_2O_2 . The doubling of the H_2O_2 (100 μL) caused the iron concentration in the sample to increase by 0.015 nM, which was then used as the H_2O_2 blank. Throughout this study, only one solution of 12 mM H_2O_2 was used. The concentration of iron in the H_2O_2 solution added to both the standards and samples was determined twice during this study and was found not to vary over time. As these samples were acidified with 6 M Q-HCl, this could also contribute to the blank. The concentration of iron in the Q-HCl was investigated by taking 10 mL of 6 M Q-HCl down to dryness and bringing it back up in 1 mL of 1.5 M HNO_3 ; the iron concentration in the Q-HCl was then determined to be 0.2 nM by graphite furnace atomic absorption spectrometry (GFAAS). Because only 250 μL is added to the 60-mL seawater sample, the contribution to the analytical signal is negligible (0.003 pM). The rinse/conditioning solution is also passed through the NTA preconcentration column and could contribute to the blank. This solution is, therefore, passed through another NTA chelating resin (cleaning column) just before loading onto the preconcentration column (Figure 1), and any iron in solution should be bound to the NTA resin in the cleaning column.

The combined blank that includes iron leached from the pump and manifold tubing upstream of the preconcentration column (manifold blank), as well as reagents loaded onto the preconcentration column for FI-DPD-NTA, was investigated

by loading acidified Milli-Q (pH 1.7) onto the preconcentration column for 1, 2, and 3 min. Extrapolating the slope of the peak area versus load time to a zero load time provides the value for this manifold blank. The manifold blank was determined with each run and varied between 0.04 and 0.11 nM over 10 days, with a mean value of 0.06 ± 0.008 nM ($n = 35$). The detection limit was estimated as the iron concentration corresponding to 3 times the standard deviation of the manifold blank, resulting in a detection limit of 0.024 nM.

Accuracy and precision—The accuracy for methods determining total dissolved iron in seawater have previously been reported using the NASS-certified reference material (National Research Council, Canada). Total dissolved iron in NASS-5 is 3.71 ± 0.63 nM, which is up to 100-fold greater than typical open-ocean iron concentrations. Instead, the accuracy of the FI-DPD-NTA method was tested by determining total dissolved iron in acidified seawater standards collected from both the surface (approximately 10 m) and 1000 m in the North Pacific as part of the SAFe project (Johnson et al., unpubl. data). Results for the surface and 1000 m acidified seawater samples analyzed by FI-DPD-NTA (mean surface Fe = 0.101 ± 0.009 nM, mean 1000-m Fe = 0.93 ± 0.04 nM) were similar to results of other shipboard FI methods and cathodic stripping voltammetry, as well as results of shore-based analysis by GFAAS after preconcentration by chelation and solvent extraction and by isotope dilution analyzed on a ICP-MS and by FI-ICP-MS (Johnson et al., unpubl. data). As the calibration curve is generated from standard addition to low-iron seawater sample, this calibration curve is also used to ensure accuracy on a regular basis. Samples that had a greater than 7% relative standard deviation on peak area for replicates were reanalyzed.

Acidification—In determining total dissolved iron, it is important that dissolved iron associated with colloids and organic fractions in the dissolved phase be detected. The length of time of acidification and degree to which the sample has been acidified are important factors in ensuring that total dissolved iron is determined. A seawater sample from 1000 m was divided into 3 aliquots and acidified to pH 1.7, 2, and 2.5; 10 $\mu\text{M H}_2\text{O}_2$ was also added to each aliquot. The concentration of iron was determined over 3 days (Figure 4). The addition of 10 $\mu\text{M H}_2\text{O}_2$ to acidified seawater samples with 19 nM Fe(II) has been demonstrated to quantitatively oxidize reduced iron on a time scale of less than 10 min (Lohan et al. 2005). Seawater samples that were acidified to pH 1.7 for 10 and 20 min were lower than those acidified for 60 min (Figure 4). After 60 min, samples acidified to pH 1.7 reached a plateau and no further increase in iron concentration was observed, whereas dissolved iron in samples that were acidified to pH 2 continued to increase over 2 h. Between 2 and 9 h, however, the dissolved iron concentration (0.76 ± 0.02 nM) in samples acidified to pH 2 was similar to that observed in samples acidified to pH 1.7 and left to stand for 60 min (0.75 ± 0.02 nM). Iron concentrations in samples acidified to pH 2.5 were lower than those acidified to pH 1.7 or 2, even after 2 days.

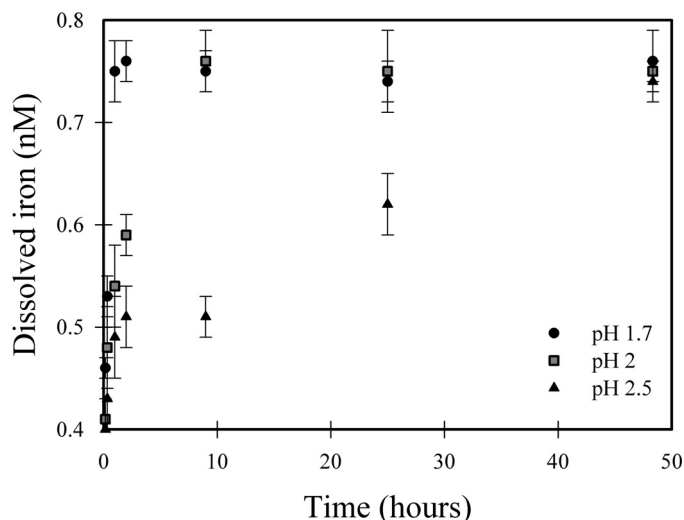


Fig. 4. Dissolved iron concentrations in seawater (pH 1.7, 2, and 2.5) monitored over 3 days.

In-line acidification—To successfully combine the FI-NTA-DPD method with a trace metal-clean surface pump sampling system, it is necessary to reduce the time taken for acid to release iron associated with the inorganic and organic colloids and organic ligands. Microwave heating of acidified samples (pH 1.7) has been reported to release iron bound to organic ligands and colloids and allow analysis of total dissolved iron within minutes of collection (Bruland and Rue 2001). Laboratory experiments were set up to investigate if heating acidified seawater samples would decrease the time needed to release iron associated with inorganic and organic colloids and organic ligands. These experiments demonstrated that heating acidified (pH 1.7) seawater samples (20 mL) to 40 °C for 5 min was sufficient to allow total dissolved iron to be detected (data not shown).

The FI-NTA-DPD method was coupled directly to a trace metal-clean surface pump sipper system based on the design of Vink et al. (2000) during a study off the coast of California in July 2004. Seawater was filtered through an acid-cleaned 0.2- μ m polycarbonate filter. This filtered seawater was continuously pumped to an acid-cleaned 60-mL LDPE bottle containing a drain inside the Class-100 Laminar flow bench. Filtered seawater was drawn from this 60-mL bottle via Teflon line to a 6-port, 2-position injection valve (VICI). This system was set up to eliminate the problem associated with degassing that occurs when very cold surface waters are pumped into a warm laboratory (Vink et al. 2000). The sample stream was acidified in-line to pH 1.7 with 0.1 M Q-HCl to yield a final acid concentration that is equivalent to 250 μ L of 6 M Q-HCl added to a 60-mL sample (as described above). The sample stream was also oxidized with H₂O₂ in-line to yield a final concentration of 10 μ M. This composite stream then passed through an 8-m mixing coil which was heated to 40 °C, allowing 7 min for this reaction to occur (using a flow rate of 1.1 mL min⁻¹). The composite stream was then loaded onto the NTA resin and analyzed as described above.

During this study, filtered samples were also collected off-line. These samples were acidified to pH 1.7, 10 μ M H₂O₂ was added, and samples were left to sit for 24 h before analysis. The total dissolved iron concentration in surface waters determined in this study ranged from 0.8 to 3 nM. A paired 2-tailed *t* test revealed that there was no significant difference at the 90% confidence level ($P = 0.1$; $n = 10$) between samples that were filtered and acidified online and those collected off-line. The successful coupling of the FI-NTA-DPD method to a clean surface pump sampling system allows the rapid, near real-time measurements of dissolved iron. A new sample was analyzed every 8 min, corresponding to a distance of approximately 1.6 km for a ship speed of ~ 7 knots (3.6 m s⁻¹), enabling excellent surface mapping of dissolved iron concentrations.

Intercomparison—Before the development of shipboard methods for determining iron in seawater, samples were traditionally analyzed using chelation and solvent extraction followed by GFAAS (Bruland et al. 1979). Samples for total dissolved iron were acidified to pH 1.7 to 2 and allowed to sit for roughly 5 months before analysis, thereby ensuring that the iron associated with colloidal fractions within the dissolved phase were detected (Bruland et al. 1979). Acidifying samples to pH 1.7 has been shown to dissociate iron bound to model organic ligands such as desferrioxamine (Lohan et al. 2005). Therefore, an intercomparison exercise was designed to investigate whether acidified samples analyzed onboard ship within 2 to 8 h of collection were comparable to those that were stored acidified for 5 months. For the intercomparison study reported here, we used a subset of 14 randomly selected individual bottles from the SAFE surface sample (surface 1 SAFE samples) and 13 from the SAFE 1000 m sample (deep 2 SAFE samples). One subset was acidified and analyzed onboard ship within 2 h of acidification by FI-DPD-NTA, and the other acidified subset was stored in acid-cleaned 60-mL LDPE bottles for 5 months until analysis by FI-NTA-ICP-MS. The analytical methods in this intercomparison study used the NTA resin for preconcentration of iron but used different detection methods. The FI-NTA-ICP-MS uses Milli-Q as the rinse step and not the 1.5 M ammonium acetate/acetic acid at pH 3.5.

Both analytical methods were calibrated daily, as described above. Results from the intercomparison study showed excellent agreement between the two analytical methods (Table 1). Mean 1000 m total dissolved iron concentrations of 0.93 ± 0.04 nM ($n = 13$) and 0.92 ± 0.08 nM ($n = 12$) were determined for the FI-NTA-DPD and FI-NTA-ICP-MS methods, respectively (Table 1). The surface mean total dissolved iron concentrations were 0.101 ± 0.009 nM ($n = 14$) and 0.098 ± 0.009 nM ($n = 13$) for the FI-DPD-NTA and FI-ICP-MS methods, respectively (Table 1). A paired 2-tailed *t* test revealed that there was no significant difference at the 95% confidence level ($P = 0.05$) between the two methods. Additional experiments carried out using a variety of methods for the determination of total dissolved iron, both onboard ship and in the laboratory, have also demonstrated that the acidified seawater samples collected

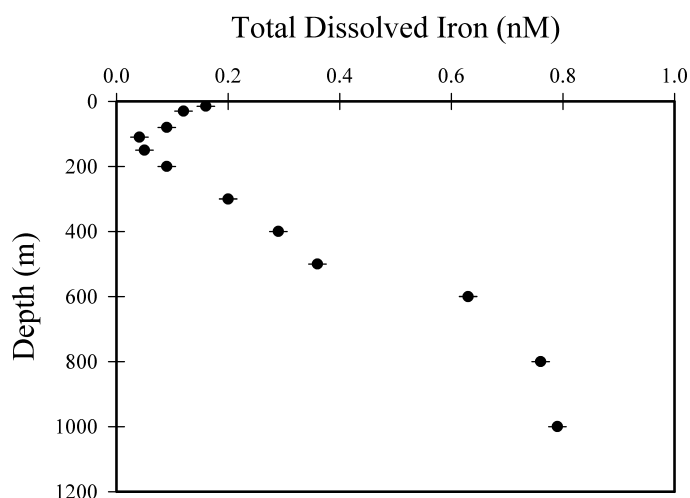
Table 1. Total dissolved iron concentrations in samples from both the deep tank 2 and the surface tank 1 from SAFe analyzed by FI-NTA-ICP-MS in the lab and by FI-NTA-DPD at sea.

Bottle	FI-NTA-ICP-MS		FI-NTA-DPD	
	Fe, nM	Bottle	Fe, nM	SD
Deep tank 2, 1000 m				
1	0.97	0.97	0.89	0.01
2	0.97	0.97	1.03	0.04
3	1.03	1.03	0.90	0.02
4	—	—	0.98	0.06
5	0.99	0.99	0.91	0.04
6	0.86	0.86	0.94	0.03
7	0.85	0.85	0.93	0.06
8	0.87	0.87	0.91	0.04
9	0.84	0.84	0.89	0.02
10	0.900	0.900	0.95	0.02
11	0.83	0.83	0.89	0.02
12	0.84	0.84	0.91	0.04
13	1.03	1.03	0.94	0.03
Mean	0.92	0.92	0.93	0.03
SD	0.08	0.08	0.04	—
Surface tank 1				
1	0.088	0.088	0.106	0.002
2	0.109	0.109	0.104	0.004
3	0.101	0.101	0.097	0.004
4	—	—	0.108	0.009
5	0.111	0.111	0.110	0.006
6	0.090	0.090	0.086	0.016
7	0.091	0.091	0.095	0.008
8	0.088	0.088	0.120	0.009
9	0.104	0.104	0.093	0.002
10	0.110	0.110	0.095	0.003
11	0.107	0.107	0.095	0.003
12	0.089	0.089	0.100	0.008
13	0.094	0.094	0.098	0.003
14	0.089	0.089	0.110	0.005
Mean	0.098	0.098	0.101	0.006
SD	0.009	0.009	0.009	—

For FI-NTA-ICP-MS at 1000 m, SD = 0.03, blank = 0.17, and detection limit = 0.09; at surface, SD = 0.0009, blank = 0.028, and detection limit = 0.027. For FI-NTA-DPD at 1000 m, blank = 0.1 and detection limit = 0.03; at surface, blank = 0.06 and detection limit = 0.024.

during the SAFe studies for surface 1 and deep 2 were homogeneous, with no significant differences observed between individual bottles (Johnson et al., unpubl. data). This indicates that samples analyzed for total dissolved iron onboard ship that have been acidified to pH 1.7 for 2 h are comparable to those that have been acidified to pH 1.7 for 5 months.

A vertical profile of total dissolved iron concentrations from the North Pacific (30°N, 140°W) was also determined onboard ship by FI-NTA-DPD and is shown in Figure 5. The seawater samples of this profile were collected by C.I. Measures and W.B. Landing using their clean rosette system and 10-L Teflon

**Fig. 5.** Vertical profile of total dissolved iron in the subtropical North Pacific (30°N, 140°W). Error bars are the standard deviation on 3 replicate measurements.

coated Go-Flo bottles. Total dissolved iron concentrations from the profile were compared those that obtained by other ship- and lab-based methods. The results from the FI-NTA-DPD method agree well with both ship- and lab-based methods (Johnson et al., unpubl. data). The higher iron concentrations observed in the surface mixed layer compared to the subsurface minimum at approximately 100 m depth are similar to what others have observed at stations in the North Pacific subtropical gyre (Bruland et al. 1994).

Comments and recommendations

Total dissolved iron determined by FI-NTA-DPD with online preconcentration on NTA chelating resin at pH 1.7 is suitable for shipboard determination of dissolved iron concentrations. The use of a commercially available resin negates the need to synthesize the 8-HQ resin and to buffer acidified samples before analyses. This method is easy to use, selective, and sensitive enough to measure the extremely low concentrations of total dissolved iron in the open ocean. The load time of acidified seawater samples onto the chelating NTA resin can be reduced by increasing the diameter of the pump tubing, thus loading the same volume onto the column at a faster rate. As Fe(III), and not Fe(II), is held on the resin at pH 1.7, this method is therefore potentially selective to discriminate between Fe(III) and total dissolved iron in seawater and determine Fe(II) by difference between samples run with and without the addition of 10 μ M H₂O₂ before analyses.

This study has also shown that seawater samples acidified to pH 1.7 that have been collected and analyzed onboard ship within 2 h of collection are similar to those stored acidified over 5 months and analyzed in the laboratory, thus validating the near real-time analysis of dissolved Fe at sea. We have also demonstrated that this method can be coupled to a clean surface pump sampling system for the determination of iron,

thereby providing rapid, near real-time measurements of dissolved iron. This will allow high-resolution spatial and temporal trends in the oceanic distribution and cycling of iron to be examined. The use of the new open-ocean iron reference materials will benefit many analysts determining total dissolved iron in seawater.

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