Improved method for shipboard determination of iron in seawater by flow injection analysis

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Received 6 March 2001; received in revised form 17 October 2001; accepted 17 October 2001

Abstract

A flow injection analysis (FIA) catalytic spectrophotometric method for the determination of dissolved iron in seawater was further developed to yield a more sensitive assay with a low detection limit. The method employs an initial sample acidification step followed by an iron pre-concentration step involving an in-line 8-hydroxy-quinoline (8-HQ) metal-chelating resin column. The copper capacity and elution efficiency, as well as the iron FIA performance of three trace-metal clean resins were compared, resulting in the selection of a clean silica gel support for the 8-HQ ligand. The concentrated sample is eluted from the resin with an acidic carrier and mixed with reagents, initiating an iron-catalyzed, color-forming reaction. Increasing the reaction temperature from 18 to 30 °C doubled the sensitivity; reaction temperature control was necessary to obtain good reproducibility in the field. Reagent blanks were as low as 0.05 nM and a detection limit of 0.016 nM was obtained from three times the S.D. of a 0.06 nM seawater sample repeated six times. A 0.06 nM detection limit was calculated from shipboard experiments where total dissolved iron was determined for 10 different samples from the same station. The instrumental sensitivity and precision evolved to the point where the blank associated with the technique is the major factor influencing its detection limit. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Flow injection analysis; 8-Hydroxy-quinoline; Dissolved iron

1. Introduction

Total dissolved iron in surface waters of oceanic regimes can range from less than 0.05 to greater than 10 nM [1,2]. Therefore, shipboard determination of iron in seawater requires a sensitive analytical technique and trace-metal clean sample handling to obtain meaningful, oceanographically consistent results. Flow injection analysis (FIA) is a robust technique for the unstable platform inherent to shipboard laboratories, with the added advantage of minimal sample handling and concomitant low exposure to contamination.

FIA has been successfully adapted for measuring iron in seawater by several laboratories [3–5]. A catalytic colorimetric FIA method [5] has recently been developed that employs the catalytic oxidation of N,N-dimethyl-p-phenylenediamine (DPD) by iron cycled with hydrogen peroxide [6]. In addition, a chemiluminescence (CL) FIA method [4] has been developed where luminol emits light when mixed with Fe(III) and H_2O_2; the number of photons produced is then related to the concentration of iron. In order to detect low iron concentrations, all of these methods use an 8-hydroxy-quinoline (8-HQ) metal-chelating resin in a column that is part of the sample injection loop. The CL technique may already be developed to its full potential by the labs currently employing this method, while there is the potential to
improve sensitivity by lowering the blank and raising the reaction temperature with the catalytic technique, therefore, this method was selected for further development. Catalytic techniques are well suited to FIA because the reaction time is controlled by the flow rate and reaction coil length, and the reaction coil itself is readily heated.

The goal was to improve the catalytic FIA method to enable reliable shipboard determination of iron in the sub-nanomolar range in seawater, particularly in regimes that could be iron limited. Lowering the blank and detection limit by decreasing reagent and system contamination and assay noise required careful attention to trace-metal clean techniques in the preparation of reagents and the design and operation of the FIA system. With reduced noise, increasing the sensitivity could be addressed through optimizing the resin and exploring higher reaction temperatures. With increased sensitivity, small but potentially significant differences between samples and sample treatments could be explored. Proper sample handling and well-defined sample treatment are paramount for ensuring that meaningful, interpretable results are obtained.

Care must be taken to define the iron fraction being measured. Iron can exist as inorganic species [7,8] of Fe(III) or Fe(II), be organically complexed [9,10], exist as colloids [11] of oxides, oxyhydroxides, or mixed with organic material, and be suspended as both biotic and abiotic particles. Which iron pools are bio-available and to what extent is debatable [12,13], but careful operational definition of what pool is being measured will allow clearer elucidation of iron’s complex biogeochemical cycle. In order to evaluate the sources and sinks of iron, clarify iron’s role as a nutrient element, and compare iron concentration results from various methods, the size fraction physically separated by filtration as well as the chemical release of iron from any bound phases within the particular size fraction needs to be carefully defined. The chemical release relates both the strength of the iron complexes to the concentration of acid and the kinetics of the release of iron from these complexes to the time and temperature of the acidification step. While the 8-HQ in the sample column binds dissolved inorganic species of iron strongly, the contact time within this column is short (4–5 s in the smallest columns) and it is unlikely that if a sample is insufficiently acidified that the 8-HQ will be able to compete quantitatively for all of the iron in the sample, as some iron may remain complexed to organics or bound in colloids.

Sample acidification is predominantly used to release Fe(III) and Fe(II) from complexes and low pH will keep the iron stabilized as labile inorganic forms. After complete release of iron from the selected fraction (or partial release if the pH, time and temperature are well defined and reproducible), the sample must be buffered just prior to analysis to a pH range where the iron will complex with the 8-HQ column. Inorganic species of Fe(III) are recovered above pH 3, and Fe(II) is completely recovered at pH 5.2 and above when using a flow-through 8-HQ column [4]. As a result, the samples must be adjusted to a pH of at least 5.2 in order to determine the sum of Fe(III) and Fe(II) released during the acidification step. At more basic pH, iron colloids may form such that iron is not fully recovered from the sample stream; the iron may even precipitate. Using a lower pH has the added advantage of providing some measure of selectivity. For example, manganese binds much more weakly to 8-HQ than iron, and only at a pH higher than 8. Thus, samples for iron analysis are acidified and then buffered in-line to a pH range of 5.2–5.4 prior to loading onto the 8-HQ pre-concentration column. This ensures the isolation of both Fe(III) and Fe(II) released during the acidification step while keeping the sample as acidic as possible to avoid precipitation of Fe(III).

In this study, 0.45 μm filtered seawater samples were acidified to pH 1.8 and microwave heated to overcome potentially slow acid release of some of the less labile forms of iron that may be present in the samples. These treated “total dissolved iron” samples were buffered in-line to pH 5.2–5.4 and pumped through the 8-HQ column to bind available iron. Resin attributes such as analyte-binding capacity, binding kinetics and release efficiency can also affect assay performance. Quantitative concentration of analyte on the column, minimal ligand leaching, efficient analyte elution and reducing the column size all contribute to increased sensitivity, improved precision and accuracy, and lower blanks. Therefore, three solid support phases for the 8-HQ ligand which have been previously utilized for iron measurements have been adapted and compared here: a trace-metal clean silica gel [4], epoxy resin [14] and a vinyl polymer resin [15].
The column is loaded for a fixed amount of time resulting in a known volume of sample sent through the column. The column is then eluted with an acid carrier that mixes with the assay reagents before traveling through a heated reaction coil. Since this is a catalytic technique, the sensitivity should improve at increased reaction temperature because the Fe(III) catalyst will be regenerated more rapidly, allowing one iron atom to oxidize more DPD molecules per time. Minimizing the reagent blank and background signal is also important for achieving the necessary low detection limit and can provide more latitude for increased sensitivity.

2. Methods

2.1. System

A Lachat QuikChem 4200 System (Zellweger Analytics) was adapted as follows (Fig. 1): a Valco Instruments Cheminert 6-port injection valve replaced the system’s original Hamilton valves. Valve switching and digital data collection were controlled by a custom program written in Microsoft QuickBasic for a Keithley Metrabyte DAS-800 A/D board connected to a STA-08PGA series module. A Darlington transistor and 5 V 1 A relay bread-boarded to the module was used to switch the valve. The reaction manifold was constructed from TFE Teflon connectors (Alltech), and 0.8 mm i.d. PFA Teflon tubing (Alltech) was used throughout. PFA Teflon can withstand higher temperatures than TFE Teflon, and PFA Teflon is also relatively clear, so bubbles and column resin break-through can easily be detected. Many systems employ 0.5 mm i.d. tubing, however, larger 0.8 mm i.d. tubing results in a longer reaction time for the same length reaction coil, is less likely to kink when made into mixing coils, and results in less back-pressure.

Columns were prepared from silicon pump tubing glued with cyclohexanone and 0.2 μm pore size, acid-washed polyethylene frit material (Bel-Arts Products). In some columns, acid-washed quartz wool was used to hold the resin in place, but the frit material allowed more reproducibility between columns. The 8-HQ column was loaded with sample in one direction of flow and eluted by pH 1 carrier in the opposite direction. A Rainin 8-channel RP-1 peristaltic pump with Pharmed 2-stop pump tubing (Cole-Parmer) provided a continuous flow of reagents and sample. In FIA, a continuous and constant flow rate is important in assuring reproducible sample injection size, as the sample column in the injection loop is loaded for a set length of time. The flow rate also controls the length of time the reaction is allowed to proceed as the reaction coil is of fixed length (2 m).

![Fig. 1. FIA schematic: black columns contain 8-HQ immobilized onto silica gel. The mixing coil is knitted 0.8 mm PFA tubing, heating coil is 0.8 mm tubing wrapped around a thermostatted aluminum heating cylinder.](image-url)
For shipboard temperature control, wrapping tubing around the aluminum heating block included with the Lachat QuikChem system to form the reaction coil was more convenient than immersing a knitted coil of tubing in a waterbath, but results in slightly more analyte dispersion [16]. The 1 cm cell, 540 nm optical filter, fiber-optic system and detector were used as supplied. Absorbance is reported with zero absorbance set with carrier alone in the flow cell. The system was flushed with 0.5N HCl daily and after any changes, such as installation of a fresh column, changing a reaction coil, etc. Rinsing the system with 1N HCl and adding 0.1 M ascorbic acid [17] had no effect on further reducing the blank nor the baseline.

2.2. Reagents

Reagents were prepared in a laminar flow hood, stored in acid-washed Teflon or polyethylene bottles, and diluted using Milli-Q water with a resistivity of >18 MΩ. The 6N HCl (Fisher Trace Metal Grade and Milli-Q water) and glacial acetic acid (Fisher Reagent Grade) were re-distilled in quartz sub-boiling stills. Ammonium hydroxide and ammonium acetate solutions were prepared by bubbling ammonia gas through a 3 M EDTA solution and then into Milli-Q water or distilled acetic acid, respectively, until saturated. The in-line sample buffer, used to bring the acidified seawater at pH 1.8 up to pH 5.2–5.4 for loading onto the column, was saturated ammonium acetate adjusted to pH 6.3–6.4 with NH₄OH. The reaction buffer was 200 ml of saturated ammonium acetate, 3 ml 15% Brj-35, and 1 ml 1% triethylenetetramine (TETA) brought to 11 with Milli-Q and adjusted to pH 6.3 ± 0.05. The 10% hydrogen peroxide was prepared from a 1:3 dilution of Ashland Terabit 30% H₂O₂ with Milli-Q water. The DPD (Fluka) was prepared fresh daily by adding 0.72 g to 75 ml Milli-Q water and 25 ml 6N HCl. The carrier was 24 ml 6N HCl and 2 ml sample buffer per liter of Milli-Q water.

Most of the reagents continually flowed through the flow cell, contributing to the baseline. The blank for each sample response peak also includes any signal from the sample buffer and the acid used for sample acidification, as well as the contribution to the signal when switching the column from loading the sample to eluting with acidic carrier. To evaluate the blank, Milli-Q water was loaded and injected as a sample and standard additions of iron were used to quantify the concentration of iron this blank represents. One second loads were used to decouple the column contribution from the reagent contribution.

2.3. Sample treatment

Shipboard samples were collected from an on-board, continuous surface sampling system (Smith and Bruland, personal communication). This system consisted of a plastic torpedo filled with lead on a weight-bearing line with the intake end of 0.75 in. rigid PFA Teflon tubing attached. The weight was suspended from a block held out over the starboard bow by a large articulating arm attached to the deck of the R/V Pt. Sur. The PFA Teflon tubing was attached to a Teflon pump on deck and from there the tubing was split off to clean sampling areas. Flow from this system was accessed in a clean area constructed of plastic sheeting enclosing a portable HEPA filter unit to keep the area under positive pressure and minimize contaminant exposure. A 0.45 μm Millipore acid-washed Teflon cartridge filter was attached to an access valve and flushed with 70.5 l of seawater prior to rinsing the sample bottles three times and taking samples. Sample bottles were Nalgene TFE Teflon or Nalgene fluorinated HDPE that had been acid washed. Bottles containing the collected samples were transported in double zip-lock bags to another clean area where they were acidified to pH 1.8 with 4 ml 6N HCl/l of sample. The samples were placed in another set of dry zip-lock bags and heated in a microwave set to high (900 W) three times for 15 s per 250 ml of sample with 1–5 min between each period of heating. This microwave procedure, to enable the determination of total dissolved iron by speeding up the acid release of bound iron, was initially validated in the lab with a seawater sample characterized for total iron by graphite furnace atomic absorption spectroscopy (GFAAS). After acidification to pH 1.8, two to three 10 s microwave cycles (high; 900 W) were sufficient to match the GFAAS value. Because natural samples can have very different iron specification, and previous work [17] describing the use of microwaving to obtain ‘total leachable iron’ employed longer heating times, the microwave time was increased for samples collected aboard ship. FIA results obtained in this manner from 14 different California coastal sample...
sites, ranging from 0.14 to 8.15 nM total dissolved iron, matched GFAAS results for the same sites within the error of the assay.

Various samples were collected and repeatedly assayed aboard ship over hours and sometimes days to assure that there was no further increase in the amount of iron detected over time. If the iron value for a particular sample continued to increase after the initial post-acidification and microwave treatment, this could indicate that not all the iron was released by this treatment. This problem was not encountered in the treated samples. Samples in the tightly closed bottles could not be allowed to boil, but sufficient heating of the acidified samples was necessary to release all the iron from various ligands and colloidal material which could otherwise keep the iron from binding to the 8-HQ in the concentrating column. This sample treatment mimics the treatment of samples collected for total dissolved iron as determined later in the laboratory by GFAAS. The samples were allowed to cool to <35°C prior to in-line buffering and column loading.

A surface seawater sample collected from the outer Monterey Bay on a previous cruise was 0.45 μM filtered and acidified and stored for a year. This was used as a control sample during one cruise, and the iron concentration value obtained was compared to the GFAAS value for this same sample. Precision and accuracy were then calculated from these results. To determine the detection limit of the entire analysis, including sample collection and treatment, seawater samples were sequentially collected in different fluminated HDPE bottles from an off-shore station, presumed to be low in iron. These samples were acidified to pH 1.8 and then microwaved. The analysis was performed the following day using a 2 min load (5 ml sample). The assay was standardized by standard additions to various seawater samples or controls, typically by adding 10 μl of an acidified 10 μM Fe stock solution to 250 ml seawater to yield a 0.4 nM Fe addition.

2.4. 8-HQ resins

Three solid supports for 8-HQ were prepared for capacity, elution efficiency and performance comparisons. The polymer resin was the Tosohaas SEC HW-40C resin (75 μm) with the 8-HQ (Fisher) attached to the resin’s hydroxyl groups by a modification of the procedure given by Landing et al. [15] (Eltrod, personal communication), with changes in the reduction step such as adding the Na2S to 0.2 M NH2Ac, pH 7.2 to help maintain neutral pH and adjusting the pH back down every 15 min with 6N HCl as necessary to ensure continued reduction. This step was allowed to proceed for 5 h. The first two rinses of the final product were with acetone to remove sulfides. The epoxy resin, by far the easiest to synthesize, was made from Tosohaas AF-Epoxy-650 M resin (65 μm) with the 8-HQ attached [14] as the dihydrochloride of 5-amino-8-HQ (Aldrich). The clean silica gel was produced as per the specifications of Obata et al. [4], but dry-sieved through a larger (150–92 μm) mesh-size range. The 8-HQ was attached as for the polymer resin mentioned previously.

Small columns (1–2.5 cm long and 0.2 cm i.d., or 30–80 μl volume) were used on the sample loop of the system. Smaller columns contribute less signal to the blank but must be large enough for quantitative recovery of iron. Slightly larger columns (4 cm long and 0.3–0.4 cm i.d., or 300 μl volume) were placed after the DPD/reaction buffer convergence for in-line DPD clean-up to minimize the baseline. Larger columns, 10 cm long with a 1 cm i.d. (8 ml resin volume), were manufactured from C-Flex tubing and Teflon swage-lock fittings and contained 8-HQ immobilized to HW-40-C, epoxy or silica resin. These columns were used to compare break-through capacity as a high concentration of metal, readily detectable by flame atomic absorption (FAA), could be used. Each column was loaded with a 20 ppm (0.315 μM) copper solution prepared from Fisher 10,000 ppm FAA Cu standard at 3–4 ml/min and the copper concentration determined by FAA (Perkin-Elmer Model 2380) for each 2–3 ml aliquot collected. To compare elution efficiency, each column was eluted with 0.5N HCl and the concentration of copper in each consecutive 2 ml aliquot were determined by FAA.

Copper was employed instead of iron in studies to determine the resin binding-site capacity so that the optimal load pH of 5.2–5.4 and relatively high concentrations of metal could be used. At pH 5.3 and I = 0, copper exists primarily as the free ion Cu^2+ with a solubility of 10^{-2} M while iron is primarily Fe(OH)_2^+ with a solubility of ~10^{-8} M [18]. The rate of water exchange (k_w) for both metal species is high [19]:
$k_{w}$ for Fe(OH)$_2$ is $\sim 10^7 \text{s}^{-1}$ and $k_{w}$ for the free copper is $10^9 \text{s}^{-1}$. As for the complexation of the metals by 8-HQ [20], both iron and copper have formation constants for the first and second 8-HQ of $\log \beta_1 \sim 12$ and $\log \beta_2 \sim 23$. Iron can accommodate a third 8-HQ, with a $\log \beta_3 \sim 34$. However, the relative load and elution efficiencies between the three resins with copper are expected to be representative for iron.

2.5. Colorimetric reaction

The DPD, reaction buffer and H$_2$O$_2$ mix briefly with the carrier after elution of the sample concentrating column at room temperature prior to entering the heated coil. Note that only the reaction mixture is heated at this stage, not the concentrating column or the individual reactants. The effect of a catalyst on the reaction rate constant, $k$, is given in the Arrhenius equation (Eq. (1)):

$$k = A e^{-\frac{E_a}{RT}}$$

where $E_a$ is the activation energy, which is lowered by the presence of the catalyst, often resulting in a 10-fold increase in rate. $T$ is temperature which, when raised, will increase the rate. DPD oxidation is greatly enhanced in the presence of the homogeneous catalyst, Fe(III) with H$_2$O$_2$, as evidenced by $\varepsilon = 9500 \text{l/mol cm}$ for the uncatalyzed reaction [21] and $\varepsilon = 1.7 \times 10^7 \text{l/mol cm}$ for the catalyzed reaction [6]. Both experiments were conducted at 25 °C, with $10^{-3} \text{M DPD}$, a 5 min reaction time and detection at 520 nm. In the presence of H$_2$O$_2$ the iron is continuously re-oxidized to Fe(III) which will in turn oxidize the DPD, while Fe(III) alone will not be recycled, and once reduced to Fe(II), will no longer oxidize the DPD. A strong pH dependence for the uncatalyzed reaction, with an optimum range of pH 2–3, was also reported [21]. This low pH would be required for the Fenton reaction (Eq. (2)):

$$\text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^- + \text{OH}^*$$

The inductance time would not affect the FIA results, as the absorbance is measured well after the reaction starts. With the reaction coil length and flow rates used in this study, the reaction was allowed to proceed for, approximately 5 min.

In this method, increased rate corresponds with more signal, as the reaction is allowed to proceed for a set length of time. Sensitivity was compared in the laboratory at slightly above room temperature (18 °C) and at 30 °C. Temperature control of the catalytic reaction, while not previously reported, is necessary for reproducible results in the field. Shipboard laboratory temperatures can fluctuate 10 °C during the same day, affecting assay sensitivity and resulting in variable quantitation of results.

3. Results and discussion

3.1. Resin optimization

The 8-HQ supports, currently in use for this application were compared directly. Copper break-through capacity studies demonstrated that the 8-HQ silica gel had the best characteristics (Fig. 2). The curves represent the concentration of copper coming through the column normalized to the concentration loaded versus micromoles of copper loaded. The dip at aliquot 8 in the otherwise continuous break-through curves is due to stopping the pump after aliquot 7 for, approximately 20 min to process samples. With the longer contact time, more copper was able to bind to the 8-HQ resin resulting in a lower value for aliquot 8 for the HW-40C and epoxy resins. The load flow rates for each resin were: HW-40C at 3.3 ml/min, epoxy at 3.4 ml/min, and silica at 3.8 ml/min, with integrated break-through capacities of: 1.0, 2.6, and 3.2 μmol Cu/cm$^3$ wet resin, respectively. Higher capacities mean smaller columns can be used to quantitatively recover analyte from the sample flow stream, thus reducing the blank and system back-pressure.

The HW-40C and epoxy resin both appeared nearly black, a good indication of high 8-HQ attachment efficiency as the darker color is related to the
amount of 8-HQ bound to the resin (Landing, personal communication). The HW-40C resin exhibits early break-through and incomplete complexation of the metal ion, therefore making it undesirable for quantitative work. There was no apparent channeling to account for the poor performance of the HW-40C. This batch of HW-40C resin, of similar to superior iron assay performance characteristics to three other batches of HW-40C resin produced, had been stored for 18 months while the epoxy and silica gels were less than a year old. Little degradation is expected for these resins as stored, in Milli-Q at 4°C in a plastic bottle in the dark. The silica resin was brick red, which is also considered acceptable for 8-HQ resin appearance [6]. The copper-binding capacity of the smallest column used on the system (1 cm × 0.2 cm or 30 μl volume) for the epoxy resin and the silica gel was calculated to be 80 and 100 nmol, respectively.

Elution profiles (Fig. 3) reveal that 90% of the copper elutes with the first column volume (estimated 25% void volume, or 2 ml) for the HW-40C and silica resin, with 80% for the epoxy resin. The silica gel has the sharpest elution profile with copper eluting from this column completely with, approximately 3 column volumes. The epoxy resin shows some tendency to hold onto a small percentage of the loaded copper. This may not be a problem with smaller columns eluted extensively between loads. When loaded with iron and compared to silica, the epoxy elution profiles were comparable to silica with complete elution of iron at 4 column volumes.

All of these columns were well rinsed with 0.5N HCl and used for several load/elution cycles, yet the HW-40C and epoxy resins continued to leach color. The initial elution aliquot of the HW-40C resin appeared light pink, while the first epoxy elution aliquot looked light yellow. Each lightened with successive elution aliquots, with color still present up to 3–4 column void volumes. Three previous batches of HW-40C resin also exhibited leaching when employed for iron determinations upon 0.5N HCl in-column washing. Leaching of color could contribute to the blank, but mainly raises the concern that the resin may not be acid stable.

Epoxy resin and silica gel were compared in performance assays with the same Milli-Q standards using 2.5 cm columns (80 μl volume). At 18°C, the sensitivity using the silica gel was 0.26 absorbance Fe with a steady reagent baseline at 0.01 absorbance and a 0.2 nM Fe Milli-Q blank. Using the epoxy resin, the sensitivity was 0.12 absorbance Fe with an increasing baseline (0.0–0.06 absorbance) and a 0.5 nM Fe blank. Using smaller
columns decreases the blank. Since the silica gel had
the highest capacity, sharpest elution profiles, and bet-
ter performance characteristics, it was selected as the
preferred resin for this assay. The iron assay involves
loading sample at pH 5.2–5.4 and eluting near pH 1;
therefore, problems with silica stability [15] at high
pH will not be encountered.

3.2. Temperature

Other researchers attempted higher temperatures
and higher signal was obtained, but the concomitant
increase in noise raised the detection limit [5,6], so
temperature control was abandoned. In this work, a
higher reaction temperature was successfully demon-
strated without impacting the detection limit. The
effect of raising the temperature by 12°C alone ac-
counted for a doubling of the sensitivity. Sensitivity
of iron standard additions to seawater samples with
the reaction coil held at 18 and 30°C were compared.
This increase in temperature doubled the sensitiv-
ity, with the added advantage of providing a stable
operating temperature. Shipboard laboratory temper-
atures ranged from 15 to 30°C; trying to operate at
a temperature lower than the highest temperature on
a given day could introduce variability in reaction
temperature and assay response.

When the temperature was increased from 18 to
30°C, the baseline doubled, but the increase in base-
line noise was comparatively small, so the detection
limit did not rise with the increasing sensitivity. Higher
temperatures should further increase the sensitivity,
but with too steep a slope, the working range would
be diminished. If higher sample through-put was de-
sired, a higher temperature with a reduced load time
and shorter reaction coil to reduce peak broadening
could be attempted; however, the reagents must be free
of iron so that the range is not diminished by a high
baseline.

3.3. Response

Fig. 4 illustrates a set of digitally collected re-
response peaks from a set of standard additions to a
0.42 ± 0.01 nM Fe seawater sample collected aboard
ship. Absorbance was recorded at 540 nm for repli-
cates of the acidified, microwaved sample, and the
same sample with its iron concentration raised by 0.4
and 0.8 nM. High magnitude, low frequency (<1%
of data points) spikes due to noise from the ship’s
electrical system were eliminated by setting those
values to the average of the point before and after
the spike. The peak shape demonstrates resolution to
baseline between peaks, efficient elution and mixing
from the compact peaks with minimal tailing, and a relatively small baseline drop at the solvent front. The baseline noise is minimal and replicate samples yield reproducible responses. The reagent blank, acidified Milli-Q run as a sample, did not increase significantly with column load time nor increasing temperature. Upon quantifying the peak obtained from injecting Milli-Q water after 1 s and 1, 2 and 4 min loads and also comparing these peaks at 18 and 30 °C, it was discovered that while the reagent blank could be made negligible, there is still a column blank that increases with column size and higher column void volume. This blank was not negligible when measuring sub-nanomolar iron levels. Sample in the column void and injection loop tubing has a different index of refraction than the carrier and, when mixed with the carrier upon column elution, contributes to the blank. Rinsing the column with Milli-Q between loading and eluting did not alleviate this problem nor did seawater carriers, pumped through either a Chelex or 8-HQ column to remove iron. Seawater carriers also had a higher contribution to the baseline noise versus the Milli-Q carrier.

The standard addition peaks shown were obtained at 30 °C with a 1 cm column and 5 ml sample load from a 2 min load, 10 min elution cycle. Sensitivity from this set of standard additions was 0.50 abs/nM Fe and could vary as much as 10% on different days depending on the particular concentrating column used, the condition of the pump tubing, and the condition of the DPD aliquot used (solid reagents must be pre-weighed and aliquotted for shipboard use). Sensitivity can be purposefully varied with longer or shorter load times. However, for higher concentration samples or longer load times, a longer elution time is necessary for complete elution and baseline resolution. Five to six

Table 1
Summary and comparison of assay performance parameters

<table>
<thead>
<tr>
<th></th>
<th>Previous work [5]</th>
<th>This work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanks (nM Fe)</td>
<td>Reported as negligible; generated as reagent spikes</td>
<td>&lt;0.05; quantified from standard additions to Milli-Q samples</td>
</tr>
<tr>
<td>Detection limit (nM Fe), six replicates of same sample</td>
<td>0.025</td>
<td>0.016</td>
</tr>
<tr>
<td>Sensitivity (abs/nM Fe)</td>
<td>Not reported</td>
<td>0.5 ± 0.05</td>
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<td>Accuracy (%)</td>
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<tr>
<td>Precision (% error)</td>
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<tr>
<td>Reaction temperature (°C)</td>
<td>Room temperature</td>
<td>30</td>
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samples per hour are optimal for the desired sensitivity when a concentrating column is used and the reaction is performed at 30 °C. This can be accomplished with the 2 min load, 8–10 min elution cycle which provides adequate sensitivity and a standard curve that is linear to 1.6 nM Fe. When samples are in the 1–12 nM iron concentration range, the load time must be reduced to 0.25–1 min. The 0.25 min load (625 μL) is linear to12 nM Fe; above such high iron concentrations, it is necessary to use an injection loop instead of a column. Greater sensitivity can be obtained by longer load times (>2 min), but this will diminish the working range and sample throughput of the assay.

The iron concentration obtained over 10 days at sea for the seawater control, 0.71 ± 0.05 nM Fe (n = 26), compares favorably with the GFAAS value of 0.755 ± 0.007 nM Fe obtained prior to this cruise for this sample (Smith, personal communication). The precision of this FIA method over these 10 days at sea at the concentration of 0.71 nM Fe was 7% (C.V.). On any single day, the precision was typically 2.5% and as low as 1.5% for this control sample. The accuracy relative to a measurement of this sample by the established GFAAS technique was within 6%. Evaluation of sub-nanomolar assay quantification performance is important for oceanographic applications, yet such low iron seawater controls are not available commercially.

The detection limit calculated from 10 samples collected consecutively from an off-shore station was 0.06 nM Fe from three times the S.D. of the 0.06 ± 0.02 nM iron concentration result. When the same bottle of this 0.06 nM sample was repeated six times, a 0.016 nM detection limit was obtained, which translates into a less than 0.003 abs S.D.

Table 1 summarizes and compares assay performance results of this study to the FIA assay [5] which was the predecessor to this work. When this assay was employed previously [5], the samples collected were unfiltered and acidified to pH 3 for an unspecified length of time. Currently, most researchers use some level (0.2, 0.45 or 2 μm) of filtration. Blanks were evaluated in previous studies [5] via spiking additional sample buffer or acid into samples. Here acidified Milli-Q, run as a sample, was used to evaluate the blank, thus loading the column using the same pH adjustment as the samples. The detection limit reported previously [5], 0.025 nM, appears to be based upon six replicates of the same sample. True replicates, where the variability from sample handling is included in the error, may be a more appropriate measure of the detection limit of the entire assay process for the stated application.

Assay parameters addressed in order to improve shipboard determination of iron were: (1) a well-defined sample pre-treatment; (2) comparison of the three 8-HQ resin supports; (3) longer load and elute times for baseline resolution; (4) acidified Milli-Q rather than acidified, cleaned seawater as the carrier for lower baseline and ease of preparation; (5) 0.8 mm i.d. PFA manifold; (6) a longer reaction time; and (7) a heated reaction coil.

3.4. Shipboard data

A near- to off-shore surface transect for iron in California coastal waters (Fig. 5a) shows high (>1 nM) iron values (Fig. 5b) over the shelf during an upwelling event, with values dropping to below the detection limit (tens of pM) in the off-shore waters. These results are oceanographically consistent [23,24], and the dissolved iron concentrations followed another upwelled nutrient, nitrate + nitrite (Fig. 5b). Since this transect was conducted from in-shore areas with high iron to off-shore areas with low iron, the Teflon filter cartridge was changed near longitude 123.75°. The Teflon filter itself would not be expected to affect any sample carry-over, but particulates entrained on the filter might. Therefore, additional care is required when samples are being filtered, whether off-line or in-line, to eliminate sample carry-over. Ideally, transects could be conducted from off-shore to on-shore, thereby sampling the low iron waters, first. This is not always feasible due to prevailing winds and sea state.

When the iron fell to a level at or below the detection limit for this assay, nitrate no longer decreased in concentration. In this off-shore segment of the transect the major nutrients, nitrate + nitrite, silicate, and phosphate were, approximately 9.8, 2.1 and 0.7 μM, respectively. These levels of nutrients are sufficient for phytoplankton growth. That the major nutrients were not drawn down further suggests another nutrient, possibly iron, was limiting growth.
4. Conclusions

From direct comparisons, trace-metal clean silica gel had the highest capacity and the steepest elution profiles of the three resins currently in use for this application, with no visible leaching of color at low pH. It was demonstrated that enhanced sensitivity provided by higher reaction temperatures can be attained if iron
contamination of the reagents is minimized. A reason-
able detection limit and oceanographically consistent
results were obtained during shipboard experiments.
Carefully defining the iron fraction being measured
will enable better comparison of field data obtained
from different laboratories.

Acknowledgements

The QuikChem 4200 analyzer was a donation
from Zellweger/Lachat Instruments QuikGrant pro-
gram. Trace-metal clean sampling was enabled by all
hands aboard the R/V Pt. Sur. Funded by NSF grant
OCE-9811114 to KWB.

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