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Determination of sub-nanomolar levels of iron in seawater using flow injection with chemiluminescence detection

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Abstract

The development of a highly sensitive system for the shipboard determination of dissolved iron at the sub-nM level is presented. The technique is based on a flow injection method coupled with luminol chemiluminescence detection. Dissolved Fe(II+III) levels are determined after Fe(III) reduction using sulphite and in-line matrix elimination/preconcentration on an 8-hydroxyquinoline (8-quinolinol) chelating resin column. The detection limit (3*s*) is 40 pM when 1.5 ml of sample is loaded onto the column, and the relative standard deviation is 3.2% (n=5) for a 1.0 nM Fe sample. One analytical cycle can be completed in 3 min. The automated method proved reliable when employed on-board the RRS James Clark Ross during Autumn 1996, mapping dissolvable Fe(II+III) levels along the Atlantic Meridional Transect from 50°N to 50°S. Data from vertical profiles through the upper water column are presented. © 1998 Elsevier Science B.V.

Keywords: Flow injection; Luminol; Chemiluminescence; Iron; Preconcentration; Seawater; Atlantic meridional transect

1. Introduction

Iron is a major component of the Earth's crust, but like other reactive trace elements, its dissolved concentration in open-oceanic waters remains very low (<1.0 nM). It is an essential micronutrient for organisms and in certain high-nutrient, low-chlorophyll areas of the world's oceans, iron appears to limit phytoplankton growth [1,2], which may have important implications for global carbon cycles [3]. Such hypotheses have recently been tested in the underproductive waters of the equatorial Pacific, where seeding an expanse of surface water with low concentrations of iron sulphate triggered a massive phytoplankton bloom [4,5], and resulted in a transient increase in the atmosphere–ocean CO_2 flux [6].

Fe(III) is the thermodynamically stable form in oxygenated seawater, existing predominantly as insoluble oxy-hydroxides or colloidal matter [7–9]. Fe(II) is a transient species in surface oxic waters, existing via chemical or photochemical Fe(III) reduction [10–12], or via atmospheric deposition [13–15]. At seawater pH, Fe(II) is oxidised rapidly by O_2 and H_2O_2 [16]. Recently, organic complexation has been thought to occur to a significant extent in marine systems [17–19]. Laboratory studies have shown that phytoplankton are only able to utilise dissolved Fe^{2+} or Fe^{3+} species, and that uptake of colloidal or particulate Fe is

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only possible via a thermal or photochemical dissolution pathway [20]. Hence the bioavailability and interaction of iron with biota is critically linked to an understanding of its thermodynamic and kinetic nature in a seawater matrix.

Major sources of iron to the world's oceans include aeolian deposition, fluvial transport, hydrothermal vents, continental shelf regeneration and upwelling of Fe-enriched subsurface waters. In remote areas, the ocean receives the majority of surface water iron from atmospheric dusts, and the true impact of suspended aerosols on trace element levels can only be made by direct in-situ measurements. Attempts to map the distribution and behaviour of iron in natural waters have been hindered by its very low concentration and ubiquitous sources of contamination in sampling equipment, research vessels and laboratories. Furthermore, the highly reactive characteristics of Fe species have hampered the elucidation of accurate iron redox models, and very little is known about the chemical nature of iron associated with various operationally defined fractions (e.g. labile, dissolved, colloidal, organically bound, acid leachable, particulate). For these reasons, coupled with the desire to evaluate the effect iron limitation may have on phytoplankton communities, there has been a demand for new analytical methodologies which can provide rapid and accurate iron determinations at pM levels on board research vessels.

Current methods for determining iron in seawater involve extractive chelation and preconcentration from large volumes (0.5–2 l) of filtered seawater [21,22]. Subsequent laboratory analysis is generally performed using graphite furnace atomic absorption spectrometry. This method suffers from many disadvantages. It is tedious, has many sample handling stages (thus increasing the risk of contamination), large sample volumes are required in order to achieve low detection limits and the precision is poor. The technique provides no information relating to the oxidation state of the metal and, without a clean container laboratory, is clearly impractical for shipboard analyses.

Recently, flow injection (FI) techniques have attracted extensive interest for the trace analysis of iron. Such systems are in many cases portable, analyses are rapid, sample handling is minimal, only small volumes are required and the precision and limits of detection are good. Batch chemiluminescence (CL) [23–25] and colorimetric [26–29] methods for sub-nM iron determinations have recently been incorporated into FI manifolds. They have comparable limits of detection when used in combination with a chelating resin preconcentration column but encounter difficulties when attempting to measure the redox state of the metal at pM concentrations.

Elrod et al. [24] measured Fe(II) (and total dissolved Fe after reduction by ascorbic acid) using the Fe(II) selective CL reagent brilliant sulfoflavin (BSF); however, this reaction gave some response in the presence of Fe(III) species. The moderate detection limit (0.45 nM) resulted in the method being used only in hydrothermal plume mapping experiments where enhanced iron concentrations are observed. Obata et al. [23] reported a sensitive CL method for Fe(III) species based on a luminol–ammonia–hydrogen peroxide reaction, but it required careful pH adjustment prior to analysis in order to determine Fe(III). Powell et al. [25] and O'Sullivan et al. [30] also exploited luminol CL for land-based Fe determinations, the latter system being incorporated into a stopped flow manifold, which required additional instrumentation and sample handling prior to detection. The spectrophotometric method of Measures et al. [26], based on the catalytic effect of iron on the oxidation of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (DPD) by hydrogen peroxide, has the potential to be incorporated into submersible chemical analysers for in-situ iron monitoring. The limit of detection is sub 0.1 nM, but the method does not distinguish between Fe(II) and Fe(III) species. Finally, cathodic stripping voltammetric methods have recently been developed [12,18], which provide information relating to speciation and organic complexation of the metal, although such systems are not as robust as spectrometric FI manifolds.

This paper reports a flow injection–chemiluminescence technique for the rapid shipboard analysis of dissolved Fe(II + III) at the sub-nM level. The system is based on the oxidation of luminol, which is catalysed by Fe ions, emitting blue light (λ_{max} ~440 nm) [31–35]. The iron is first reduced to Fe(II) and then extracted from its sea-salt matrix and preconcentrated in-line using a micro-column containing 8-hydroxyquinoline immobilised on a vinyl polymer gel. Elution may be performed with a weak acid prior to detection

of the light on mixing with the CL reagent system. The method requires very little sample handling, the reaction is simple, throughput is high (a 3 min analytical cycle, seawater sample quantified within 25 min) and the shipboard limit of detection is 40 pM, which is below Fe concentrations generally found in open-ocean field studies. Preliminary results from the shipboard determination of vertical iron distributions in the upper water column of the North and South Atlantic using the FI–CL monitor are also presented.

2. Experimental

All plasticware was cleaned by first soaking in hot 5% (v/v) micro detergent (DECON®; Merck BDH) for 24 h, followed by 1 week in 50% (v/v) hydrochloric acid (AnalaR; Merck BDH) and 1 week in 50% (v/v) nitric acid (AnalaR; Merck BDH). Labware was thoroughly rinsed with ultra high purity (UHP) deionised water (18.2 MΩ cm^{-1}, Elgastat Maxima). All reagents and standards were of analytical grade, supplied by Merck BDH, unless stated otherwise, and prepared in UHP water. Wherever possible, in order to reduce airborne contamination, experimental work was carried out in a class-100 laminar flow bench (Bassaire model A3VB) contained in a class-1000 clean air room. Reagent solutions and the FI–CL system were regulated at 20°C in the clean air laboratory in order to prevent the generation of air bubbles in the FI tubing and to maintain a steady baseline from the PMT detection unit. For shipboard analyses, reagents and samples were contained within a sealed perspex cabinet unit (350×345×380 mm), and PTFE tubing was used to transfer the solutions to the FI analyser which was positioned in the ship's laboratory.

2.1. Reagents

2.1.1. Iron standards

A 0.01 M Fe(II) standard was prepared by dissolving 0.3921 g of $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in 100 ml of a 0.1 M Q-HCl (sub-boiled, quartz-distilled) solution. This stock was prepared monthly. Other standards were prepared daily in 0.01 M Q-HCl by serial dilution. Fe(II) standards were pre-purged with nitrogen to avoid oxidation. Fe(III) working standards were prepared by dilution of a 1000 ppm Fe(III) atomic absorption standard (Spectrosol). These Fe(III) solutions were prepared in low iron seawater (see below) and kept at pH 8 for 1 h to oxidise any Fe(II) present in the stock solution and then acidified to pH 1 with Q-HCl (9 M). These conditions ensured the integrity of the redox state of the iron standards.

2.1.2. Luminol/carbonate buffer

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Fluka) was used as received. A 0.1 M Na_2CO_3 buffer was prepared by dissolving 5.3 g in 500 ml of UHP water. A stock solution of ca. 2 M NaOH was prepared by dissolving 8 g in 100 ml of UHP water. A stock solution of luminol (0.01 M) was prepared by dissolving 0.177 g in 100 ml of carbonate buffer followed by sonicating for 30 min. A working luminol reagent solution (1×10^{-5} M) was prepared by diluting 0.5 ml of the stock solution in 500 ml of 0.1 M Na_2CO_3 and adjusting to pH 12.2 with sodium hydroxide solution. This reagent was passed through a column containing the Chelex-100 chelating resin just prior to use in order to reduce the baseline noise generated by impurities in the reagents. On mixing with the HCl eluent stream in the FI manifold, an effluent pH of 10.4 allowed optimum chemiluminescent emission. Luminol solutions provided maximum and stable sensitivity after a storage period of 24 h and therefore were prepared a minimum of 1 day in advance. Luminol solutions were stable for at least 1 month.

2.1.3. Acids/ammonia

Purified hydrochloric acid (Q-HCl, 9 M) and purified acetic acid (Q-acetic acid, 17.5 M) were prepared by a single distillation of the analytical grade acids in a quartz-finger, sub-boiling distillation apparatus. An ammonia solution (Q-NH_3, ca. 6 M) was purified using isopiestic distillation and nitric acid (HNO_3, Aristar; Merck BDH, 15.5 M) was used as received.

2.1.4. Ammonium acetate buffer

Ammonium acetate stock solution (2 M) was prepared by adding 22.2 ml of Q-acetic acid to 90 ml of Q-ammonia solution and diluting to 200 ml with UHP water. A working sample buffer (0.4 M) for in-line pH adjustment of the sample was prepared by diluting 20 ml stock to 100 ml with UHP water, and adjusting to pH 5.5 with Q-acetic acid. This buffer was cleaned

in-line using two 8-HQ columns (see Section 2.4) in series.

2.1.5. Reducing reagent

A stock solution (0.04 M) of reducing reagent for Fe(III) was prepared by dissolving 0.1008 g of Na_2SO_3 (extra pure, Merck BDH) in 15 ml of UHP water, adding 5 ml of 0.4 M ammonium acetate buffer (pH 5.5) and sonicating for 10 min. This stock reagent was cleaned by passing the solution through two sequential 8-HQ columns (see Section 2.4) just prior to use. 2.5 μl aliquots were added per ml of acidified seawater to achieve a final sulphite concentration of 100 μM in the sample. The reducing reagent was allowed to react with the acidified sample for 4 h.

2.1.6. HCl carrier

The eluent solution (0.09 M) was prepared by diluting 5 ml of Q-HCl (9 M) to 500 ml with UHP water.

2.1.7. Acid wash

An acid wash solution (0.6 M Q-HCl/0.16 M HNO_3) was prepared by diluting 7 ml of Q-HCl (9 M) and 1 ml of HNO_3 (15.5 M) to 100 ml with UHP water.

2.1.8. Low iron seawater

Seawater used to optimise the FI–CL manifold and perform matrix interference experiments was collected from the English Channel (near Eddystone Rocks), transferred to a high density polyethylene (HDPE) container and acidified to pH 2 using Q-HCl. The seawater was then buffered to pH 5 via the addition of 2.5 ml of 2 M ammonium acetate buffer (pH 5.5) per 100 ml of seawater. The water was passed through two sequential 8-HQ columns (see Section 2.4) at ca. 1 ml min^{-1} in order to remove iron from the matrix. The eluent seawater was once again acidified to pH 2 with Q-HCl and stored in the dark.

2.2. Sample collection and handling

Bottles were cleaned as described above, and stored in 0.01 M Q-HCl. Prior to use, they were thoroughly washed with UHP water and rinsed with the seawater sample. Samples were collected from 10 l modified Niskin bottles (General Oceanics) designed for trace metal work mounted on an epoxy-coated stainless steel rosette frame. Stringent clean sample handling techniques were followed. Unfiltered samples were collected in HDPE bottles and acidified to pH 2 with 250 μl of Q-HCl (9.0 M) per 250 ml of sample. The Fe(III) reducing reagent was added to acidified samples (final sulphite concentration 100 μM) and allowed to react for at least 4 h. The samples were analysed and calibration performed by standard additions. Three 10 ml aliquots of reduced sample were transferred to clean 25 ml polystyrene screw-capped vials (Sterilin, Merck BDH). Sub-sample 1 was left alone, whilst sub-samples 2 and 3 were spiked with a small volume (20–50 μl) of Fe(II) standard (variable concentration) to achieve standard additions ranging from 0.2–5.0 nM. The sub-samples were mixed and analysed in order of increasing concentration.

2.3. Instrumentation

The flow injection–chemiluminescence manifold used for this work is shown in Fig. 1. Three peristaltic pumps (Pump A: Gilson Minipuls 3, Pumps B, C: Ismatec Mini-S 820) were used to deliver the reagents, sample/buffer and wash solutions, respectively, to the injection valve, preconcentration column and CL detector. All manifold tubing was PTFE (0.75 mm i.d., Fisher) except for the peristaltic pump tubing, which was flow-rated silicone (Elkay), and the 8-HQ columns. A six-port rotary injection valve (Rheodyne, model 5020) operated by a Universal Valve Switching Module (Anachem) was used to transport the sample to the detector. Three 3-way PTFE solenoid switching valves V1, V2 and V3 (Cole Palmer, model E-01367-72) enabled sample, UHP water and acid wash solutions to pass sequentially through the 8-HQ preconcentration column. The load, wash and inject cycles of the FI monitor were computer controlled using a device control and data acquisition card (Brainboxes, model PC AD 1211L) housed in a PC (Viglen 486-DX).

A quartz glass spiral flow cell C (1.1 mm i.d., 130 μl internal volume) positioned behind a mirror in a light-tight housing enabled the CL reaction to be monitored. The detection system consisted of an end-window photomultiplier tube (THORN EMI, 9798QA) contained in a μ-metal shield for magnetic insulation (MS52D), a built-in current-to-voltage amplifier (C634), an ambient temperature rf shielded housing

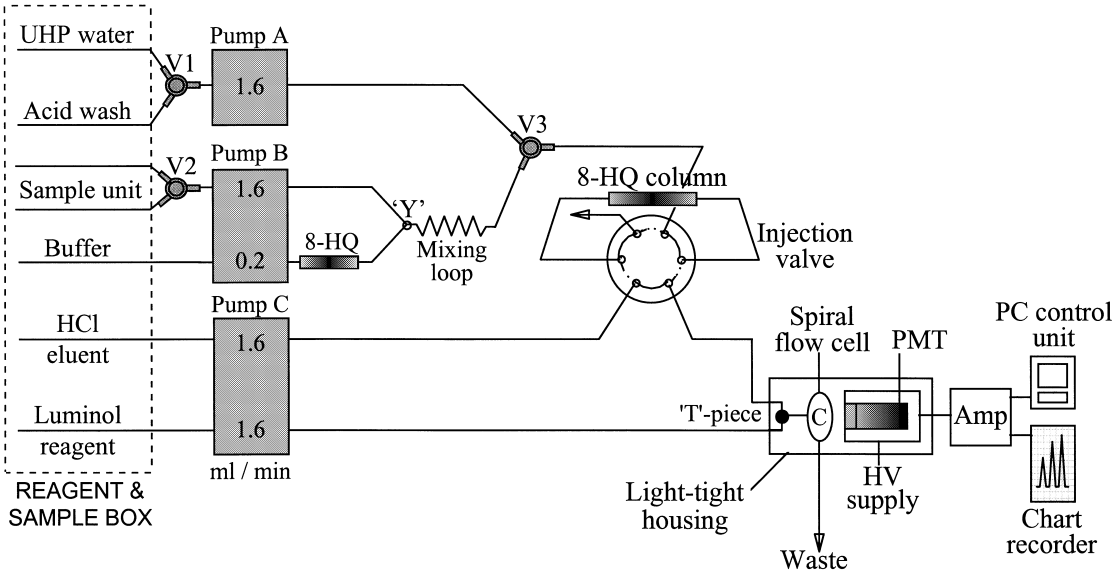

Fig. 1. Flow injection manifold used for the chemiluminescence based determination of Fe in seawater; PMT=photomultiplier unit, C=spiral flow cell, V1, V2 and V3=switching valves, A=wash pump, B=sample pump, C=reagents pump.

(B2F/RFI) and a 1.1 kV power supply (THORN EMI, PM28B). The amplifier was supplied with 15 V from an independent power supply (BBH products). Once powered, the PMT detection system remained switched on and took approximately 1 h to stabilise and give low dark current and voltage signals. Peak detection and quantification was achieved using a flat-bed chart recorder (Kipp and Zonen, BD111).

2.4. Preconcentration

Preliminary studies showed that generally more than 70% of the CL signal was suppressed in standards prepared in solutions containing Ca^{2+} and Mg^{2+} ions at their typical seawater concentrations, compared to UHP water standards. Furthermore, synthetic UHP solutions containing halide ions showed significant increases in the CL signal (e.g. 30% enhancement on addition of Cl^{-} at seawater concentration). Elimination of the sea-salt matrix prior to introduction of the buffered CL reagent would also prevent any precipitation of such sea-salt ions in the FI manifold at the reaction pH (ca.10.4). Furthermore, Fe(II) must be separated from other heavy metals and concentrated to achieve the pM detection limits required for open-ocean analyses.

A chelating resin of 8-hydroxyquinoline (8-HQ) immobilised on a hydrophilic vinyl co-polymer (TSK gel, Toyopearl HW-75F, 30–60 μm, fine, Toso-Haas, supplied through Anachem) was prepared according to a modified procedure of Landing [36,37]. The columns were constructed from 2.4 mm i.d. PTFE tubing, as illustrated in Fig. 2. The column ends were fabricated using 0.89 mm i.d. Tygon pump tubing, which was connected to the 0.75 mm i.d. PTFE manifold tubing. A plug of quartz wool was placed at either end in order to retain the resin inside the column. The columns were cleaned with 0.5 M Q-HCl for at least 4 h, followed by UHP water 1 h prior to use. The 8-HQ columns were also used for in-line purification of the ammonium acetate buffer and Fe(III) reducing reagent prior to analysis.

2.5. Procedures

One analytical cycle was completed within 3 min. Firstly, the seawater sample was pumped through the manifold where it was buffered in-line to pH 5.0 as it passed through the 0.5 m knitted mixing coil. The combined flow then passed through the 8-HQ column

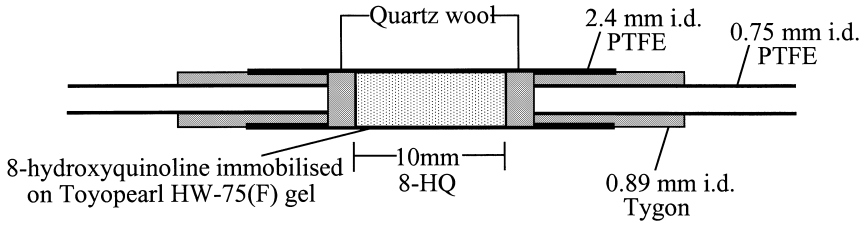

Fig. 2. Schematic diagram of the 8-HQ chelating resin preconcentration column.

at a rate of 1.5 ml min^{-1} for typically 60 s, during which time the iron was preconcentrated from its sea-salt matrix. Longer load times could be used to increase the sensitivity of the system. By switching the solenoid valve V3, UHP water was subsequently passed through the column (30 s) to remove any residual matrix ions present. The injection valve was then switched to the elute position and 0.09 M Q-HCl flowed through the column in a reverse direction (60 s) carrying the iron to the detection system where it mixed with the buffered reagent stream and generated a signal in the form of a narrow peak (ca. 10 s at half height). The elution volume was approximately 0.38 ml, resulting in a theoretical concentration factor of 4. The injection valve was then returned to the load position and washed with UHP water (30 s) to remove residual hydrochloric acid before the next load sequence commenced. The analysis of one sample in triplicate with two standard additions each in triplicate could be completed in 25 min. All responses were blank corrected for reagent impurities using the response for clean UHP water. Prior to use, the FI manifold, the PTFE flow lines, fittings and connectors were cleaned with 0.5 M Q-HCl and UHP water for several hours.

3. Results and discussion

3.1. CL reaction optimisation

The Fe(II)-catalysed chemiluminescence emission from luminol oxidation in the absence of added oxidant has been well documented [31–33]. Key variables, namely reagent flow rate, sample load volume, luminol concentration, reaction pH and PMT voltage, were evaluated using a modified simplex algorithm written in-house. For these studies no preconcentration of the analyte was performed and the 8-HQ column in Fig. 1 was replaced by a short length of PTFE tubing (internal volume 120 μl). Standards prepared in 0.01 M Q-HCl were used throughout. The variables, conditions and optimum results for the simplex optimisation are shown in Table 1. These conditions were valid for Fe(II) standards ranging from 10 nM–1 μM prepared in UHP water, and were reached after 12 iterations of the simplex. Subsequent experiments used these optimum conditions; however, under a system containing an 8-HQ preconcentration column, variables such as flow rates and sample load volume required re-optimisation. These experiments confirmed that the reaction is highly pH dependant and

Table 1
Results from the simplex optimisation of the FI–CL method for Fe(II) standards (10 nM–1 μM) prepared in UHP water

Variable	Unit	Range	Step size	Starting conditions	Optimum conditions
Flow rate [a]	ml min^{-1}	0.8–3.3	0.1	1.3	3.0
Sample loop volume [a]	μl	25–400	25	100	120
[Luminol]	M	10^{-7}–10^{-3}	variable	10^{-7}	10^{-5}
Reaction pH	—	8–12.5	0.2	11.8	10.4
PMT voltage	kV	0.8–1.2	0.05	1.0	1.1

[a] No preconcentration column was used for the initial optimisation of the CL reaction system. Hence these conditions are not optimal for a manifold containing an 8-HQ column.

requires a strongly alkaline (ca. pH 10.4) buffered reagent for maximum sensitivity.

3.2. Fe(III) reduction

Experiments were performed with various reducing agents. Ascorbic acid concentrations as low as 1 μM resulted in total suppression of the luminol CL emission. Additions of hydroxylammonium chloride also reduced the signals and generated poor, irreproducible FI peaks which did not return to the baseline. Sulphite (Na_2SO_3; S(IV)) produced the best results with no interference on the analytical method. A complete study of the reduction kinetics of Fe(III) by sulphite in natural waters has been reported by Millero [13]. The reaction is first order with respect to Fe(III) and S(IV):

$$d[Fe(III)]/dt = -k[Fe(III)][(S(IV)]$$

In seawater at pH 2, Millero reported a half-life $t_{1/2}$=12 min at [S(IV)]=1 mM. Fe(III) standards and samples were treated with reducing reagent ([S(IV)]=100 μM, S=35 psu, t=20°C, pH=2) for 4 h prior to analysis. A Fe(III) reduction by S(IV) versus time profile in seawater is shown in Fig. 3. Greater than 98% reduction of Fe(III) species was observed after 4 h, and standard additions to seawater samples containing Fe(II) and Fe(III) showed no significant difference in calibration gradient up to an Fe concentration of 10 nM.

3.3. Preconcentration optimisation

The complexation of iron by the chelating resin is influenced by many parameters. 8-HQ complexes strongly with Fe(II) ions, with a stability constant (log K_1) of 8.71 [38]. In a dynamic flow injection system, however, the exchange times are much reduced and the reaction may not reach equilibrium. Therefore, the experimental parameters of sample pH, column internal diameter and length and eluent concentration need to be optimised for maximum chelating efficiency. The sample, wash solution and reagent flow rates were also optimised once again after insertion of the 8-HQ column in the manifold, in order to maintain adequate loading rates without excessive back pressures.

3.3.1. Sample pH

Fig. 4 illustrates the retention of Fe(II) ions on an 8-HQ column as a function of sample pH. Fe(II) was quantitatively retained from seawater in the pH range 4.5–8.0. High sample pH values result in solutions in which the analyte becomes unstable, Fe(II) oxidation takes place and precipitation may occur. Indeed, Fe(II) analyte loss was noted after 1 h from seawater samples which were buffered to pH 4.5 prior to analysis. Therefore, all samples were acidified (0.01 M Q-HCl), and the pH was only adjusted in-line using ammonium acetate buffered at pH 5.5 immediately prior to adsorption onto the 8-HQ column. This

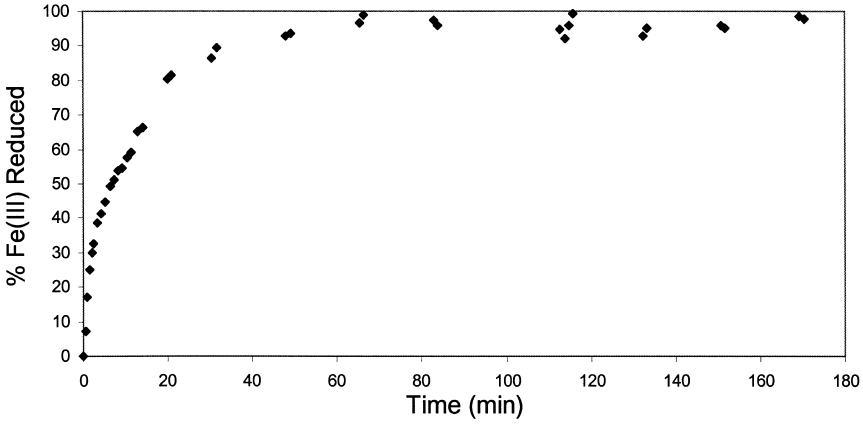

Fig. 3. Plot illustrating time course of Fe(II) formation resulting from the reduction of Fe(III) by S(IV) in seawater ($[Fe(III)]_0$=10 nM, $[S(IV)]_0$=100 μM, S=35 psu, T=20°C, pH=2).

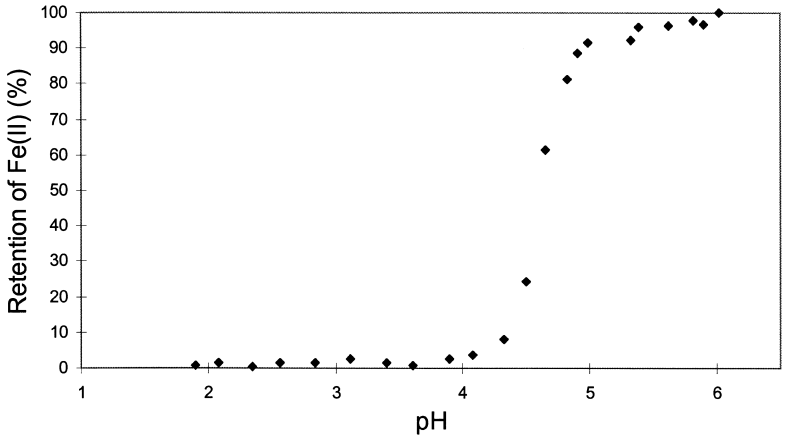

Fig. 4. Graph illustrating the retention of Fe(II) ions on an 8-HQ column as a function of pH ([Fe(II)]=2.5 nM).

resulted in a final sample pH of ca. 5.0, which maintained a balance between loading efficiency and Fe(II) oxidation rate.

3.3.2. Column construction

Various columns were constructed with a range of lengths and diameters. Initially, PTFE tubing of 1.5 mm i.d. was used to pack the resin. Such a narrow diameter resulted in increased back pressure in the FI manifold and a reduced flow rate for loading sample was necessary, increasing the overall analysis time. Increasing the column to lengths greater than 30 mm showed no improvement in sensitivity and resulted in increased dead volumes and back pressures and less reproducible peaks. Columns were therefore constructed from 2.4 mm i.d. PTFE tubing and a 10 mm length of resin was used (volume of resin= 45 μl). The low concentration of Fe ions in the sample, coupled with high stability constants, explains why such a small volume of resin can be used.

3.3.3. Elution

The acidic eluent must be sufficiently strong to rapidly desorb the iron from the 8-HQ resin, but mild enough not to suppress the highly pH dependent CL reaction, and therefore 0.09 M Q-HCl was used. Periodically, during the analysis of a sequence of seawater samples, the CL peaks did not return to the baseline. This had a minimal effect on the precision for these samples but the tail was eliminated by switching valve V1 and rinsing the column with the acid wash solution (0.6 M HCl / 0.16 M HNO_3, 60 s) and UHP water (60 s). On elution of Fe(II), a small negative dip in the signal was noted prior to a sharp positive emission. This was due to a pH change in the solution transported to the CL cell, produced by the mixing of UHP water in the dead volume of the 8-HQ column with the luminol reagent. After such a dip, Fe(II) ions contained in the acidic eluent reached the detector and produced the measured signal.

3.3.4. Column breakthrough capacity

In a dynamic column operation, the breakthrough capacity is the most useful parameter for characterising the column. This parameter is defined as the maximum amount of metal ion that can be chelated per unit mass of solid under the existing operating conditions, before the analyte is detected in the column eluent. A modified flow injection manifold was used to measure the capacity of the column. Operating conditions were as outlined in Section 2.5. A fresh TSK-8HQ column (internal volume=45 μl) was attached directly in-line with the detector and acidified seawater (pH 2) containing 10 μM Fe(II) was buffered in-line (pH 5) and passed continuously through the column. Initially, the CL reading was at the baseline, indicating the effective complexation of the Fe ions onto the resin. As the analyte began to break through the column and mix with the luminol reagent, the signal increased until a plateau was reached, indicat-

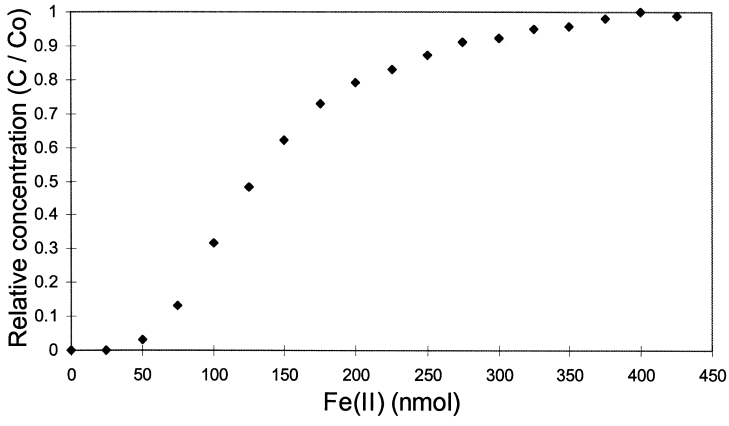

Fig. 5. Fe(II) breakthrough curve for a 45 μl TSK-8HQ column; C/C_0 is the effluent/influent concentration ratio ([Fe(II)]=10 μM, [S(IV)]=100 μM, pH=2, flow rate=1.5 ml min^{-1}).

ing that the chelating sites on the TSK-8HQ column were saturated.

An Fe(II) breakthrough curve is shown in Fig. 5. The breakthrough capacity of the column can be interpolated from the curve, based on the ratio of the concentration in the effluent (C) to the total concentration in the sample (C_o), at the point where C/C_o=0.05 [36]. At breakthrough, the column retained 56 nmol (3.1 μg) of Fe(II), which is significantly higher than the 1–2 pmol of Fe recovered from loading a 1.5 ml sample containing 1 nM Fe, typical of ocean waters. Since the dry mass of the resin contained in a 45 μl TSK-8HQ column was 54.7 mg, this represents a total capacity of 1.02 μmol of Fe(II)/g of resin.

3.4. Interferences

Experiments were conducted in seawater at pH 2 with Fe(II) concentrations of 1.0 nM. Individual metal ions were added at 1, 10 and 100 times the Fe(II) concentration and the change in CL emission was compared to the signal for 1.0 nM Fe(II) only. Possible interfering metal ions investigated were Co(II), Cr(III), Ni(II), Mn(II), Cu(II), Ag(I), Pb(II) and Zn(II), and the results are presented in Table 2.

With the exception of Co(II), none of the elements exhibited a significant interference at any of the concentrations tested, when loaded onto the 8-HQ column at pH 5.0, but problems were encountered above this level at ambient seawater pH. Co(II) showed a strong positive interference at all concentrations, and is known to be an efficient catalyst for the luminol CL system [32]. However, the maximum Co(II) concentrations in open-ocean waters typically range between 100–300 pM [39] and, in additional experiments, no interference to the analytical method was noted at concentrations up to 500 pM Co(II). The addition of 1000 pM Co(II) to a 1.0 nM Fe(II) standard yielded a 28% increase in CL signal.

Table 2
Responses for interfering metal ions in the presence of 1.0 nM Fe(II) in seawater, normalised to the response for 1.0 nM Fe(II) in seawater [b]

Ratio	Metal conc. (nM)	Co(II)	Cr(III)	Ni(II)	Mn(II)	Cu(II)	Ag(I)	Pb(II)	Zn(II)
1×	1.0	128±4	101±3	102±2	104±5	102±5	100±3	97±4	99±2
10×	10.0	244±5	99±2	101±4	98±6	97±5	105±4	101±4	97±1
100×	100.0	398±4	99±3	103±4	94±7	98±6	104±5	101±3	100±1

[b] The data values are percent signal intensity relative to the signal intensity of 1.0 nM Fe(II) in the absence of other added metals. Uncertainties represent two standard deviations (95% confidence) for n=4.

Table 3
CRM results for dissolved Fe(II+III) for trace metal certified seawater solutions [c]

Standard solution	Fe concentration (nM)	
	Certified	FI–CL
NASS-4	1.88±0.29	2.01±0.12
CASS-2	21.49±2.15	23.61±0.94

[c] Uncertainties represent 95% confidence interval for an individual sub-sample.

3.5. Analytical figures of merit

The calibration for iron in the optimised system was linear in the concentration range 0.04–10 nM (r^2=0.9976), and the relative standard deviation was 3.2% (n=5) for a 1.0 nM Fe(II) standard. The limit of detection in low iron seawater (three times the standard deviation) was 40 pM for a preconcentration time of 1 min. This could be improved by increasing the loading time, although this would require further purification of the sample buffer and reducing reagents to reduce the blank signal. The sample throughput for complete analyses in triplicate including standard additions was 3 h^{-1}.

The accuracy of the flow injection–chemiluminescence technique was ascertained by analysing seawater certified reference materials obtained from the National Research Council of Canada, Marine Analytical Chemistry Standards Programme. The results (Table 3) for North Atlantic Surface Seawater (NASS-4) and Coastal Atlantic Surface Seawater (CASS-2) show good agreement (t-test) with certified values.

3.6. Shipboard analyses

The system was extensively applied at sea on-board the RRS James Clark Ross, traversing the Atlantic Meridional Transect from Portsmouth (UK) to Stanley (Falkland Islands) (September 16th to October 25th 1996). A vertical distribution of dissolved Fe(II+III) in unfiltered samples collected in the upper water column (7–200 m) is shown in Fig. 6(a), together with the chlorophyll fluorescence, salinity and temperature profiles in Fig. 6(b). The location of the sampling station was in the sub-tropical eastern Atlantic Ocean, at 24°N, 21°W. The analysis of unfiltered samples using the FI–CL method is likely to have resulted in the detection of an important fraction of colloidal and particulate Fe which dissolved during acidification.

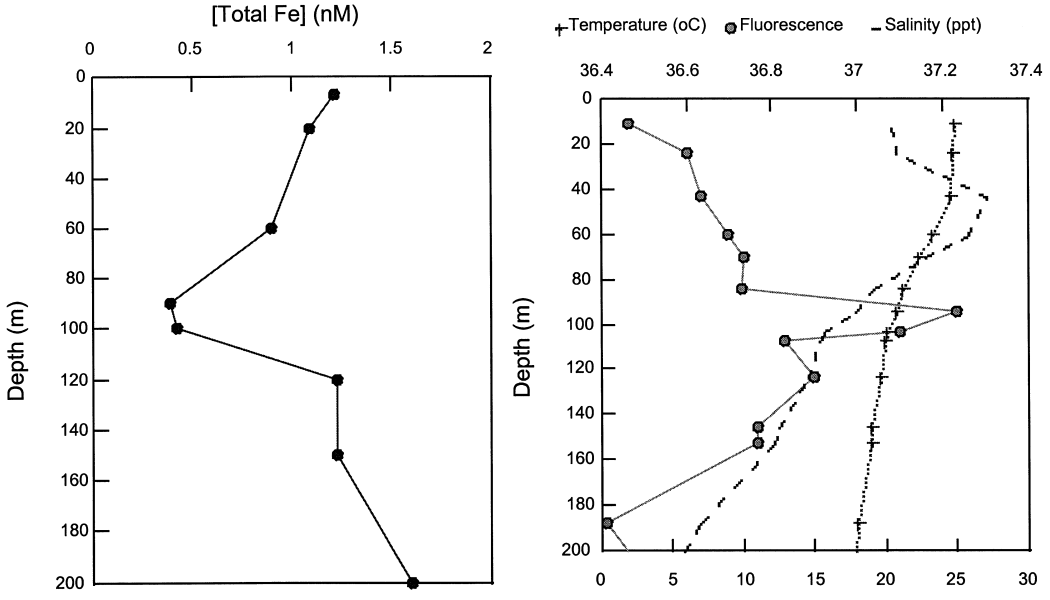

Fig. 6. (a) Vertical profile of dissolved Fe(II+III) distribution in NE Atlantic seawater sample; (b) Corresponding temperature, salinity and chlorophyll fluorescence profiles (measured by Neil Brown CTD sensors); AMT-3 CTD station 8, SDY 273, 24°N, 21°W.

Enhanced surface water concentrations at 7 m depth (1.2 nM) were possibly due to Fe-laden atmospheric deposits. Levels decreased with depth through the euphotic zone and reached a minimum (0.4 nM) coinciding with the fluorescence maximum. Fe concentrations showed an increase below 100 m depth which may have been caused by release upon breakdown of Fe-containing detritus. The fluorescence measurements are indicative of chlorophyll levels and therefore the Fe minimum at ca. 100 m depth may be caused by uptake by photosynthesising organisms.

A latitudinal surface water profile of dissolved Fe(II+III) between the UK and the Falkland Islands was obtained, in addition to 27 daily depth profiles in the upper water column down to ca. 200 m depth. Samples were analysed on-board ship using the method of standard additions. Dissolved Fe levels across the N and S Atlantic were found to range from 0.1–4.5 nM. The system required less than 100 ml of seawater (including rinses) for Fe quantification and was operated continually for up to 16 h per day. The data illustrate the reliability of the FI–CL technique for mapping Fe concentrations through a range of biogeochemical oceanic regions with contrasting conditions ranging from polar to tropical and from productive shelf waters to oligotrophic mid-ocean gyres. The full data set from AMT-3 cruise Fe analyses will be published elsewhere.

4. Conclusions

The analytical figures of merit and operational performance of the FI–CL system during two months of continual shipboard analyses demonstrate the practicability and accuracy of this simple technique for sub-nM Fe(II+III) determinations. The ability of the method to map Fe in open-ocean waters will aid in the elucidation of Fe input mechanisms and distributions in remote oceanic ecosystems. Further development of the method is planned in order to differentiate the redox forms. This will be achieved by incorporating two sample flow lines, one for Fe(II) and the other in which the sample is reduced in-line for Fe(II+III). Experiments will also partition between different operationally defined Fe(II+III) fractions (e.g. colloidal, organically bound, particulate).

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