Direct Determination of Iron(II) in Acidified Seawater using a Matrix Matched Flow Injection Manifold with Chemiluminescence Detection

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Abstract

A flow-injection method is reported for the determination of iron(II) in acidified seawater (pH 3.0) based on selective chemiluminescence detection using the luminol reaction with no added oxidant and no preconcentration column. The limit of detection (3 x standard deviation of the blank) was 0.1 nM and the time between injection and detection was 12 s. The calibration graph was linear (r^2 = 0.993, n = 5) over the range 2 – 10 nM with relative standard deviations (n = 4) in the range 1.0 – 3.7%. Analysis of a coastal water certified reference material (CASS-3) for iron(II), after reduction of iron(III) with sodium sulphite, gave good results (18.5 ± 5.2 nM compared with the certificate value of 22.56 ± 3.04 nM).

Keywords Flow injection, iron(II), total iron, chemiluminescence, seawater.

1. Introduction

Iron is an important element in biogeochemical processes. It is an essential micronutrient for living organisms and plays a key role in oceanic plankton productivity. Iron also has a regulatory role in micro-organisms as an inhibitor and promoter of citrate and antibiotic syntheses respectively [1, 2].

Despite the high abundance of iron in the earth's crust (5.6%) its concentration in coastal waters is usually in the nM range (e.g. 0.4 - 28 nM in the North Sea) and is typically very low in open ocean environments (0.05 - 2 nM) [3]. In areas such as the Southern Ocean iron limits phytoplankton growth [4] and this has important implications for the air - sea exchange of carbon dioxide [5]. The speciation of iron in aquatic systems is very important for biogeochemical studies because of the influence of its chemical form on bioavailability, mobility and toxicological properties [6 - 8].

Flow-injection with chemiluminescence detection (FI-CL) has been widely used for the determination of trace metals in environmental, pharmaceutical, clinical, biochemical, food and beverage samples at very low (nM) concentrations [9 – 12]. Major advantages of FI-CL methods include robustness, nb portability and low cost of the instrumentation, rapid analysis, low contamination risk, low (nM) detection limits and excellent sensitivity.

A number of FI-CL methods have been reported for dissolved iron species in seawater. Elrod et al. [13] and Hirata et al. [14] utilised the iron(II)-specific reagent brilliant sulfoflavin (4-amino-*N*-*p*-tolyl)-naphthalimide-3-sulfonate) to determine iron(II). Elrod et al. preconcentrated iron on an 8-HQ microcolumn whilst Hirata et al. used an Amberlite XAD-4 resin functionalised with *N*-hydroxyethylenediamine (HEED). Total dissolved iron was measured by adding a reducing agent (ascorbic acid [13] or hydroxylammonium chloride [14]) to samples prior to analysis.

Luminol, (5-amino-2,3-dihydro-1,4-phthalazinedione) which is catalytically oxidised by iron to an excited state 3-aminophthalate di-anion, has also been widely used. The type of oxidant used with luminol influences which redox species of iron catalyses the reaction. Obata et al. [15, 16] used hydrogen peroxide as an added oxidant to determine iron(III). Samples were acidified to pH 3.0 and iron(III) was selectively preconcentrated on an 8-HQ column before detection. Determination of iron(II) required initial removal of iron(III) from the sample using this column and increasing sample pH to 6 in order to preconcentrate iron(II). Powell et al. [17] and Bowie et al. [18] utilised the oxidation of luminol by dissolved oxygen present in the reagents to selectively determine iron(II). Iron(III) was reduced to iron(II) using sulphite and then preconcentrated on-line using an 8-HQ microcolumn before measurement of total dissolvable iron in unfiltered samples. O'Sullivan et al. [19] also utilised this reaction with a stopped flow technique without preconcentration. Luminol chemiluminescence reactions offer the lowest detection limits for iron in seawater.

In the present study, we describe a FI-CL method for the rapid and selective determination of Fe(II) in acidified seawater by its catalytic effect on the oxidation of luminol in the absence of added oxidant and without a preconcentration column. The manifold used is similar to previously reported methods [20, 21] but uses a seawater carrier stream (rather than 0.7 M NaCl at pH 7.0 [20] or ultra-pure water [21]). Manifold parameters have

been optimised, several buffers have been investigated and the method applied to the determination of Fe(II) in a coastal seawater certified reference material.

2. Experimental

2.1. Materials and Methods

All plasticware used during the experiments and for storage of reagents and standards was cleaned with 50% HCl for 48 h, thoroughly rinsed with ultra high purity (UHP) deionised water (18.2 MΩ cm^{-1}, Elgastat, Maxima, England) and stored in re-sealable plastic bags to prevent contamination. All reagents and standards were of analytical grade (supplied by VWR unless stated otherwise), were prepared in UHP water and further diluted immediately prior to use. Low nutrient seawater (LNS, salinity 35) was obtained from Ocean Scientific International, Wormley, England and used as received for the optimisation and interference studies. For the calibration and accuracy experiments LNS was pre-cleaned to reduce trace metal contamination as described in section 3.3.

An iron(II) stock solution (0.01 M) was prepared by dissolving 0.196 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 50 mL of HCl (0.01 M, prepared every 15 days). The standards were prepared daily in LNS adjusted to pH 3.0 to prevent oxidation of Fe(II). Standard solutions of Mn(II), Cu(II), Ni(II), Zn(II), Pb(II), Co(II) and Cr(III) were prepared from atomic absorption standards (Spectrosol, BDH, England) in LNS (pH 3.0). Iron(III) standard was prepared directly from $NH_4Fe(SO_4)_2.12H_2O$.

Luminol stock solution (0.01 M) was prepared by dissolving 0.089 g of luminol (5-amino-2, 3-dihydro-1, 4-phthal-zinedione, Fluka) in 50 mL of borate buffer followed by sonicating for 30 min. A working luminol solution (1 x 10^{-3} M) was prepared by diluting 10.0 mL of the stock solution in 100 mL of borate buffer (0.1 M) and adjusting to pH 10.4 with sodium hydroxide (2 M). The following buffer solutions were used for luminol solution preparation; NH_3/NH_4, Tris/NaOH, carbonate/NaOH and borate/NaOH (all 0.1 M, pH 10.5).

2.2. Instrumentation and Procedures

The FI-CL manifold used for the determination of Fe(II) is shown in Fig. 1. A 4 channel peristaltic pump (Minipuls 3, Gilson, France) was used to propel the sample carrier and reagent solutions at a flow rate of 2.0 mL min^{-1}. A rotary injection valve (Rheodyne 5020, UK, 270 µL sample loop) was used to inject Fe(II) standards into the LNS (pH 8.0) stream and was merged at a T-piece with the CL reagent stream. The merged streams travelled 3.0 cm before passing through a quartz glass spiral flow cell (1.1 mm i.d., 130 µL internal volume) placed directly in front of an end window photomultiplier tube (PMT, Thorn EMI, 9798QA). Aluminium foil was placed behind the coil to reflect light onto the photo-cathode. The PMT, glass coil and T-piece were enclosed in a light tight housing [18]. The PMT was attached to a 1 kV power supply (Thorn EMI, PM20NS, England) and an integral amplifier was powered from an independent 15 V power supply (BBH Power Products, England). The detector output was recorded using a chart recorder (Kipp & Zonen, Delft, The Netherlands).

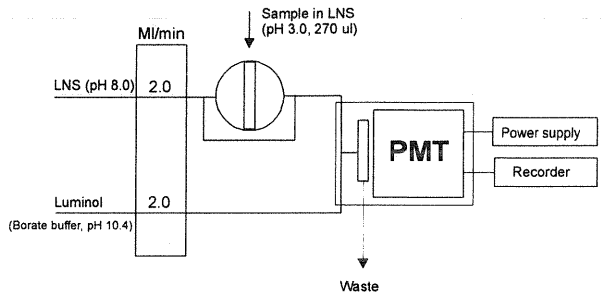


Fig. 1. Flow injection with chemiluminescence detection (FI-CL) manifold for the determination of Fe(II).

3. Results and Discussion

3.1. Optimisation of FI manifold

In order to establish optimum conditions for the determination of Fe(II), various parameters were investigated including buffer concentrations and pH, reagent concentrations, sample volume, reagent flow rates and photomultiplier voltage. All these studies were performed using a 100 nM Fe(II) standard. A univariate strategy was deliberately adopted in order to understand the effect of each variable on the reaction chemistry and the system response.

The efficiency of luminol chemiluminescence is particularly dependent on reaction conditions. In the proposed FI-CL system, different buffers were investigated (NH_3/NH_4, Tris/NaOH, carbonate/NaOH and borate/NaOH; 0.1 M, pH 10.5). The CL responses for 100 nM Fe(II) using these buffers were 5.0, 15.0, 20.0 and 350 mV (n =4) respectively. LNS formed a precipitate with the carbonate buffer and CL signals were irreproducible with an unsteady baseline. The CL responses were very small when using ammonia and Tris-HCl buffers and maximum CL response was obtained with borate buffer. The optimum pH for the luminol reaction with borate buffer was therefore investigated in the range 9.8 – 10.8. Maximum CL emission was observed at pH 10.4, as shown in Fig. 2, and was therefore used for all subsequent studies.

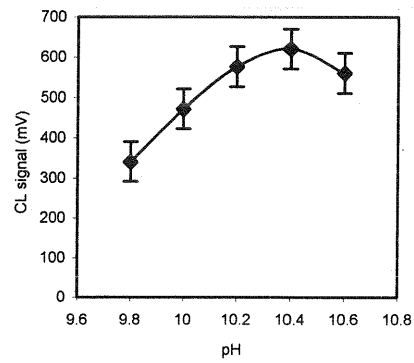
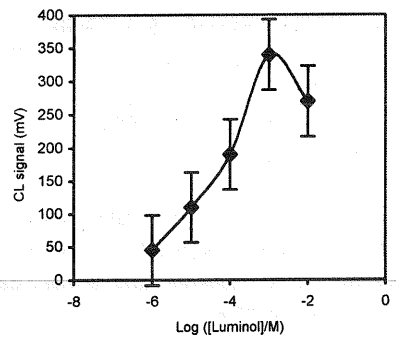


Fig. 2. Effect of borate buffer pH on the peak response of the luminol CL reaction.

The effect of the luminol concentration was then studied over the range 1x10^{-2} – 1x10^{-6} M using the optimised buffer conditions. As shown in Fig. 3, the CL response increased up to

$1x10^{-3}$ M luminol (used in all subsequent experiments), above which the response decreased due to photon quenching. Variation in reagent sensitivity was observed over time as the luminol solution aged, as found by other workers [20], and therefore it was always prepared 24 h before use.

Fig. 3. Effect of luminol concentration on the determination of Fe(II).



The effect of flow rate of the carrier and reagent streams was studied over the range 1.0–3.0 mL min^{-1} in terms of CL response, speed of analysis and reagent consumption (it is important to match the flow rates of the two streams for maximum efficiency). At a flow rate of 2.0 mL min^{-1} (used for all subsequent experiments), maximum CL intensity was observed with a steady base line and reproducible peak heights (Table 1). The effect of the sample volume on CL response was studied in the range of 45 – 450 µL. Maximum CL response was obtained at 270 µL (Table 1) and was selected for all subsequent experiments.

The effect of LNS pH on the determination of Fe(II) was investigated in the range 1.0 – 5.0. There was an increase in CL intensity up to pH 3.0 but a higher pH decreased the CL intensity (Table 1). Therefore, LNS at pH 3.0 was used for the preparation of all Fe(II) standard solutions to prevent oxidation of iron(II).

The effect of PMT voltage over the range 900 – 1300 V was optimised for maximum CL signal-to-noise (Table 1). CL response increased linearly with PMT voltage but 1050 V was used for all subsequent studies because it gave the best signal-to-noise ratio.

Table 1. Effect of various parameters on the CL peak response for the determination of 100 nM Fe(II). For each parameter the optimised conditions for all other parameters were used, i.e. flow rate 2.0 mL/min; sample volume 270 µL; PMT voltage 1050 V; LNS pH 3.0.

Flow Rate (mL/min)	1.0	1.5	2.0	2.5	3.0
CL signal (mV)*	300	450	760	755	750
Sample volume (µL)	90	180	270	360	450
CL signal (mV)*	250	460	510	500	500
PMT voltage (V)	900	1000	1100	1200	1300
CL signal (mV)*	32	90	250	580	1400
LNS pH	1.0	2.0	3.0	4.0	5.0
CL signal (mV)*	390	410	450	430	400

* Mean of four injections

3.2. Interferences

The effect of foreign metal ions on the determination of Fe(II) was studied by preparing standards at elevated concentrations (compared with typical seawater) in LNS (pH 3.0). The results are shown in Table 2. Cr(III), Ni(II), Pb(II) and Zn(II) had negligible effect, Cu(II) and Mn(II) had a small suppressive effect (similar to that reported previously by O'Sullivan et al. [19]) and Fe(III) and Co(II) had a small positive effect, However, the maximum Co(II) concentration in open sea waters typically range between 100 – 300 pM [22]. If necessary, Fe(III) and Co(II) can be masked by adding desferrioxamine B (1.0 µM, in UHP water) [14] and dimethylglyoxime (100 µM in methanol) [18], respectively, to the luminol stream.

Table 2. Effect of foreign ions on the CL peak response for the determination of Fe(II).

Metal ion	Concentration (nM)	CL signal (mV)*	Conc. (nM)	CL signal (mV)*
LNS	----	6.0 ± 1.0	----	6.0 ± 1.0
Fe(II)	10	24 ± 2.2	10	24 ± 2.2
Fe(III)	10	7.5 ± 1.4	100	9.2 ± 1.3
CoII)	100	7.8 ± 1.2	1000	9.5 ± 1.5
Cu(II)	100	4.6 ± 1.5	1000	3.2 ± 1.3
Mn(II)	100	3.8 ± 1.0	1000	2.4 ± 0.8
Cr(III)	100	6.8 ± 1.0	1000	7.0 ± 1.2
Ni(II)	100	6.4 ± 1.1	1000	6.8 ± 1.0
Pb(II)	100	6.6 ± 1.0	1000	6.6 ± 1.1
Zn(II)	100	6.7 ± 1.2	1000	7.0 ± 1.0

* Mean of four injections

3.3. Calibration

To obtain the analytical blank for the calibration graph, LNS (pH 5.0), was first passed through a pre-cleaned 8-hydoxyquinoline column (8-HQ, 2.5 mm x 3.0 cm, washed with HCl (0.5 M) for 24 h, followed by a UHP water rinse) [18] at a flow rate of 0.3 mL min^{-1}. Thereafter, the LNS was adjusted to pH 3.0 with HCl (2.0 M) and used as a blank for the proposed FI-CL system. The blank signal was reduced by approximately 50% compared with the signal from untreated LNS at pH 3.0. This residual blank signal is due to (i) release of complexed iron from the seawater matrix on adjusting the pH of the LNS down to 3.0 and (ii) the pH gradient at the sample/carrier stream interface.

Using the optimum conditions described above, the calibration data of CL response versus Fe(II) concentration over the range 2.0 – 10 nM is shown in Table 3. The correlation coefficient was 0.993 (n=5) and the regression equation was $y = 2.65x - 2.10$ [y = CL response (mV), x = concentration (nM)]. The relative standard deviation of the method was 1.0 – 3.7% (n=4) over the range studied. The limit of detection (3 x standard deviation of the blank) was 0.1 nM Fe(II). The time between injection and detection with the optimised system was 12 s, which makes the method suitable for high resolution in situ monitoring of Fe(II) in marine waters.

Table 3. Calibration data (blank value 3.0 mV; all data are blank subtracted).

Iron Standards (nM)	2.0	4.0	6.0	8.0	10.0
CL intensity (mV)*	4.0	8.0	13.0	19.0	25.0
RSD (%)	3.7	2.7	3.7	1.0	1.3

* Mean of four injections

3.5. Accuracy

The accuracy of the proposed method was ascertained by analysing CASS-3 (Coastal Atlantic Surface Seawater) certified sea water obtained from the National Research Council of Canada (Marine Analytical Chemistry Standards Program). This solution was analysed using the proposed FI-CL system after addition of a 100 μM solution of high purity sodium sulphite for 4 h at room temperature (20 °C) and pH 3.0 to reduce Fe(III) into Fe(II). A value of 18.5 ± 5.2 nM was obtained for Fe(II) which is in good agreement with the certified value of 22.56 ± 3.04 nM. CASS-3 was also analysed without the reduction step and gave a result of 16.8 ± 4.5 nM Fe(II) which shows that the majority of the iron in the CRM is in the reduced Fe(II) form.

4. Conclusions

This manual FI-CL method is simple and rapid, with an analysis time of 12 s and a limit of detection of 0.1 nM for Fe(II) in acidified seawater. The method is based on enhancement of the luminol CL reaction with no added oxidant and does not need a preconcentration / matrix removal column.

The method was validated by quantifying total dissolved iron (Fe(II) +Fe(III)) in a certified reference coastal seawater (CASS-3) after reduction with sulphite. The result, 18.5 ± 5.2 nM, was in good agreement with the certified value 22.56 ± 3.04 nM. The speciation of iron in CASS-3 was over 90 % in the Fe(II) form. This method could therefore be used to examine iron speciation in stored, acidified samples as an aid to understanding the mechanisms of iron release from dissolved, colloidal and particulate material.

This method also has the potential to be used for real time Fe(II) monitoring in the field using natural seawater at ambient pH as the carrier stream. The use of a matrix matched carrier stream (after Fe(II) removal) should minimise analytical artefacts caused by mixing gradients at the sample/carrier stream interface.

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