QUANTIFICATION OF IRON IN SEAWATER AT THE LOW PICOMOLAR RANGE BASED ON THE OPTIMIZATION OF THE BROMATE-AMMONIA-DIHYDROXYNAPHTALENE SYSTEM BY CATALYTIC ADSORPTIVE CATHODIC STRIPPING VOLTAMMETRY

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ABSTRACT

A new voltammetric method for the challenging analysis of total dissolved iron at the low picomolar level in oceanic waters suitable for onboard analysis is presented. The method is based on the adsorptive properties of the iron-2,3-dihydroxynaphthalene (DHN) complexes on the Hanging Mercury Drop Electrode with catalytic enhancement by bromate ions. Although based on a previously proposed reagent combination, we show here that the addition of an acidification/alkalinisation step is essential in order to cancel any organic complexation and that an extra increment of the pH to 8.6-8.8 leads to the definition of a preconcentration free procedure with the lowest detection limit described up to now. For total dissolved iron analysis, samples were acidified to pH 2.0 in the presence of 30 μM DHN and left to equilibrate overnight. A 10 mL sample was subsequently buffered to pH~8.7 in the presence of 20 mM bromate: a 60 seconds deposition at 0V led to a sensitivity of 34 $nAnM^{-1}min^{-1}$, a 4 fold improvement over previous methods, that translated in a limit of detection of 5 pM (2-20 fold improvement). Several tests proved that a non reversible reaction in the time scale of the analysis, triggered by the acidification/alkalinisation step, was behind the signal magnification. The new method was validated onboard via the analysis of reference material and via intercalibration against FIA-chemiluminescence on Southern Ocean surface samples.

INTRODUCTION

Despite being one of the most abundant elements in the Earth´s crust (5%), iron concentrations in seawater are particularly low (picomolar to nanomolar range) due to a combination of minute solubility[1], effective removal caused by biological uptake[2] and particle scavenging[3]. Moreover, coprecipitation with flocculating organic matter at intermediate salinities in coastal water[4] drastically reduces potential inputs from rivers and run-off waters [5]. The accurate measurement of iron concentrations is essential to understand the distribution of biomass in vast areas of the ocean where it is a limiting oligonutrient [6]. The onboard determination of dissolved iron concentrations in open ocean waters is one of the most challenging problems in environmental analysis. Whereas ultraclean sampling gear and protocols that offer confidence in the collection of samples from research vessels have been developed and intercalibrated in the last two decades[7], improvements in the performance and reliability of analytical methods are actively sought [7a, 8]. Currently, iron concentrations in the open ocean are mainly measured by chemiluminescence[9], spectrophotometry[10] and ICP-MS[11] after preconcentration by: coprecipitation with $Mg(OH)_2$, liquid/liquid extraction or strong acid elution following preconcentration in columns packed with different resins. Adsorptive cathodic stripping voltammetry (AdCSV), on the other hand, offers the possibility to reach the lower end of natural iron concentrations, around 0.02 nM[11b], without a preconcentration step. Previous efforts to determine iron concentrations via AdCSV made use of the following commercial ligands: 2,3-dihydroxynaphthalene (DHN)[12], salycilaldoxime (SA)[13], 1-nitroso-2-naphthol (NN)[14] and 2-(2-Thiazolylazo)-p-cresol (TAC)[15] with limits of detection close or below the lowest iron concentrations reported for open ocean waters. However, difficulties associated with the stability of the Hanging Mercury Drop Electrode (HMDE) on a moving lab surface, the challenging cleaning of reagents needed to reach a blank at the pM level, and the inconvenient of spiking reagents to an open cell have undermined the applicability of voltammetry for iron analysis at picomolar levels and its use in ocean waters has been scarce[16], being nowadays abandoned to the best of our knowledge.

Here, we based our method on a previous work on the AdCSV determination of iron using DHN as a ligand in the presence of bromate as a catalytic agent[12]. After significant modification of the protocol i.e.: the need for prior acidification and a new optimization of pH caused by the presence of bromate, we obtained a 4 fold improvement of the sensitivity based on an irreversible transformation of one of the reagents in the measurement time scale that translated in the preconcentration free most sensitive method for iron determination. The limit of detection (LOD) obtained (5pM) was significantly better than those obtained with other preconcentration-free techniques and close to the lowest LOD previously described for methods requiring of preconcentration to work at open ocean concentrations. The method was validated with certified reference material and during a Southern Ocean cruise by intercalibration against the standard flow injection method with detection by chemiluminescence.

MATERIALS AND METHODS

Equipment and reagents for voltammetry. The voltammetric apparatus included a 663 VA stand (Metrohm AG) with a hanging mercury drop electrode (HMDE), a glassy carbon counter electrode, and an Ag–AgCl reference electrode, controlled by a μAutolab voltammeter (Eco Chemie B.V.). Engine vibrations during onboard analysis were attenuated fixing the VA stand to a PVC platform suspended by an elastic rope.

Ultrapure water used for the preparation of solutions and rinsing of electrodes was purified using an Elix/Milli-Q apparatus (Millipore). Hydrochloric acid (Merck), and ammonia (UltraTrace, Sigma) were of the maximum commercially available purity. Iron standards were prepared by dilution (pH= 2.0) of an atomic absorption spectrometry standard solution (BDH, 1 mgL^{-1}). Acidification and neutralization were obtained via addition of pure hydrochloric acid or a 50% ammonia solution. DHN was prepared in acidified ultrapure water (pH~1.8) at a concentration of 10 mM. Catalytic effect and pH control were achieved by addition of a combined solution of piperazine-N,N'-bis-(2-hydroxypropanesulfonic) acid (POPSO, Sigma-Aldrich), potassium bromate (AnalaR, BDH) and ammonia. A 500 μL addition of this buffer/bromate solution to 10 mL sample made BrO_3^- and POPSO concentrations 20 mM and 5 mM respectively. The ammonia concentration was such that pH_{NBS}=8.0. One BrO_3^-/POPSO solution was prepared replacing ammonia with NaOH (Merck) at the same pH (see below). Contaminating iron in all reagents (but DHN) was removed by adsorption on a MnO_2 suspension subsequently retained by gravity filtration (0.2 μm).

UV-digested seawater (UVSW) was prepared using a home-built system with a 150W, high-pressure, mercury vapour lamp. Seawater filling 30-mL quartz tubes was placed around the lamp at a distance of 10 cm for an irradiation time of 2 hours.

Sampling. Samples used for intercalibration were collected from the upper 300 meters of the water column by means of 8 metal free GOFLO bottles attached to a Kevlar line during the EDDY PUMP cruise in waters of the Southern Ocean (Jan-Mar 2012) onboard the research vessel Polarstern. Samples were immediately filtered online by 0.2 μm by means of filtration sterile capsules (Sartobran 300) and collected in LDPE bottles.

Analytical procedure for the determination of the total concentration of iron. For onboard samples two 60 mL LDPE bottles were filled and immediately acidified by addition of 12μl HCl (30%) per 10 ml seawater for a pH of 2.0 (NBS). The bottle destined for CSV-DHN analysis was spiked also with DHN to a final concentration of 30 μM. After seating for a minimum of 24 hours at room temperature, both samples were analyzed by CSV-DHN and FIA-CL.

For AdCSV analysis the following sequence of solutions was mixed in an empty quartz cup in a quick succession: 500 μl of the BrO_3^-/POPSO solution, the volume of a NH_4OH (15%) solution required to raise the pH to ~8.7 and 10 mL of the mix sample+HCl+DHN. The method requires the strict following of this sequence as DHN would be quickly oxidized at

high pH and adding bromate to an acidic solution would instantly produce bromine vapours. The analytical sensitivity was determined for every sample by two standard additions.

The measurements shown in 0.7 M NaCl and ultrapure water as a function of pH were repeated in two independent laboratories to ascertain that differences with respect to prior works were not due to errors introduced by equipment, reagents or the analyst.

AdCSV settings were as follow: 20-90 s deposition at 0 V, quiescence period of 7 seconds, potential scan in the range -0.1 to -1.15 V at 50 mVs^{-1} (step increment of 5 mV and 10 steps s^{-1}).

Reagent blank was determined by analysis of ultrapure water tripling the concentrations of the following individual solutions: BrO_3^-/POPSO mix (typical contamination 50 pM Fe per 500 μL addition), DHN (for 30 μM < LOD) and the combination of the HCl and NH_4OH solutions (typical contamination of ~20 pM for acidification to pH 2.0 and alkalinisation to pH 8.8).

Equipment for FIA-CL. The FIA-CL system used for intercalibration (software and hardware) was cloned from a model repeatedly used for the determination of dissolved iron in open ocean waters[9b, 17] based on the original analytical procedure[9a]. Samples were measured following the same acidification protocol shown before. The accuracy of the method was verified using the following certified reference seawater: SAFe (0.097±0.043 nM certified, 0.084±0.020 nM determined, n=3) and Geotraces (0.52±0.07 nM certified, 0.53±0.01 nM determined, n=3).

pH dependence experiments. The pH was varied by adding either small volumes of 20-fold diluted acid (HCl) or base (NH_4OH) solutions kept air tight in between experiments. Buffering capacities were reported as pH increment per volume added of those solutions. A pH thin electrode (Slimtrode, Hamilton) attached to a pHmeter (mivropH2002, Crison) was inserted in the cell to allow continuous monitoring of pH. The electrode was calibrated using NBS (National Bureau of Standards) solutions. Iron concentrations were determined before the beginning of the experiments by two standard additions. The stability of the measurement and the pH were checked before proceeding to the next acid or base addition.

RESULTS

Background and iron lability in the presence of DHN. The determination of the total iron concentration by AdCSV at circumneutral pH might be strongly affected by the non lability of the fraction that could not be outcompeted by the artificial ligand (AL) added to the sample. This problem cannot be circumvented increasing several orders of magnitude the AL concentration because an AL excess forces a substantial decrease of the sensitivity by saturation of the HMDE surface. Moreover, the slow dissociation kinetics of natural complexes could hinder the ligand exchange reaction leading to unreliable results. The removal of organic complexation prior to analysis is usually achieved by a period of strong acidification, digestion by UV irradiation or both. This is also the case for the determination of many other trace metals[18]. The use of DHN presents a clear advantage with respect to other voltammetric methods based on different AL (NN, TAC and SA): the possibility to increase the DHN concentration about 30 times (from 1 μM to 30 μM) with respect to the concentration used for speciation studies[12, 19]. The rest of AL operate for complexation studies at the upper limit of the AL concentration linear range. This DHN concentration is the equivalent to a log $\alpha_{Fe'-DHN}$ of 4.6 ($\log K'_{Fe-DHN}$= 9.1)[20], a side coefficient 1 to 2 orders of magnitude higher than those reported for the other AL. Possibly, that strong Fe-DHN complexation was behind the reason to keep untested the recovery achieved in the absence of sample acidification in previous uses of the DHN/BrO_3^- pair[12].

Iron recovery in open ocean seawater after 24 hours equilibrium with 30 μM DHN at pH 8.0 without further treatment was measured as a percentage with respect to the iron recovered if the sample was acidified for the same period at pH 2.0. Figure S1A shows that in those experimental conditions only 42 ± 7 % of the total dissolved iron was labilized indicating the requirement for an acidification prior to analysis. This is in agreement with the reported presence of strong binding ligands in all open ocean waters[16]. This test is not definitive in order to validate the method as the pH could not be acidic enough to break all natural complexes. Moreover, the pH neutralization prior to analysis could lead to the restoration of those Fe complexes with natural ligands strong enough to outcompete DHN leading to underestimations of the iron concentration. For that purpose, the same sample was measured with and without UV digestion in order to cancel any organic complexation. Figure S1B shows the iron recovery caused by the acidification to pH 2.0 as a function of the acidification time prior to the analysis at pH 8. The result was a full recovery after 2.5 hours that was unaffected for 24 hours. We decided to keep an acidification period of at least overnight in order to follow recommendations presented in other publications.

The effect of acidification/ neutralization on the sensitivity. In their work, Obata and van den Berg described a maximum of the sensitivity of ~8 $nAnM^{-1}min^{-1}$ at pH 8 with a steady decrease to a constant sensitivity of 40% the maximum at pH<7 and a significant decrease up to pH 8.5 but never presented this dependence in the presence of bromate. We found a similar response in the absence of bromate. However, once seawater was acidified for 24 hours and neutralized immediately before analysis we observed that the sensitivity showed a significant increase (Figure 1). Our Fe-DHN peak in the presence of bromate at pH=8.0 without prior

acidification for 0.26 nM iron is a well defined shoulder that once calibrated gives a sensitivity of 14.0 $nAnM^{-1}$ after 90 s deposition (9.3 $nAnM^{-1}min^{-1}$). Obata and van den Berg reported a well defined peak for a lower concentration (0.089 nM) instead of the shoulder we obtained here despite using a higher concentration. Because the magnitude of the current baseline was not reported, preventing any comparison, we ascribed the difference to a higher labile vanadium concentration (released by the acidification step) interfering with the iron peak. V-DHN complexes were the cause of the high peaks found at -1.0 V[12, 21]. When seawater was acidified to pH=2.0 and the pH restored to 8.0 immediately before analysis (black line in Figure 1, [Fe]=0.19 nM) we obtained a similar shoulder. However, in this case the sensitivity had grown significantly to 17.7 $nAnM^{-1}min^{-1}$. When the pH was further increased to a value in the range 8.5-8.8 we observed a considerable improvement of the performance of the method. The red line in Figure 1 is the result of the analysis at pH=8.7 after 24 hours acidification of a sample 0.14 nM in iron. The resulting scan gave a well defined peak and calibration resulted in an improvement of the sensitivity by a factor of ~2 and ~4 with respect to previous conditions (to 33.7 $nAnM^{-1}min^{-1}$).

Effects of varying the pH. Reproducibility in the pH range 8-9. Figure 1 shows the scans obtained from the analysis with internal calibration of the same ocean water at two different pH where there is an obvious increase of the sensitivity and a moderate broadening of the Fe-DHN peak. In order to discard a negative effect of pH we analyzed the same sample in the pH range 8-9 after an acidification/neutralization step. Figure S2 shows 3 raw scans (90 s deposition time) from the same sample analyzed at pH=8.1, 8.5 and 8.7 (Fe-DHN peak magnified in insert plot). The pH shift moved the Fe-DHN peak towards more negative potentials and substantially reduced the V-DHN peak: as a consequence, the peak changed from a poorly resolved shoulder to a well defined peak. The magnitude of the peak increased from 11.8 nA (pH=8.1) to 17.4 nA (pH=8.7) but the sensitivity (determined after two 0.3 nM additions) increased accordingly from 30.4 to 54.7 $nAnM^{-1}$. Iron concentrations determined in 5 different aliquots were: 0.39±0.02 (pH=8.1), 0.36±0.01 and 0.31±0.01 (pH=8.4), 0.35±0.01 (pH=8.5), and 0.32±0.02 (pH=8.7). Therefore, the performance and accuracy of the method were not a function of the pH in the range 8-9.

At pH=>9 we found in some samples serious difficulties to define the end of the Fe-DHN peak in its intersection with the residual V-DHN signal that advice against its use for analysis (Figure S3).

Effect of varying the pH. Sensitivity dependence as a function of the pH. The effect of pH on the sensitivity was thoroughly investigated in the range 7-9 to find the optimum pH for the determination of Fe-DHN complexes. pH played a major role in defining the sensitivity of the method, as already mentioned. Figure 2A shows the dependence of the sensitivity at increasing pH for ultrapure water, 0.72M NaCl (the ionic strength of seawater) and UV digested seawater at the same concentration of DHN and BrO_3^-. The Fe-DHN signal increased steadily as a function of the pH in the whole range of study with the exception of the response in 0.72M NaCl (Figure 2A) that followed the behaviour of seawater up a maximum at pH 7.8 with a nearly constant value at higher pH until equalling the sensitivities found for ultrapure water at pH>8.4. The sensitivity increased by a factor of 2 in NaCl, 5 in

seawater and 12 in ultrapure water. It is interesting noting that this effect was completely different to that observed in the Obata and van den Berg paper [12] where they found a maximum response at pH=8.0 (~8 $nAnM^{-1}min^{-1}$) as they only checked the effect of pH in the absence of bromate. In this study, the sensitivity in seawater grew up to 30 $nAnM^{-1}min^{-1}$ (an improvement by a factor of 4 with respect to previous settings) whereas for ultrapure water and 0.7 M NaCl the maximum was around 10 $nAnM^{-1}min^{-1}$. Because the addition of the HCl/NH_4OH pair improved substantially the sensitivity (Figure 1) and further NH_4OH additions increased additionally the sensitivity, as a first hypothesis we pointed to NH_4OH as the direct cause of the signal enhancement. Nevertheless, when the experiment was repeated with Ultrapure water and seawater after an acidification/alkalinisation cycle by consecutive additions of HCl and NH_4OH prior to the analysis (~3x the original NH_4OH concentration provided by the POPSO/BrO_3^-/NH_4OH reagent) the relation sensitivity vs pH barely changed, reaching for seawater again a maximum of ~30 $nAnM^{-1}min^{-1}$ at pH 8.9 (data not shown). This ruled out, any significant effect caused by ammonia.

This direct proportionality in between sensitivity and pH was tested reversing the experiment via acidification aiming at understanding the mechanism causing this sensitivity increase with unexpected results. We repeated for ultrapure water and seawater the experiment by acidification via HCl additions (after a prior ammonia spike to shift the pH close to 9). The sensitivities obtained (Figure 3B) followed a completely different pattern from the one reported in Fig 2A. For seawater, the sensitivity increased slightly from 25 to again 30 $nAnM^{-1}min^{-1}$ at pH 8.4 remaining constant down to pH 7.7 where it started to grow exponentially. However, at pH < 7.8 the V-DHN peak is so huge that the Fe-DHN peak becomes a poorly defined shoulder of no analytical value. For ultrapure water, the sensitivity plot took a domed shape with a maximum value in the pH range 7.7-8.4 of again ~10 $nAnM^{-1}min^{-1}$. In this case, two final NH_4OH additions showed that now the system became reversible to pH changes and at pH 8.0 and 8.4 the sensitivity came back to that obtained during the acidification (see arrows in Fig 3B). It is clear from Figures 2A and 2B that a non-reversible transformation of the DHN/BrO_3 system takes place at high pH in a time scale of minutes and lasts at least for a time scale of many hours. To study the specific effect of NH_4OH we repeated the experiment in seawater replacing it by NaOH in all solutions. Figure 2B shows that in the pH range of analytical interest (8.0-8.9) the absence of NH_4OH did not lead to any significant difference. However, the exponential rise of sensitivity found at pH<8.0 seemed to be related to the presence of NH_4OH in solution.

Effect of varying the pH. Buffering capacity in the analytical range. POPSO is characterized by a buffering interval of 7.2-8.5 (pK_a = 7.80). The pH range where we found optimum analytical conditions (8.5-8.8) was at the edge and beyond that interval. Borate, a better suited buffer (pKa = 9.2) commonly used in AdCSV was discarded as borate additions suppressed the Fe-DHN/BrO_3^- peak.

Figure S4 shows the buffering capacity as a function of pH for ultrapure water and seawater after alkalinisation in the presence of 5 mM POPSO buffer (initial [NH_4OH]~6mM from the BrO_3^-/POPSO solution). Buffer capacities (as μL NH_4OH per pH increment) did not decrease as the pH exceeded 8.5, but there was a steep increase up to the end of the pH range tested

(7.2-9) that became steeper when the experiment was repeated at a higher [NH_4OH]. This is proof of the formation of NH_4OH/NH_4Cl buffer (pK_a= 9.25) that complements POPSO at the upper end of the experimental pH range.

Effect of varying the pH. Scan rate. Changes in the nature of the reaction with pH could be inferred from the dependence of the Fe-DHN peak height as a function of the scan rate. For that purpose, the effect of the scan rate in UV digested seawater in the presence of DHN and BrO_3 was studied before and after shifting its pH from 8.0 to 8.8. Figure 3 shows how at pH 8.0 the sensitivity as a function of the scan rate followed the expected increase in a less than linear fashion observed before[12]. This is caused by the limitation of the catalytic reagent to diffuse to the surface of the HMDE on the diminishing scale time of the stripping step as scan rates become faster[22]. However, at pH 8.8 the trend is opposite with a decrease up to a rate of 40 mVs^{-1} where reaches a constant Fe-DHN signal. This is characteristic of surface catalytic systems where the relative weight of the catalytic reaction is strongly accentuated with respect to the redox reaction controlling the overall kinetics[23].

We selected a scan rate of 50 mVs^{-1}. Figure 3 shows that slower scan rates could improve slightly the sensitivity; however, the stripping period would be increased to the order of minutes damaging the reproducibility during onboard analysis.

Reaction mechanism. The irreversibility of the system with pH changes and the different dependence with the scan rate shows that the CSV reaction of the Fe/DHN/BrO_3^- system on the HMDE is incompatible with the reaction mechanism described before[12]. In that work, the CSV current was described as the electrochemical reduction of the iron forming part of adsorbed Fe(III)-DHN complexes with a catalytic effect purely caused by bromate forcing the immediate reduction of the Fe(II) freshly created on the surface of the HMDE. For such a simple reaction mechanism, pH changes should be fully reversible. The reaction mechanism is identical to that described for the CSV determination of Fe-humic substances (HS) complexes in the presence of BrO_3^-[20]. For 1 mgL^{-1} Suwannee River Fulvic Acid, the mechanism was corroborated by the perfect reversibility of the sensitivity with acidification followed by alkalinisation in the pH range 7.5-9 (Figure S5). This is proof that the reaction mechanims of the Fe/DHN/BrO_3^- system is more complex.

In order to give an approach to the processes involved, we investigated the relative weight of the kinetics of the two main reactions involved, redox surface and catalysis, making use of square wave voltammetry[23-24]. Peaks in the absence of bromate at increasing frequencies (Figure S6A) clearly showed that the kinetics of the redox surface reaction were slower at pH 9 than pH 8 in agreement with[12] (where at pH >8 a decrease in sensitivity was observed in the absence of bromate). With respect to the kinetics of the catalytic mechanism, bromate increments at pH 8 and 9 (100 Hz) showed that (Figure S6B) at pH 9 the slope of the signal vs. [BrO_3^-] is higher; a clear indication that the catalytic mechanism is more efficient at higher pH. Figure 3 could therefore be explained as a combination of both trends: at a higher pH the diminishing redox component of the current becomes a small fraction of the catalytic constituent.

However this could not explain the non reversibility to alkalinisation. Several possible mechanisms leading to an irreversible transformation of the chemical species involved were investigated. Ammonia could be oxidised to hydroxylamine and/or brominated amines (NH_2Br and $NHBr_2$) by the action of bromate ions which are strong oxidising (E^{θ} = +1.5V) and possible brominating agents. Hydroxylamine was recently shown to be a good catalytic reagent[25]. However, the formation of these oxidation products can be discarded as none of them could be detected in UV digested seawater by UV-vis spectrophotometry at pH 8 and 9 (see[26] for the UV-vis spectra of these species). The possible transformation of DHN is not the same caused by the reported slow oxidation of DHN to a pink by-product at natural pH[19]. The variation suffered by the visible spectrum of 50 μM DHN after two days of slow oxidation at room temperature is not reproduced by a rise of pH to 8.8 (Figure S7).

Understanding the intimate chemical mechanism involved revealed itself as a difficult task, we could not find the process that would explain the irreversible behaviour with respect to pH changes and the differences found in between ultrapure water, NaCl and seawater that cannot be ascribed to the presence of ammonia (Figure 2). Further tests requiring non electrochemical techniques were beyond the scope of this paper.

Vanadium interference and peak height vs peak area. During the analysis of reference material we observed a persistent trend to obtain slightly higher concentrations than the certified ones. Careful inspection of the CSV scans obtained before and after iron spikes showed that as the Fe-DHN peak grew and broadened, the increasing overlapping caused by the V-DHN peak lifted the right end of the Fe peak and introduced a bias in the calculation of its height (detailed in Figure S8) in the form of an underestimation of the sensitivity. At pH > 8.6 and despite its declining, the V-DHN signal still constitutes a serious interference. This effect could be minimized by the use of the peak area. Table S1 gives examples of the extent of the enhancement of the accuracy obtained for the analysis of different samples and reference materials. The use of peak area always led to lower estimations for all CRMs, values that were closer to the certified value.

In order to prove that the effect was caused by the V-DHN peak we studied the recovery via analysis of fortified ocean and ultrapure (V free) waters (Table S1). Fe concentrations before fortification were determined as 0.12±0.01 (ultrapure water) and 0.23±0.02 (ocean sample) respectively averaging the results obtained using peak height and peak area. Both samples were subsequently fortified to 2.12 and 4.23 nM respectively bringing the uncertainty on the iron concentration caused by selection of the peak to less than 1%. After a new internal calibration, the iron recovery in ultrapure water was very close to 100% independently of the use of peak height or area. For seawater, again the peak area gave a lower and significantly better estimate of the Fe concentration.

Limit of detection, limit of quantification and precision. The LOD (as 3x the standard deviation of repeated analyses) for the determination of iron in seawater using DHN/BrO_3^- at pH=8.0 without previous acidification/neutralization was determined at 13 pM elsewhere [12]. This LOD, considering the reported sensitivity of 7.9 nA nM^{-1} (using 60 seconds deposition) results in a LOD equivalent to a ~0.1 nA peak. Despite being determined by established

methods, this limit is clearly unrealistic. A 0.1 nA peak approximately equals the common level of noise in an unsmoothed scan working in optimum conditions and is much lower than the common baseline of 2-4 nA. Visual inspection of plot 6 in[12] clearly shows that a 0.1 nA peak would be hard to resolve.

In our case, after acidification and alkalinisation to a pH in the range 8.5-8.8, sensitivities were in the range 25-35 nA $nM^{-1}min^{-1}$ which is a major improvement (~4 fold) at no cost of baseline or noise enhancement. Repeated analysis of the same sample gave a LOD in seawater (n=5; [Fe]=0.098 nM; pH=8.8) of 0.005 nM Fe, i.e.: a peak of 0.45 nA height/0.073 nA^2 area that would translate in a limit of quantification (as 10x standard deviation) of 0.018 nM (deposition time of 90 s). LOD and LOQ could be easily improved increasing the bromate concentration.

The precision of the method, calculated from the average of the standard deviations of duplicates of samples analyzed during a Southern Ocean cruise across the concentration range 0.06-2.45 nM Fe (n=148) was of 13%.

Analysis of Certified Reference Material and samples with consensus values. The performance of the analytical method was assessed by analysis of Nearshore Certified Reference Material (CASS-5, National Research Council, Canada) and of three of the seawater Reference Standards produced in the framework of the SAFe (Sampling and Analysis of Fe)[7a] and GEOTRACES programs (updated consensus values in: http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html). For convenience, the nearshore seawater was diluted 5 times with ultrapure water (pH 2.0). Reference values and the result of our analysis at pH 8.7 are shown in Table 1. In all cases the values obtained were in excellent agreement with the target concentrations.

Comparison of the CSV-DHN method with FIA-CL analysis. In order to further validate the method we also carried out an intercalibration against the most used method for onboard analysis, chemiluminescence after FIA. During a Southern Ocean cruise, the upper 300m of the water column was sampled at the same location in the time span of three weeks. The oceanographic, meteorological and biological conditions did not suffer dramatic changes and significant variability of the dissolved iron profiles was not expected. Water column profiles obtained by both methods are shown in Figure 4. Despite a few minor discrepancies, there is an elevated agreement in between methods. All common features could be observed in both sets of results: nearly constant concentrations in the mixing layer (range 0.07-0.15 nM, down to 100-120 m) with slightly lower values in the range 60-100 m and a significant constant increase at depths >100m .

Comparison to other analytical methods. Table 2 presents a compilation of the performance of the different techniques available for the determination of iron at picomolar level in seawater. Our LOD of 5 pM is actually only bested by the double $Mg(OH)_2$ co-precipitation method[11b] where they reached a LOD of 2 pM. Among methods not requiring preconcentration of the sample (and/or matrix exchange), all of them voltammetric, our method gives a 2 to 20 fold improvement of the LOD. According to data in Table 2, the Fe-

SA method offers a close performance, however those figures of merit were obtained using a mercury drop of 5.6 times the drop surface used in this work, i.e: the same correction factor should be obtained for the sensitivity on those conditions (at a similar cost on the baseline current). Moreover, our method is the only one currently under use for the measurement of total dissolved iron concentrations in ocean waters.

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The authors declare no competing financial interest

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Table 1. Results of the cathodic stripping voltammetry (ACSV) analysis with DHN/BrO_3^- at pH=8.7 of Certified Reference Material. All concentrations in nM.
*after 5 fold dilution in acidified ultrapure water

CRM	$[Fe]_{declared}$	$[Fe]_{DHN/BrO3}$	n
SAFe-S	0.097 ± 0.043	0.12 ± 0.04	5
SAFe-D2	0.91 ± 0.17	1.00 ± 0.02	3
GEOTRACES-S	0.52 ± 0.07	0.47 ± 0.07	2
CASS-5*	25.8 ± 2.0	27.2 ± 0.8	3

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Table 2. A comparison of detection limit of available methods for iron analysis in seawater

Analytical method	LOD	Cite
Preconcentration-free methods (AdCSV)		
CSV-TAC	100 pM	[15]
CSV-SA	10 pM	[13]
CSV-DHN/BrO_3 (pH=8)	13 pM	[12]
CSV-NN	90 pM	[27]
CSV-DHN/BrO3 (pH=8.7)	5 pM	This study
Methods requiring a preconcentration step		
ICPMS after $Mg(OH)_2$ co-precipitation	2 pM	[11b]
GFAAS after APDC/DDDC solvent extraction	30 pM	[28]
ICPMS after concentration on NTA	6-28 pM	[29]
Chemiluminiscence luminol/H_2O_2 after concentration in oxine	50 pM	[9a]
Catalytic spectrophotometry after concentration in oxine	25 pM	[30]

Figure 1. Raw voltammetric scans obtained in different seawater samples under the following conditions: all samples 30 µM DHN, 20 mM BrO_3^-, 90 seconds deposition at 0V. In all cases calibration by two additions of 0.3 nM Fe. Blue line: equilibrated and analyzed at pH=8.1 (0.26 nM Fe); black line: equilibrated pH=2.0 and analyzed at pH=8.0 (0.19 nM Fe); red line: equilibrated for 24 hours at pH=2.0 and analyzed at pH=8.8 (0.16 nM Fe). Blue scans were brought down 15 nA for the sake of clarity.

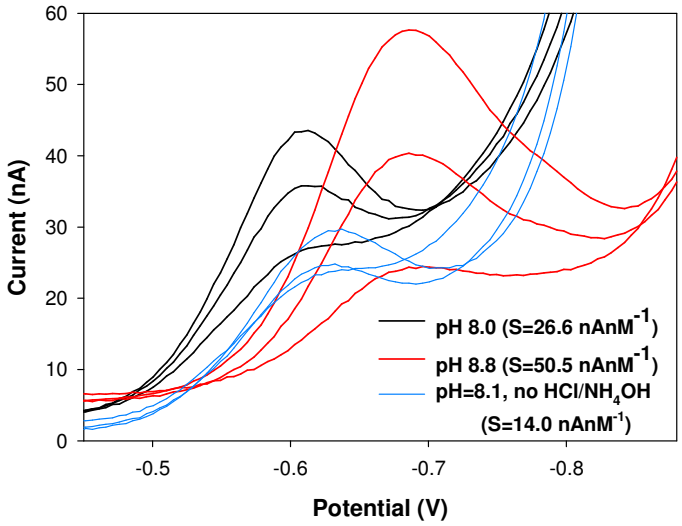

Figure 2. A: effect of pH on the sensitivity of the AdCSV of Fe-DHN complex in the presence of BrO_3^- in three different solutions: ultrapure water, NaCl (0.72) and Southern Ocean UV digested seawater. pH moved initially to 7.2-7.4 by a HCl addition and increased by successive NH_4OH additions. B: effect of pH on the sensitivity of the AdCSV of Fe-DHN complexes in ultrapure water and seawater. pH changed by HCl additions afer an initial NH_4OH addition to bring the pH close to 9. Red line: experiment in seawater repeated in the absence of NH_4OH, substituted by NaOH. Arrows show the result to spike some NH_4OH at the end of the experiment. All solutions 20 mM bromate, 5 mM POPSO and 30 μM DHN.

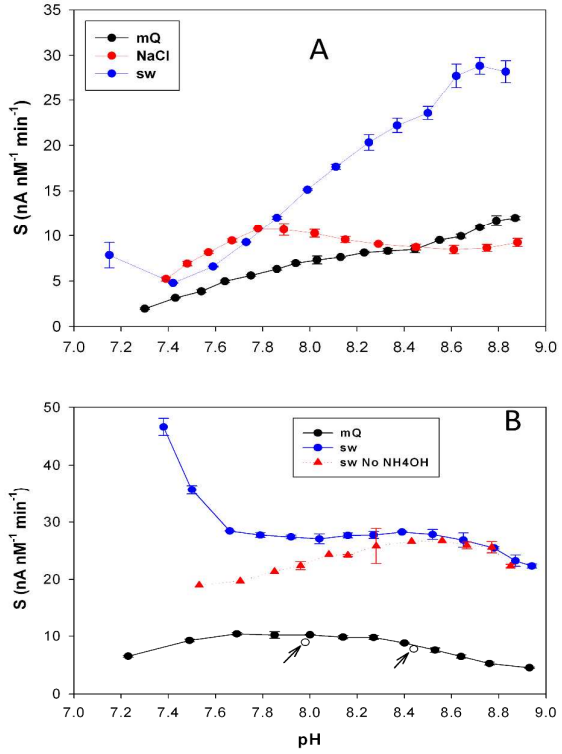

Figure 3. Effect of the scan rate on the peak height of Fe-DHN complexes in seawater (30 μM DHN, 20 mM BrO_3^- and 5 mM POPSO buffer) at pH=8.0 (full circles) and at pH=8.8 (open circles).

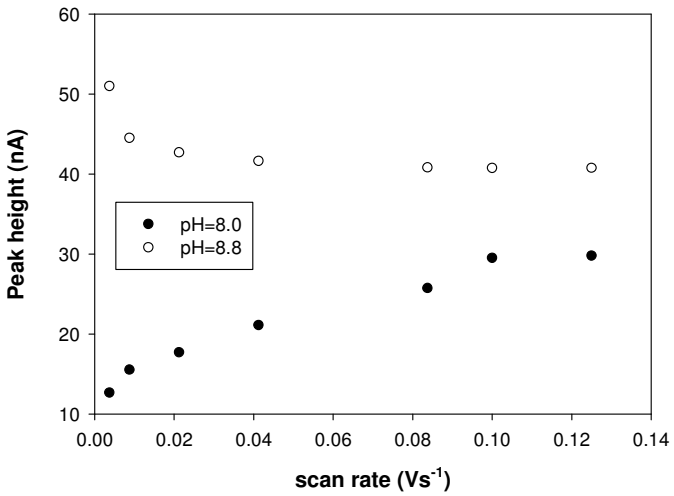

Figure 4. Determination of the concentration of Fe-DHN and FIA-CL in filtered seawater samples collected during the EDDY PUMP cruise in waters of the Southern Ocean.