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Determination of Picomolar Levels of Iron in Seawater Using Catalytic Cathodic Stripping Voltammetry

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A new procedure for the direct determination of picomolar levels of iron in seawater is presented. Cathodic stripping voltammetry (CSV) is preceded by adsorptive accumulation of the iron(III)−2,3-dihydroxynaphthalene (DHN) complex from seawater, containing 20 μM DHN at pH 8.0, onto a static mercury drop electrode, followed by reduction of the adsorbed species. The reduction current is catalytically enhanced by the presence of 20 mM bromate. Optimized conditions include a 60-s adsorption period at −0.1 V and a voltammetric scan using sampled dc modulation at 10 Hz. In these conditions, a detection limit of 13 pM iron in seawater was achieved which can be lowered further by extending the adsorption time to 300 s. The new catalytic CSV method is ∼5 times more sensitive than existing CSV methods and was tested on samples from the Atlantic Ocean.

Intensive studies to reveal the biological role of iron in the ocean have been carried out since it was recognized that iron can be a limiting nutrient for phytoplankton growth in high-nutrient, low-chlorophyll (HNLC) regions such as the equatorial Pacific, the Subarctic Ocean, and the Southern Ocean.[1−4] Recently the iron hypothesis has been tested by mesoscale iron enrichment experiments in the equatorial Pacific Ocean[5,6] and the Southern Ocean.[7] The iron distribution in the world oceans has also been investigated by many researchers. Mapping of the iron distribution in the world ocean is crucial to clarify the oceanic geochemical iron cycle. However, the concentration range of iron in open ocean is low, especially in the euphotic zone (less than 0.1 nM), which has made it difficult to obtain accurate iron data, causing much uncertainty and discussion regarding the validity of these data.[8] For these reasons, it is important to develop a sensitive and relatively simple analytical method to determine subnanomolar iron in seawater that can also be used onboard ship.

Several methods have been used to determine iron in seawater; flow analysis with direct chemiluminescence detection,[9] chemiluminescence detection combined with chelating resin preconcentration,[10−15] and colorimetric detection with column preconcentration[16,17] are successful methodologies to determine subnanomolar iron in seawater, but these methods required relatively complicated systems.

Low levels of iron in seawater can also be determined electrochemically by cathodic stripping voltammetry (CSV) preceded by adsorptive collection of electroactive complexes of iron. This technique has been used with catechol,[18] solochrome violet RS,[19] 1-nitroso-2-naphthol (1N2N),[20−23] salicylaldoxime (SA),[24] *N*-benzoyl-*N*-phenylhydroxylamine (BPA),[25] and 2-(2-thiazolylazo)-

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4-methylphenol (TAC).[26,27] The advantage of the voltammetric method is that it can be used to determine the chemical speciation of iron as well as its concentration. Although low levels of iron in seawater can be detected with the CSV method, it is difficult using most existing procedures to determine subnanomolar iron in uncontaminated seawater without a long deposition time (>5 min). Additionally (as will be shown here) the presence of interfering background peaks can cause a bias in the determined iron concentration at subnanomolar iron levels.

The voltammetric sensitivity can be greatly improved by catalysis. Usually an electroactive complex of iron(III) is adsorbed onto the mercury drop and is reduced to iron(II) during the potential scan, and the reduction current is measured. The reduction current can be enhanced catalytically if the reduction product is chemically reoxidized in the presence of an oxidant, causing very high sensitivity.[21,23,25]

Preliminary experiments to determine low iron levels in seawater using the existing CSV methods revealed the presence of interfering peaks that overlapped with the iron peak; the peaks could not be resolved and were found to produce serious systematic errors (overestimates) at subnanomolar iron concentration. Several ligands were evaluated during preliminary experiments to find a ligand without a background peak and with high sensitivity for iron. Best results were obtained using 2,3-dihydroxynaphthalene (DHN) taking advantage of the catalytic effect of the Fe(II)/Fe(III) redox couple on the reduction of bromate to enhance the sensitivity.

EXPERIMENTAL SECTION

Apparatus. The voltammetric experiments were carried out using a μAutolab voltammeter (Ecochemie) connected to a hanging mercury drop electrode (HMDE, Metrohm model 663VA). The reference electrode was double junction, Ag/AgCl, saturated with a salt bridge filled with 3 M KCl, and the counter electrode was a glassy carbon rod. Solutions in the voltammetric cell were stirred by a rotating Teflon rod. For low-level iron determinations, a Teflon voltammetric cell was used. The mercury was triple-distilled quality, and the largest drop size of the HMDE was selected. The potentiostat was computer-controlled using a compiled Basic program (GPES 3.2 from Ecochemie).

Seawater samples and reagent solutions were stored in high-density polyethylene (HDPE) containers (Nalgene). The containers were cleaned sequentially by soaking 24 h in 5% sodium dodecyl sulfate (SDS, BDH) solution and 2 M HNO_3 (BDH), followed by soaking (12 h) with hot 1 M distilled hydrochloric acid and hot Milli-Q water. The voltammetric cells were cleaned by soaking (12 h) with hot 1 M distilled HCl for 12 h and hot Milli-Q water.

The electrode stand was situated under a laminar-flow clean hood with filtered air where sample manipulations and reagent preparation were also carried out.

Reagents. Hydrochloric acid, nitric acid, and methanol were redistilled using a coldfinger silica distillation apparatus by mild heating with a lamp. High-purity aqueous ammonia (J. T. Baker) was used without further purification. Water used for dilution of the reagents and for rinsing containers and voltammetric cells was purified by reverse osmosis (Milli-RO) and deionization (Milli-Q).

DHN (Fluka) was recrystallized from methanol (~10 g in 20 mL) and 0.1 M hydrochloric acid (200 mL added to 20 mL of the methanolic solution). The precipitate was filtered using acid-washed filter paper (Whatman), and the residue was dried in a vacuum desiccator.

Bromate was prepared by adding 50 g of potassium bromate (BDH) to 200 mL of hot MQ water; the supernatant was filtered using an acid-washed membrane filter (3-μm Isopore, Millipore). The filtered solution was acidified with 0.1 M nitric acid and placed at 4 °C for several days. The precipitate was filtered through acid-washed paper filter (Whatman), and the residue was dried in the vacuum desiccator. Brown vapor after acidification indicated that some of the bromate was reduced to bromine in the acid, but more than 50% was recovered as apparent from its catalytic effect on the iron determination.

A 20 mM stock solution of DHN was prepared in redistilled methanol in a 20-mL HDPE bottle. The stock solution was kept at 4 °C when not in use. A 0.4 M potassium bromate solution was made up in Milli-Q water. A 1 M HEPPS (*N*-(2-hydroxylethyl)-piperazine-*N*′-3-propanesulfonic acid, Fluka)/0.5 M NaOH buffer (pH 8.0) was made up in Milli-Q water and purified by equilibration with 50 μM MnO_2[28] and filtration using an acid-washed membrane filter (Whatman, cellulose nitrate, 0.2-μm pore size).

A stock solution of 0.1 mM iron(III) was prepared from a BDH standard solution for atomic adsorption spectrometry in 0.1 M distilled HCl. Iron standard solutions were prepared from the stock solution by dilution with 0.05 M redistilled HCl. Seawater (0.2 μm filtered) from the North Atlantic was used for the development work.

Procedure To Determine Iron in Seawater. An aliquot of 10 mL of seawater was pipetted into the voltammetric cell. A 100-μL aliquot of HEPPS pH buffer (final concentration 0.01 M, pH 8), 10 μL of the DHN solution (final concentration 20 μM), and 0.5 mL BrO_3^- (final concentration 20 mM) were added. The solution was deaerated by purging for 5 min with water-saturated nitrogen gas prior to analysis. A new mercury drop was extruded, and the deposition potential was set to −0.1 V for a period of 60 s, while the solution was stirred at 2500 rpm. Then the stirrer was switched off, and a quiescence time of 10 s was allowed. The potential scan was carried out from −0.1 to −0.8 V using sample dc mode with a scan rate of 24 $mV \cdot s^{-1}$ and 2.4-mV potential step at 10 Hz. The measurement was repeated with two standard additions of iron(III) of appropriate concentration to calibrate the sensitivity.

RESULTS AND DISCUSSION

Several ligands were tested to evaluate their use for the detection of iron. They were selected because they had been used before in voltammetric methods or because of chemical similarity. Several of these ligands produced a peak with iron; the experimental conditions to detect iron were optimized, and it was checked whether catalytic effects could be used to improve the

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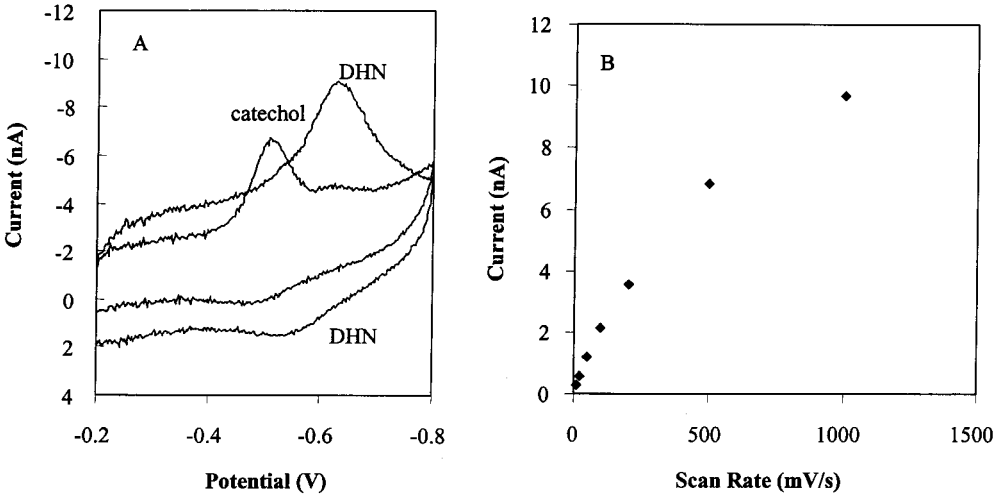

Figure 1. (A) Cyclic voltammograms of 50 nM Fe in seawater in the presence of HEPPS pH buffer and 20 μM DHN or 200 μM catechol at pH 8.0 (without bromate); the scan rate was 100 mV·s^{-1}. (B) Effect of the scan rate on the peak height of the iron−DHN complex in the same condition.

Table 1. Compounds Tested and Giving a Peak for Iron Using CSV[a]

compound	pH	sensitivity (nA·nM^{-1}·min^{-1})
catechol	7	0.14
N-benzoyl-*N*-phenylhydroxylamine (BPA) and hydrogen peroxide	8	0.14
4-(2-pyridylazo)resorcinol (PAR)	7	0.67
1-(2-pyridylazo)-2-naphthol (PAN)	5	0.21
salicylaldoxime (SA)	8	0.051
Solochrome violet RS (SVRS)	5	0.029
1-amino-2-naphthol (AN)	8	0.065
pyrogallol	7	0.47
2-nitroso-1-naphthol (2N1N)	7	0.047
cupferron	7	0.13
mandelic acid	8	0.50
1N2N and bromate	8	1.6
TAC	8	2.2
DHN and bromate	8	7.9

[a] Oxidant concentrations where used are indicated, as well as the pH and the sensitivity for iron; peak height standardized for 1 nM iron after 1-min adsorption.

sensitivity for iron. The sensitivity for iron using these ligands can be compared in Table 1. The best results in terms of sensitivity and lack of bias were obtained using DHN. The experiments optimizing this method to determine iron follow below.

Characteristic of the Iron(III)−Dihydroxynaphthalene Complex. Cyclic voltammetry (CV) was used to investigate the electrode reaction in seawater containing 20 nM iron(III), 20 μM DHN, and 0.01 M HEPPS buffer (pH 8.0). The scan was preceded by 60-s adsorption at −0.1 V. The effect of DHN on the iron reduction peak can be compared to that of catechol in Figure 1A. The scan for the iron−DHN showed a reduction peak at ∼−0.60 V, with a smaller reoxidation peak; the reduction peak was at a much more negative potential than the oxidation peak, indicating electrochemical irreversibility of the electrode reaction. The low height of the reoxidation peak may be caused by slow kinetics of formation of the complex of iron(III) with DHN, by desorption of iron(II) from the HMDE, or by a combination of these. The reduction peak current was found to increase with the adsorption time, indicating that the iron(III)−DHN complex is adsorbed on the electrode surface.

Variation of the scan rate showed that the reduction current of iron−DHN increased linearly with the scan rate between 10 and 100 mV; however, at higher scan rates, between 100 and 1000 mV·s^{-1}, the peak height increase diminished (Figure 1B). A linear increase is to be expected for the reduction of an adsorbed species as the contribution due to diffusion current is then negligible; the less-than-linear increase at scan rates of >100 mV·s^{-1} indicates that the reduction process became electrochemically irreversible at those rates. The irreversibility caused the peak potential to shift in a negative direction with the increasing scan rate (Figure 1B), indicative of the increasing nonconformance with the theory for reversible reactions.[29] A scan rate of 24 mV·s^{-1} (10 Hz, sampled dc, 2.4-mV potential steps) was selected for further preliminary experiments as a balance between sensitivity and resolution.

Catechol is similar to DHN in that it also contains two hydroxy groups, but it differs in having only one benzene ring; a peak is also obtained for the iron−catechol complex[18] (Figure 1A), which has a more positive reduction peak than with DHN, suggesting greater stability of the iron−DHN complex although the shift could also be caused by greater adsorption stabilization (however, such a large shift would be unlikely). Catechol is easily oxidized in solution and tends to cause reduction of iron(III) to iron(II), whereas methanolic DHN solutions were stable for at least several weeks and the aqueous voltammetric cell solutions showed no sign of discoloration due to DHN oxidation.

Effect of Varying the DHN Concentration and the pH. To optimize the analytical conditions of CSV using DHN, the concentration of DHN and the solution pH were varied. The peak height for iron was found to increase with the DHN concentration until ∼5 μM (Figure 2A), the increase decreasing at higher levels; the peak height reached a plateau at DHN concentrations between 5 and 40 μM. An optimal DHN concentration of 20 μM was selected for further experiments.

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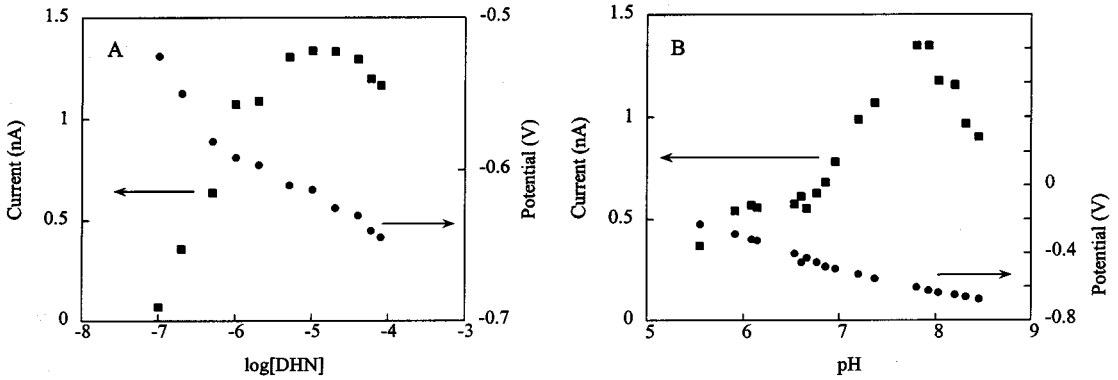

Figure 2. Effect of varying the DHN concentration (A) and pH (B) on the CSV sensitivity for 10 nM iron in seawater without bromate. Iron−DHN complexes were adsorbed at −0.1 V for 60 s in the presence of 20 μM DHN and 0.01 M HEPPS (at pH 8.0), unless indicated otherwise.

The potential of the iron(III)−DHN reduction peak shifted ~50 mV in a negative direction when the DHN concentration was increased from 0.5 to 20 μM, reflecting the increasing stabilization of the iron(III)−DHN complex with the higher ligand concentrations.

An increase of the pH from 6.8 caused the CSV peak height to increase until pH 7.9, above which it decreased again (Figure 2B) presumably due to competition for iron by hydrolysis reactions. The peak potential for iron−DHN was found to shift toward more negative potentials with the increasing pH, with a slope of 160 mV/pH unit as a result of complex stabilization due to decreased proton competition. HEPPS was selected to buffer the experimental solutions because it is an effective pH buffer at pH 8.0 where the sensitivity is high, and it did not interfere with the detection of iron in seawater.

Catalytic Effects. Bromate and hydrogen peroxide were tested as oxidants to enhance the sensitivity. Both oxidants caused a catalytic effect in the iron−DHN system and amplified the peak height. Variations of the oxidant concentration showed that the sensitivity for iron increased almost linearly with the oxidant concentration (Figure 3A and B). The peak current for the iron(III)−DHN complex was linearly related to the bromate concentration between 5 and 60 mM (Figure 3A), and at 20 mM bromate, the sensitivity was 290× greater than without bromate. Variation of the hydrogen peroxide concentration between 1 and 500 μM also caused an increase in the sensitivity (Figure 3B), but the background current increased as well (Figure 3D), as a result of the reduction of hydrogen peroxide itself. Comparison of the scans in the presence of either bromate or hydrogen peroxide (Figure 3C and D, respectively) showed a much improved baseline and peak shape in the presence of bromate. The baseline is reasonably flat next to the iron peak in the presence of bromate as the major distortion caused by hydrogen peroxide is absent. Bromate was therefore chosen as oxidant.

Bromate is poorly soluble in water (the solubility is 0.48 M), restricting the maximum concentration that can be added from a stock solution to the voltammetric cell without significantly diluting the sample. A final concentration of 20 mM BrO_3^- (0.5 mL of 0.4 M BrO_3^- to 10 mL of seawater in the cell) was selected to minimize any contributions of iron from the reagents to the iron blank.

Cyclic Voltammetry in the Presence of Bromate. Cyclic voltammetry was used to confirm the reaction mechanism of the

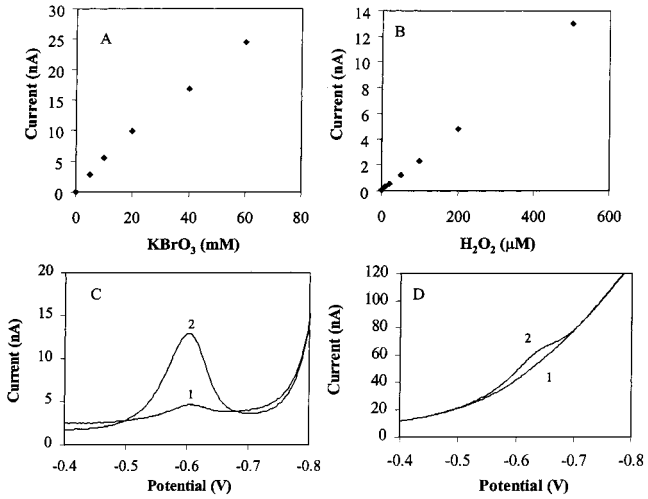

Figure 3. Effect of varying the bromate concentration (A) and hydrogen peroxide concentration (B) on the CSV sensitivity for 1 nM iron in seawater in the presence of 20 μM DHN and 0.01 M HEPPS (pH 8.0). The deposition time was 60 s at −0.1 V. CSV scans of seawater containing 20 μM DHN and 0.01 M HEPPS (pH 8.0) with 20 mM bromate (C) and 200 μM hydrogen peroxide (D). Before (1) and after addition of 1 nM iron (2).

electrode reaction of the iron(III)−DHN complex with bromate (Figure 4). CV scans showed that the reduction peak was much enhanced (100 nA, as compared to 3 nA without bromate (Figure 1A)) in accordance with a catalytic reaction. The reverse scan yielded a cathodic rather than the anodic peak without bromate (Figure 1A). Such behavior has been observed before in previous studies[23,30,31] and is typical for catalytic systems; it indicates that the chemical oxidation of iron(II)−DHN to iron(III)−DHN by bromate was complete so there is negligible electrochemical reoxidation of the iron(II)−DHN that is produced by electrochemical reduction at potentials negative of the iron−DHN peak. The reduction current apparent during the reverse (positive going) scan is due to the continued diffusion of iron(III)−DHN and bromate to the electrode; the peak shape of this current indicates that the catalytic reaction is terminated, or inhibited, at potentials more negative than the reduction peak; the inhibition could be due to diffusion of iron(II)−DHN away from the electrode or to

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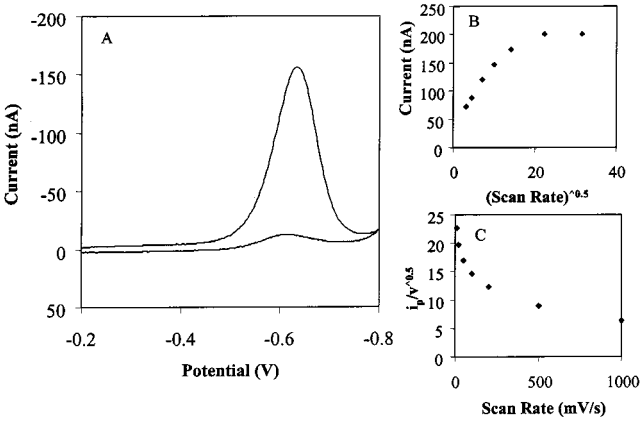

Figure 4. (A) Cyclic voltammogram of 1 nM Fe in seawater in the presence of 20 μM DHN at pH 8.0 with 20 mM bromate; scan at 100 mV·s^{-1}. (B) The reduction current versus the square root of the potential scan rate. (C) Diagnostic test for catalytic mechanism.

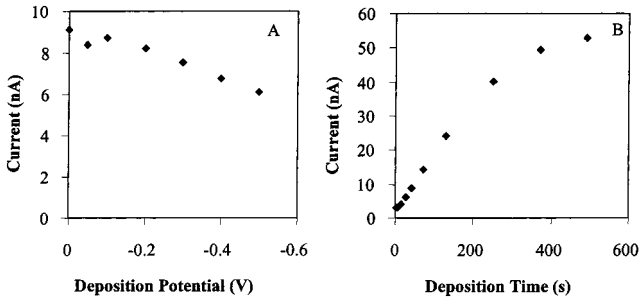

Figure 5. Effect of varying the adsorption potential at a fixed adsorption time of 60 s (A), and of varying the adsorption time at a fixed adsorption potential of −0.1 V (B), on the CSV sensitivity for 1 nM iron in seawater containing 20 μM DHN, 0.01 M HEPPS (pH 8.0), and 20 mM bromate.

poor adsorption at the more negative potentials. Cyclic voltammograms at increased scan speed showed increasing reduction asymmetry due to slow kinetics of the electrode process.

The reduction current of adsorbed species should increase linearly with the scan rate as the reducible charge is constant, whereas a linear relationship with the square root of the scan rate indicates that diffusion currents dominate.[29] A plot (Figure 4B) of the peak height of the iron peak as a function of the square root of the scan rate was linear at scan rates of <50 mV·s^{-1}, indicating that the diffusion of bromate to the electrodes controls the peak height for iron at these scan rates. The linearity breaks down at greater scan rates giving less peak height than expected, presumably due to the slow kinetics of the catalytic process.

The catalytic nature of the iron(III)/(II) redox reaction in the presence of bromate is confirmed by means of a diagnostic test[32] utilizing a plot of ($i_p v^{1/2}$) versus v (Figure 5B), where i_p is the peak current of the iron peak and v the scan rate. At low values of v, a comparatively large amount of bromate diffuses toward the electrode surface, reoxidizing the iron(II), and leading to higher currents than expected from the reduction of iron(III) alone, thus causing the shape of the plot shown in Figure 4C which is typical for catalytic processes.[32]

Reaction Mechanism. Iron(III) forms a complex with DHN [Fe(DHN)$_n$], which subsequently adsorbs on the mercury drop electrode during the deposition step at −0.1 V. During the voltammetric scan, the iron(III) in the complex is reduced to iron(II), which is then reoxidized chemically to iron(III) by bromate. The reoxidized iron(III) then contributes again to the reduction current, causing a greatly improved sensitivity. The potential of the catalytically enhanced peak is at the same location as that in the absence of bromate, indicating that the freshly reoxidized iron is complexed with DHN. In view of the very short available reaction time (milliseconds in the diffusion layer close to the electrode surface), it is likely that the iron(II) remains complexed with DHN throughout the redox process. Therefore, the following reaction mechanism is likely:

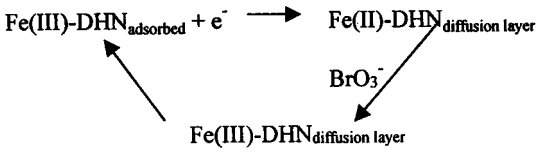

Table 2. Peak Potentials and Sensitivities for CSV of Complex of Various Metals with DHN in Seawater without and with Bromate[a]

	without $KBrO_3$		with $KBrO_3$	
metals	peak potential (V)	sensitivity (nA nM^{-1} min^{-1})	peak potential (V)	sensitivity (nA nM^{-1} min^{-1})
Cu	−0.33	0.029	−0.33	0.024
V	−0.86	0.031	−0.87	3.7
Pb	−0.50	0.032	nd[b]	nd
Sb	−0.73	0.031	nd	nd
Fe	−0.61	0.034	−0.60	9.0

[a] Conditions: 20 μM DHN, pH 8.0 (HEPPS buffer), and 1-min adsorption at −0.1 V. [b] nd, not determined.

Effects of Varying the Adsorption Potential and Time. The adsorption potential was varied and each CSV scan was initiated after a 60-s stirred adsorption and a 10-s equilibration time (Figure 5A). The peak height was highest at adsorption potentials between 0 and −0.2 V and gradually decreased at more negative adsorption potentials. This effect may indicate that the iron(III)−DHN complex is adsorbed better when the electrode is positively charged (the mercury electrode has a zero point of charge at ∼−0.5 V in chloride electrolytes), suggesting that the iron−DHN complex is negatively charged.

Variation of the deposition time showed that the sensitivity increased linearly until a deposition time of 2 min and nonlinearly thereafter (Figure 5B). The sensitivity for iron was improved ∼3.5-fold by increasing the deposition time from 1 to 6 min.

Interferences. Other metal ions can interfere if they form electroactive complexes with DHN and adsorb on the mercury drop electrode with a peak close to that for iron. Several metal ions were added in high concentration to seawater, containing 20 μM DHN and 0.01 M HEPPS buffer without bromate, to establish whether any such interferences could occur. No interfering effect was detected from aluminum (100 nM), cobalt (100 nM), cadmium (100 nM), manganese (100 nM), molybdenum (100 nM), nickel (100 nM), and zinc (100 nM) (all except for molybdenum at much greater than normal levels). Copper produced a peak at −0.33 V,

(32) Greef, R.; Peat, R.; Peter, L. M.; Pletcher, D.; Robinson, N. J. *Instrumental Methods in Electrochemistry*; Ellis Horwood: London, 1990; p 197.

lead at −0.53 V, antimony at −0.74 V, and vanadium at −0.88 V (Table 2). The peaks were large enough to interfere in the absence of bromate. The peak heights of copper, lead, and antimony were not increased by the bromate addition, and these metals do not interfere with the iron determination under optimized conditions as they normally occur in much lower concentrations than those used here. The peak for vanadium was enhanced by the bromate addition, indicating a catalytic effect analogous to that seen here for iron, consistent with previous work using the vanadium−DHN complex with bromate at pH 5[33] and for the vanadium−catechol complex with bromate at pH 6.6.[31] The mean concentration of vanadium in the open ocean is ∼40 nM,[34] and our work shows that unpolluted oceanic seawater produces a large peak for vanadium at the conditions optimized for iron at pH 8.0. However, the iron peak is fully resolved as the vanadium peak is 250 mV more negative than that for iron. It is likely that vanadium can be determined simultaneously with iron, but this was not further investigated.

Possible interference of copper, lead, antimony, and vanadium with the iron determination was tested again by addition of 10 nM of these metals to seawater containing 1 nM iron under optimized conditions. The peak height for iron was not affected by these additions.

Linear Range, Accuracy, Sensitivity, and Detection Limit. The linear range for iron under the optimized method was evaluated at two adsorption times (15 and 60 s) by increasing the concentration of iron in seawater. The peak height increased linearly with the iron concentration over the entire concentration range tested (up 50 nM iron; ∼100-fold higher than typical oceanic iron concentrations). It is expected that the increase of the peak height will become nonlinear and flatten off at higher iron concentrations due to saturation of the mercury drop electrode; however, this experiment was not continued to higher iron concentrations to avoid contamination of the electrode.

The accuracy of the method for iron was verified by replicate analyses of certified seawater (NASS-5). This sample is supplied acidified to pH 1.65 with nitric acid and is certified to contain 3.71 ± 0.63 nM iron. It is not a good standard for ocean water as iron levels are normally much less (5−100-fold), and it was used here only to indicate that the right concentration is obtained at the rather high iron concentration of this preserved seawater; also, the water has been heavily acidified, necessitating the addition of ammonia for pH neutralization. Iron in aliquots of this reference material was quantified in the optimized conditions using a deposition time of 30 s. An iron concentration of 3.89 ± 0.08 nM (n = 12, RSD = 2.2%) was found, within the certified range.

Surface seawater of the north Atlantic Ocean was used to establish the limit of detection of this method at an adsorption time of 60 s; this seawater had been stored at the natural pH and room temperature in a 50-L HDPE container, and the iron level was probably lowered by adsorption on the container walls. Using the optimized conditions, a sensitivity of 7.85 nA·nM^{-1}·min^{-1} was calculated from the slope of a linear regression of standard additions of 0.05 and 0.1 nM iron. The iron scans are shown in Figure 6. The background iron in this seawater corresponded to an iron concentration of 86 ± 4 pM (n = 11, RSD = 5.1%). The detection limit calculated from 3σ (σ being the standard deviation of the measurement) was 13 pM. This detection limit can be reduced further by increasing the deposition time or increasing the bromate concentration.

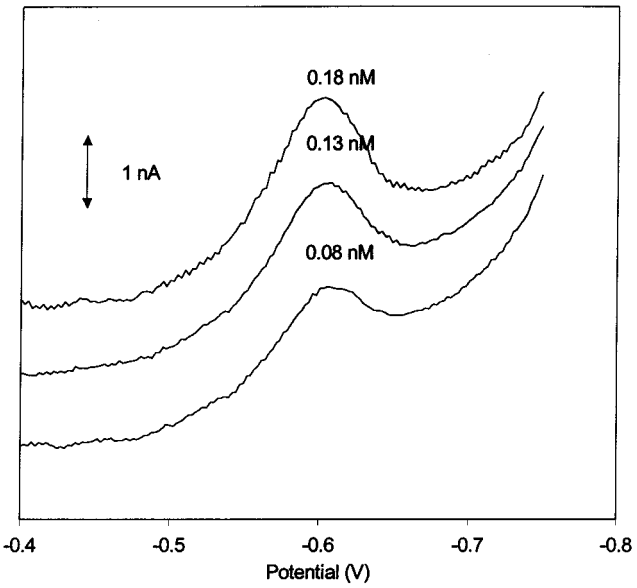

Figure 6. CSV scans for low iron levels in seawater in the presence of 20 μM DHN, 0.01 M HEPPS (pH 8.0), and 20 mM bromate after 60-s adsorption. Scan a, 0.08 nM iron; scan b, addition of 0.05 nM iron to (a); scan c, addition of 0.1 nM iron to (a).

Table 3. Comparison of the Sensitivity, Detected Iron Concentration, and Detection Limit for the Determination of Iron in Seawater by CSV Using Various Ligands

ligand	oxidant	sensitivity[a] (nA nM^{-1} min^{-1})	detected iron concn[b] (nM)	detection limit[c] (nM)
TAC		2.2	0.16	0.11
1N2N	0.04 M $KBrO_3$	1.6	0.67	0.066
DHN	0.02 M $KBrO_3$	7.9	0.09	0.013

[a] Sensitivities were calculated from the slope of a linear regression in standard addition. [b]Iron concentrations were estimated by dividing the peak height of the North Atlantic surface seawater by the sensitivities. [c]Detection limits were calculated by dividing 3σ in the measurements of the North Atlantic surface seawater (n = 8) by the sensitivity.

Comparison to Other Ligands. Previously 1N2N[23] and TAC[26,27] were used to determine low iron levels in seawater; these methods were compared to the DHN method using our equipment to obtain a comparison unbiased by differences in equipment. The sensitivity, the background peak, and the detection limit of each method are shown in Table 3. A background peak presented a problem for the 1N2N method as the peak height was greater than expected for the ambient iron concentration, thus causing a positive bias at low iron concentrations; careful analysis in the absence of bromate and at low iron concentrations showed that a second peak precedes, and overlaps with, the iron peak, causing an overestimate at low iron concentrations. It is possible to separate the two peaks using a slow-frequency square-wave modulation,[22] but the sensitivity is relatively poor and the peaks have the tendency to merge. Addition of bromate to increase the sensitivity causes both peaks to appear merged: the background

(33) Li, H.; Smart, R. B. *Anal. Chim. Acta* **1996**, *333*, 131−138.
(34) Nozaki, Y. *EOS* **1997**, *78*, 221.

peak is then smaller compared to the catalytically enhanced iron peak, but it is not possible to differentiate the two peaks.

The effect of background peaks on the determination of iron using 1N2N and TAC was estimated by comparing the iron concentration in the same surface seawater from the North Atlantic as determined using these ligands. In the case of 1N2N, the background peak is caused by an unknown interfering peak (could be due to free 1N2N), but in the case of the other ligands, contributions to the background peak may also be due to reagent impurity (iron or otherwise). Filtered seawater was adjusted to pH 8.0 (HEPPS) for all the methods. The 1N2N method was in the presence of bromate, using 20 μM 1N2N and 40 mM bromate.[23] The TAC method was reoptimized, and a TAC concentration of 10 μM was used; the potential scan was from -0.4 to -0.8 V using the square-wave modulation with a scan rate of 100 mV$\cdot$s^{-1}, 20-mV plus height, 2-mV step height, and a frequency 50 Hz, preceded by stirred adsorption at -0.4 V for 1 min.

The voltammetric sensitivities were calculated from the slope of a linear regression of standard addition of 0.5 and 1 nM iron. The background peaks were converted to iron concentrations by dividing the peak height by the sensitivity.

The sensitivity of the DHN method was $\sim$5$\times$ greater than that with 1N2N; using the same concentration (40 mM) of bromate as that in the 1N2N method, the sensitivity of the DHN method was increased to 13.5 nA$\cdot$nM$^{-1}\cdot$min^{-1}, which is 8$\times$ greater than using the 1N2N method. In addition to having the highest sensitivity, the DHN method produced the lowest iron concentration, indicating lowest levels of any interference (if any). The low background peak for the DHN method was achieved after purification of the DHN, bromate, and HEPPS; increases in the reagent concentrations showed that the residual iron contribution from the reagents was negligible at $<$10 pM.

The 1N2N method showed the highest background peak although the apparent detection limit was reasonably low. The high background peak was found even though the same HEPPS buffer, bromate, and seawater were used, and the peak was not removed by recrystallization of the 1N2N.

The TAC method showed similar sensitivity to the 1N2N method and a lower background peak. However, a large peak caused by the ligand itself preceded the iron peak and interfered with the determination of low levels of iron, causing the relatively high detection limit. Purification of TAC to lower the background peak was difficult due to its high solubility in water.

(35) Martin, J. H.; Fitzwater, S. E.; Gordon, R.M.; Hunter, C. N.; Tanner, S. J. *Deep-Sea Res. II* **1993**, *40*, 115−134.

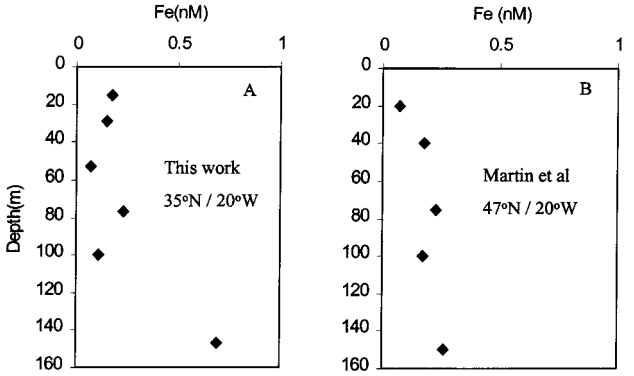

Figure 7. (A) Distribution of dissolved iron in the surface layer of the North Atlantic Ocean determined using the optimized conditions with a 60-s adsorption period. (B) Data from Martin et al.[35] for a station 12° further north.

Sample Analysis. The new method was tested on samples from the North Atlantic, collected during *Challenger* cruise 76/90 (March 1991) at 35°29′ N, 19°58′ W from 0 to 200 m depth. The samples had been stored deep frozen. The concentrations of iron in two aliquots of 10 mL of each sample were determined by CSV using a 60-s adsorption time. The distribution of iron in the water column is shown in Figure 7 and can be compared to data from Martin et al.[35] The concentrations of iron from 15 to 100 m were less than 0.25 nM (average 0.15 $\pm$ 0.04 nM) and the lowest value was at 53 m depth, at the depth of the fluorescence maximum corresponding to chlorophyll a. The low concentrations in the euphotic zone are probably due to uptake by phytoplankton. The low concentrations in the euphotic zone are in good agreement with data from Martin et al.[35] for the NE Atlantic with an average of 0.16 $\pm$ 0.05 nM iron for the top 100 m; their station was 12° further north so the degree of agreement could be fortuitous and our data are insufficient to provide the iron distribution in the NE Atlantic. However, these data show that the new CSV method is suitable for the determination of low iron levels in seawater.

It is the intention to convert the new method for flow analysis in further work.

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