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COLORIMETRIC FLOW-INJECTION ANALYSIS OF DISSOLVED IRON IN HIGH DOC WATERS

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Abstract—An iron flow-injection analysis system has been optimized for the analysis of iron in waters high in dissolved organic carbon. The method detects either dissolved iron(II) or total dissolved iron with a detection limit of 10 nM, precision of 0.65% at 1 μ M, and a dynamic range of four orders of magnitude. There are minimal interferences (<1%) from other metals at environmental concentrations. The iron(II) method measures iron(II) in the presence of excess iron(III) with less than 1% interference. When used with pre-acidified samples, the total dissolved iron method agrees well with electrothermal atomic absorption spectrometry for a variety of natural waters with a range of dissolved organic carbon (3–36 mg C/L) and iron (1–28 μ M) concentrations. When used with samples at their ambient pH, the total dissolved iron method detects dissolved iron, but not colloidal iron (size fraction 0.05–0.45 μ m).
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Key words—iron, flow-injection analysis, ferrozine, colloid, redox, fresh water

INTRODUCTION

A critical requirement for the analysis of iron in many environmental samples is the discrimination between iron(II) and iron(III). Atomic spectrometry is both sensitive and selective, but does not meet this criteria. Electrochemistry and colorimetry have been used to gain this redox state sensitivity during the analysis of iron (Pehkonen, 1995). However, iron(III) hydrolysis and dissolved organic matter (DOM) sorption to electrodes hamper electrochemical measurements (Pehkonen, 1995). DOM complexation and hydrolysis of iron(III) complicate the use of colorimetric analysis methods, causing incomplete recovery of atomic spectrometry measured iron. This has prompted the use of operationally defined iron fractions (Box, 1984). DOM has also been reported to reduce iron(III) during colorimetric analysis using iron(II)-specific chromophoric ligands, leading to positive errors in the measurement of iron(II) (Box, 1984). The chromophores themselves have also been noted to reduce iron under some conditions (Box, 1984; Cowart *et al.*, 1993). Pehkonen provides a review of the problems associated with measuring the

oxidation state of iron in natural waters (Pehkonen, 1995).

Flow-injection analysis (FIA), the automation of a wet chemical analysis method in a non-segmented continuous flow apparatus, has the potential to overcome these problems. FIA provides controlled conditions that allow complicated reaction sequences to take place in a reproducible manner, greatly increasing their reliability (Ruzicka and Hansen, 2000). The approach of this study is to use FIA to automate and better control the colorimetric analysis of iron in high dissolved organic carbon containing natural water samples.

FIA has previously allowed researchers to measure sub-nanomolar amounts of iron and/or iron(II) in seawater using iron-catalyzed chemiluminescence (Elrod *et al.*, 1991; Measures *et al.*, 1995; O'Sullivan *et al.*, 1995). Unfortunately, the chemiluminescent FIA methods would not work well on freshwater samples. DOM, usually quantified as dissolved organic carbon (DOC), interferes with the analysis (O'Sullivan *et al.*, 1995; Voelker and Sulzberger, 1996) and is present in higher concentration in freshwater (2–40 mg C/L) than in seawater (≤ 0.5 –1.2 mg C/L).

Researchers have also used FIA to automate traditional colorimetric methods for the analysis of iron in aqueous samples. However, the numerous published methods are often unsuitable for high DOC natural waters. For example, studies tested only low carbon samples (To *et al.*, 1999), required

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the digestion of high carbon samples (Mortatti *et al.*, 1982), or analyzed samples without giving the carbon content (Pascual-Reguera *et al.*, 1997). Additionally, many of the methods have inadequate detection limits (Schnell *et al.*, 1998), metal ion interferences (Yamane and Yamada, 1995), or use absorbance detection at a wavelength where DOM also absorbs (Whitman *et al.*, 1988). No previously reported colorimetric iron FIA method has been optimized for the analysis of high DOC natural waters, such as freshwater streams and lakes.

An FIA method used for the analysis of iron in these waters should meet a set of requirements determined by the nature of the samples. Because the chemical behavior of iron is strongly affected by its redox state, the method should distinguish between iron(II) and iron(III). Iron concentrations vary widely among natural waters, so the method should have both a low detection limit (< 100 nM) and a wide dynamic range (3–4 orders of magnitude). Good precision (1%) will allow the measurement of small relative changes in iron concentration, important in time course studies. The method should not be affected by large amounts of DOM (up to 40 mg C/L) or other metals at environmental concentrations and should be fast and easy to use. This paper reports the development of an iron FIA method that meets these criteria.

METHODS

Materials

All solutions used deionized water supplied by an Ionpure 150 deionizer. Glass and polymeric labware were soaked overnight in 10% HNO₃ and rinsed with deionized water. Except where noted, all reagents were purchased from the Aldrich Chemical Company and used without further purification. Sodium acetate/acetic acid buffer (pH 4.0) was made from trace metal grade glacial acetic acid (Fisher Scientific) and sodium hydroxide (99.999%). MES (2-[N-morpholino]ethanesulfonic acid) buffer (pH 6.0) was made from its hemisodium salt (Sigma Chemicals). Phthalate buffer (pH 4.0) was made from primary standard grade potassium hydrogen phthalate (Acros Organics). Primary standard grade citric acid monohydrate was also obtained from Acros. Copper, cobalt, and nickel solutions were made from the following salts: CuCl₂·2H₂O (Sargent-Welch Scientific), CoCl₂·6H₂O, and Ni(NO₃)₂·6H₂O. Suwannee River fulvic acid was provided by J. Leenheer, US Geological Survey. Iron(II) solutions were made fresh daily

from Fe(NH₄)₂(SO₄)₂·6H₂O (Certified ACS, Fisher) that was warmed in an oven at 45°C (to remove sorbed water) for one hour and stored in a desiccator. Iron(III) solutions were made by diluting a 1000 ppm atomic absorption standard. Unless noted, all iron solutions were made in pH 1.0 HNO₃, from concentrated acid (99.999% purity, used throughout). Polypropylene volumetric flasks and Teflon beakers were always used for the preparation and handling of iron containing solutions.

Electrothermal AAS

Electrothermal atomic absorption spectrometry (ETAAS) of iron at 248.3 nm. (0.20 nm slit) was conducted on a Perkin-Elmer 5100PC spectrometer with Zeeman background correction and a HGA-600 graphite furnace attachment using Perkin-Elmer graphite tubes and platforms. The temperature program is given in Table 1. Standards were made by diluting a 1000 ppm atomic absorption standard (Aldrich) in pH 1.0 nitric acid. The response was non-linear, even over the short range of 0.1–1.0 μM, and quantitation used a second-order polynomial. This method was found to give a good recovery of 0.5 μM iron in the presence of up to 25 mg/l dissolved organic carbon.

Natural water samples

Water was collected from Triangle Bog Lake, a small, acidic *Sphagnum* moss bog (2) (Kent, OH) on 4 September, 1997. Cuyahoga River water was collected from near the Main St. bridge in Kent, OH, on 13 December, 1997, 2 April and 8 April, 1998. Suwannee River water was collected near Fargo, GA on 20 March, 1996. The Triangle Bog Lake, Cuyahoga River, and Suwannee River samples were 0.45 μm filtered (Gelman Sciences, 47 mm GN-6 Metrical filters). The Cuyahoga River samples required prefiltration using Type A/E glass fiber filters (Gelman). The first 50 ml of each filtration step was discarded. Samples were also collected from a variety of sites in the McDonald's Branch Basin in the New Jersey Pinelands (Lebanon State Forest). The area, including the sampling sites we used, is well described elsewhere (Johnsson and Barringer, 1993). An additional sample was collected from nearby Atsion Lake (an impoundment) in Wharton State Forest, New Jersey. Because large volumes of filtered water were needed as part of another study, all the Pinelands samples were filtered using three high volume cartridge filters in series (20 μm, Omnifilter; 1 μm, Parker Filtration; 0.4 μm, Nucleopore). Samples were collected from sites S1, S2, and S10 on 5/12/97 and from S10, QWH1A, and Atsion Lake on 10/29/97. All the samples were stored in dark refrigeration until use and acidified to pH 1.0 using the high-purity nitric acid several days before iron analysis, except where noted.

FIA reagents

In this study, iron(II) is detected using ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,P'*-disulfonic acid).

Table 1. Electrothermal atomic absorbance spectrometry temperature program

Step	Ramp time (s)	Temperature (°C)	Hold time (s)
Drying of 5 μL matrix modifier ^a	3	120	20
Drying of 15 μL sample	5	120	60
Ashing	1	1400	60
Cool down	1	20	15
Atomization, read signal ^b	0	2500	10
Cleaning	1	2600	10

^a 1000 mg/L Mg(NO₃)₂.

^b GAS flow off.

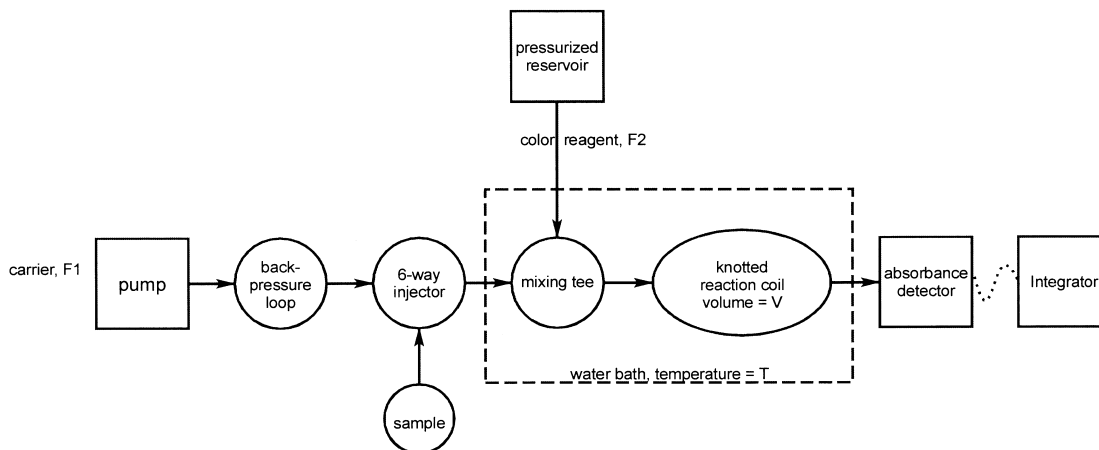


Fig. 1. A diagram of the flow-injection analysis system. $F_1 = 1.0$ ml/min, $F_2 = 0.1$ ml/min. *Iron(II) system*: Carrier = 0.1 M MES (pH 6.0), color reagent = 0.1 M MES (pH 6.0) + 0.01 M ferrozine, $V = 375$ μ L, $T = 25^\circ\text{C}$. *Total iron system*: Carrier = 0.1 M sodium acetate/acetic acid + 1 mM citric acid (pH 4.0), color reagent = 0.1 M sodium acetate/acetic acid (pH 4.0) + 0.1 M ascorbic acid + 1 mM citric acid + 0.01 M ferrozine, $V = 1200$ μ L, $T = 65^\circ\text{C}$.

Ferrozine was chosen because its iron(II) complex has a suitable molar absorptivity ($27,900 \text{ M}^{-1} \text{ cm}^{-1}$ at 562 nm), it has a large iron(II) binding constant ($\log \beta_3 = 15.6$), and the complex is stable over a wide pH range (4–9) (Gibbs, 1976). Additionally, ferrozine has not been shown to bind iron(III), has high water solubility, and is commercially available. Ferrozine is described in detail elsewhere (Gibbs, 1976; Carter, 1971; Stookey, 1970; Thompson and Mottola, 1984). Total dissolved iron is detected by adding a chemical reductant to the reaction mixture with ferrozine. In studies where a variety of reductants were tested for use in iron analysis, ascorbic acid was found to have the highest relative reaction rate (Box, 1984; Elrod *et al.*, 1991) and is used here. The reaction pH is buffered at 6.0 using MES or at 4.0 using acetic acid (discussed in detail below).

FIA apparatus

The FIA system (Fig. 1) uses a pH buffered carrier to move the sample from the injection valve sample loop to the mixing tee, where the sample, carrier, and color reagent are mixed and the complex forming reaction begins. The mixture moves through the reaction coil where the reaction proceeds to completion. The amount of colored complex is measured using the absorbance detector with the signal recorded and processed using an integrator.

The FIA system was assembled from commercially available components. The carrier pump is a Dionex GP-40 HPLC pump with a metal-free flow path (flow rate = F_1). To minimize pump-generated baseline noise, the back pressure observed by the GP-40 was increased to ~ 900 psi by inserting 10 ft of 0.005" ID PEEK tubing (Upchurch Scientific) between the pump and the injector and the pump was operated in "constant pressure" mode. The injector is a Rheodyne 9125 biocompatible 6-way injection valve. To eliminate sample contact with steel syringe needles, the injector is equipped with a needle suction adapter (Rheodyne) so that the sample is pulled through a waste port (PEEK tubing) into the sample loop. The color reagent is supplied using a Dionex PC10 reagent delivery module, an apparatus that uses pneumatic pressure (up to 100 psi) to push the color reagent from a reservoir into the carrier flow stream, with the flow rate (F_2) controlled by the gas pressure. The carrier and color reagent streams are mixed using a 3.1 μ L dead volume fritted static mixing tee (Upchurch). We used the knotted reaction coil supplied

with the PC10 (375 μ L) as well as coils of various geometry and volume (V) that were made by hand from 0.020" ID PTFE tubing (Upchurch). The mixing tee and reaction coil are immersed in a Lauda RM-6 variable temperature (T) water bath. The absorbance detector is a Dionex AD-20 with a 10 mm path length PEEK flow cell, used at 562 nm. 0.020" ID PEEK tubing is used to make the connections from the carrier pump up to the mixing tee with 0.020" ID PTFE tubing used for all other connections. PEEK "fingertight" fittings (Upchurch) are used throughout. A Hewlett Packard 3395 integrator determines peak areas and heights. Helium pressurizes the carrier reservoir and the PC10 module. The FIA reagents were degassed by stirring under a vacuum or by vacuum filtration (0.45 μ m Gelman GN-6) prior to use.

FIA optimization

The reaction time (the time between mixing with the color reagent and detection) is controlled by the carrier flow rate, the color reagent flow rate and the volume of the reaction coil and connecting tubing. For this study, the carrier flow rate remained constant at 1.0 ml/min and the color reagent flow rate and reaction coil volume were varied. The reaction temperature, pH, and the compositions of the carrier and color reagent were also optimized. Selectivity of the iron(II) method was determined by measuring the response of iron(III) stock solutions. Recovery of the total dissolved iron method was optimized by maximizing the response of iron(III) stock solutions, iron(III)-carboxylic acid complexes, and iron(III)-Suwannee River fulvic acid mixtures relative to iron(II) stock solutions. The effect of varying the sample loop volume was also examined.

FIA testing

The precision, detection limit, and linear range of the FIA methods were measured using repeated injections of pH 1.0 iron(II) (iron(II) method) and iron(III) (total dissolved iron method) stock solutions. Accuracy of the total dissolved iron method was tested by analyzing iron(III)-Suwannee River fulvic acid mixtures and natural water samples by both FIA and ETAAS. Due to the limited dynamic range of the atomic absorbance method, the natural water samples had to be diluted with pH 1.0 nitric acid to ≤ 1 μ M before analysis. The diluted and undiluted

samples were also analyzed by FIA. To further test the recovery of the method, fractions of the diluted natural water samples were spiked with enough iron(III) to double their original concentration and reanalyzed by FIA and ETAAS. The dissolved organic carbon concentration of the natural water samples was measured by acidifying the samples, purging with CO₂-free air, and analyzing using a Shimadzu TOC-5000, with quantitation using potassium hydrogen phthalate (Acros) solutions.

The effect of sample pH on the total dissolved iron FIA system response was tested using 0.45 µm filtered Cuyahoga River water (2 April, 1998). 50 ml aliquots of the sample were measured into Teflon beakers, adjusted to the desired pH using HNO₃, and analyzed by FIA within 5 min. The aliquots were stored for 24 h in the dark at room temperature, reanalyzed by FIA, and the pH values were re-measured. Next, 5 ml of each sample was filtered through 0.05 µm Nucleopore filters (Corning Separations) using 10 ml plastic syringes and Nalgene Swin-Loc filter holders. Prior to use, the 0.05 µm filters, filter holder, and syringe were rinsed using 10 ml pH 2.0 HNO₃, 10 ml water, and 1 ml sample. The filtrate was acidified to pH 1.0 using HNO₃ and analyzed by ETAAS. Percent recovery calculations for the total dissolved iron FIA analysis as well as the ETAAS analysis of the 0.05 µm filtered samples are based on the ETAAS analysis of an acidified aliquot of 0.45 µm filtered water.

To further examine the pH dependence of the total dissolved iron method, two 50 ml aliquots of Cuyahoga River water (18 April, 1998) were again measured into Teflon beakers. One aliquot was adjusted to pH 1.1 using HNO₃, the other was left at its original pH (7.9). The samples were stored for 24 h in the dark at room temperature, then analyzed by total dissolved iron FIA and their pH re-measured (1.2 and 8.2 respectively). The samples were 0.05 µm filtered as above. The filters were saved for analysis by atomic force microscopy (AFM) and the filtrate was acidified to pH 1.0 and analyzed for iron by ETAAS. An aliquot of the 0.45 µm filtered sample was acidified to pH 1.0 and also analyzed for iron by ETAAS. The 0.45 µm filtered, pH adjusted aliquots and their 0.05 µm filtered counterparts were acidified to pH 1.0 and analyzed for dissolved organic carbon. To provide a control for the AFM analysis, deionized water was treated with the same filtration procedure as the river water samples (glass fiber, 0.45 µm, 0.05 µm), with the 0.05 µm filter saved for AFM.

Atomic force microscopy

A Digital Instruments, Inc. MultiMode NanoScope III atomic force microscope with tapping mode and phase imaging capabilities was used in this study. Specialized Tesp etched Si probes (tips) having 125 µm cantilever length and resonant frequency in the range of 284–355 kHz were employed. All imaging was conducted under ambient conditions, in air. The images shown in this paper were collected using tapping mode AFM (TMAFM™, Digital Instruments, Inc.). With TMAFM, two data types are collected simultaneously: height mode and amplitude mode. While height mode gives more quantitative topographic information (height of sample's *z* axis), amplitude mode gives data that are essentially the first derivative of the height mode data. For relatively rough, particulate-containing surfaces, amplitude mode results in images that are substantially easier to interpret with the human eye. All images shown here were collected in the amplitude mode. The *z*-axis data are greatly distorted in amplitude mode and hence they are not reported in images shown here. A description of AFM and its application to similar experiments can be found in Maurice (1996) and Maurice and Lower (1998).

RESULTS AND DISCUSSION

Optimization of the iron(II) FIA system

Initially, the buffer used for the carrier and color reagent was 0.1 M sodium acetate/acetic acid at pH 4.0. However, this promoted the reduction of iron(III), giving a false positive signal. This agrees with the results of Box (1984) who reported the reduction of iron by a variety of iron(II) chromophores, including ferrozine, in the presence of acetate at pH 4.6. When the acetate buffer was replaced with 0.10 M MES at pH 6.0 the reduction of iron(III) was decreased to <1%, making iron(III) undetectable at concentrations ≤ 1.0 µM and only a very minor interference at higher concentrations. Lower concentrations of MES (0.010 M) increased the reduction of iron(III) to 45%. This is due to the diminished buffer capacity of the carrier and color reagent, presumably leading to the same low pH reduction seen with the pH 4.0 buffer.

To ensure an excess of ferrozine in the final reaction mixture, the ferrozine concentration in the color reagent was 0.010 M. The color reagent flow rate was 0.10 ml/min, this gives a final ferrozine concentration of 1.0 mM in the reaction mixture, sufficient to detect up to 0.10 mM iron(II). The reaction coil was the 375 µl knitted coil provided with the PC10 reagent delivery module, giving a reaction time of 25 s. The reaction temperature was 25°C.

The iron(II) system required no optimization beyond these initial conditions. The iron(II)—ferrozine forward reaction proceeds quickly, $k = 3 \times 10^{11} \text{ M}^{-3} \text{ s}^{-1}$ (Thompson and Mottola, 1984). Reduction of the reaction rate by iron(II) hydrolysis is not expected at environmental pH values and DOM complexes of iron(II) are either fast-reacting or non-existent (Langford *et al.*, 1977). As a result, increasing the reaction time by adding a larger reaction coil only increased band broadening, presumably due to increased longitudinal diffusion. Increasing the reaction temperature, the concentration of ferrozine and the color reagent flow rate did not increase response, but did increase the baseline noise.

Reaction coil geometry

The effect of reaction coil geometry on FIA response was examined using the iron(II) system. The peak area, height, and width were measured for different geometries of a 1200 µl piece of tubing. The tubing was used uncoiled, loosely coiled (5 cm diameter loops), tightly coiled around a 4 mm diameter rod, and knotted in a series of connected square knots. No difference in FIA response was seen between the uncoiled, loosely coiled, and tightly coiled configurations. However, as predicted by the theory of Tijssen (1980), the knotted conformation greatly decreased the band broadening and therefore increased the sensitivity via peak height, relative to

Table 2. The effect of reaction coil geometry on flow-injection analysis peak characteristics^a

Geometry	Average peak width ^b (min)	Peak height sensitivity ($\times 10^{10}$)	Peak area sensitivity ($\times 10^{11}$)
Uncoiled	0.40	1.0	2.4
Looped (5 cm)	0.42	0.96	2.4
Coiled (4 mm)	0.39	1.0	2.4
Knotted	0.13	3.1	2.4

^aSensitivity was determined from the linear regression of peak height or area against injected iron(II) concentration for four standard solutions. Reaction coil volume and system flow rates remained constant throughout.

^b1 μ M Fe(II) injections.

the other configurations (Table 2). This is in disagreement with the results of Chalk and Tyson (1994), who report that 6 cm loops gave the best response, relative to knotted and uncoiled configurations. The knotted geometry was used throughout the rest of the study.

Optimization of the total dissolved iron FIA system

Since reduction of iron(III) was observed to occur with the pH 4.0 acetate buffer, this system was adopted for the total dissolved iron method. The carrier initially consisted of 0.10 M acetate at pH 4.0 and the color reagent contained 0.10 M acetate, 0.10 M ascorbic acid, and 0.010 M ferrozine. This gave 75% recovery of iron(III), relative to iron(II). Increasing the temperature from 25°C to 65°C and the reaction coil volume from 375 to 1200 μ l further improved recovery to 85–90%, while additional increases in temperature and reaction coil volume (to 80°C and 1800 μ l) gave no further improvement.

Since the total dissolved iron FIA method should give the same response for iron(II) and iron(III), further optimization was warranted. The reductant was both increased in concentration and changed to hydroxylamine HCl, with no improvement. The buffer was changed to 0.10 M phthalate at pH 4.0 with only a slight increase in recovery. Finally, adding a variety of carboxylic acid ligands to the iron(III) standard solutions improved the recovery of iron(III) to 100%. Partial complexation of iron(III) by the buffer was suspected. In all further experiments, the addition of 1 mM citric acid to the carrier and color reagent improved the recovery of iron(III) solutions to 100%, relative to iron(II) standards. Apparently the acetate buffer complexed \sim 10% of the iron(III), preventing its reaction with the ascorbic acid and/or ferrozine. The citric acid prevented the complexation by acetate and formed a complex that was easily reduced by the ascorbic acid.

The final conditions for the total dissolved iron FIA systems are as follows: The carrier solution is 0.10 M acetate/acetic acid and 1.0 mM citric acid at pH 4.0. The color reagent is 0.10 M acetate/acetic acid, 0.10 M ascorbic acid, 0.010 M ferrozine, and 1.0 mM citric acid at pH 4.0. The reaction coil is 1200 μ l and the reaction temperature is 65°C. The carrier flow rate is 1.0 mL/min and the color reagent

Table 3. The effect of simple organic acids on the recovery of the total dissolved iron FIA system

Ligand	[Ligand] (M)	pH	Recovery (%) ^a
Citric acid	0.01	6	99.7
Oxalic acid	0.01	6	101.3
Malonic acid	0.01	4	99.8
Salicylic acid	0.01	4	101.5
NTA	0.01	6	102.9
EDTA	1×10^{-6}	6	95.9
	1×10^{-5}	6	95.4
	1×10^{-4}	6	83.5
	1×10^{-3}	6	37.7

^a1.0 μ M total iron.

flow rate is 0.10 mL/min, giving a reaction time of 70 s.

Total dissolved iron FIA system recovery

To further test the recovery of the total dissolved iron system, solutions containing carboxylic acids ligands (citric acid, oxalic acid, malonic acid, salicylic acid, nitrilotriacetic acid (NTA), ethylenediamine tetraacetic acid (EDTA)) and iron(III) were analyzed. The concentration of ligand and pH in the test solutions (Table 3) were optimized using computerized equilibrium calculations to give \geq 99% complexation of 1.0 μ M iron. The calculations used Titrator (Cabaniss, 1987) and thermodynamic constants from the NIST database (Smith *et al.*, 1993). The constants were entered at 0.10 M ionic strength and not varied. Complete recovery, relative to iron(II) standards, was observed for all the ligands tested at concentrations well above those found in the environment (Table 3). At concentrations of EDTA that were in 100 and 1000 fold excess of that needed for 99% complexation (0.10 and 1.0 mM), the recovery of iron decrease. However, these EDTA concentrations are much greater than observed in natural waters and will not represent an interference to our method.

The effect of Suwannee River fulvic acid on total dissolved iron recovery was also examined. Iron(III) from an acidic stock solution was added to pH 2.0 solutions of Suwannee River fulvic acid, adjusted to the desired pH (Table 4), mixed well, and let stand for 24 h in the dark at room temperature. The solutions were then analyzed using the total dissolved iron FIA system and ETAAS. All the solutions

Table 4. The effect of Suwannee River fulvic acid on the recovery of the total dissolved iron FIA system

Added [Fe] (μM)	[Fulvic acid] (mg/L)	pH ^a	FIA measured [Fe] (μM)	Precision ^b (μM)	ETAAS measured [Fe] (μM)	Precision ^b (μM)
0.4	10	2.3	0.473	0.007	0.413	0.004
0.4	40	2.3	0.647	0.004	0.521	0.004
1.0	10	2.3	1.079	0.009	1.021	0.005
1.0	40	2.2	1.263	0.005	1.142	0.009
0.4	10	6.8	0.410	0.006	0.393	0.002
1.0	40	7.3	0.59	0.01	0.523	0.006
0.4	10	7.3	0.88	0.02	0.858	0.003
1.0	40	7.2	1.129	0.003	1.121	0.008
0.4	10	6.8 ^c	0.419	0.004	0.415	0.003
1.0	40	7.3 ^c	0.617	0.007	0.504	0.004
0.4	10	7.3 ^c	0.903	0.008	0.950	0.001
1.0	40	7.2 ^c	1.155	0.004	1.13	0.01

^aSee text for experimental details. Measured after 24 h incubation.

^b1 σ , $n=3$.

^cAcidified to pH 2 prior to analysis.

contained more iron than added during the experiment, presumably a result of the iron content of the Suwannee River fulvic acid. Good agreement was seen between the two methods. For the total dissolved iron FIA system, samples that were incubated at pH 2 gave higher iron concentrations than those incubated at pH 7. When the pH 7 samples were acidified to pH 2 prior to analysis, some of this difference was eliminated. Given the low solubility of inorganic iron(III) at non-acidic pH values, this observation is not surprising. The effect of sample pH on the total dissolved iron FIA measurement was examined in detail using natural water samples and is discussed below.

Comparison to ETAAS

The analysis of natural water samples with a range of DOC and iron concentrations by the total dissolved iron FIA method gave good agreement to ETAAS (Table 5). The total dissolved iron FIA system gave good agreement between the diluted and undiluted samples, indicating that dilution is not necessary for analysis by total dissolved iron FIA (in contrast to analysis by ETAAS). The precision for the analysis of the diluted samples was similar for both methods. The FIA analysis of the undiluted samples gave improved precision compared to the diluted samples, 0.1–1% relative standard deviation versus 1–2% RSD for the latter. The spike recovery for the total dissolved iron FIA system was 97–100%, compared to 101–114% for ETAAS.

Effect of sample pH on the total dissolved iron FIA response

The recovery of the total dissolved iron FIA system is pH dependent (Fig. 2a). Increasing amounts of iron are detected as the pH decreases, reaching 100% recovery at pH 1.3 after 24 h. However, when the 0.05 μm filtered and acidified samples were analyzed by ETAAS, the results were

within 10% of the FIA analysis for all pH values (Fig. 2b). This indicates that the total dissolved iron FIA system measures only iron smaller than 0.05 μm . Iron in the size range 0.05–0.45 μm is detected by ETAAS but not FIA.

Atomic force microscopy images show the particles retained on the 0.05 μm filters (Fig. 3). The pH 1.2 sample filter contained well-defined particles, possibly clays and/or iron oxides. The pH 8.2 sample contained both particles and bacteria. The bacteria in the pH 1.2 sample were apparently destroyed during acidification. No particles or bacteria were found on the distilled water control filters (Fig. 3).

The pH effect is attributable to a number of factors. The most obvious is the dissolution of iron oxide particles, favored at low pH. Iron could also be released from the surfaces of particles, such as aluminosilicates. Additionally, iron could be freed from both the interior and exterior of bacterial cells on acidification. The DOC analysis indicates that the carbon content of the water samples decreased significantly when 0.05 μm filtered. The pH 1.2 sample decreased from 8.6 to 7.1 mg/L and the pH 8.2 sample decreased from 10.5 to 7.6 mg/L. This suggests that the iron could be associated with bacteria and/or colloidal humic material. In any case, a substantial percentage of the iron in a water sample could be found in this small particle fraction (40% in this instance).

When using the total dissolved iron FIA system, the operator needs to decide whether this small particle fraction is to be detected. If its measurement is desired, the samples should be acidified to pH 1 and incubated for at least 24 h at room temperature prior to analysis. The results of the pH dependent study as well as the data shown in Table 5 indicate that the total dissolved iron FIA system measurements on 0.45 μm filtered and acidified samples are equivalent to those obtained using ETAAS. If the investigator does not want to measure iron associated with small (0.45–0.05 μm) particles, the samples should be 0.45 μm filtered and analyzed immediately

Table 5. The analysis of natural water samples by total dissolved iron flow-injection analysis and electrothermal atomic absorbance spectrometry^a

Sample	[DOC] (mg/L)	Precision (mg/L) ^b	Undiluted samples						Diluted samples					
			Flow Injection Analysis			Electrothermal AAS			Flow Injection Analysis			Electrothermal AAS		
			[Fe] (μM)	Precision (μM) ^b	[Fe] (μM)	Precision (μM) ^b	Spike recovery (%)	[Fe] (μM)	Precision (μM) ^b	Spike recovery (%)	[Fe] (μM)	Precision (μM) ^b	Spike recovery (%)	
S10, Fall 1997 ^c	3.13	0.04	1.086	0.005	1.07	0.01	98	1.10	0.02	114				
Atsion lake ^d	4.8	0.2	7.27	0.05	7.23	0.07	97	7.6	0.1	104				
S10, Spring 1997 ^e	8.5	0.2	2.705	0.002	2.69	0.02	99	2.69	0.04	106				
Cuyahoga River ^e	9.0	0.1	1.488	0.008	1.48	0.02	99	1.74	0.01	101				
QWH1A ^e	14.9	0.4	28.49	0.04	28.2	0.2	100	29.0	0.8	105				
Triangle Bog ^e	16.1	0.4	6.481	0.005	6.42	0.05	99	6.7	0.1	103				
S1 ^c	24.0	0.3	13.04	0.02	13.11	0.05	100	13.08	0.08	103				
S2 ^c	28.6	0.1	16.74	0.03	16.9	0.1	99	17.41	0.03	103				
Suwannee River ^f	35.5	0.2	4.57	0.02	4.79	0.03	99	4.9	0.1	110				

^a See text for details.^b $1\sigma, n = 3$.^c McDonalds Branch basin, Lebanon State Forest, NJ.^d Wharton State Forest, NJ.^e Kent, OH.^f Sampled near Fargo, GA.

at their ambient pH. Using the total dissolved iron FIA system in this manner prevents the detection of very small particles without the difficulties associated with using small pore size filters. As evident from the AFM results, the method reported here provides a much better estimate of the iron in the true dissolved phase than the commonly employed method of atomic spectrometry in combination with 0.45 μm filtration. While a 0.05 μm filter could be used to exclude colloidal iron from detection by any total iron method, this awkward and time consuming step is eliminated by use of the FIA method.

Clearly, using a filter to separate colloidal and dissolved material has its limitations (Gustafsson and Gschwend, 1997). However, a recent study of submicron particles used field-flow fractionation to measure average particle sizes of 58–60 nm in natural water samples (Vaillancourt and Balch, 2000). This suggests that a 0.050 μm cutoff is a reasonable approximation of the division between dissolved and colloidal iron.

Metal ion interferences

Since ferrozine is reported to form colored complexes with copper, cobalt(II), and nickel(II) (Kundra *et al.*, 1974; King *et al.*, 1991), we analyzed pH 2.0 solutions of each using both the total dissolved iron and iron(II) FIA systems. Copper is reported to be bound by ferrozine in its reduced +1 state. However, since the total dissolved iron FIA system uses a reductant, it was tested using copper(II) solutions. Since copper(I) can be produced in the environment by similar processes as iron(II), we tested the iron(II) system using copper(II) with and without reductant (0.10 M ascorbic acid) added to the copper solutions.

Copper(II) without reductant was detected by the total dissolved iron FIA system at 9.5% the sensitivity of iron, but gave <1% response with the iron(II) system. However, copper(II) with reductant was detected at 11.5% the sensitivity of iron by the iron(II) FIA system, presumably because the ascorbic acid reduced the copper to its +1 state.

Copper is generally found at much lower concentrations than iron in the environment. Thus, in typical fresh waters ($\text{Cu}_T \leq 100 \text{ nM}$), the interference from copper is negligible. In systems where copper is found at similar concentrations to iron, neocuproine could be added to the color reagent to complex copper. This would shift the maximum absorbance of the interfering species from 470 nm for the ferrozine-copper complex to 454 nm for the neocuproine-copper complex, compared to 562 nm for the iron(II)-ferrozine complex (Kundra *et al.*, 1974; King *et al.*, 1991). Cobalt and nickel were also detected by both systems, but only with 2.2 and 0.17% the sensitivity of iron respectively, insignificant at typical environmental concentrations of those metals.

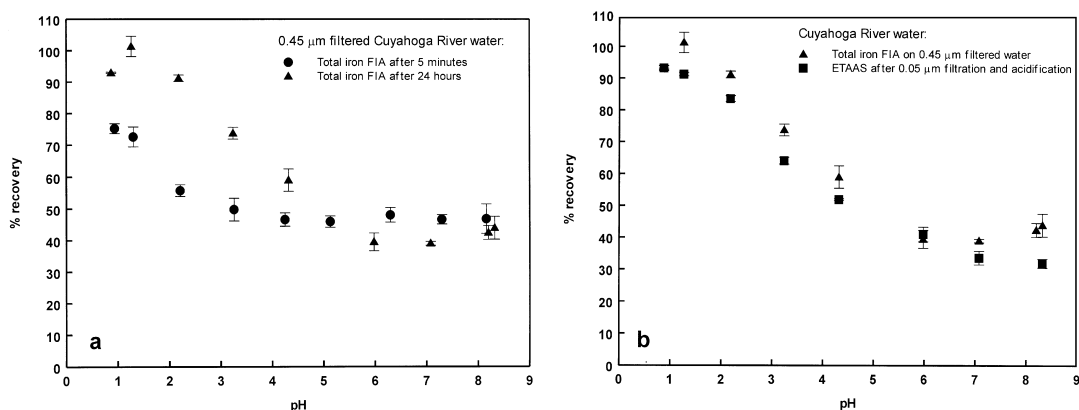


Fig. 2. The effect of sample pH on the recovery of the total iron FIA system. (a) Total iron FIA response to 0.45 µm filtered Cuyahoga River water at various pH values 5 min (●) and 24 h (▲) after pH adjustment. (b) Total iron FIA response to 0.45 µm filtered Cuyahoga River water at various pH values 24 h after pH adjustment (▲) and ETAAS analysis of the same samples after 0.05 µm filtration and acidification (■). The error bars represent 2σ for three injections.

Analytical characteristics

The analytical characteristics for the iron(II) and total dissolved iron FIA systems are nearly identical, so the total dissolved iron system figures will be reported. Quantitation using peak height gave better precision than using peak area and was used throughout this study. The relative standard deviation of five replicate injections is 2.6% at 0.10 µM, at 0.65% at 1.0 µM, and 0.34% at 10 µM. The theoretical detection limit determined as three standard deviations ($n = 5$) in the blank is 10 nM. The least concentrated standard solution that could be reliably differentiated from a blank injection was 25 nM, giving a relative standard deviation of 11.6% ($n = 5$). The system response was linear up to 300 µM. These figures of merit were determined using a 20 µl sample loop. While slight improvements in sensitivity were seen using 100 and 200 µl sample loops, the 20 µl loop provides measurements well in the range of iron concentrations found in fresh waters.

Application

The flow-injection analysis methods reported here provide significant improvements over atomic absorbance for iron analysis. The detection limit for the FIA methods were similar to that measured for ETAAS in our lab using 15 µl injections. However, the dynamic range of the FIA systems was much larger, eliminating the need for a time-consuming dilution step often required by ETAAS. This will reduce error from contamination and losses due to sorption to container walls, as well as decrease overall time of analysis. Additionally, analyzing samples at higher iron concentration provides better precision, as shown in Table 5. Furthermore, 45 and 60 injections an hour can be processed using the total dissolved iron and iron(II) FIA systems respectively,

compared to 10 per hour for the ETAAS. Finally, the FIA approach is able to provide speciation information not available using atomic spectrometry, including measurements of iron(II).

The FIA methods reported here also have advantages over other colorimetric techniques. The iron(II) FIA method does not reduce iron(III). The total dissolved iron FIA method gives good recovery in high DOC samples. It also has the unique ability to differentiate between dissolved and colloidal (0.45 to 0.05 µm) iron. The total dissolved iron FIA system should be adaptable for use as a iron-specific HPLC detector, a subject of future research.

CONCLUSIONS

The flow-injection analysis methods presented here meet the requirements for the analysis of iron in high dissolved organic carbon natural waters outlined in the introduction. The methods can discriminate between iron(II) and iron(III) as well as between dissolved and colloidal iron. The method has a low detection limit (10 nM), a wide dynamic range (10^4), and excellent precision (<1%). Environmental levels of DOC or other metals do not interfere in the analysis. These FIA methods will provide researchers interested in the behavior of iron in a wide range of environmental and treatment systems with an important tool for better understanding this important element.

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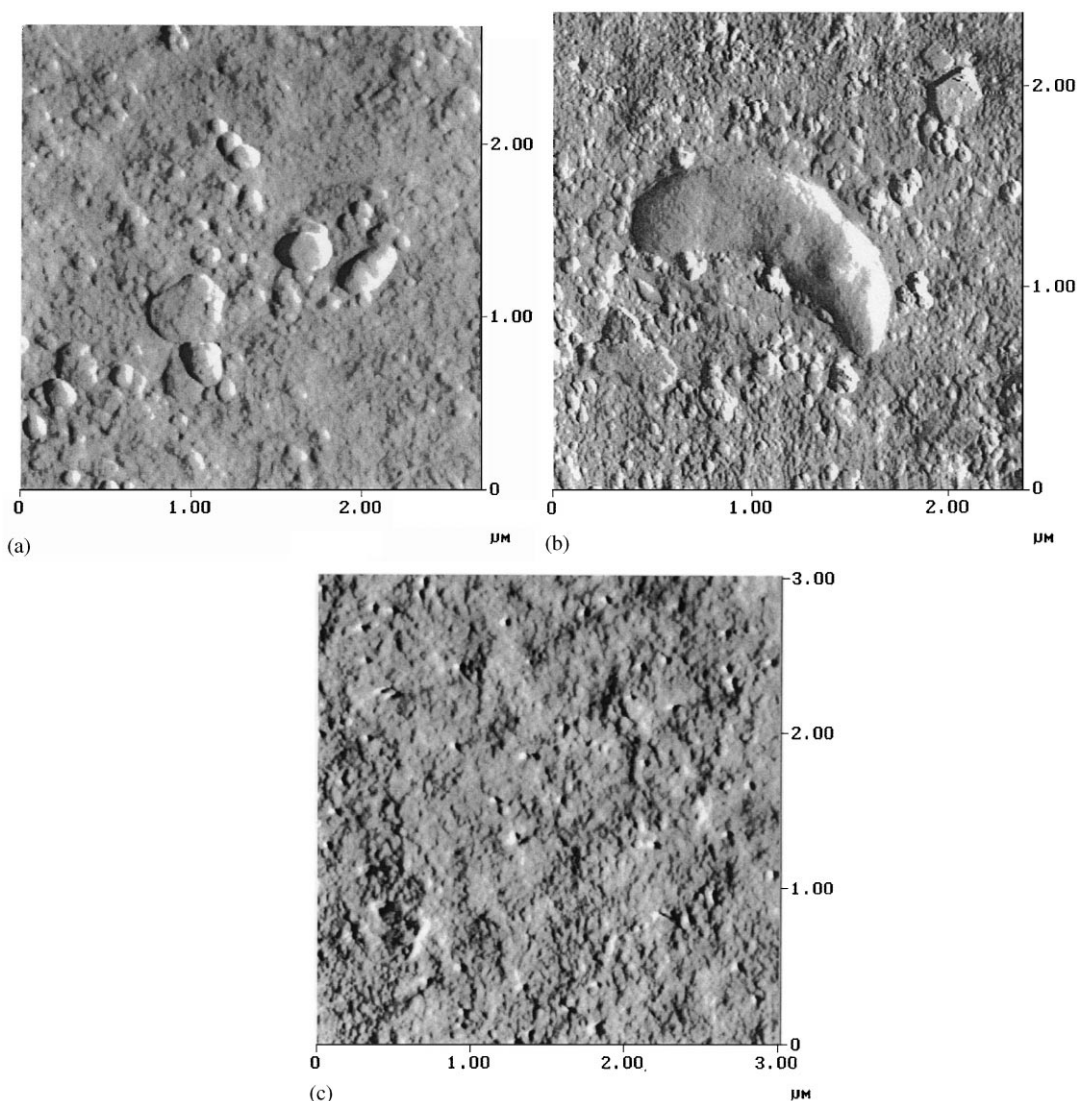


Fig. 3. Atomic force microscopy images of natural particles. $0.45\ \mu\text{m}$ filtered water from the Cuyahoga River was adjusted to pH 1.2 (a) and 8.2 (b) and then filtered using Nucleopore $0.05\ \mu\text{m}$ filters after 24 h. Tapping mode AFM images of the $0.05\ \mu\text{m}$ filters were collected in air and are displayed here in amplitude mode. The total iron FIA system does not measure iron associated with particles in this size range unless the sample is acidified prior to analysis. The moon shaped particle (b) is probably a bacterium. (c) As a control, distilled water was treated in the same manner as the river water, the $0.05\ \mu\text{m}$ pores are plainly visible.

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