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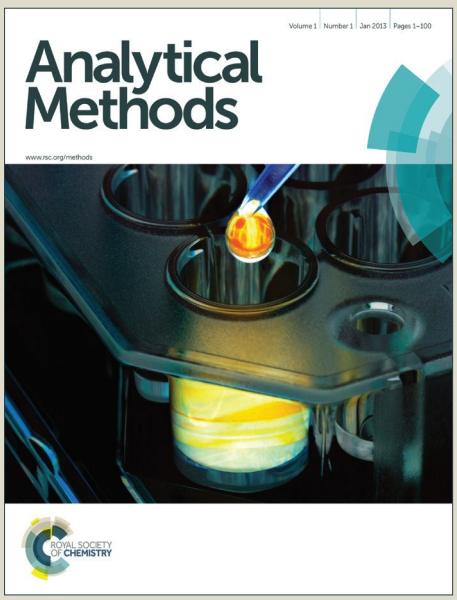

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ARTICLE

Solid phase extraction coupled with a liquid waveguide capillary cell for simultaneous redox speciation analysis of dissolved iron in estuarine and coastal waters

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A portable automatic flow injection (FI) based system incorporating on-line C18 solid phase extraction (SPE) cartridges and a 2-m long liquid waveguide capillary cell (LWCC) was established for simultaneous redox speciation analysis of dissolved iron in estuarine and coastal waters. Utilization of the SPE preconcentration and the LWCC enhanced the sensitivity of the ferrozine method for Fe(II) analysis. The Fe(II)-ferrozine complex was formed and extracted onto a C18 cartridge, and eluted with an HCl-ethanol solution for spectrophotometric detection with an LWCC. The determination of total Fe(II+III) was realized in a parallel flow channel after reduction of Fe(III) to Fe(II) with ascorbic acid. The optimal combination of pre-eluting solution and eluent was investigated to eliminate the Schlieren effect. Parameters of the FI-SPE-LWCC system were optimized based on a univariate experimental design. The effect of salinity on method sensitivity was low enough to apply the system in both estuarine and coastal waters without adjustment. The limit of detection was 0.056 nmol L^{-1} for Fe(II) and 0.096 nmol L^{-1} for Fe(II+III). A linear determination range of 0.5-50 nmol L^{-1} iron was obtained with a sample loading volume of 5 mL and a sample throughput of 6 h^{-1}. The system was successfully applied *in situ* in Wuyuan Bay, Xiamen, for the continuous monitoring of dissolved iron species for 20 h.

1. Introduction

Iron limitation constrains phytoplankton growth not only in oceanic high-nutrient, low chlorophyll regions,[1, 2] but also in coastal waters.[3, 4] Iron in seawater is traditionally differentiated into dissolved and particulate iron with a 0.2-0.45 μm filter membrane.[5] Dissolved iron (DFe) concentration in coastal waters is higher than in the open oceans. However, estuaries and coasts, which contain comprehensive processes of iron introduction and scavenging, lead to a large iron concentration gradient at the land-sea margin.[6, 7] DFe exists in two oxidation states, Fe(II) and Fe(III), in natural waters. Although Fe(III) is relatively insoluble at typically seawater pH,[8] it persists owing to its high affinity towards dissolved organic ligands.[9] In surface coastal waters, Fe(II) is maintained from the photoreduction and bioreduction of Fe(III),[10, 11] atmospheric deposition[12] and diffusion from sediments,[13] while it tends to be oxidized to the more thermodynamically stable Fe(III) in oxygenated seawater.[14] The variation of Fe(II) and Fe(III) is affected by the source, precipitation-dissolution process, and reduction-oxidation rates. To understand the role of iron in marine ecosystems, techniques for iron determination and redox speciation are important.

Traditional methods for determining iron in seawater can be classified into land-based (e.g. graphite furnace atomic absorption spectrometry,[15] inductively coupled plasma mass spectrometry[16]) and ship-based (e.g. chemiluminescence,[17] spectrophotometry,[18] cathodic stripping voltammetry[19]) analyses. The half-life time of Fe(II) in seawater is only several minutes.[20] Therefore, iron speciation analysis immediately after underway sampling is required to obtain real-time data with temporal and spatial resolution and to minimize potential contamination and redox state change. Major in-field methods for iron speciation are based on flow injection (FI) analysis, because of its easy automatic operation, high sample throughput and high precision, and include chemiluminescence,[17] N,N-dimethyl-p-phenylenediamine dihydrochloride (DPD) catalytic spectrophotometric detection[7] and ferrozine colorimetric detection.[21, 22]

Ferrozine, particularly, has been widely employed in Fe(II) determination as a strongly selective Fe(II) chelator to form a stable Fe(II)-ferrozine colored complex. Thus, Fe(II) concentration is measured in offshore waters of Peru using the ferrozine method with a preconcentration step;[13] ferrozine impregnated C18 solid phase extraction (SPE) cartridges with the FI technique is used for on-line preconcentration and determination of Fe(II) and Fe(II+III), where Fe(II+III) is the sum of the Fe(II) in the original sample and the reduced Fe(III);[21] a submersible osmotically pumped analyzer using

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ferrozine as the colorimetric reagent is adapted to obtain long-term *in situ* concentration variation of Fe(II) and Fe(II+III) in deep-sea hydrothermal vents;[23] and the sensitivity of the ferrozine method is enhanced and Fe(II) and Fe(II+III) analyzed in waters with a 2-m liquid waveguide capillary cell (LWCC) and a gas-segmented continuous flow auto-analyzer.[22]

An automatic FI-SPE-LWCC spectrophotometric system, utilizing the complexation of ferrozine and Fe(II), for simultaneous determination of Fe(II) and Fe(II+III) in estuarine and coastal waters is presented in this article. A pair of commercial C18 cartridges was installed in a 10-port valve to extract ferrozine complex with Fe(II) and Fe(II+III), respectively. A 2-m LWCC was adopted to further enhance the sensitivity of spectrophotometric detection. The instrument was controlled with software written with LabVIEW (National Instruments, USA). The results of the system performance, analytical figures of merit, and a 20 h *in situ* application in Wuyuan Bay, northeast of Xiamen, are described.

2. Experimental

2.1 Standards and reagents

Ultrapure (UP, electrical resistivity>18 MΩ cm) water used for making solutions was obtained from a Milli-Q water purification system (Millipore, USA). To eliminate contamination, a Class-100 air flow hood was set up for the preparation of reagents and standards. Reagent and standard solutions, and water samples were stored in low/high-density polyethylene bottles (LDPE/HDPE, Nalgene, USA), which were washed following the reported protocol.[5] The cap of each bottle was drilled with two small holes, one equipped with a 0.22 μm filter for particle-free air inflow and the other one for solution outflow, to prevent contamination of the solutions in use even without the clean bench.[7]

HCl solutions were prepared by diluting certain amounts of 30% (v/v) HCl (Suprapur®, Merck, Germany) in UP water. The 0.01 mol L^{-1} Fe(II) stock standard solution was made by dissolving $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (puriss p. a., 99.0%, Fluka®, Sigma-Aldrich, USA) in 0.1 mol L^{-1} HCl solution and prepared monthly. The 1000 mg L^{-1} Fe(III) stock solution (CertiPUR®, Merck, Germany) was an iron atomic absorption standard. Fe(II) and Fe(III) standard solutions were obtained from appropriate dilutions of the stock standard solutions in 0.01 mol L^{-1} HCl or acidified low iron seawater (LISW, salinity 35), which was collected from the surface of the South China Sea, filtered through a 0.2 μm membrane filter (Millipore, USA) and acidified to pH 1.7.

The 0.01 mol L^{-1} ferrozine stock solution was prepared by dissolving ferrozine ($C_{20}H_{13}N_4NaO_6S_2$, ≥97.0%, Sigma-Aldrich, USA) in UP water. For pH adjustment of the ferrozine solution, a 2.5 mol L^{-1} ammonium acetate buffer stock solution was prepared by mixing 39 mL 25% (v/v) ammonia solution (Suprapur®, Merck, Germany) and 28.5 mL glacial acetic acid (Suprapur®, Merck, Germany), adjusting the pH with 6 mol L^{-1} HCl solution and making up to 100 mL with UP water. The pH of this buffer stock solution was approximately 5.5. A 0.01 mol L^{-1} ascorbic acid solution obtained by dissolving ascorbic acid (A.R. grade, Sinopharm Co., China) in UP water was prepared weekly and stored in the dark at 4°C. The ferrozine working solution for determination of Fe(II), named FZ, containing 750 μmol L^{-1} ferrozine and 0.125 mol L^{-1} ammonium acetate was made by dilution of the ferrozine and buffer stock solution with UP water. Aliquots of 1 L FZ were added with 2.5 mL ascorbic acid solution as the reducing agent[21] to achieve another ferrozine working solution, named as FZ_{AA}, for the determination of Fe(II+III).

A 50% (v/v) ethanol (A.R. grade, Sinopharm Co., China) was used for SPE cartridge conditioning. UP water and 0.15 mol L^{-1} HCl were provided as the rinsing solution. The pre-eluting solution, named as pre-eluent, was prepared by mixing ethanol and 6 mol L^{-1} HCl to the solution containing 12.5% (v/v) ethanol and 0.8 mol L^{-1} HCl. The eluent contained 30% (v/v) ethanol and 0.3 mol L^{-1} HCl.

2.2. Apparatus

In this study, the reported FI-SPE system[24] was adopted and developed. An FIA 3110 flow injection analysis processor (Beijing Jitian Instruments Co., China) equipped with two peristaltic pumps (P1 and P2) was used to deliver samples and reagents. An 8-position selector valve (V1) and a 10-port, 2-position injection valve (V2), were obtained from VICI, Valco Instruments Co., USA. V2 was equipped with two 360 mg Sep-Pak® C18 cartridges (C1 and C2, Waters Co., USA) for the preconcentration and extraction of Fe(II) and Fe(II+III). A 2-m LWCC with 550 μm internal diameter and approximately 500 μL internal volume (Type-II, World Precision Instruments Inc., USA) was connected to a tungsten halogen lamp (LS-1-LL, Ocean Optics Inc., USA) and a miniature multichannel wavelength spectrophotometer (USB2000+, Ocean Optics Inc., USA) via two fiber optic cables.

0.8 and 1.6 mm i.d. silicone tubing (Beijing Jitian Instruments Co., China) was furnished as pump tubing. 1.0 mm i.d. polytetrafluoroethylene tubing (VICI, Valco Instruments Co., USA) was utilized as the manifold tubing and mixing coils (MC1 and MC2).

To achieve *in situ* application, an on-line sampling system was connected to the developed system. It was constructed using a 1-channel peristaltic pump (P3, MASTERFLEX®, Cole Parmer Instrument Co., USA) for delivering the surface water, a 0.45 μm membrane filter (Millipore Co., USA) for filtration, and an on-line sample acidification device for providing a 0.3 mol L^{-1} HCl solution.

2.3. Manifold and procedures

As shown in Fig. 1, the iron-ferrozine complex was continuously formed in two parallel channels. Fe(II) in S1 was merged with FZ in MC1 to form the Fe(II)-ferrozine complex, while Fe(II+III) in S2 was mixed with FZ_{AA} in MC2, reduced to Fe(II) with ascorbic acid and formed the Fe(II+III)-ferrozine complex. By shifting V1, a relevant reagent was selected and introduced to C1 or C2. V2 controlled the alternation of the sample loading and extraction procedures in each channel. When V2 was at position A (solid line in Fig. 1), reagents flowed through C1, and Fe(II+III)-ferrozine was loaded onto cartridge C2; and when at position B (dashed line in Fig. 1), the reagents were shifted to C2, and Fe(II)-ferrozine was loaded onto C1. Table 1 shows the operational procedure of the system.

The eluted iron-ferrozine complex was propelled through the LWCC for detection at 563 nm. The system was automatically controlled and the continuous output signal was recorded with software programmed in LabVIEW. Concentration of Fe(II) or Fe(II+III) was evaluated from the peak height arising during the elution step together with the calibration curve prepared. Each batch of 20 samples was inserted with a 10 nmol L^{-1} standard solution to check the measurement deviation. The Fe(III) concentration was worked out by subtracting the concentration of Fe(II) from that of Fe(II+III).

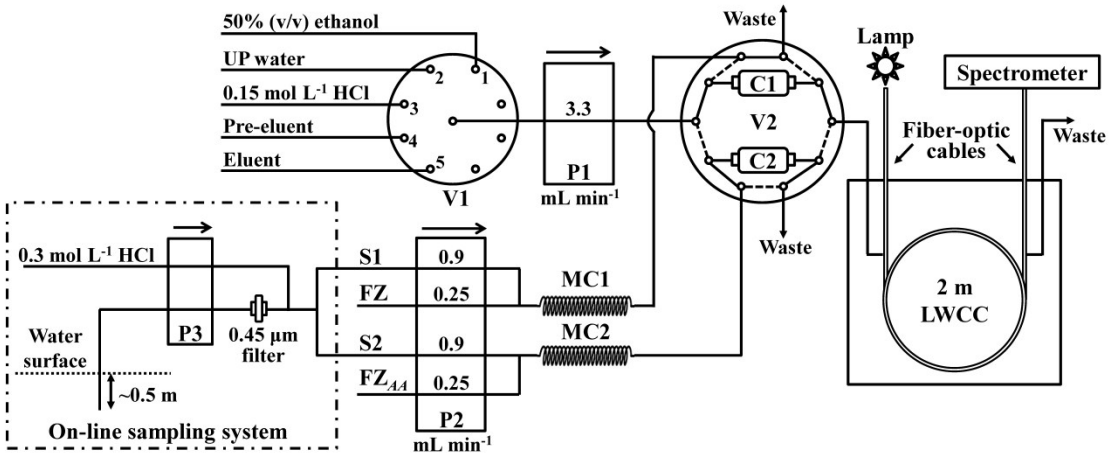

Fig. 1 Manifold configuration of the proposed method for on-line determination of Fe(II) and Fe(II+III) in waters. S1, sample or standard for Fe(II) measurement; S2, sample or standard for Fe(II+III) measurement; FZ and FZ_{AA}, ferrozine working solutions; P1-P3, peristaltic pumps; V1, 8-position selector valve; V2, 10-port, 2-position injection valve; MC1 and MC2, mixing coils; C1 and C2, C18 cartridges. The solid line in V2 represents the valve in position A, and the dashed line represents it in position B.

Table 1 Operational procedure of the FI-SPE-LWCC system

Step	Time	V1	V2	Description of C1	Description of C2
1	60 s	4	A	Pre-eluting with pre-eluent	
2	80 s	5	A	Eluting with eluent, Obtaining Fe(II)-ferrozine peak	Loading Fe(II+III)-ferrozine
3	60 s	1	A	Conditioning with 50% (v/v) ethanol	
4	60 s	2	A	Rinsing with UP water	
5	60 s	3	A	Rinsing with 0.15 mol L^{-1} HCl	
6	60 s	4	B		Pre-eluting with pre-eluent
7	80 s	5	B		Eluting with eluent, Obtaining Fe(II+III)-ferrozine peak
8	60 s	1	B	Loading Fe(II)-ferrozine	Conditioning with 50% (v/v) ethanol
9	60 s	2	B		Rinsing with UP water
10	60 s	3	B		Rinsing with 0.15 mol L^{-1} HCl

The rinsing procedure for the system was as follows: (1) At the beginning and the end of each working day, the system was rinsed with UP water; (2) Before measurement, the system ran with the solutions to condition the C18 cartridges; and (3) The LWCC was sequentially rinsed thoroughly with 1 mol L^{-1} NaOH, 1 mol L^{-1} HCl and UP water, each for 2 min, after use.[25]

The *in situ* analysis was conducted with the combination of a on-line sampling system. The P3 (see Fig. 1) was set at a proper speed to deliver the surface water sample. A 0.45 μm membrane filter was fixed in the tubing line for on-line filtration. Meanwhile, a stream of 0.3 mol L^{-1} HCl was propelled by P3 to mix with and acidify the filtered water sample. In this way, the acidified filtered water sample was continually introduced to the determination system.

3. Results and discussion

3.1 Significance of pre-eluting

The solutions for the C18 cartridge pre-eluting and iron-ferrozine complex elution have been studied and optimized.[24] However, while the FI section was altered to connect with the LWCC, the air-bubble formation and Schlieren effect at the interface of pre-eluent and eluent should be taken into consideration. The Schlieren effect caused by the refractive index differences between the reagents with different characters would introduce errors in the low concentration quantification. A suitable pre-eluent, which would neither elute the iron-ferrozine complex from the C18 cartridge nor generate air-bubbles with the eluent, was required to eliminate the problem.

Several solutions were tested as the pre-eluent. Fig. 2 illustrates the Schlieren effect signal with different groups of pre-eluent and eluent. The results indicated that the Schlieren effect was eliminated to a great extent when Group (c) was applied. As a result, a solution containing 12.5% (v/v) ethanol and 0.8 mol L^{-1} HCl was chosen as the pre-eluent.

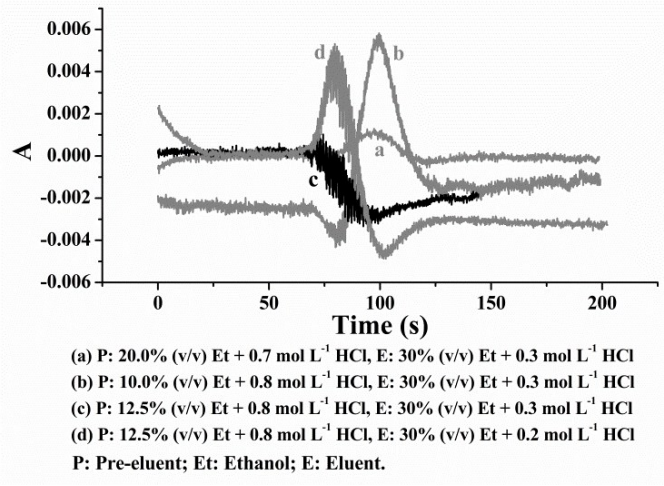

Fig. 2 Schlieren effect with different groups of pre-eluent and eluent

3.2 Optimization of method parameters

Various parameters were investigated based on a univariate experimental design with concern for the sensitivity, precision, sample throughput and peak shape to obtain the optimal operation of the developed system. A blank and a 10 nmol L^{-1} Fe(III) standard solution in 0.01 mol L^{-1} HCl were used as test samples. The parameters studied included length of mixing coil, sample loading flow rate, eluting flow rate, concentrations of ferrozine, ammonium acetate and ascorbic acid in FZ_{AA}.

The sample volume was decided based on the sample loading flow rate and time. The eluting flow rate would theoretically affect the signal peak height. With a loading sample of 5 mL, the effect of sample loading and eluting flow rate was investigated in the range 0.45-1.80 mL min^{-1} and 1.5-4.4 mL min^{-1}. Within the tested ranges, the flow rate almost did not affect the signal peak height. Finally, flow rates of 0.9 mL min^{-1} for sample loading and 3.3 mL min^{-1} for eluting were chosen to balance the column pressure and sample throughput.

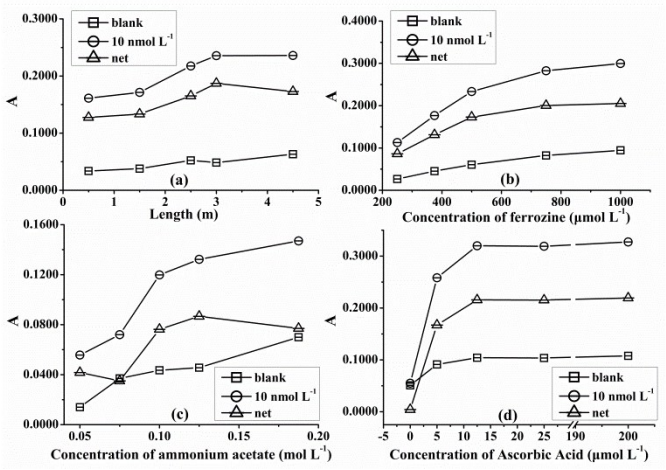

Fig. 3 Effect of (a) length of mixing coil, (b) concentration of ferrozine, (c) concentration of ammonium acetate, and (d) concentration of ascorbic acid in FZ_{AA} on the signal intensity

Fig. 3(a) reveals that increasing the length of the mixing coil from 0.5 to 3.0 m improved the signal intensity, but the absorbance did not further increase with the 4.5-m mixing coil. This was probably due to the fact that the longer mixing coil not only improved the reaction, but also increased the dispersion of the iron-ferrozine complex. For the highest net signal, a 3.0-m mixing coil was selected. In terms of the effect of the concentration of ferrozine and ammonium acetate, Fig. 3(b) and Fig. 3(c) illustrate that the higher absorbance values were obtained with higher concentrations of ferrozine and ammonium acetate. Therefore, a FZ_{AA} solution containing 750 μmol L^{-1} ferrozine and 0.125 mol L^{-1} ammonium acetate was used as the optimum for the most acceptable method sensitivity. Fig. 3(d) shows that the signals were maintained at a high value with concentrations of ascorbic acid higher than 12.5 μmol L^{-1}, indicating that this was enough to completely reduce the Fe(III) in the samples.

3.3 Interference

Before applying the system in both coastal and estuarine regions, it was necessary to study the effect of salinity on the analytical method. Two sets of calibration solutions were prepared with 0.01 mol L^{-1} HCl and LISW, and each was spiked with a series concentration of Fe(III) standards. The ratio of the slopes obtained from the two curves was used to evaluate the interference from salinity. The linear equation with a 0.01 mol L^{-1} HCl curve was A = 0.02336 C_{spiked} + 0.05378 (R^2 = 0.9994, n = 6), while the curve prepared with LISW was A = 0.02226 C_{spiked} + 0.05576 (R^2 = 0.9994, n = 6), where C was in nmol L^{-1}. The ratio of the two slopes was 0.9539, suggesting that the detection sensitivity was impervious with different sample salinities.

Co(II), Ni(II) and Cu(I) are metal ions which potentially interfere the Fe(II) determination using ferrozine. Co(II) and Ni(II) contribute a detectable inference signal only when the concentrations much exceeded Fe(II) concentration,[26, 27] which is not usual in estuarine waters.[28, 29] Although Cu(I) shows an intensive interference with the Fe(II) determination,[21, 26, 27] it is

extremely unstable and comprises only 5-10% of the total copper in natural waters.[30] The average dissolved copper concentrations in ocean and river water is at 3.9 and 23 nmol L^{-1} (0.25 and 1.5 μg L^{-1}).[31] Since in estuarine and coastal waters Cu(I) is at trace level, its interference is negligible in the normal situation. In typical cases, it is suggested that neocuprine be added into the ferrozine solution to eliminate Cu(I) interference.[21, 26, 27]

The presence of Fe(III) has no detectable interference to the reaction of Fe(II) and ferrozine.[27] Additionally, previous work[26] proves that there is no effect on the accuracy of Fe(II) determination, even when the concentration of Fe(III) is 700 times higher than Fe(II).

3.4 Analytical figures of merit

Calibration curves for Fe(II) and Fe(II+III) were obtained with the optimized parameters. The upper limit of the linear range was 50 nmol L^{-1}, which was satisfactory for the determination of most estuarine and coastal waters. The limit of detection (LOD, n = 5) was defined as the analytical concentration corresponding to three times the standard deviation of the absorbance of replicate measurement blanks. The details of merit are summarized in Table 2. Fig. 4 illustrates the elution curves of various Fe(II+III) concentrations ranging from 0 to 25.0 nmol L^{-1}.

Table 2 Analytical figures of merit of the proposed method

Characteristic	Fe(II)	Fe(II+III)
MDL (3σ, nmol L^{-1})	0.056	0.096
Linear range (nmol L^{-1})	0.5-50	0.5-50
Slope of calibration curve (with iron concentration in nmol L^{-1})	0.0204 ±0.0002	0.0216 ±0.0001
Intercept	0.0523	0.0779
Correlation coefficient (R^2, n = 5)	0.9998	1.0000

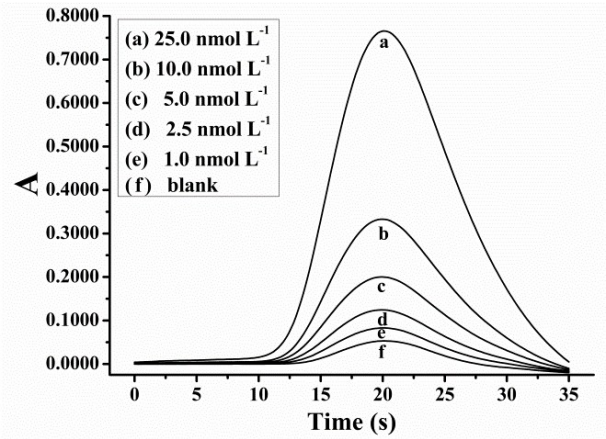

Fig. 4 Elution curves of different concentration of Fe(II+III)

The precision and parallelism of the method were evaluated through relative standard deviations (RSDs) based on repetitive determinations of water samples with different concentrations. The RSDs (n = 3) were 0.26-0.86% for 0.9-10.0 nmol L^{-1} Fe(II) and 0.50-1.94% for 0.9-24.0 nmol L^{-1} Fe(II+III). The time for sample analysis of Fe(II) and Fe(II+III) was 640 s, i.e. the sample throughput was approximately 6 h^{-1}. The performance of C18 cartridges remained steady for at least 120 environmental water sample analyses.

3.5 Validation of the proposed method

LISW and water samples collected from the Jiulongjiang Estuary were used to study the recovery. The sample salinity was measured on board. Estuarine water was collected with a towed fish sampling system previously described,[7] acidified to pH 1.7 with 6 mol L^{-1} HCl after 0.45 μm filtration, sealed in LDPE bottles and stored at 4 °C in the dark. Fe(II) standard solutions were spiked into LISW for testing Fe(II) recovery, and Fe(III) was added to the estuarine samples for Fe(II+III) recovery. The results presented in Table 3 suggested negligible matrix influence and good recovery.

Table 3 Recoveries of Fe(II) and Fe(II+III) from LISW and estuarine waters (EW) (n = 3)

Sample	Salinity	Added species	Added (nmol L^{-1})	Found (nmol L^{-1})	Recovery (%)
LISW	35.0	Fe(II)	0	0.13	—
			0.75	0.86±0.02	97.28±1.19
LISW	35.0		4.00	3.94	—
			10.00	9.61±0.21	94.42±1.71
EW	29.6	Fe(III)	0	3.64	—
			5.02	8.56±0.11	97.90±2.37
EW	24.6		0	7.33	—
			9.76	17.11±0.03	100.1±0.37

The accuracy and precision of the method were assessed using two seawater certified reference materials obtained from National Research Council of Canada: Coastal Atlantic Surface Seawater (CASS-5) and North Atlantic Surface Seawater (NASS-6). The results of the t-test shown in Table 4 indicated that the measured values well agreed (t-test) with the certified values.

Table 4 Analytical results of CASS-5 and NASS-6 (n = 3)

Sample	Certified value (nmol L^{-1})	Measured value (nmol L^{-1})	Performed t-value	Critical t-value (P=0.95)
CASS-5	25.71 ±1.96	23.97 ±0.13	1.53	2.78
NASS-6	8.84 ±0.82	8.10 ±0.07	1.56	2.78

3.6 Application

The *in situ* performance of the proposed method was carried out on a platform (N24°31′48″, E118°10′47″) located in Wuyuan Bay, Xiamen, on 12-13 August, 2013. The day-night variation of Fe(II) and Fe(II+III) in surface water is shown in Fig. 5. The calibration curves for Fe(II) and Fe(II+III) were prepared in-field before *in situ* analysis. A quality control sample was inserted every two or three hours to confirm the accuracy. The concentrations of Fe(II) and Fe(II+III) were calculated after the

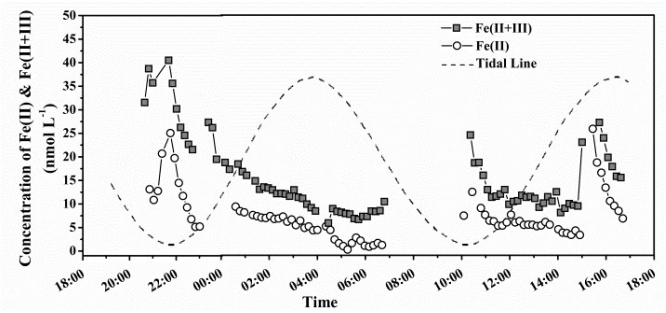

Fig. 5 Concentration variation of Fe(II) and Fe(II+III) in Wuyuan Bay. The broken line was due to clogging of the filter membrane and its changing and to instrument debugging.

whole experiment. The Fe(II) and Fe(II+III) concentrations appeared highest at low tide and decreased quickly with the rising tide. In addition to the dilution with fresh seawater, the loss of colloids could be another reason causing low concentration of DFe at high tide. It is suggested that the seawater cations neutralize the negatively charged iron oxide-organic matter colloids, resulting in flocculation and precipitation of DFe.[32] In our study, the organic matter in the bay water was rich during low tide, which kept DFe at a high concentration. During high tide the outer bay seawater with massive cations rushed into the bay, and flocculation and precipitation of iron colloids occurred and the DFe concentration decreased. The irregular concentration jump at 15:00 was caused by a visible influx of near-shore sewage.

The concentration ratio of Fe(II) to Fe(II+III) ranges from 0.03 to 0.37 in oceanic surface waters,[33] and the high fraction of Fe(II) in coastal areas could be due to photochemical and biological reduction of Fe(III),[10, 11] atmospheric deposition,[12] and diffusion from the sediments.[13] Our study revealed a range of 0.03-0.78 for 70 samples, among which the ratio was higher than 0.4 from 51 samples. Even though there was lack of other supporting information for identification of the Fe(II) source since the present study was focused on the development of iron redox speciation analysis, the data obtained supported previous results that the concentration ratio of Fe(II) to DFe increases in the surface seawaters of Boston Harbor (0.50-0.74) and Massachusetts Bay (0.35-0.46).[34]

3.7 Comparison with other ferrozine-based methods

Table 5 Summary of the ferrozine-based methods for iron determination

Analyte	Technique	Detection [a]	LOD (nmol L^{-1})	Analytical range (nmol L^{-1})	Ref.
Fe(II)/ Fe(II+III)	FIA	Vis	Fe(II): 0.6×10^3 Fe(II+III): 0.7×10^3	Fe(II)/Fe(II+III): $0.3\text{-}100\times10^3$	[35]
Fe(II+III)	*in situ* osmotic analyzer	Vis; 0.7 cm cell	0.1×10^3	50×10^3	[23]
Fe(II)	off-line 8-HQ SPE	Vis; 10 cm	not reported	$0\text{-}5\times10^3$	[13]
Fe(II)	off-line ferrozine-immobilized C18 SPE	Vis	0.6	0.6-185	[27]
Fe(II)	off-line ferrozine-immobilized C18 SPE; HPLC	UV-Vis	0.1-10	$1\text{-}5\times10^3$	[36]
Fe(II)	off-line ferrozine-immobilized C18 SPE	GFAAS	5.4	not reported	[37]
Fe(II)	off-line	Vis; 4.5 m LWCC	0.2	0.5-10	[38]
Fe(II+III)	off-line	Vis; 10 m LWCC	0.1	not reported	[39]
Fe(II)	off-line	Vis; 1/5 m LWCC	0.4	not reported	[40]
Fe(II)	off-line ferrozine-immobilized C18 SPE	Vis; 5 m LWCC	1.0	not reported	[14]
Fe(II)/ Fe(II+III)	GSCFA [b]	Vis; 2 m LWCC	Fe(II): 0.12-0.14 Fe(II+III): not reported	Fe(II) [c]: 2-50 Fe(II+III) [c]: 6-50	[22]
Fe(II)/ Fe(II+III)	reverse FIA	Vis; 2 m LWCC	Fe(II): 0.1 Fe(II+III): 0.2	Fe(II) [c]: 0.4-200 Fe(II+III) [c]: 0.8-287	[26]
Fe(II)/ Fe(II+III)	on-line C18 SPE	Vis; 3 cm cell	Fe(II): 0.1 Fe(II+III): 0.3	Fe(II): 0-10 Fe(II+III): 0-4	[21]
Fe(II+III)	multi-syringe FIA; on-line NTA Superflow	Vis; 1 m LWCC	0.89	1.4-140	[41]
Fe(II+III)	multi-syringe FIA; on-line Chelex 100	Vis; 1 m LWCC	3.6	15-268	[41]
Fe(II)/ Fe(II+III)	on-line C18 SPE; simultaneously	GFAAS	Fe(II): 1.38 Fe(II+III): 1.87	Fe(II)/Fe(II+III) [c]: 2.5-25	[24]

Table 5 (continued)

Analyte	Technique	Detection [a]	LOD (nmol L^{-1})	Analytical range (nmol L^{-1})	Ref.
Fe(II)/ Fe(II+III)	on-line C18 SPE; simultaneously	Vis; 2 m LWCC	Fe(II): 0.056 Fe(II+III): 0.096	Fe(II)/Fe(II+III): 0.5-50	This method

[a] Vis, Visible spectrophotometry; UV-Vis, Ultraviolet and visible spectrophotometry; GFAAS, Graphite furnace atomic absorption spectrophotometry.
[b] GSCFA, Gas-segmented continuous flow analysis.
[c] A broadened analytical range can be obtained by altering some parameters.

Table 5 presents a comparison of ferrozine-based methods for the determination of iron. The present work is one of the only two methods for the simultaneous determination of Fe(II) and Fe(II+III). Compared with the "land-based" method,[24] the proposed spectrophotometric method was portable and suitable for *in situ* application. Although the manifold of the FI-SPE in the present work was similar to the one in the previous study[24], the detection limit of the present one was lower by about 20 times. The proposed method combined the FI and LWCC technique, and was automatic and convenient compared with the off-line methods.[13, 14, 27, 36-40] Among the FI spectrophotometric detections with or without preconcentration,[21-23, 26, 35, 41] our work provided a reliable system with the combination of the on-line preconcentration and LWCC technique, and obtained the lowest LODs for Fe(II) and Fe(II+III).

4. Conclusions

The developed FI-SPE-LWCC system was suitable for simultaneous determination of Fe(II) and Fe(II+III) in surface estuarine and coastal waters, and salinity interference was negligible. The method had a low detection limit, good precision, and a large linear range, and the *in situ* application of the method was successful, demonstrating that the real-time redox speciation analysis of DFe using the system was practicable.

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