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Fe analysis by the ferrozine method: Adaptation to FIA towards in situ analysis in hydrothermal environment

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Abstract

The target of this study is the adaptation of the ferrozine method to flow injection analysis (FIA) to perform iron analysis in situ using an in situ chemical analyser in hydrothermal environments. The adaptation of the method to FIA was followed by its optimisation using an experimental design screening method. The goals of the optimisation steps were to decrease the detection limit and to increase the measuring range to cope with the constraints of in situ analysis. The method allows the determination of iron in the mixing zone of hydrothermal fluid, enriched in iron, and seawater. A single manifold gives the possibility to analyse either Fe(II) or ΣFe [Fe(II) + Fe(III)] in situ, or ΣFe in the lab on hydrothermal seawater samples. The measuring range of the method was increased to up to 2000 μM, which is coherent with the study of the chemical environment of communities associated with deep-sea hydrothermal activity. Finally, the method was applied in situ using the chemical analyser ALCHIMIST during the ATOS cruise on hydrothermal vent fields on the Mid Atlantic Ridge.

Keywords: Iron determination; Hydrothermal; Seawater; FIA; Experimental design; In situ analysis

1. Introduction

The chemical environment of the communities associated with deep-sea hydrothermal activity is a fluctuating medium with constant switching of cold oxygenated seawater and hot hydrothermal fluid [1–3]. Iron is a major component of the hydrothermal fluid, its concentration in pure hydrothermal fluids encountered along the Mid Atlantic Ridge (MAR) ranges from 0.002 mM in the Menez Gwen vent field to 24 mM in the Rainbow one [4]. It is removed from the medium by precipitation processes within the mixing zone, predominately associated with sulphides in plumes, to build chimneys and on conduit surfaces [5,6]. The iron particles can scavenge significant amounts of other elements including As, Pb, Po, ... [7,8]. A first approach of the metallic environment of the hydrothermal vent fauna showed a high metallic enrichment regarding to seawater and a great variability [9,10]. This metallic load can also produce an important total particle flux with high sulphur contribution and a potential effect on the organisms communities [11].

Iron appears to be a key parameter in hydrothermal environment. Its behaviour can be studied as an indicator of the chemical reactions occurring within the mixing zone and can help us in understanding the processes governing the metal bioavalability in hydrothermal ecosystems and its influence on the biological community structure in these extreme aquatic environments [12].

The ferrozine method that could detect iron up to nanomolar concentrations after preconcentration on resins [13], has been used in situ for hydrothermal plume studies [14,15] and for long term monitoring in deep-sea hydrothermal vents [16] after adaptation to flow analysis (FA). The objective of this study is the adaptation and optimisation of the ferrozine method to flow injection analysis (FIA) to perform iron analysis in situ using the chemical analyser ALCHIMIST [17], and in hydrothermal seawater samples. The targeted environment is

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the zone of mixing of hydrothermal fluid with seawater where hydrothermal organisms are thriving. The hydrothermal input in this part of the mixing zone ranges from close to 0 up to 15%, corresponding to estimated total Fe concentrations (from [4]) greater than 3 mM in the more concentrated sites of the Mid Atlantic Ridge.

The adaptation of the method was performed in two steps: the first one allowed the adaptation of the flow analysis method developed by Chin et al. [14] to FIA, the study of the selectivity of the method and the possible interferent. The optimisation steps focussed on two goals: a low detection limit to describe areas where the hydrothermal influence is low, and a wide measuring range to cope with the large chemical gradients encountered in situ and the impossibility to change the manifold or reagent concentration on an in situ analyser during at sea operation. Finally, the method was applied in situ using the chemical analyser Alchimist implemented on the French ROV Victor 6000 during the ATOS cruise on hydrothermal vent fields of the Mid Atlantic Ridge.

2. Experimental

2.1. Instrumental set up

The laboratory FIA manifold used is presented in Fig. 1. The reagents are carried by a Gilson peristaltic pump (minipuls 3 M312, 8 channels, 5 rounds/min). Tubing is made of PTFE or PEEK (Upchurch, 0.8 mm i.d., 1/16″ o.d. with PEEK Upchurch flangeless ferrules). PEEK housing injection and selection valves also come from Upchurch (V451 and V241). The detection cell is home made (polymethyl metacrylate with glass windows, $L = 3$ cm). The detector is a fibre optic spectrometer (PC1000, Ocean Optics) used with a LS-1 tungsten halogen light source (Ocean Optics). The system control, signal acquisition and processing are performed using a home made software (LabVIEW$^{\text{TM}}$). As described in Daniel et al. [18] and applied in [17,19], a pseudo-absorbance FIA signal based on a dual-wavelength measurement was calculated to overcome the Schlieren effect, produced by refractive index variation at the sample/carrier interface. Peak height was used for quantitative analysis.

The Alchimist (Analyseur chimique in situ) is a submersible chemical analyser based on FIA with colorimetric detection and in situ calibration. The principle, design and analytical performance of the instrument are described in [17]. The Alchimist, implemented on the French submersible Nautile or ROV Victor 6000, allows the simultaneous in situ measurements of two parameters (nitrate, total sulphide) in deep-sea hydrothermal environment at a rate of analysis of 22 samples per hour with a precision around 1%.

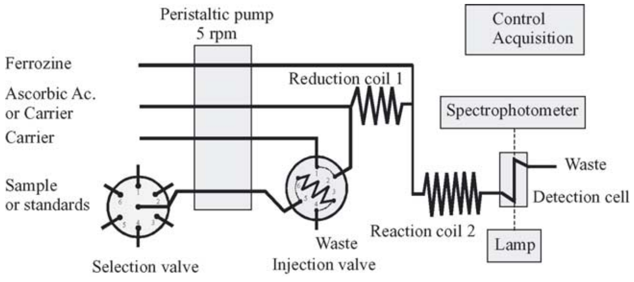

Fig. 1. FIA manifold used for the analysis of iron by the ferrozine method.

2.2. Reagents

All reagents are prepared in ultrapure water (18 MΩ, Elga Maxima or Millipore). The carrier solution is NaCl 35 g L^{-1} (Sigma, 99.5%). The ferrozine stock solution (10^{-2} M) is prepared from ferrozine disodium salt (Sigma) in an acetate buffer (0.15 M). The working solutions (10^{-3} M) are daily diluted in the acetate buffer. The acetate buffer (0.15 M) contains acetic acid (4.3 mL, Sigma, 99%) and sodium acetate (10.2 g, Sigma, minimum 99%) in 500 mL ultrapure water. The ascorbic acid solution (0.03 M) is prepared from Sigma (minimum 99%) reagent in ultrapure water. The Fe(II) stock solution (10^{-2} M) is prepared with $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (Aldrich, 99%) in ultrapure water and 1% (v/v) HCl 30% (Fluka). The Fe(III) stock solution (10^{-1} M) is prepared with $Fe(NH_4)(SO_4)_2{\cdot}12H_2O$ (Panreac) in ultrapure water and 1% (v/v) HCl 30% (Fluka). The working solutions are daily prepared by dilution in the carrier acidified to pH 2 with 1% (v/v) HCl 1 M. The interference of Mn, Zn, Cu(I) and Cu(II) were studied using the following 10^{-2} M stock solutions diluted in the carrier: $MnCl_2$ (Panreac 98%), $ZnCl_2$ (Sigma 98%), CuBr (Fluka 98%), $CuSO_4{\cdot}5H_2O$ (Merck 99%). The masking agent tried is neocuproine 10^{-3} M (Sigma). The sulphide stock solution ($Na_2S{\cdot}9H_2O$, Prolabo rectapur 98%) is 60 mM in ultrapure water. Suprapure nitric acid (Merck, 65%) is used for the storage of water samples (1/1000 v/v).

2.3. Method

Ferrozine [FZ, disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] is a complexing agent of Fe(II) and will form a magenta complex $Fe(II)(FZ)_3$ with a maximum absorbance at 562 nm. This reagent is specific to Fe(II) but the use of a reducing agent can extend the method to total Fe [$\Sigma Fe = Fe(II) + Fe(III)$]. The method has been applied to the determination of iron in freshwater [20], seawater [13,21] and in hydrothermal environments [14–16]. Possible interference are observed [22] with Cu, Co and Ni.

3. Results and discussion

3.1. Adaptation of the FA method to FIA

The FIA method presented hereafter is an adaptation of the flow analysis ferrozine method used by Chin et al. [14]. The FIA manifold for the analysis of total Fe is presented in Fig. 1 with an injection volume of 100 μL. The manifold for the measurement of Fe(II) is obtained by replacing the ascorbic acid solution by a seawater-like solution of NaCl 35 g L^{-1}. The measuring wavelength is 560 nm (the measuring wavelength of the in situ analyser Alchimist is 560 ± 5 nm), the reference one is chosen at 810 nm outside the absorption spectra of the FZ complexes [13]. The carrier used was similar to seawater to limit the differences of refractive index between the sample and carrier (NaCl 35 g L^{-1}), at a flow rate of 0.8 mL min^{-1}, the ferrozine reagent (10^{-3} M in a 0.15 M acetic/acetate buffer) was flowing at 0.4 mL min^{-1}, the ascorbic acid reducing solution was 0.03 M at 0.4 mL min^{-1} and the sample flow rate was 0.8 mL min^{-1}. These concentrations did not result from a straightforward adaptation of the concentrations used by Chin et al. [14] (FZ = 9.72 mM, buffer 2 M) that lead to noisy FIA signals typical of a non-homogeneous mixture in the reaction coil. The use of more diluted reagents overcame this problem. In a continuous flow system, potential iron contamination in the reagent stream does not contribute directly to the analytical blank, its only possible contribution is to raise the baseline signal, which was not observed in our study. After this adaptation step, the calculated limit of detection (LD) was 0.6 μM ΣFe, the repeatability and reproducibility were respectively, 2 and 1.4% for concentrations ranging from LD to 100 μM.

3.2. *Interference studies*

The possible interference of Mn, Zn, Cu(I) and Cu(II) were tested by adding these metals at the respective concentrations of 100 μM, 1 mM, 50 and 50 μM in Fe(III) standard solutions of 25–100 μM. The relative concentration of the potential interfering agent was set superior to the possible concentration encountered in different hydrothermal environments [4,23]. The analysis were done in triplicate with or without the interfering agent. The mean responses were compared by pair (with and without interfering agent) using a Fisher test and Student's *t*-test ($P = 0.05$). As reported by Chapin et al. [16], no significant interference was determined for Mn and Zn at the respective concentrations of 0.1 and 1 mM. As previously observed by King et al. [13] an interference was significant for Cu(I) and Cu(II) at the concentrations of 50 μM studied. The addition of neocuproine 10^{-3} M, reported by King et al. [13] as a masking agent, in the ferrozine reagent did not reduced significantly the Cu interference. This positive interference lies in the range 2–5% and should be negligible in natural conditions where the ratio Fe/Cu ranges from 30 to 170 in hydrothermal fluids collected in the Mid Atlantic Ridge, [4] whereas the ratio studied was 0.5–2.

The selectivity of the method towards labile FeS particles was tested by adding sulphide (0–500 μM) to Fe(II) solutions (50 μM prepared in NaCl 35 g L^{-1}). The results are presented in Fig. 2. From 0 to 100 μM of sulphide, the signal remains constant. For sulphide concentrations greater than 100 μM, the absorbance decreases rapidly and reaches a plateau. This concentration of 100 μM of sulphide also corresponds to the appearance of a black precipitate in the solution, presumably a mixture of FeS and $Fe(SH)_2$ with a fast precipitation reaction [24]. The experiment was repeated for unstirred, stirred and 0.45 μm filtered solutions. The results are identical suggesting that the method is selective to dissolved iron (<0.45 μm) and does not analyse the black precipitate of iron sulphide formed.

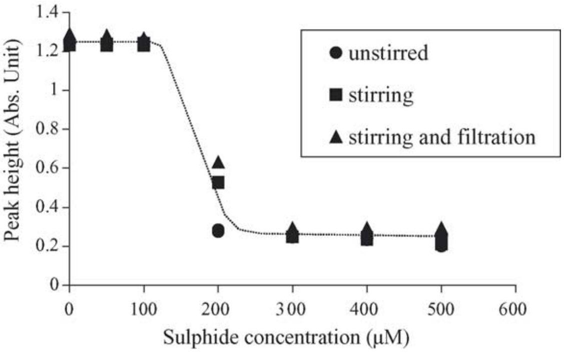

Fig. 2. Absorbance signals obtained for the injections of a solution containing 50 μM of Fe(II) (three replicates for each sulphide concentration) with increasing concentration of sulphide. The solution was unstirred, stirred or stirred and filtrated before injection.

The effect of temperature on the method was tested at 2 and 25 °C by keeping the reagents, standards and manifold in a thermostated bath. The observed effect is a slight increase of the signal (4%) when increasing the temperature from 2 to 25 °C which is coherent with the positive effect of temperature on the overall complexation rate constant of Fe(II) by Ferrozine in seawater [25]. This limited effect does not justify the in situ use of a thermostated manifold.

3.3. *Optimisation of the method*

After the first adaptation step, an experimental design was run to screen the effects of 10 factors potentially affecting the experimental response using the ΣFe manifold. Screening experimental designs are an efficient way, with a minimal number of runs, of determining the important factors influencing a method. This approach has been efficient for the adaptation of the procedure for the analysis of total sulphide by FIA on the ALCHIMIST [19]. The dual aim was to decrease the detection limit and to enlarge the measuring range with a single manifold and reagent concentrations. The software used was Statgraphics®plus. The optimisation was done using a screening design (64th fraction) with 10 experimental factors and three response variables. The design has to be run in three blocks, corresponding to 57 randomised runs (39 error degrees of freedom). The levels of the 10 experimental factors are presented in Table 1. Central points were used to estimate the repeatability and the possible drift of the method. Each run consisted in the injection of 25 and 75 μM Fe(III) standard solutions. The peaks obtained were processed to obtain three response variables: the standard deviation of the baseline signal (*Baseline* S.D., indicative of the noise of the signal, calculated on 10 points), the slopes (maximum peak

Table 1
Description and levels of the experimental factors studied through the experimental design

Experimental factor	Unit	High level	Low level	Central point
Ferrozine concentration	M	5×10^{-3}	5×10^{-4}	2.75×10^{-3}
Buffer concentration	M	0.5	0.1	0.3
Ferrozine flow rate	mL min^{-1}	1.1	0.44	0.92
Ascorbic acid concentration	M	0.1	0.01	0.05
Ascorbic acid flow rate	mL min^{-1}	1.1	0.44	0.92
Carrier flow rate	mL min^{-1}	1.1	0.44	0.92
Injection volume	μL	200	100	150
Sample pH		2	1	1.5
Reaction coil 2 length	cm	150	50	100
Reduction coil 1 length	cm	150	50	100

height divided by concentration) corresponding to the signal obtained after the injection of 25 and 75 μM Fe(III) standard solutions (respectively, *Slope* 25 and *Slope* 75).

During the course of the experiment, the standard deviation of the baseline and the slopes ranged from 0.0005 to 0.087 absorbance unit and 0.006 to 0.031 absorbance unit μmol^{-1}, respectively. These values highlight the variability of the response when changing the experimental conditions with an increase up to five-fold of the method sensitivity. The effects were calculated (Table 2) and their statistical significance tested by the software using an analysis of variance. The bold values in Table 2 pointed out the significant effects at the 95% confidence level. The average is the mean of the 57 observed responses. When changing an experimental factor from its low level to its high level, a negative effect will decrease the average response of its estimated value whereas a positive one will increase it.

The *concentration of ferrozine* has a significant effect on the baseline (−171%) and on the sensitivity of the method (+8 to 18%). Though the overall complexation rate constant of Fe(II) by ferrozine is not dependant on the initial ferrozine concentration, Lin and Kester [25] observed that an excess of complexing reagent will assure a complete Fe(II) complexation and avoid a decrease in Fe(II)(FZ)$_3$ complex due to dissociation.

The *buffer concentration* has a positive effect on the *Baseline* S.D. This increase in the signal noise can be related to differences in refractive index of concentrated solutions not corrected by the two-wavelength method or by incomplete mixing in the reaction coils. The low level buffer concentration used is sufficient to keep the pH of the reaction mixture around a measured value of 5.1, i.e. below 7.5 where the overall complexation rate constant of Fe(II) by ferrozine is roughly constant [25].

The *Ferrozine* and *ascorbic acid flow rates* have negative effect on the sensitivity of the method by dilution of the iron sample in the reagent flow. Conversely, The *carrier flow rate* has a positive effect on the signal obtained, by increasing this time the ratio sample/reagent. This ratio sample (in the carrier)/reagent ranges from 0.33 when all the flow rates are equal (0.44 mL min^{-1}, low level) to 0.55 when the Ferrozine and ascorbic acid flow rates are set to the low level (0.44 mL min^{-1}) and the carrier one to the high level (1.1 mL min^{-1}). Unfortunately, this positive effect was offset by a negative effect on the baseline signal. High flow rates might decrease the homogeneity of the mixture or the stability of the flow.

The *concentration of ascorbic acid* has no significant effect on the responses studied, suggesting that its amount is sufficient to reduce the Fe(III) present.

Table 2
Estimated effects for each experimental factor

	Baseline S.D.		*Slope* 25		*Slope* 75		Optimum value
	Effect	%	Effect	%	Effect	%	
Average	0.0062		0.0167		0.0125		
Ferrozine conc.	**−0.0106**	**−171**	**0.0013**	**8**	**0.0022**	**18**	5×10^{-3} M
Buffer conc.	**0.0118**	**190**	−0.0007	−4	−0.0005	−4	0.1 M
Ferrozine flow rate	0.0021	34	**−0.0014**	**−8**	−0.0003	−2	0.44 mL min^{-1}
Ascorbic acid conc.	0.0021	34	0.0000	0	−0.00006	−1	0.01 M
Ascorbic acid flow rate	−0.0013	−31	**−0.0034**	**−20**	**−0.0023**	**−18**	0.44 mL min^{-1}
Carrier flow rate	**0.0105**	**169**	**0.0050**	**30**	**0.0029**	**23**	1.1 mL min^{-1}
Injection volume	**−0.0119**	**−192**	**0.0072**	**43**	**0.0049**	**39**	200 μL
Sample pH	−0.0014	−23	**−0.0056**	**−34**	−0.0004	−3	2
Reaction coil 2 L	**−0.0122**	**−197**	0.0007	4	0.0009	7	150 cm
Reduction coil 1 L	**−0.0106**	**−171**	0.0006	4	−0.00004	0	150 cm

The bold values represent significant effects at the 95% level (2σ). The standard errors (1σ) are 0.0029 for the *baseline* S.D., 0.0005 for the *Slope* 25 and 0.0006 for the *Slope* 75. The relative effect (%) is related to the average value.

The *injection volume* has an expected positive effect [26,27] with a signal increase close to 40% when injecting 100–200 μL of sample.

The *sample* pH has a significant negative effect (−34%) only on the slope obtained when injecting a 25 μM standard. The percentage of Fe(II) complexed by the ferrozine was observed to be dependant of the FZ concentration and the sample pH [25] whereas a lowering of the sample pH could increase the yield of the complexation reaction. However, as sample pH lower than 0.9 led to an important peak distortion and as water samples are stored acidified at pH 2 prior analysis, we stayed at this working pH.

The length of the *reaction and reduction coils* have no significant effect on the signal as observed yet by [27]. The increase of the dispersion (flattening of the peak) produced by the increase of residence time in the reaction coil may be offset by an increase of the percentage of Fe(II) complexed by the FZ. The increase of the dispersion is a negative effect when speaking of the signal but a positive one when speaking of the noise with a significant decrease of the standard deviation of the baseline.

The best values of the experimental factors decreasing the standard deviation of the baseline and increasing the sensitivity are highlighted in Table 2. Opposite effects were observed for the factor "*carrier flow rate*", the sensitivity of the method obtained with the high level was privileged.

A closer investigation of the two factors showing a significant effect on the response (ferrozine concentration and carrier flow rate) was performed using a "response surface design" to map the absorbance versus the influent factors and to determine which combination of the factors will lead to the best response. The design was performed with seven triplicate injections of a 50 μM Fe(III) standard solution at various FZ concentrations (2.5–12.5 mM) and carrier flow rates (1.1–2.2 mL min^{-1}) tested through a Simplex approach. An analysis of variance on the results confirmed the statistical significance of the effect of each factor (95% confidence level) with no significant interaction between the two factors. The estimated response surface is presented in Fig. 3, the R-squared statistic obtained indicates that the model as fitted explains 99.9% of the variability in the absorbance. From this model, the combination of factor levels which will maximize the absorbance over the studied region is a FZ concentration of 11 mM and a carrier flow rate of 1.9 mL min^{-1}. The observation of the standard deviation of the baseline was done in the same time with no significant effect estimated on the studied region.

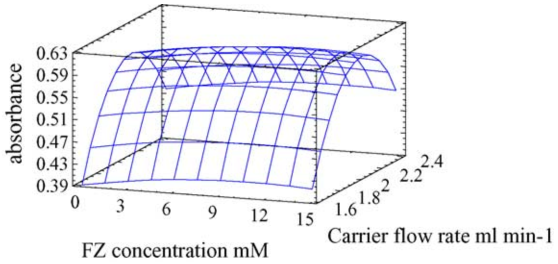

Fig. 3. Estimated absorbance surface vs. FZ concentration and carrier flow rate obtained from the optimisation experiment.

The analytical figures of merit before and after the optimisation step are compared in Table 3, line (1), (2) and (3), respectively. The optimisation steps resulted in a significant increase in the performance of the method regarding both detection limit and sensitivity. The detection limit of the method has been slightly decreased compared to [16,27]. The method is not linear on the whole studied range, both for Fe(II) and ΣFe measurements as observed by [16,27] but non-linear calibration can be performed. The same conditions were applied for the analysis of Fe(II) by removing the ascorbic acid channel and replacing it by NaCl 35 g L^{-1} with identical results (Table 3). The removal of this NaCl channel in a manifold dedicated to the analysis of Fe(II) could limit the dilution of the sample by merging NaCl possibly resulting in a lower detection limit for this parameter.

Table 3
Analytical figure of merit of the method after the adaptation and optimisation steps

	Manifold	LD[a] (μM)	LoQ[b] (μM)	Repeatability[c] (%)	Reproducibility[d] (%)	Range (μM)
(1)	Initial ΣFe	0.6	–	2	1.4	LoQ-100
(2)	ΣFe	0.07	0.3	0.8	0.6	0.3–100
(3)	Fe(II)	0.06	0.3	0.2–0.4	0.6	0.3–100
(4)	Fe(III)[e], 2/37 s	–	–	0.8–5		Up to 2000
(5)	Fe(II)[f] or ΣFe	0.1			<2	LD-50
(6)	ΣFe[g]	0.2	0.7	0.3		LoQ-109

[a] The limit of detection (LD) was calculated after the analysis of 20 blanks followed by a calibration experiment. LD = 3 × S.D. (20 blanks) × slope of the calibration curve.

[b] The limit of quantification (LoQ) was estimated by injections ($n = 5$) of standards with decreasing concentrations. The LoQ corresponds to a signal greater or equal to three times the blank signal.

[c] $n = 5$.

[d] Injections of five solutions of 50 μM [Fe].

[e] Injection 2 s, processing of the peak height at 37 s.

[f] [16] using a 0.7 cm pathlength.

[g] [27] in fresh water.

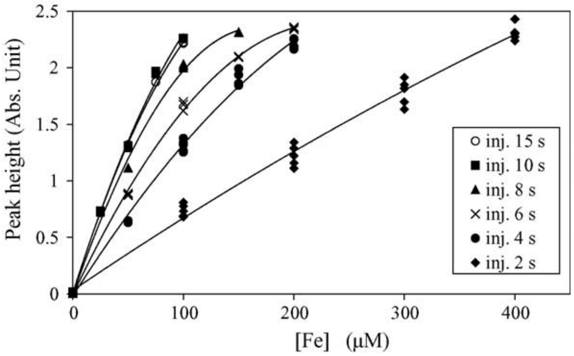

Fig. 4. Calibration experiments ($n = 5$ injections for each concentration) with partial injection of the sample loop.

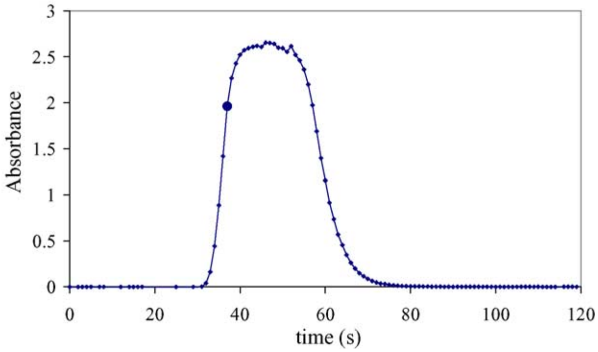

Fig. 5. Absorbance vs. time obtained for the partial injection of a 1500 μM Fe(II) standard solution. The bold dot corresponds to the absorbance value 37 s after injection.

3.4. Optimisation of the measuring range

The method used with the experimental conditions presented in this paper is characterised by its short measuring range ([16,27]), which appears to be a limiting property when dealing with in situ analysis. Furthermore, the operational constraints of work in the deep-sea using submersible prevent to modify in situ the chemical manifold or the reagents concentration. To describe the chemical gradients encountered in hydrothermal ecosystems, the requirement is to work with a single manifold (e.g. without changing the sample injection volume) and operating conditions, at least during a single submersible dive. Saturation of the detection signal is observed for iron concentrations around 120 μM. To increase the measuring range and thus, the efficiency of the in situ analyser, two alternative methods were tested using the same manifold and experimental conditions. The first one is based on the partial injection of the sample loop [19]. The injection duration was set from 2 to 15 s (corresponding to 30 and 100% respectively, of the total volume injected) during calibration experiments (0–500 μM). Two seconds is the minimum time required to allow a correct switching of the injection valve. The partial injection allowed to increase the measuring range of the method up to 400 μM corresponding to an injection lasting 2 s (Fig. 4). The side effect of this technique is an increase of the standard deviation observed for five injections from 0.4 to 5%. The use of a sample loop guarantees the repeatability of the volume injected whereas the partial injection introduces an additional uncertainty related to the injection time, characteristic of the instrumental set up.

The second method is based on the alternate processing of saturated peaks. The response variable used is no longer the peak height but the value of absorbance at a determined time after the injection in the unsaturated ascending or descending part of the peak. The absorbance was measured for the partial injection (injection duration 2 s) during calibration experiments (0–2000 μM) on the ascending part of the peak 37 s after the injection (Fig. 5). The results are presented in Fig. 6. For a 400 μM injection, the peak was saturated at its maximum whereas the absorbance measured at 37 s were still exploitable up to 2000 μM and can be fitted to a polynomial regression curve. The relative standard deviation ($n = 5$) varied on the whole range from 0.8 to 5%. The use of the signal in the ascending part of the peak decreases the repeatability of the method but greatly enhances its measuring range.

The combination of those two methods based on partial injection and processing the unsaturated part of the peak allowed to increase the measuring range of the method from LoQ-100 μM to at least 2000 μM without changing the manifold or experimental conditions which is particularly valuable for in situ measurements. The figure of merit of the method are presented in Table 3(4). This work allowed a significant improvement of the method both in term of detection limit and measuring range when compared to the method used by [27] in wastewater or in identical environment by [16].

3.5. Method validation

Finally, discrete samples obtained during the ATOS cruise were analysed in duplicate using the laboratory FIA method and ICP-AES (Vista Pro, VARIAN, Centre Commun d'Analyses, Université de La Rochelle, France). The cross

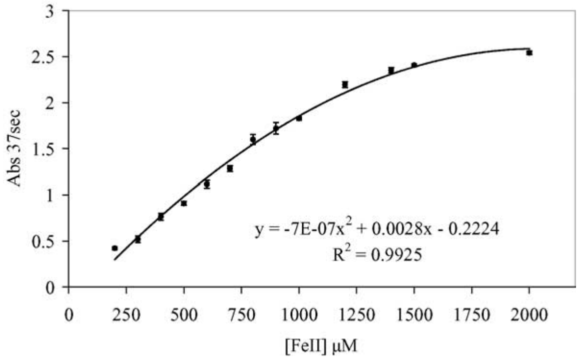

Fig. 6. Calibration experiments ($n = 3$ injections for each concentration, partial injection 2 s.). The response variable used is the absorbance measured 37 s after the injection in the ascending part of the peak. The error bars represent the standard deviation for five injections.

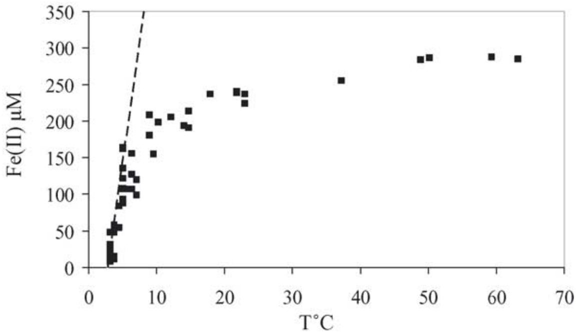

Fig. 7. in situ Fe(II) concentrations vs. temperature obtained with the ALCHIMIST on the Rainbow hydrothermal vent field. Temperature is used as a semi conservative tracer of dilution. The dotted line represents the estimated concentration obtained by a single dilution of the pure fluid (derived from [4]) by seawater.

comparison of the results obtained was linearly fitted on the two concentration ranges studied ($[\mathrm{Fe}]_{\mathrm{FIA}} = a \times [\mathrm{Fe}]_{\mathrm{ICP}} + b$, $a = 0.991$ and 1.09, $b = -0.42$ and -7.5, $R^2 = 0.991$ and 0.999, concentration range 0–0.2 and 0–2 mM dissolved ΣFe, respectively) and validates the accuracy of the data obtained by the Ferrozine method.

3.6. Application

The method was tested in situ during the ATOS cruise on the N/O L'Atalante [28]. This cruise was part of an interdisciplinary study focussed on the fluid/seawater mixing zone on the walls of sulphide edifices. The study was performed on three active hydrothermal vent fields distributed from 36°13′N to 37°51′ on the Mid Atlantic Ridge. The in situ chemical analyser ALCHIMIST implemented on the ROV Victor 6000, enabled to determine simultaneously total sulphide and Fe(II) contents. The analyser sample inlet was associated to the manipulated temperature probe of Victor.

The Fe(II)/T gradient obtained using the ALCHIMIST on the Rainbow hydrothermal vent field, is presented in Fig. 7, bearing in mind that the chemical species measured by the ALCHIMIST is the reduced form of iron whereas the concentrations given in the literature are total iron. Temperature (as pH or dissolved manganese) should be physically unaffected during the mixing process and is often used as a semi conservative tracer of the dilution of the hydrothermal fluid by seawater.

The data obtained allowed us to characterize the Fe(II)/temperature gradients prevailing in the studied site. The Fe(II)/T gradient obtained in Rainbow is lower than the theoretical one derived from a simple dilution process, especially in the hot part of the mixing zone. This departure from the dilution process can account for an important Fe(II) loss in the mixing zone. Iron is lost in the near field plume mainly as a result of large grained iron sulfide particle fall out [7,29]. The chemical characteristics of the habitat cannot be estimated directly from the dilution of the pure vent fluid in seawater. This estimation would not take into account the biotic and abiotic processes occurring in the subsurface and within the highly reactive mixing zone characterized by non-equilibrium and pressure/temperature dependant chemical systems [9,12,30–33]. In situ characterisation of the medium surrounding the communities thus provides the more relevant way to describe and compare the different habitats and to understand the chemical gradients encountered in hydrothermal vents.

4. Conclusion

The method described in this study allows the determination of iron [Fe(II) and ΣFe] in the mixing zone of hydrothermal fluid, enriched in iron, and seawater. A single manifold gives the possibility to analyse either Fe(II) or ΣFe in situ or ΣFe in hydrothermal seawater samples. The measuring range of the method was increased to LoQ-2000 µM, which is coherent with the study of the chemical environment of communities associated with deep-sea hydrothermal activity. This increase of the measuring range, keeping the same manifold and experimental conditions, appears to be of prime importance when dealing with in situ analysis in hydrothermal environment.

The method was applied in situ using the ALCHIMIST on the Rainbow hydrothermal vent on the Mid Atlantic ridge to characterize the mixing zone. Further processing of the data are in progress to understand the chemical processes governing the Fe/S reactions occurring as well as the characterisation and analysis of the dissolved and particulate fractions of water samples collected on the same areas that will enrich our understanding of the metal behaviour and bioavailability in the mixing zone.

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