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The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters

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Abstract

The original ferrozine method has been modified to sequentially determine the Fe(II)/Fe(III) speciation in small volumes of fresh and marine water samples, at the submicromolar level. Spectrophotometric analyses of the Fe(II)–ferrozine complex are performed on a single aliquot before and after a reduction step with hydroxylamine. The procedure is calibrated using Fe(III) standards stable under normal conditions of analysis. It is shown also that the presence of high concentrations of dissolved NOM (natural organic matter) do not create any significant artifacts. The method was used to measure Fe(II) and Fe(III) depth distribution in salt marsh pore waters and in a stratified marine basin.

Keywords: Iron speciation; Fresh and marine water; Spectrophotometry; Ferrozine

1. Introduction

Determination of the concentration distribution of soluble reactive species is key to understanding biogeochemical processes in natural settings. Iron is one of the most reactive elements in aquatic environments, and its cycling is coupled to that of the major biogeochemical elements (C, O, S and P) and trace elements such as heavy metals (Tessier and Campbell, 1988). It is present in the hydrosphere under two oxidation states, II and III, which are thermodynamically stable under anoxic and oxic conditions, respectively. Fe(III) forms complexes with organic acids and oxyhydroxide colloids. The latter may aggregate into larger particles (Davison, 1993). Fe(III) oxyhydroxides undergo reductive dissolution in most aquatic sediments (Van Cappellen and Gaillard, 1996). The reductive dissolution can be coupled directly to the oxidation of organic matter by specialized bacteria (DiChristina and Delong, 1993), or it may proceed via abiotic reactions with inorganic or organic reductants (Van Cappellen and Wang, 1996).

The usual methods for collecting sediment pore waters (dialysis, core extraction, diffusive equilibration in thin films) for the determination of dissolved chemical parameters (Carignan, 1984; Janhke et al., 1986; Davison et al., 1994) typically yield small sample volumes (200 μl to 20 ml). The spectrophotometric measurements of Fe(II) and total Fe (i.e. Fe(II)+-

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Fe(III) species) are generally performed on two separate sample fractions (Fadrus and Maly, 1975). Electroactive Fe(II) concentration is also measured by voltametric methods complemented by an independent determination of total Fe (Buffle et al., 1987). More recently, Elrod et al. (1991) developed a method of detection based on the work of Yamada et al. (1985) to measure subnanomolar levels of Fe(II) and Fe(III) (after reduction to Fe(II) with ascorbic acid) in ocean water by flow injection analysis, coupled to a chemiluminescence detector. A major drawback of these approaches is the requirement for a separate analysis of dissolved Fe(II) and total dissolved Fe (Fe(III) is calculated by difference).

The concentrations of total Fe measured on filtered samples (0.45 μm pore size) from anoxic environments are generally interpreted as representing the concentrations of Fe(II) species (e.g. Albrechtensen and Christensen, 1994). However, O_2 contamination during sampling and filtration may induce fast precipitation of Fe(III) oxyhydroxides and decrease the dissolved Fe(II) concentration. Moreover, when collecting natural waters, contamination by colloidal particles may occur. The preservation of samples by acidification may cause the dissolution of these particles, and hence increase the total dissolved Fe concentration. These two effects are not reproducible and emphasize the need for an immediate speciation measurement, preferably on a single sample fraction.

The authors have designed an experimental protocol to work with sample volumes of 1 ml or less in order to determine the dissolved concentrations of Fe(II) and Fe(III) at the submicromolar level. The method, suitable for field work, consists of recording the absorbances of an Fe(II) colored complex before and after an Fe(III) reduction step.

2. Theory

The ferrozine (monosodium salt hydrate of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid) reagent proposed by Stookey (1970) which reacts with divalent Fe to form a stable magenta complex species is used. The maximum absorbance is recorded at 562 nm and yields, between pH 4 and 9, a molar absorption coefficient close to 30,000 l mol^{-1} cm^{-1}. When Fe(III) is also present in solution (as a true dissolved complex or as colloids with diameters less than the filtration membrane pore size) it can react with ferrozine, thereby interfering with the coloration of the ferrous complex (Siffert, 1989). A mixture of dissolved Fe(II) and Fe(III) reacting with the ferrozine leads to the following absorbance:

$$A_1 = \varepsilon_{\mathrm{Fe(II)}} l C_{\mathrm{Fe(II)}} + \varepsilon_{\mathrm{Fe(III)}} l C_{\mathrm{Fe(III)}} \quad (1)$$

where A_1 is the measured absorbance before the reduction step, $\varepsilon_{\mathrm{Fe(II)}}$ and $\varepsilon_{\mathrm{Fe(III)}}$ are molar absorption coefficients, l is the optic path length and $C_{\mathrm{Fe(II)}}$ and $C_{\mathrm{Fe(III)}}$ are the Fe species concentrations.

After addition of a reducing agent and a buffer, Fe(III) reduces to Fe(II), thereby increasing the concentration of Fe(II)–ferrozine complex in solution. The additive property of the Lambert–Beer law yields the absorbance:

$$A_2 = \varepsilon_{\mathrm{Fe(II)}} l (C_{\mathrm{Fe(II)}} + C_{\mathrm{Fe(III)}}) \alpha \quad (2)$$

where A_2 is the measured absorbance after the reduction step and α is the dilution factor due to addition of the reducing agent and buffer. The simple linear system of Eqs. (1) and (2) is solved for the Fe(II) and Fe(III) concentrations:

$$C_{\mathrm{Fe(II)}} = \frac{A_1 \varepsilon_{\mathrm{Fe(II)}} l\alpha - A_2 \varepsilon_{\mathrm{Fe(III)}} l}{\varepsilon_{\mathrm{Fe(II)}} l\alpha (\varepsilon_{\mathrm{Fe(II)}} l - \varepsilon_{\mathrm{Fe(III)}} l)} \quad (3)$$

$$C_{\mathrm{Fe(III)}} = \frac{A_2 - A_1 \alpha}{\alpha (\varepsilon_{\mathrm{Fe(II)}} l - \varepsilon_{\mathrm{Fe(III)}} l)} \quad (4)$$

Calibration curves with Fe(III) standards allow one to derive the values of $\varepsilon_{\mathrm{Fe(II)}} l$ at A_1 and $\varepsilon_{\mathrm{Fe(II)}} l\alpha$ at A_2 (Fig. 1). Due to the successive additions of reagents during the procedure, the uncertainty on the calculated value of α may become important. However, an accurate value of α can be obtained directly during the procedure: one has to repeat the reduction step for the standards and record A_2'. α is the coefficient of proportionality between A_2' and A_2.

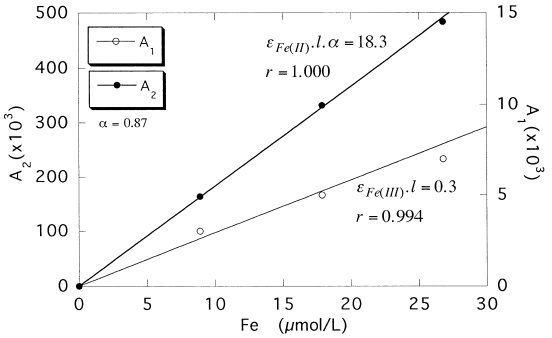

Fig. 1. Calibration curves (absorbance vs. concentration) with Fe(III) standards determined before A_1 and after the reduction step A_2.

3. Experimental

3.1. Reagents

All solutions were prepared in water purified with a NANOpure II deionization system (Barnstead Corp.). The following reagents were used as received:

1. *Ferrozine* (FW 492.47, 97%, Aldrich #16,060-1): 10^{-2} mol/l prepared in an *ammonium acetate* (CH_3COONH_4, Aldrich #37,233-1, 99.999%) solution of 10^{-1} mol/l.
2. Reducing agent — *hydroxylamine hydrochloride* ($H_2NOH.HCl$, 99.9999%, Aldrich #37, 992-1): 1.4 mol/l prepared in a solution of analytical grade hydrochloric acid 2 mol/l.
3. Buffer — *ammonium acetate*: a 10 mol/l solution adjusted to pH 9.5 with a solution of *ammonium hydroxide* (28–30%, NH_4OH, JT Baker #9721-02).

Standards are prepared from a 1000 μg ml^{-1} Fe(III) stock solution (1.786 × 10^{-2} mol/l of $FeCl_3$ in HCl 10^{-2} mol/l) diluted in a NaCl solution matching sample salinity (only deionized water in case of freshwater samples).

3.2. Procedure

(1) A_1 is recorded after adding 1 ml of filtered sample or standard to 100 μl of reagent A. (2) Reduction step: a known volume (e.g. 800 μl) of the mixture is then added to 150 μl of reagent B. The solution is allowed to react for 10 min to complete the reduction of Fe(III). (3) 50 μl of reagent C is added and A_2 is recorded. (4) Steps (2) and (3) are repeated for the standards; A_2' is measured and α is calculated. In the procedure above α is close to 0.8.

In the experiment, the sample volume of 1 ml is constrained by the volume necessary for the quartz spectrophotometric cell. It can be considerably reduced by immediate sample dilution at step (1), as long as the Fe concentration is sufficient.

4. Results and discussion

4.1. Verification of the theory

Fe(III) has long been considered to interfere with Fe(II) determinations (Siffert, 1989) and was consequently masked by addition of strong ligands such F^-, NTA or EDTA. Fig. 1 shows calibration curves with Fe(III) standards for steps (1) to (3) of the procedure (recording of A_1 and A_2). Though the A_1 absorbance measurements are small, the response of the Fe(III)–ferrozine mixture is linear within the range of the standards, hence making it possible to account for the contribution of Fe(III) to the absorbance.

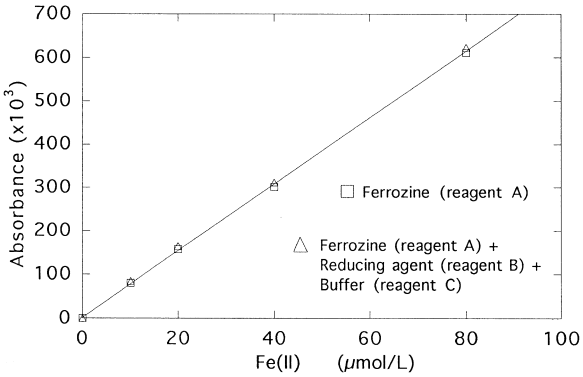

Fig. 2. Calibration curves of Fe(II) standards performed with (reagents A, B and C) and without (only reagent A) the reduction step. The dilution factor (α) is the same for both cases. Reagents B and C do not interfere with the Fe(II)–ferrozine complex.

It was also verified that the ferrozine complex is not affected by the successive additions of reagents. Standards made with $FeSO_4$ prepared in deaerated water were added in the same volume proportion to either the ferrozine reagent (A) alone, or the ferrozine reagent (A) + the reducing agent (reagent B) + the buffer (C). The two calibration lines superpose almost perfectly indicating no significant interfering effects (Fig. 2).

In a 1:1 Fe(II)–Fe(III) mixture, successive measurements over a period of 90 min showed an increase of 3% over initial absorbance (Fig. 3). Hence, better accuracy is achieved in the first step of the procedure by recording the absorbance within 10 min of adding the ferrozine reagent. Variation of the absorbance A_1 of the Fe(II)–ferrozine complex was found to remain under 1% for periods of up to 3 h.

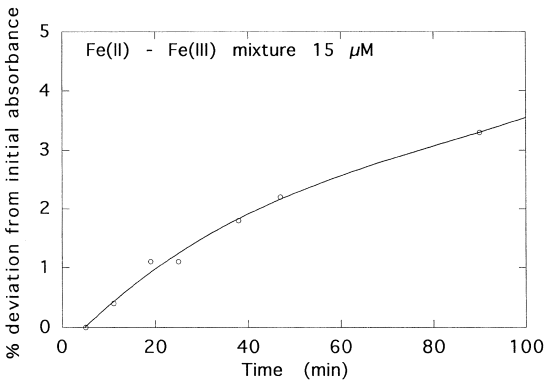

Fig. 3. Relative change of the absorbance of a Fe(II)/Fe(III) mixture with time.

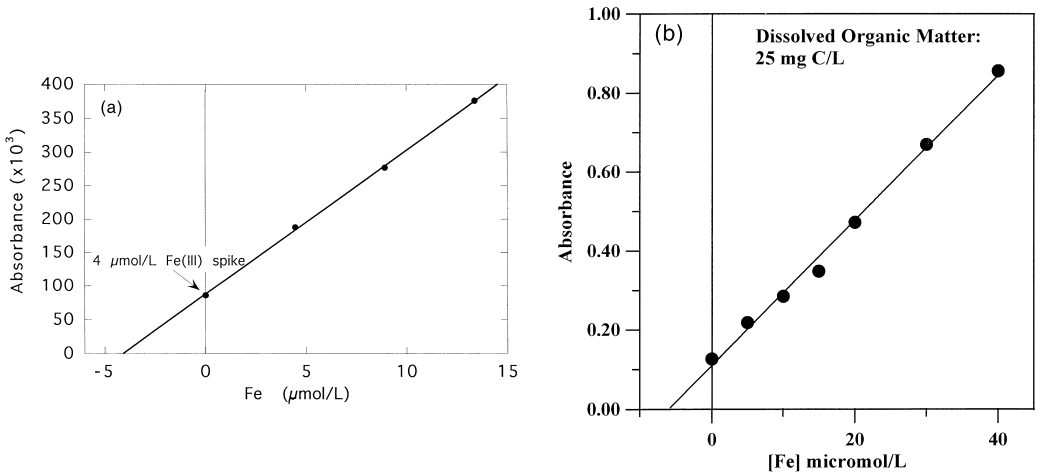

Fig. 4. (a) Standard addition of Fe(III) to an organic-rich water sample (experimentally derived salt marsh pore water). (b) Standard addition of Fe(III) to an organic-rich water sample (25 mg C/l from Suwannee River, GA).

A potential problem with the classical ferrozine method is the incomplete reduction of organically complexed Fe(III), as pointed out by Luther et al. (1996). These authors also attribute a poor recovery of total Fe to the precipitation of Fe(III) and Fe(II) humic complexes upon acidification of the sample by hydroxylamine hydrochloride. In order to test the reliability of the proposed analytical protocol in the presence of organically complexed Fe(III), standard additions of Fe(III) were performed to two water samples rich in dissolved organic matter (NOM).

A pore water sample was extracted under anaerobic conditions from a flow-through reactor containing an undisturbed core section of a salt marsh sediment (Roychoudhury et al., 1998). The presence of relatively high levels of dissolved NOM was apparent from the yellow to light brown color of the pore waters. Dissolved organic C (DOC) concentrations in marshes are among the highest found in natural environments, typically in the range 15 to 30 mg C/l (Leenheer, 1994). A second water sample was prepared by adding NOM extracted by reverse osmosis from the Suwannee River (Georgia, USA) (SR) to deionized water (1409 mg C/l SR-NOM sample provided by E.M. Perdue). The original total dissolved Fe content (363 ± 26 μmol/l) was determined using graphite furnace atomic absorption (GFFAS) with 800 times sample dilution. The final DOC concentration of the solution was 25 mg/l. The SR-NOM was chosen because of its high abundance of strong cation-binding functional groups (Cantrell et al., 1990; Gu et al., 1996; Leenheer, 1998).

In both NOM-rich solutions, excellent linearity of the standard additions was observed (Fig. 4). The extrapolation of the calibration curve to X axis leads to 337 ± 18 μmol/l for the original SR-NOM Fe content. These observations demonstrate the reliability of the reduction step and the absence of NOM-bound Fe precipitation. Though reduction of the ferric ferrozine complex in the presence of humic substances could potentially lead to an artefact, no significant change in absorbance with time was observed in the experiment. Thus, the proposed method can be used to measure Fe(II)/Fe(III) speciation, even in organic-rich natural waters. The method detection limit (MDL) is estimated to 0.3 μmol/l with a 1 cm cell. Tests on a 20 μmol/l Fe (III) solution prepared in NaCl 0.7 M lead to 20.1 ± 0.2 μmol/l Fe(III) and 0.1 ± 0.1 μmol/l Fe(II) ($n = 5$). Only slight absorbance differences occur for salinity above 5‰. Salinity, when above this latter value, should be known within ±20%. Interferences with major inorganic ions are described in Stookey (1970).

4.2. Applications

The proposed method was used to measure the depth distributions of Fe(II) in anoxic pore waters retrieved by dialysis from a salt marsh (Skidaway Island, Georgia), and in the water column of the Orca Basin (Gulf of Mexico) whose deeper part contains an anoxic brine (salinity 250‰) (Shokes et al., 1977).

Iron(II) concentrations show the expected depth distribution in the salt marsh pore waters (Fig. 5). Iron(II) produced by reductive dissolution right below the sediment–water interface is removed from the pore waters by formation of FeS in the lower part of the profile. The amounts of Fe(III) are negligible except for 4 samples in the top of the profile. These anomalous concentrations are explained by contamination of the samples by sediment particles sticking to the dialysis membrane. These particles dissolve after addition of the acidic reagent leading to a release of Fe(III) in the

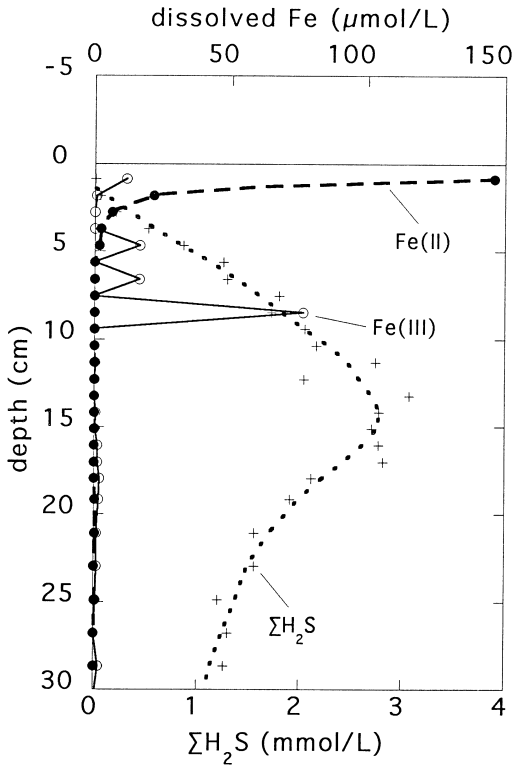

Fig. 5. Vertical distributions of dissolved Fe(II), Fe(III) and ΣH_2S in a salt marsh of Skidaway Island, GA.

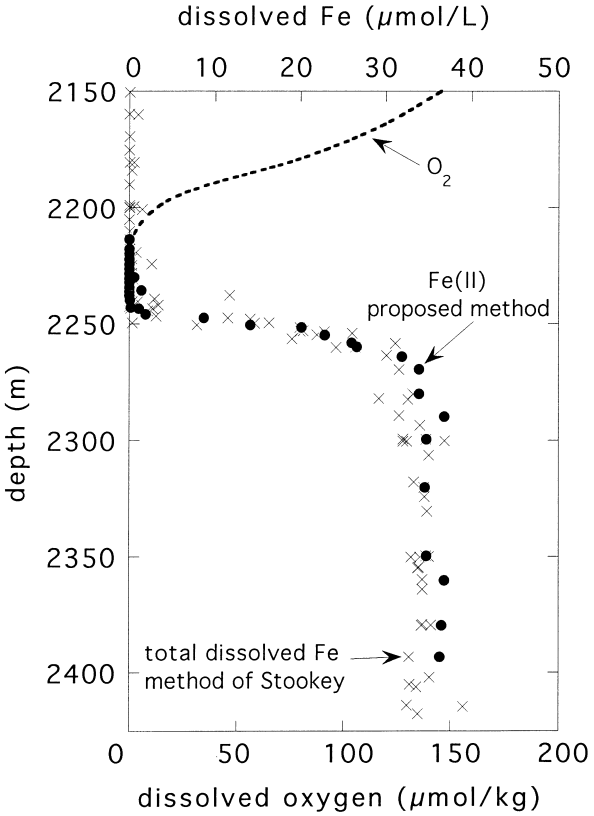

Fig. 6. Comparison between Fe(II) concentrations in the Orca basin (Gulf of Mexico) determined by the proposed method and total Fe concentrations determined with the original ferrozine method by a research group at the University of Texas at Austin.

solution. Visual observation of the progressive development of a thin magenta veil in one of the reaction tubes strongly supports this hypothesis. The identification of the nature of the spurious Fe(III) concentrations would not have been possible by measuring only total Fe or performing a speciation on different sample fractions.

The authors determined Fe(II) concentrations in the stratified water column of the Orca Basin, by the proposed method, while an independent research group (University of Texas at Austin) used Stookey's (1970) total Fe procedure (i.e. without intermediate absorbance reading A_1). A comparison of the results (Fig. 6) shows excellent agreement between the two methods and confirms that all the observed dissolved Fe in the anoxic brine is at the oxidation state II, and that no significant oxidation of Fe(II) occurred after sample filtration.

5. Conclusions

The proposed protocol offers a means to check Fe(II) measurements for O_2 or solid contamination, and to determine the Fe(II)/Fe(III) speciation in natural waters across redox interfaces. The method was used in marine waters of different salinities and was proven to be reliable. Though not yet tested, the method should also apply to freshwater environments. Future developments include: (1) the addition of an inert dye to directly estimate α for each sample or standard, and (2) adaptation of the method for continuous or segmented flow spectrophotometry, flow injection analysis, and in situ measurement techniques (Viollier et al., 1993).

References

Albrechtensen, H.-J., Christensen, T., 1994. Evidence for microbial iron reduction in a landfill leachate-polluted aquifer. Appl. Environ. Microbiol. 60, 3920–3925.

Buffle, J., Zali, O., Zumstein, J., De Vitre, R., 1987. Analytical methods for the direct determination of inorganic species: seasonal changes of iron, sulfur and pedogenic and aquagenic organic constituents in the eutrophic Lake Bret, Switzerland. Sci. Total Environ. 1–2, 41–59.

Cantrell, K.J., Serkiz, S.M., Perdue, E.M., 1990. Evaluation of acid neutralizing capacity data for solutions containing natural organic acids. Geochim. Cosmochim. Acta 54, 1247–1254.

Carignan, R., 1984. Interstitial water sampling by dialysis: methodological notes. Limnol. Oceanogr. 29, 667–670.

Davison, W., 1993. Iron and manganese in lakes. Earth Sci. Rev. 34, 119–163.

Davison, W., Zhang, H., Grime, G.W., 1994. Performance characteristics of gel probes used for measuring pore waters. Environ. Sci. Technol. 28, 1623–1632.

DiChristina, T.J., Delong, E.F., 1993. Design and application of rRNA-targeted oligonucleotide probes for the dissimilatory iron- and manganese-reducing bacterium *Shewanella putrefaciens*. Appl. Environ. Microbiol. 59, 4152–4160.

Elrod, V.A., Johnson, K.S., Coale, K.H., 1991. Determination of subnanomolar levels of iron(II) and total dissolved iron in seawater by flow injection analysis with chemiluminescence detection. Anal. Chem. 63, 893–898.

Fadrus, H., Maly, J., 1975. Suppression of iron(III) interference in the determination of iron(II) in water by the 1,10-phenanthroline method. Analyst 100, 549–554.

Gu, B., Mehlhorn, T.L., Liang, L., McCarthy, J.F., 1996. Competitive adsorption, displacement, and transport of organic matter on iron oxide, II. Displacement and transport. Geochim. Cosmochim. Acta 60, 2977–2992.

Janhke, R.A., Emerson, S.R., Cochran, J.K., Hirschberg, D.J., 1986. Fine scale distributions of porosity and particulate excess ^{210}Pb, organic carbon and $CaCO_3$ in surface sediments of the deep equatorial Pacific. Earth Planet Sci. Lett. 77, 59–69.

Leenheer, J.A., 1994. Chemistry of dissolved organic matter in rivers, lakes and reservoirs. In: Baker, L.A. (Ed.), Environmental Chemistry of Lakes and Reservoirs, Am. Chem. Soc. Advances in Chemistry Series, vol. 237, pp. 195–222.

Leenheer, J.A., 1998. Models of metal binding structures in fulvic acid from the Suwannee River, Georgia. Environ. Sci. Technol. 32, 2410–2416.

Luther III, G.W., Shellenbarger, P.A., Brendel, P.J., 1996. Dissolved organic Fe(III) and Fe(II) complexes in salt marsh porewaters. Geochim. Cosmochim. Acta 60, 951–960.

Roychoudhury, A.N., Viollier, E., Van Cappellen, P., 1998. A plug flow-through reactor for studying biogeochemical reactions in undisturbed aquatic sediments. Appl. Geochem. 13, 257–268.

Shokes, R.F., Trabant, P.K., Presley, B.J., Reid, D.F., 1977. Anoxic, hypersaline basin in the Northern Gulf of Mexico. Science 196, 1443–1446.

Siffert, C., 1989. L'effet de la lumière sur la dissolution des oxydes de fer(III) dans les milieux aqueux, Ph.D., ETH-Zurich, Switzerland.

Stookey, L.L., 1970. Ferrozine: a new spectrophotometric reagent for iron. Anal. Chem. 42, 779–781.

Tessier, A., Campbell, P.G.C., 1988. Partitioning of trace metals in sediments. In: Kramer, J.R., Allen, H.E. (Eds.), Metal Speciation, Theory, Analysis and Application. Lewis Publishers, pp. 183–199.

Van Cappellen, P., Gaillard, J.-F., 1996. Biogeochemical dynamics in aquatic sediments. In: Lichtner, P.C., Steefel, C.I., Oelkers, E.H. (Eds.), Reactive Transport in Porous Media, Reviews in Mineralogy, vol. 34, pp. 335–376.

Van Cappellen, P., Wang, Y., 1996. Cycling of iron and manganese in surface sediments: a general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron and manganese. Am. J. Sci. 296, 197–243.

Viollier, E., Michard, G., Sarazin, G., Choquier, A., Roziere, G., Trameson, P., 1993. A photometric probe for in situ analysis: principle, method, first trials. Rev. Sci. Eau 6, 395–410.

Yamada, M., Sudo, A., Susuki, S., 1985. Chem. Lett. (Chem. Soc.), 801–804.