Marine Chemistry 234 (2021) 104003

Contents lists available at ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

The role of gentisic acid on the Fe(III) redox chemistry in marine environments

M.L. Arreguin, A.G. González, N. Pérez-Almeida, V. Arnone, M. González-Dávila, J. M. Santana-Casiano *

Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, ULPGC, 35017 Las Palmas de Gran Canaria, Spain

ARTICLE INFO

Keywords:
Iron
Gentisic acid
Kinetics
Seawater
Complexation

ABSTRACT

Phenolic compounds excreted by marine microalgae are part of the ligand pool in natural waters. The effect of the polyphenol gentisic acid (GA; 2,5 dihydroxybenzoic acid) on the Fe(III) reduction as a function of organic ligand concentration (100 nM – 1000 nM) and the pH (7.00–8.01) was investigated in seawater. Major seawater ions interactions with both GA and Fe(III) were also considered using 0.7 M NaCl +2 mM $NaHCO_3$ solutions with each major ions (Mg^{2+}, Ca^{2+}, SO_4^{2-}, K^+, F^-, Sr^{2+}) at the same ratio than that in a 35 salinity seawater. The Fe(III)-GA complex in solution was able to produce Fe(II) in a pH-dependent process that was faster in seawater than in NaCl-$NaHCO_3$ solution. The addition of each major ion affected the Fe(III) reduction process. At pH 7.5, the presence of Mg^{2+} and Ca^{2+} contributes to accelerate the reduction rate constant of Fe(III) to Fe(II). The K^+, F^-, Sr^{2+}, and SO_4^{2-} ions slow down the Fe(III) reduction rate constant. At pH 7.8, the effect of the ions is counteracted. Increasing the pH to 8, the effect on the solubility of Fe(III) is more important, being greater in NaCl and no reduction of Fe(III) was detected, compared with seawater.

In this study, the formation and dissociation of the Fe(III)-GA complex in seawater was studied, resulting in $k_f = 1.19(\pm0.18)\bullet10^4$ (M^{-1} s^{-1}), $k_d = 1.86$ (±0.53)$\bullet10^{-4}$ (s^{-1}), and conditional complexation constant of $\log K_{Fe^{3+}L}^{Cond} = 17.81 \pm 0.05$ in seawater.

Accordingly, phenolic compounds like GA influence the Fe marine biogeochemical cycles promoting the formation of the bioavailable Fe(II) in solution. Taking this into account, the Fe(III) reactions with phenolic compounds have to be considered to improve our understanding of the global iron cycle.

1. Introduction

Iron (Fe) is an essential micronutrient of great importance to marine environments and the organisms, influencing global biogeochemical cycles. Fe availability is known to control phytoplankton productivity, and in turn its community structure and ecosystem functionality in large areas of the open ocean and coastal regions (Gledhill and Buck, 2012).

Fe can be found in the ocean in two oxidation states (Fe(II) and Fe (III)). In oxygenated seawater, Fe(II) is quickly oxidized (Millero et al., 1987; Santana-Casiano et al., 2005) to Fe(III) being this species the most thermodynamically stable. However, the solubility of Fe(III) in seawater is very low due to the Fe(III) precipitation as oxides and oxy-hydroxides (Millero et al., 1995). The presence of organic ligands increase this solubility (Liu and Millero, 2002). More than 99% of dissolved Fe (dFe) is bound to organic ligands (Rue and Bruland, 1995; van den Berg, 1995). Therefore, organic binding ligands play a key role on the Fe biogeochemical cycles, controlling the spatial and temporal distribution and the speciation of Fe in seawater, as well as determining the bioavailability of Fe to organisms (Croot e al., 2011). The ligand pool is produced by cellular exudation, viral lysis, zooplankton grazing and bacterial breakdown of organic matter (Granger and Price, 1999; Hutchins et al., 1999; Maldonado and Price, 2001). Those ligands are commonly classified into three classes; strong ligands or L1-type ligands ($\log K'_{Fe'L} > 12$) and the weak ligands L2 and L3-type ligands ($\log K'_{Fe'L}$ 11–12 and $\log K'_{Fe'L} < 11$) (Gledhill and Buck, 2012), although the ligand pool is a mixture of different size classes with varying Fe binding capacities (Town and Filella, 2000). Other nomenclature is also used limiting to L1 ($\log K'_{cond} > 22$) and L2-type ligand ($\log K'_{cond} < 22$) (Rue and Bruland, 1995).

The microorganisms have developed a number of strategies in order to acquire the scarce Fe in the ocean (Morel and Price, 2003) being even able to compete aggressively to acquire iron. This behaviour has been

* Corresponding author.
E-mail address: magdalena.santana@ulpgc.es (J.M. Santana-Casiano).

https://doi.org/10.1016/j.marchem.2021.104003
Received 4 August 2020; Received in revised form 2 March 2021; Accepted 23 May 2021
Available online 16 June 2021
0304-4203/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

described as a "chemical warfare" (Bruland and Lohan, 2003). Eukaryotic phytoplankton as diatoms, for example, have been shown to be able to utilize cell-surface reductase systems to reduce the Fe(III) bound to the organic ligands and form Fe(II), that can become available either as Fe(II) or be reoxidized into Fe(III) and become available for assimilation (Hudson, 1989; Hudson et al., 1992; Hudson and Morel, 1989, 1990, 1993; Shaked et al., 2004, 2005; Shaked and Lis, 2012). Certain microorganisms as cyanobacteria are able to utilize their own high affinity Fe ligands called siderophores (Reid et al., 1993), but also assimilate numerous siderophores produced by other species. Eukaryotic diatoms are thought not to have the receptor sites to assimilate Fe(III) siderophores directly, but they growth in the presence of this compounds (Hutchins et al., 1999). An abiotic reduction of Fe(III) to Fe(II) has been demonstrated to occur in the presence of organic compounds that have catecholate groups in their structures (Santana-Casiano et al., 2010, 2014), similar to that found in some siderophores. Moreover, it has been demonstrated the phytoplankton cells are able to exudate polyphenols compounds with this property (López et al., 2015; Rico et al., 2013).

There is a lack of information about the functional groups which form the ligand pool in the ocean and their individual role on Fe chemistry. In order to fully understand the Fe redox chemistry which takes place in natural waters, further investigation must be made on the Fe(III) reduction process in seawater by different functional organic groups. Among all the possible organic compounds, polyphenols are relevant because will form weak complexes with Fe, modifying its chemical speciation and bioavailability (Andjelković et al., 2006; Brown et al., 1998; Lodovici et al., 2001; Mira et al., 2002; Re et al., 1999; Sroka and Cisowski, 2003). Polyphenols have recently been defined as an important organic compounds group excreted by marine diatoms and green algae, particularly under metal stress conditions acting as barrier against the toxicity of copper or as a ligand source sequential to the complexing and the reduction of Fe(III) to Fe(II) (González et al., 2019; López et al., 2015; Rico et al., 2013; Santana-Casiano et al., 2010, 2014). Polyphenols can also reach the ocean through terrestrial supply and can be considered as model ligand within humic substances (Blazevic et al., 2016; Krachler et al., 2005, 2010, 2012, 2015, 2016, 2019; Orlowska et al., 2016, 2017a, 2017b; Rathgeb et al., 2017). Polyphenols react with oxygen and reactive oxygen species both abiotically and enzymatically (De et al., 2018; Rahaman et al., 2019), by superoxide (Joshi et al., 2012; Velika and Kron, 2012) and hydroxyl radical (Udenfriend et al., 1954). Polyphenols have been considered as complexing ligand for dFe in natural waters (Andjelković et al., 2006; Brown et al., 1998; González et al., 2019; Hynes and O'Coinceanainn, 2004; Khokhar and Apenten, 2003; Mira et al., 2002). The high Fe-binding capacity of polyphenols has been related to the position of the OH^- group within the benzene ring, forming Fe-complexes, with those polyphenols having at least two OH^- groups in consecutive positions (Wu et al., 2016).

Gentisic acid, 2,5 dihydroxybenzoic acid, (GA) (Fig. 1) is an hydroxybenzoic acid type polyphenols and has been found as exudates in marine microalgae cultures of *Phaedactylum tricornutum* and *Dunaliella tertiolecta* under different laboratory conditions (López et al., 2015; Rico et al., 2013). GA is an important Fe-binding ligand in eukaryotic cells (Devireddy et al., 2010). The implication of GA in biological processes has been strongly studied (Devireddy et al., 2005, 2010; Liu et al., 2012; Richardson et al., 2010), however, the chemical reaction between Fe and GA remain poorly known, mainly in seawater solutions, that is essential to understand the implications in the Fe speciation as well as its links with the uptake mechanisms.

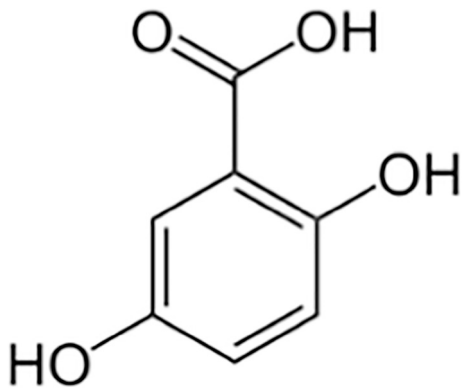

Fig. 1. Molecular structure of gentisic acid (GA).

GA has been proved to chelate Fe(III) in aqueous solution at micromolar concentration range acting as a mode of siderophore binding interaction (Porwal et al., 2015). They supported a model in which GA can form 1:1, 1:2 or 1:3 Fe(III):complex when high GA concentration is present. However, UV–Vis titration data (Porwal et al., 2015) demonstrated that only a 1:1 complex was formed at equivalent concentrations even when additional multimers formed at higher concentrations. These complexes will suffer, through single electron oxidative transformation (leading to quinone forms), oxidative processes which may cause reduction of Fe(III) in solution.

GA is also an oxidation product of salicylic acid (Kalyanaraman et al., 1993; Kuzma et al., 2018) and has been studied extensively in medicine for this reason (Joshi et al., 2012; Lack, 1959; Yano and Arima, 1958). GA has been shown to have antioxidant properties (Galato et al., 2001; Sawyer et al., 1995) and defined as an important intermediate in the biodegradation of mono-, poly-cyclic and hetero-aromatic compounds through microorganisms (Feng et al., 2012; Harpel and Lipscomb, 1990).

The objective of this study was to investigate the effects produced and enhanced by GA in the Fe(III) reduction to Fe(II) in seawater and hence act as a source of Fe(II) in the ocean, allowing its persistence to be assimilated by marine microorganisms. The Fe complexation and reduction by polyphenols increase the presence of Fe(II) in solutions, slowing down the oxidation and the precipitation of Fe, thus allowing dFe to be found in natural waters for longer periods. For this aim, the Fe (III) reduction by GA was studied as a function of GA concentration (100 nM – 1000 nM) at the pH range 7.00 to 8.01 both in seawater and NaCl-$NaHCO_3$ solutions. The role of major ions of seawater on the Fe(III) reduction rate constant was also studied at two pH (7.50 and 8.01). Finally, the Fe(III)-GA complex in seawater was kinetically characterized via its formation and dissociation rate constants in order to know the residence time of Fe(III) in seawater in the presence of GA.

2. Experimental

2.1. Chemicals

The Fe(II) stock solution ($4\bullet10^{-4}$ M) was prepared using ferrous ammonium sulfate hexahydrate (Sigma) and prepared in acidified (pH 2; adding HCl suprapur, VWR) Milli-Q water (18 MΩ, Millipore). The gentisic acid, GA (Sigma-Aldrich), was prepared on a weekly basis, in HPLC grade methanol at 10^{-2} M. Before each experiment, a secondary stock was prepared in Milli-Q water at $2\bullet10^{-4}$ M. The 0.7 M NaCl (Sigma-Aldrich) with 2 mM $NaHCO_3$ (Sigma-Aldrich) solution was prepared in Milli-Q water.

The 0.01 M TAC (2–2(2-thiazolylazo)-*p*-cresol) solution (Sigma-Aldrich, in HPLC grade methanol) was prepared once every two weeks and stored in the fridge when it was not in use. The 1.0 M EPPS buffer solution (N-(2-hydroxyethyl)piperazine-N′;2-propanesulfonic acid; SigmaUltra) was prepared in 1.0 M NH_4OH (ultrapure, VWR) at pH 8.05. Contaminating metals were removed from the buffer solutions by using 100 μM MnO_2 (van den Berg, 1982), equilibrated overnight and filtered through acid-cleaned 0.45 μm pore-size filters (polysulfone, Whatman).

The seawater used in all the experiments was collected from the ESTOC Time Series station (29° 10′ N 15° 30′ W), an oligotrophic area situated in the North Atlantic subtropical gyre, using a trace metal clean Teflon pump (PFD2 316F, AstiPure®) and in line filtered by 0.2 μm dual

pore-size trace metal clean filters (Acropack™) and kept in darkness to remove any reactive oxygen species (ROS) present in the seawater.

2.2. Fe(III) reduction experiments

Reactions were studied in 200 mL glass thermostated vessel at a constant temperature (25 °C) controlled by an AG-2™ bath. The redox experiments were always carried out under darkness to ensure there were no light effects taking part in the Fe(III) reduction reactions. The pH of the solution was determined using the pH-free scale (Millero, 1986) and adjusted for each experiment by adding small aliquots of 0.01 and 0.1 M HCl; pH was recorded during each experiment with a maximum variation of ±0.02. Samples were aerated with pure air prior and during the experiment, as well as stirred with a Teflon coated magnetic stirrer.

Following the previous reported studies (Santana-Casiano et al., 2010, 2014), 100 nM of Fe(II) was added to 100 mL of solution (seawater or NaCl-$NaHCO_3$) in the reaction vessel and fully oxidized for one hour under oxygen saturation conditions. Under such conditions, equilibrium for the hydrolysis of Fe(III) species and organic ligands naturally present in the seawater was achieved. Then, 10^{-4} M Ferrozine (FZ) was added, due to the fact that Fe(II) and FZ form a complex (1:3 ratio) with an absorption peak at 562 nm. Fe(II) was never detected previous to the addition of GA indicating both that all Fe(II) was oxidized and that no Fe(III) was reduced with any ROS species generated in the Fe(II) oxidation process or naturally present in solution. Blanks were always realized before the addition of the organic ligand and for each experimental condition. GA was then added, which was accounted as the time zero of the reaction. The experiments were carried out at different pH values (range 7.00 ± 0.01–8.01 ± 0.02) and GA concentrations (100 nM – 1000 nM). In the presence of the organic ligand, any interference to the Fe(II)-FZ_3 peak was observed. Experiments were carried out in duplicate or triplicate.

The determination of regenerated Fe(II) was done in a 5 m long waveguide capillary flow cell LWCC (Liquid Wavelength Capillary Cell) from World Precision Instruments connected to the UV detector S4000 (Ocean Optics). Spectra were recorded using OOIBase software by Ocean Optics. The light employed was a halogen light source HL-2000-FHSA (Mikropack). The sample was then pumped into the column with the use of a peristaltic pump (EXPETEC Perimax 12). The solution was measured in an open-system where the seawater pass through the column and it was never back to the reaction vessel to avoid photoreactions. The detection limit of this technique was 0.1 nM at pH 8.0 to 0.21 nM at pH 7.0, with a limit of quantification that ranged from 0.3 nM to 0.7 nM at pH 8.0 and 7.0, respectively. The accuracy of the Fe(II) determination was measured against a standard solution freshly prepared as indicated above and was always less than 6% (in triplicate). The response of the system was linear over two orders of magnitude Fe(II) (González-Dávila et al., 2005; Santana-Casiano et al., 2005).

The effect of the ionic composition of seawater on the Fe(III) reduction was also studied by using 0.7 M solutions with NaCl and each major ion present in seawater (Millero, 1996), keeping constant 2 mM $NaHCO_3$. These experiments were carried out at two different pH values (7.50 and 8.01) at constant temperature (25 °C).

According with Santana-Casiano et al. (2010), the apparent Fe(III) reduction rate constant was obtained measuring the appearance of Fe (II). The disappearance of [Fe(III)] = $[Fe(III)]_0$ - [Fe(II)] was determined as a function of time under pseudo first-order conditions (GA in excess at a constant pH), the subscript zero denotes the initial concentration of Fe (III).

$$\frac{\partial[Fe(III)]}{\partial t} = -k'[Fe(III)] \quad (1)$$

where $k' = k_{app}[GA]$.

2.3. Fe speciation by differential pulse cathodic stripping voltammetry

Dissolved Fe (dFe), labile Fe (inorganic Fe; Fe^{3+} and all the Fe(III)-inorganic complexes), and dFe-binding ligands (L_{Fe}) concentrations were determined by differential pulse cathodic stripping voltammetry (DP-CSV), according to Croot and Johansson (2000), where TAC was the competitive ligand. An Epsilon voltammeter (Basi, Inc) connected to a hanging mercury drop electrode (CGME, Basi, Inc) was used. The reference electrode was Ag/AgCl with a salt bridge filled with 3 M KCl, with a platinum auxiliary electrode. The solution was mixed during the deposition time and purged by bubbling with pure nitrogen. The samples were always processed in a Class 100 clean laboratory at room temperature. Fe speciation was studied in seawater with and without GA.

Labile Fe was measured in 10 mL seawater samples by adding 100 μL of EPPS (final concentration 10 mM EPPS buffered to pH 8.05) and 10 μL of 0.01 M TAC (final concentration 10 μM).

Dissolved Fe concentrations were measured in UV-treated seawater (UV photoxidation unit 7900–74 Ace Glass). The UV-irradiation lasted 4 h in quartz tubes. These tubes were soaked for one day in 10% HCl (suprapur, VWR) and washed with MQ-water 5 times prior to use. They were also rinsed one more time with natural seawater before being used. The dFe concentration was determined by three standard additions.

The voltammetric measurement was always the same for all the analysis. Deposition Potential at −400 mV for 120 s, Quiet Time for 10 s, Initial Potential at −400 mV and Final Potential at −650 mV. The amplitude and step were 50 and 2 mV respectively while the pulse time and period were 10 and 100 ms respectively.

2.4. Fe complexation through kinetic approach

Dissolved Fe complexation by GA was measured through kinetic measurements following the procedure proposed to study the complexation of Fe with organic ligands in the marine environment (Croot and Johansson, 2000; Gerringa et al., 2007; González et al., 2019; Witter et al., 2000; Witter and Luther, 1998; Wu and Luther, 1995). Briefly, the experiments were performed with an excess of dFe (10 nM) compared to model ligand (5 nM) in order to have enough dFe to saturate the binding sites in GA and to achieve the maximum complexing capacity. Samples were prepared in different Teflon vials by adding 10 mL of UV-seawater, 100 μL of EPPS, 10 nM of dFe and 5 nM of GA. The addition of the GA corresponds with the initial time (t_0). In the formation experiments, TAC (10 μL of 0.01 M in 10 mL of sample) was added during the purge, 20 s before starting the deposition time. The measured Fe in the formation kinetics was considered as labile Fe (Gerringa et al., 2007). In the dissociation experiments, the addition of TAC is after reach the equilibrium and corresponds with the t_0. Here, the measured Fe is TAC labile Fe (Gerringa et al., 2007). All the experiments were carried in triplicate and the Teflon vials were conditioned 5 times with UV-treated seawater.

The formation and dissociation rate constant can be studied both in natural waters and for a model ligand considering the mass-balance:

$$Fe' + nL \xrightarrow{kf} Fe'L_n \quad (2)$$

Where k_f is the formation rate constant. Then,

$$\frac{\partial[Fe'L_n]}{\partial t} = k_f[Fe'][L]^n \quad (3)$$

In our experiments, 10 nM of Fe was added to the UV-treated seawater and at desired times TAC was also added. Then, TAC labile Fe was measured. L was computed from the amount of free ligand present at the equilibrium. Even when GA forms several multiligand complexes ($Fe'L_n$), it was assumed that $n = 1$. Initially, complexation occurs at 1:1 ratio (Porwal et al., 2015) and higher-order complexes are found at higher concentrations, being the FeL_3 the most accepted complex in

ligand excess at micromolar levels.

In the dissociation rate constant (k_d) determination using TAC, k_d for weak ligands and all Fe recovered can be computed from

$$Fe'L + 2(TAC) \overset{kd}{\rightarrow} Fe' + L + 2(TAC) \overset{k2}{\rightarrow} Fe'(TAC)_2 + L \quad (4)$$

where an adjunctive mechanism is likely to occur (70–90% of the overall rate even at 10 μM TAC (Croot and Heller, 2012):

$$Fe'L + 2(TAC) \leftrightarrow Fe'(TAC)_2L \overset{kd}{\rightarrow} Fe'(TAC)_2 + L \quad (5)$$

When a pseudo-equilibrium (steady state approximation) is considered for the formation of the ternary complex $Fe(TAC)_2L$, the adjunctive mechanism, while occurring, is not rate determining and the reaction is only disjunctive in character (Croot and Heller, 2012).

The overall reaction rate is pseudo-first order in [TAC] due to the range of concentration.

$$-\frac{\partial[Fe'L]}{\partial t} = \frac{\partial[Fe'(TAC)_2]}{dt} = k_{obs}[Fe'L][TAC]^2 \quad (6)$$

Integrating:

$$ln[Fe'L] = -k_{obs}[TAC]t \quad (7)$$

According to Wu and Luther (1995), Witter and Luther (1998) and Witter et al. (2000),

$$-\frac{\partial[Fe'L]}{\partial t} = \frac{\partial[Fe'(TAC)_2]}{\partial t} = k_d[Fe'L] \quad (8)$$

In addition, K_{FeL}^{Cond} can be estimated (Witter and Luther, 1998; Wu and Luther, 1995) from k_f and k_d

$$K_{FeL}^{Cond} = k_f/k_d \quad (9)$$

The equilibrium constant expressed as a function of the free Fe, Fe^{3+}, is

$$K_{Fe^{3+}L}^{Cond} = \alpha_{Fe'} K_{FeL}^{Cond} \quad (10)$$

where $\alpha_{Fe'} = 10^{10}$ (Hudson et al., 1992; Sunda and Huntsman, 2003), is commonly used for pH 8 seawater. The conditional stability constant with respect to Fe^{3+} ($K_{Fe^{3+}L}^{Cond}$) can be calculated by assuming that the second-order rate constant is $k_{f,\ Fe^{3+}L} = 3.02 \bullet 10^{11}\ M^{-1}\ s^{-1}$, previously estimated (Luther et al., 1997).

3. Results

3.1. Fe(II) formation from Fe(III) by GA

The formation of Fe(II) after Fe(III) reduction at different GA concentrations was studied in the range 100 to 1000 nM GA in seawater and was followed as the amount of Fe(II)-FZ_3 complex formed. The initial Fe (III) concentration was 100 nM. Fig. 2 shows the spectra of the Fe(II)-FZ_3 complex in the presence of GA at pH 7.00, after 60 min of reaction. The Fe(II)-FZ_3 signal peak with maximum absorbance at 562 nm was observed to increase with the concentration of GA. A small peak was observed at around 450 nm related to the oxidation of the Fe(III)-GA complex to benzoquinone through the semiquinone radical (Santana-Casiano et al., 2010). No peak/shoulder was observed at 590 nm due to the formation of Fe(III)-GA complexes (Porwal et al., 2015), probably due to the low concentration range considered in our studies, even when it could be present, and due to the different experimental conditions used in their studies.

The Fe(III) reduction in presence of GA (from 100 nM to 1000 nM) was studied at two different pH conditions (7.00 and 8.01) in seawater. Fig. 3 represents the measured absorbance of the Fe(II)-FZ_3 complex versus time for each experimental condition. The plot of ln [Fe(III)] (determined as indicated in Eq. (1)) versus time followed a linear relationship and from the slope a pseudo-first order Fe(III) reduction rate

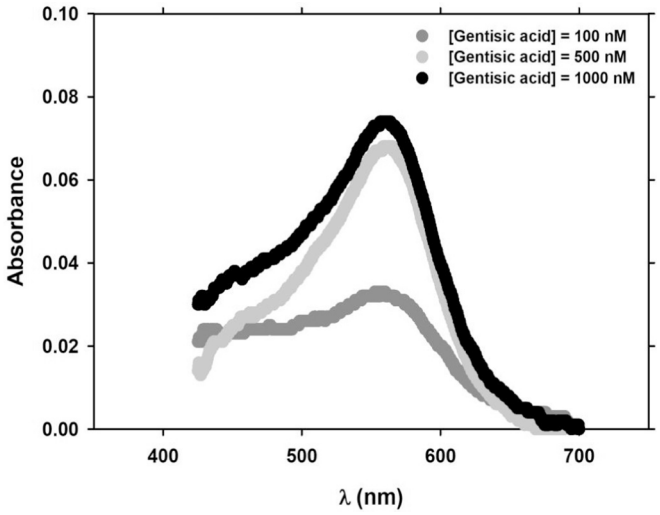

Fig. 2. Fe(II)-FZ_3 peak in the presence of gentisic acid from 100 nM to 1000 nM in seawater after 60 min experiments at constant pH 7.00 ± 0.01. Experimental conditions: 100 nM Fe(III), 10^{-4} M FZ, $T = 25$ °C darkness conditions.

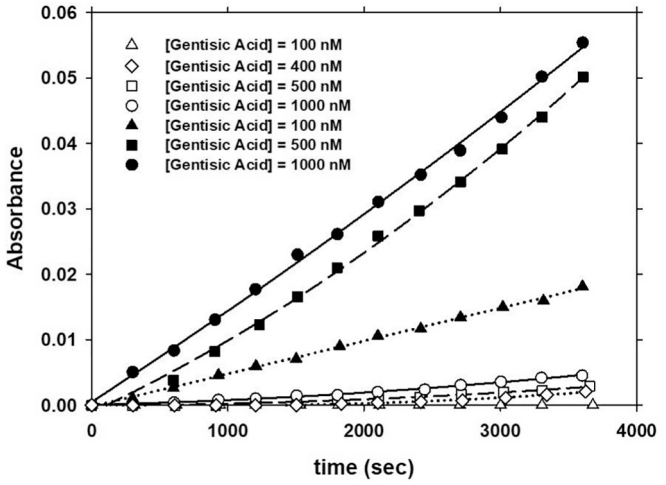

Fig. 3. Fe(II) formation from Fe(III) by gentisic acid at different concentrations (100 nM – 1000 nM) at pH 7.00 ± 0.01 (filled symbols) and 8.01 ± 0.02 (open symbols). Experimental conditions: 100 nM Fe (III), 10^{-4} M FZ, T = 25 °C, darkness conditions. The absorbance was measured at 562 nm.

constant (k, in s^{-1}) was obtained (Table 1). At pH 8.01 and 100 nM GA, the Fe(II)-FZ_3 peak was not detected over time but it was detected at pH 7.0. In addition, at higher GA added, the values of the oxidation rate constants were higher at pH 7.0 than at 8.01 for the same concentration of GA. The log k' value was statistically similar for 500 and 1000 nM of GA, at pH 7.00. The amount of Fe(II) formed was higher at pH = 7 than

Table 1
Fe(III) reduction rate constant (k', s^{-1}) in seawater by the presence of 100–1000 nM gentisic acid at constant temperature (25 °C) and different pH.

pH	[gentisic acid] (nM)	k' (s^{-1})	log k'
8.01 ± 0.02	1000	$1.08(\pm0.01)\bullet10^{-6}$	−5.97 ± 0.01
	500	$6.68(\pm0.1)\bullet10^{-7}$	−6.18 ± 0.01
	400	$6.17(\pm0.9)\bullet10^{-7}$	−6.21 ± 0.06
	100	nd	nd
7.00 ± 0.01	1000	$1.14(\pm0.01)\bullet10^{-5}$	−4.94 ± 0.01
	500	$1.14(\pm0.02)\bullet10^{-5}$	−4.94 ± 0.01
	100	$3.83(\pm0.04)\bullet10^{-6}$	−5.42 ± 0.01

at high pH values (Fig. 3 and Table 1). The log k' value at pH 7.00 ranged from −4.94 ± 0.01 to −5.42 ± 0.01 for 100 nM GA and 1000 nM GA, respectively.

To check whether the major ions of seawater exert any influence on the formation of Fe(II) from the reduction of Fe(III) by GA, the experiments were repeated in simple solutions of NaCl-$NaHCO_3$ and NaCl-$NaHCO_3$ with the characteristic major ions of seawater. The results of the reduction of 100 nM Fe(III) to Fe(II) by 1000 nM GA in seawater and NaCl-$NaHCO_3$ solutions in the pH range from 7.00 to 8.01 are shown in Fig. 4 and Table 2. In both solutions, experiments done at pH 7.00 showed a greater Fe(III) reduction rate constant than those done at pH 8.01. Moreover, the experiments in seawater showed a greater Fe(II) formation than those done in NaCl-$NaHCO_3$ solutions. In seawater and after 60 min of reaction, the Fe(III) reduction was ~90% higher at pH 7.00 than at pH 8.01. At pH 7.00, the Fe(III) reduction in NaCl-$NaHCO_3$ solutions was 40% less than in seawater (Fig. 4). Therefore, the experimental data indicated that 1000 nM GA was able to regenerate 4.3 nM of Fe(II) at pH 7.00 and 0.42 nM at pH 8.01 in seawater medium, after 60 min of reaction.

The pH dependence of the Fe(III) reduction rate constant (expressed as log k) is depicted in Fig. 5 (data and range of pH in Table 2) for seawater and NaCl-$NaHCO_3$ media. In both solutions, log k' followed a linear relationship with the pH (Eqs. (11)–(12)), with R^2 higher than

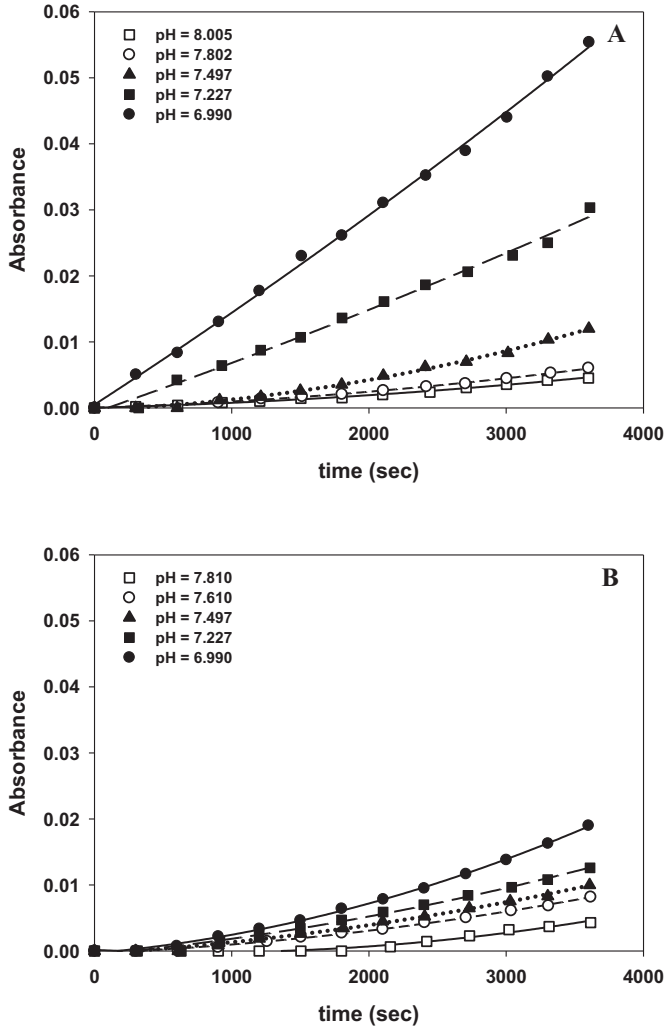

Fig. 4. Fe(II) formation from Fe(III) by gentisic acid in seawater (A) and NaCl-$NaHCO_3$ (B) solutions as a function of pH. Experimental conditions: 1000 nM GA, 100 nM Fe(III), 10^{-4} M FZ, T = 25 °C, darkness conditions. The absorbance was measured at 562 nm.

Table 2
Values of log k' (s^{-1}) for Fe(III) reduction in seawater, 0.7 M NaCl +2 mM $NaHCO_3$ and 0.7 M solutions NaCl +2 mM $NaHCO_3$ + each major seawater ions (Millero, 1996) as a function of pH. The concentration of GA was 1000 nM, FZ 10^{-4} M and the initial Fe(III) concentration was 100 nM. Concentration of each major ion is in molal.

Media	pH	k' (s^{-1})	log k'
Seawater	8.01	1.08(±0.10)•10^{-6}	−5.97 ± 0.04
	7.81	1.36(±0.03)•10^{-6}	−5.87 ± 0.01
	7.50	2.61(±0.20)•10^{-6}	−5.58 ± 0.03
	7.23	6.24(±0.10)•10^{-6}	−5.21 ± 0.01
	7.00	1.14(±0.01)•10^{-5}	−4.94 ± 0.01
0.7 M NaCl + 2 mM $NaHCO_3$	8.01	nd	nd
	7.81	1.84(±0.01)•10^{-6}	−5.74 ± 0.01
	7.61	1.97(±0.09)•10^{-6}	−5.71 ± 0.02
	7.50	2.29(±0.06)•10^{-6}	−5.64 ± 0.01
	7.23	2.95(±0.05)•10^{-6}	−5.53 ± 0.01
	7.00	3.76(±0.40)•10^{-6}	−5.42 ± 0.05
Mg^{2+} (5.33•10^{-2} m)	7.50	5.24(±0.30)•10^{-6}	−5.28 ± 0.02
SO_4^{2-} (2.82•10^{-2} m)		5.67(±0.20)•10^{-7}	−6.25 ± 0.01
K^+ (9.90•10^{-3} m)		1.26(±0.06)•10^{-6}	−5.90 ± 0.02
F^- (5.26•10^{-5} m)		1.01(±0.01)•10^{-6}	−6.00 ± 0.01
Sr^{2+} (9.13•10^{-5} m)		5.57(±0.01)•10^{-7}	−6.25 ± 0.01
Ca^{2+} (1.03•10^{-2} m)		3.75(±0.30)•10^{-6}	−5.43 ± 0.04
Mg^{2+} (5.33•10^{-2} m)	8.01	1.12(±0.01)•10^{-6}	−5.95 ± 0.03
SO_4^{2-} (2.82•10^{-2} m)		3.43(±0.10)•10^{-7}	−6.47 ± 0.20
K^+ (9.90•10^{-3} m)		8.19(±0.04)•10^{-7}	−6.09 ± 0.02
F^- (5.26•10^{-5} m)		5.64(±0.05)•10^{-7}	−6.43 ± 0.04
Sr^{2+} (9.13•10^{-5} m)		3.71(±0.10)•10^{-7}	−6.43 ± 0.10
Ca^{2+} (1.03•10^{-2} m)		9.03(±0.04)•10^{-7}	−6.04 ± 0.01

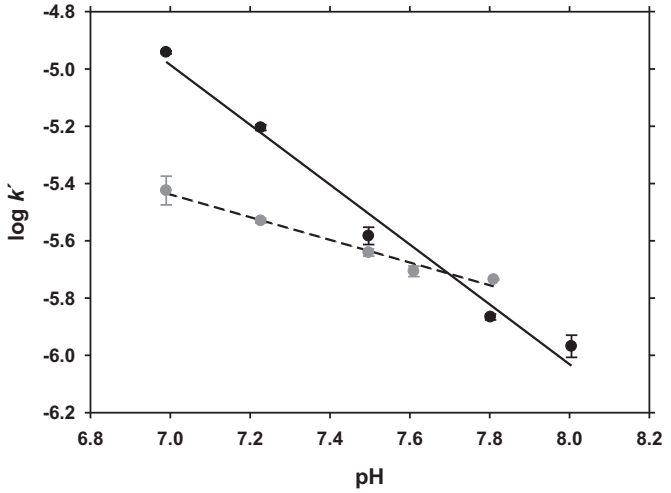

Fig. 5. The dependence of Fe(III) reduction rate constant (log k', s^{-1}) as a function of pH in seawater (black symbols) and NaCl-$NaHCO_3$ (grey symbols) mediums. Experimental conditions: 1000 nM GA, 100 nM Fe(III), 10^{-4} M FZ, T = 25 °C, darkness conditions.

0.99 and a standard error of estimation of 0.07 and 0.02, respectively.

$$logk'_{sw} = 2.3(\pm 0.6) - 1.05(\pm 0.07)pH \quad (11)$$

$$logk'_{NaCl} = -2.67(\pm 0.3) - 0.40(\pm 0.02)pH \quad (12)$$

The pH slope indicates a higher influence of pH on the Fe(III) reduction by GA in seawater than in NaCl-$NaHCO_3$ solutions. It could be related to effects of the major ions present in seawater on the Fe(III) reduction which also increase the Fe(III) solubility in seawater due to both shielding effect and specific iron interaction. To elucidate this aspect, studies were prepared in 0.7 M NaCl-2 mM $NaHCO_3$ solutions, with each of the most representative major ion in seawater, at two pH 7.5 and 8.01. When the spectrum for each solution is represented, it was

observed that the Fe(II)-FZ_3 peak was present but the signal of the Fe(II)-FZ_3 changed with respect to NaCl or seawater solution (Fig. 6). At pH 7.5 (Fig. 7 and Table 2) the log k' in NaCl-$NaHCO_3$ and seawater was quite similar. However, at pH 8.01 the Fe(III) reduction was not observed in NaCl-$NaHCO_3$ solutions. The ions K^+, F^-, Sr^{2+} and SO_4^{2-} presented log k' always lower (slower oxidation rate constant) than in NaCl solution at the two pH and in the order $K^+ < F^- < Sr^{2+} < SO_4^{2-}$. At pH 7.5, log k' was -6.25 ± 0.01 for SO_4^{2-} and Sr^{2-}, while at pH 8.01 the value increased to -6.47 ± 0.20 and -6.43 ± 0.10, respectively. The ions Mg^{2+} and Ca^{2+} showed a different behaviour. At pH 7.5 the oxidation rate constants were faster (higher log k) than in both NaCl and seawater solution. At pH 8, the presence of those ions (also for all other major ions) allows to measure Fe(II) in solution with a value for the oxidation rate constants similar to that determined in seawater. Increasing the pH to 8, decreases the Fe(III) solubility being the effect greater in NaCl-$NaHCO_3$ solutions than in seawater. The formation of the non-reactive $Fe(OH)_3$(s) species strongly compete with the Fe(III)-GA complex (Millero et al., 1995; Porwal et al., 2015). At pH 7.5, the effects of the different ions are counter-balanced and the values in NaCl and in seawater are close. At pH 8, the major ions increase the Fe(III) solubility and the Fe(III):GA formation, therefore Fe(II) was formed and detected.

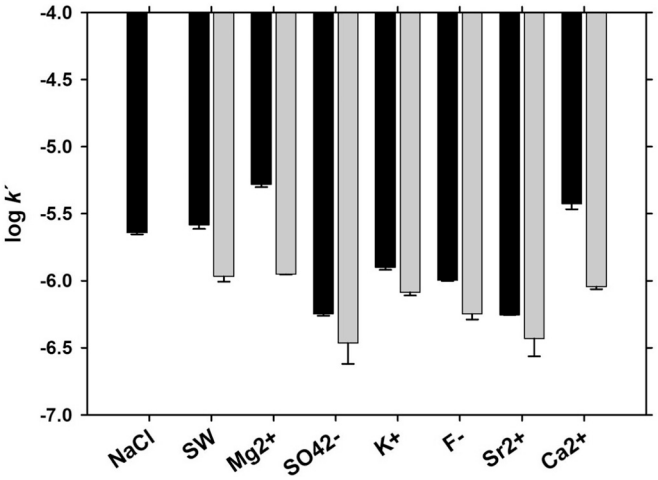

Fig. 7. Fe(III) reduction rate constant (k', sec^{-1}) measured in 0.7 M NaCl (+2 mM $NaHCO_3$), in seawater and in NaCl-$NaHCO_3$ + each major seawater ions, at pH 7.50 (black symbol) and 8.01 (grey symbol). Experimental conditions: 1000 nM GA, 100 nM Fe(III), 10^{-4} M FZ, T = 25 °C, darkness conditions.

3.2. Fe(III)-GA complex in seawater via the kinetic approach

Once the Fe(III) reduction by GA was demonstrated, the formation of the Fe(III)-GA complex was studied through a kinetic experiments in order to determine the equilibrium constant for the complex Fe(III)-GA in seawater at the nanomolar Fe(III) concentration used in this work. The initial dFe concentration of the UV-treated seawater was 0.49 ± 0.10 nM ($n = 10$). The ligand concentration was determined in natural seawater and it was 1.20 ± 0.2 nM. This iron is recoverable by the added TAC though so no apparent loss was observed because the height peak (the dFe concentrations signal) after several additions to reach the final added concentration of 10 nM of dFe was linear and the value was stable for more than 24 h in the UV-irradiated seawater.

The kinetic of formation and dissociation of Fe with GA, assuming a complex 1:1 formed at the very low GA concentration (5 nM) against Fe (III) (10 nM) (Porwal et al., 2015) is showed in Fig. 8. The computed formation rate constant was $k_f = 1.19(\pm 0.18)\bullet 10^4$ (M^{-1} s^{-1}) and a dissociation rate constant of $k_d = 1.86$ (± 0.53)$\bullet 10^{-4}$ (s^{-1}) with the conditional stability constant of Fe(III)-GA complex of $\log K'^{Cond}_{Fe'L} = 7.81$

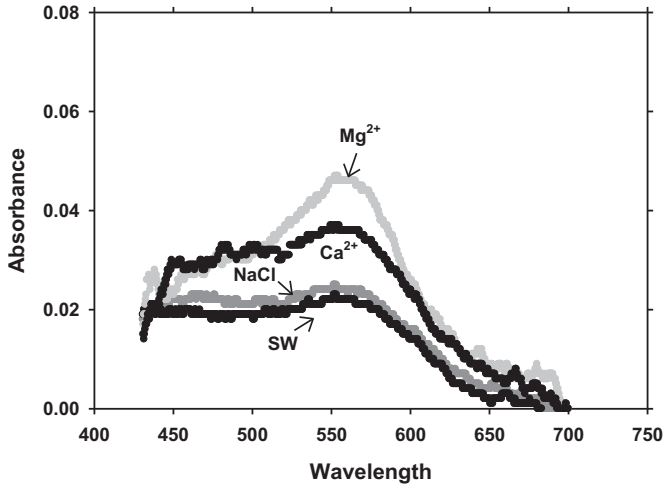

Fig. 6. Fe(II) formation due to the Fe(III) reduction by gentisic acid in seawater, 0.7 M NaCl (+ 2 mM $NaHCO_3$), and also the same solution with $+MgCl_2$ or $+ CaCl_2$, at pH 7.5. Experimental conditions: 1000 nM GA, 100 nM Fe(III), 10^{-4} M FZ, T = 25 °C, darkness conditions. The absorbance was measured at 562 nm.

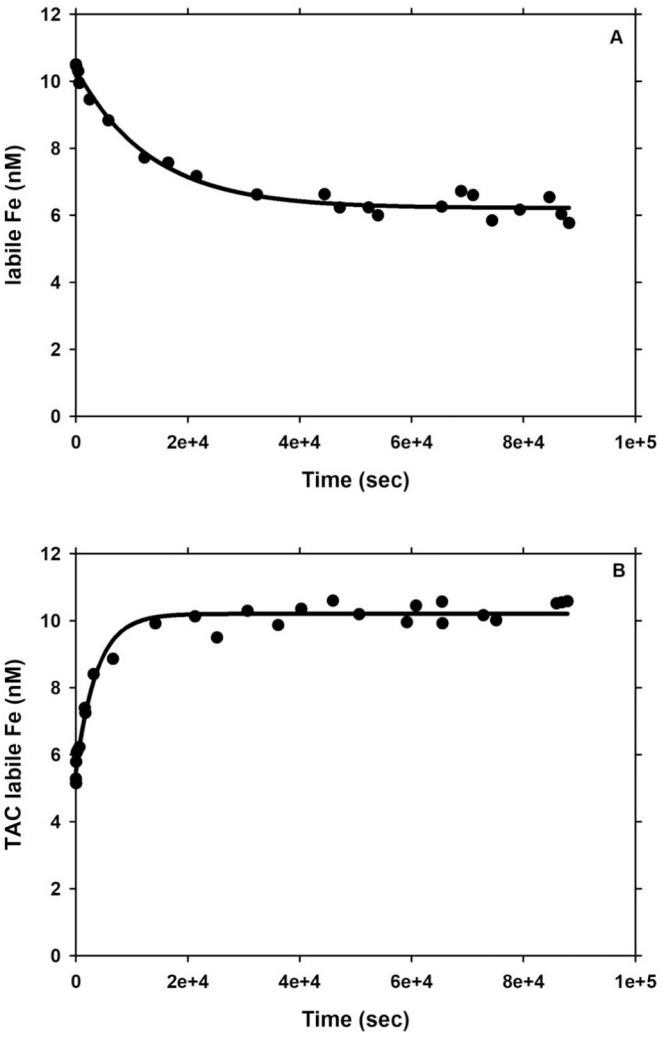

Fig. 8. Kinetic of formation (A) and dissociation (B) of Fe complex with gentisic acid in seawater. The concentration of dFe is expressed as labile Fe due to its complex with TAC. Here is plotted the data of three experiments. Line represents the fitting of the experimental data.

± 0.05 (Table 3 and Fig. 8). Using a side reaction coefficient for the Fe^{3+} specie of $\alpha_{Fe'} = 10^{10}$ (Hudson et al., 1992; Sunda and Huntsman, 2003) in seawater, the $\log K_{Fe^{3+}L}^{Cond} = 17.8 \pm 0.5$. In a 0.1 M sodium perchlorate solution (Jahagirdar, 1974) a value of 13.65 has been obtained for the 1:1 complex, with lower values determined for the complexation of a second and a third ligand (1:3 Fe(III):GA). Previously reported values for other individual ligands in seawater are listed in Table 3. GA can be considered as L3-type ligand (Gledhill and Buck, 2012). According to the dissociation rate constant, the half-life time of the complex in terms of Fe′ was 62.1 ± 14.0 min. Moreover, the half-life times increased to 18.25 days (0.05 years) in terms of Fe^{3+} speciation. This difference in values is due to the calculation of half-life time for $Fe^{3+}L$ complexes, assuming an upper diffusion control limit to the formation of the complex rather than the activation control for Fe'L (Witter et al., 2000). The presence of organic ligands, such as GA, enhances Fe solubility by favouring the formation of small Fe soluble species (Kuma et al., 1996).

The percentage of Fe complexed by GA was computed from the $K_{Fe^{3+}L}^{Cond}$ measured in this paper using the Eq.

$$\alpha_{FeL} = \frac{[FeL]}{[Fe^{3+}]} = K_{Fe^{3+}L}^{Cond}[L] \quad (13)$$

where $K_{Fe^{3+}L}^{Cond} = 10^{17.81}$ (Table 3). As a first estimation it was assumed that both all ligands were available for complexation and the total ligand was free ([L] is the concentration of GA added; 5 nM). The α_{FeL} results to be $3.23 \cdot 10^9$. Taking into account the relationship between total Fe and FeL (Eq. (14) and (15)):

$$\frac{[FeL] + [Fe']}{[FeL]} = 1 + \frac{[Fe']}{[FeL]} = 1 + \frac{\alpha_{Fe'}}{\alpha_{FeL}} = \frac{\alpha_{Fe'} + \alpha_{FeL}}{\alpha_{FeL}} \quad (14)$$

$$\frac{[FeL]}{[Fe_T]} = \frac{\alpha_{FeL}}{\alpha_{Fe'} + \alpha_{FeL}} \quad (15)$$

The FeL, [Fe(III)-GA], respect to the Fe_T was 0.244 (24.4%). Applying this value to iteratively compute the real free ligand concentration, it was obtained that the maximum iron complexed by the GA is 17.4%. This is a weak complex but not negligible. At the pH of our study, 100% of the GA is in the basic form (pKa of 2.97 at 25 °C). The amount complexed was close to 100% for stronger Fe-binding ligands such as Protoporphyrin IX (Table 3).

4. Discussion

The presence of organic compounds, such as GA, can affect the Fe redox cycle as well as its organic speciation in marine environments (Gerringa et al., 2007; Gledhill and Buck, 2012; González et al., 2019; Rose and Waite, 2003; Rue and Bruland, 1995; Santana-Casiano et al., 2010, 2014; Theis and Singer, 1974). This study revealed the capacity of GA to reduce Fe(III) to Fe(II) in seawater and in NaCl-$NaHCO_3$ solutions in a pH-dependent process. The Fe(III) reduction to Fe(II) increased with the decreasing of pH from 8.01 to 7.00 in both seawater and NaCl-$NaHCO_3$ solutions. The lower pH allowed high levels of H^+ increasing the iron solubility with a higher contribution of $Fe(OH)_2^+$ in respect to $Fe(OH)_3$ (Millero et al., 1995). The addition of 1000 nM GA in the presence of 100 nM Fe(III) (ratio 1:10 Fe(III):GA) can favour the formation of Fe(III):GA complexes, with 1:3 stoichiometry (Porwal et al., 2015) and the amount of Fe(II) regenerated in solution was 4.3 nM of Fe(II) at pH 7.00 and 0.43 nM at pH 8.01. These Fe(II) levels are important within the values measured in oceanic waters (Roy et al., 2008; Sarthou et al., 2011). In fact, by considering that the intracellular pH for marine phytoplanktonic cells is around 7.2 (Taylor et al., 2012), although the vacuolar pH can decrease to 4.9–5.1 (Kurkdjian and Guern, 1989), these lower pH conditions should allow the reduction of Fe(III)-GA complexes to Fe(II) through single electron oxidative transformation within the phytoplankton cells or in surface cell walls.

The differences between the Fe(III) reduction rate in seawater and NaCl-$NaHCO_3$ solutions can be explained by both the solubility of Fe(III) and the role of the major ions in seawater. The presence of Mg^{2+} and Ca^{2+} accelerates this reduction rate while the presence of SO_4^{2-}, K^+, F^- and Sr^{2+} decrease it at both pH 7.50 and 8.01 (in respect to that in NaCl-$NaHCO_3$ solutions). Shielding effects and complexation with major ions in solution affect the iron solubility. Moreover, the presence of major ions may affect the ligand steric distribution and polarization of the ligand with changes in the π bonding donor-acceptor character of the ligand and Fe(III) (Jones et al., 1958) resulting in a higher single electron oxidative processes of the complex and higher Fe(II) formation. This behaviour is different to that observed for catechol, catechin and sinapic acid (Santana-Casiano et al., 2010, 2014), where the presence of Mg^{2+} and Ca^{2+} blocked the Fe(III) reduction at higher pH than 7.5. In these studies (Santana-Casiano et al., 2010), a higher reduction rate was observed in NaCl-$NaHCO_3$ solutions and the presence of major ions all decreased this reduction rate. The molecular structure of GA plays an important role. Capelle et al. (1996) demonstrated that Fe(III) could help the oxidation of GA and could explain the one-electron oxidation of GA to semiquinone free radicals. In addition, Fe can be bound by two oxygen via peroxo-iron-gentisate (one from the carboxylic group and another from the OH group), keeping one more OH group available (De et al., 2018). Organic ligands containing carboxyl groups are also able to complex major seawater ions such as Ca^{2+} (Kalinowska et al., 2016). In that study, they reported a Ca^{2+}-GA complex, where Ca^{2+} was bound by two monodentate ligands both in solid state and in solution. These complexes, together with Na^+ and Cu^{2+} were also studied by Regulska et al. (2014). They reported how the aromaticity and molecular properties of GA were altered by the presence of Na^+, Ca^{2+} and metals such as Cu^{2+} and Zn^{2+}, as well as the impact of the intramolecular hydrogen

Table 3
Formation and dissociation constants, conditional stability constant and half-life time determined using the kinetic method.

Ligand	k_f x 10^5 (M^{-1} s^{-1})	k_d (s^{-1})	$\log K_{FeL}^{Cond}$	$\log K_{Fe^{3+}L}^{Cond}$	Fe′ $t_{1/2}$ (min)	Fe^{3+} $t_{1/2}$ (years)
Gentisic acid	0.12 ± 0.02	$1.86(\pm0.53)\bullet10^{-4}$	7.81 ± 0.05	17.81	62.1	0.05
(±)-Catechin*	4.2 ± 1.8	$2.43(\pm0.03)\bullet10^{-4}$	9.2 ± 0.1	19.20	47.5	1.26
Sinapic acid*	3.2 ± 0.7	$4.4(\pm0.3)\bullet10^{-4}$	8.86 ± 0.04	18.86	26.3	0.53
Gallic acid*	3.1 ± 0.4	$3.2(\pm0.1)\bullet10^{-4}$	9.01 ± 0.02	19.01	36.1	0.71
Protoporphyrin IX**	6.2 ± 0.8	$0.7(\pm0.7)\bullet10^{-6}$	11.9 ± 0.5	21.9	$1.65\bullet10^4$	645
Protoporphyrin IX**	15.3 ± 0.2	$0.2(\pm0.9)\bullet10^{-6}$	13.0 ± 0.2	23.0	$5.78\bullet10^4$	5866
Phaeophytin**	12.2 ± 0.1	$12.3(\pm16.8)\bullet10^{-6}$	11.0 ± 1.2	21.0	$9.39\bullet10^2$	72
Apoferritin**	0.93 ± 0.3	$0.08(\pm0.04)\bullet10^{-6}$	12.1 ± 0.1	22.1	$1.44\bullet10^5$	820
Phytic acid**	12.8 ± 0.1	$0.51(\pm0.28)\bullet10^{-6}$	12.4 ± 0.2	22.4	$2.27\bullet10^4$	1820
Alterobactin A**	3.8 ± 0.8	$0.17(\pm0.04)\bullet10^{-6}$	12.3 ± 0.4	22.3	$6.80\bullet10^4$	1620
Alterobactin B**	8.0 ± 0.6	$0.25(\pm0.02)\bullet10^{-6}$	12.5 ± 0.3	22.5	$4.62\bullet10^4$	2320
Ferrichrome**	4.6 ± 2.9	$0.05(\pm0.04)\bullet10^{-6}$	12.9 ± 0.1	22.9	$2.31\bullet10^5$	6700
Desferrioxamine**	19.6 ± 10.1	$1.5(\pm1.8)\bullet10^{-6}$	12.1 ± 0.6	22.1	$7.70\bullet10^3$	952

* González et al. (2019).
** Witter et al. (2000).

bonds on the oxygen-metal distances. These changes can also explain the differences in the reactivity of GA with Fe in solution compared with other polyphenols.

A study carried out on the complexation and reduction of Fe by phenolic substances in peatlands at pH 8.03 also found that while GA was able to reduce Fe(III), gallic acid and caffeic acids were the most efficient (Wan et al., 2018). This difference was explained considering that polyphenols containing galloyl groups, such as gallic acid, have a superior ability to chelate ions than those with catechol moiety (Andjelković et al., 2006). The chelating capacity of GA may be linked to the high nucleophilic character of its aromatic ring, whereas for catechol it may be attributed to the presence of their pyrogallol-type structure (Alcalde et al., 2019; Moran et al., 1997; Saeki et al., 2000; Spiegel et al., 2020). The presence of organic ligands increases the generation of *OH* and the O_2^- scavenging and have to be considered (Miller et al., 2012). The electron donor properties of the hydroxyl group at position 5 in the GA molecule has been shown to increase the equilibrium constant for Fe(III) complexation (Jones et al., 1958). Moreover, this *para*- hydroxyl arrangement can also undergo an electron transfer oxidation to carboxybenzoquinone (Sawyer et al., 1995) and reduces Fe(III) in solution (Galato et al., 2001). However, the Fe(III)-L complex as one of the steps to reduce Fe(III) to Fe (II) should first be considered and has been studied in this current investigation. The formation rate constant $k_f = 1.19(\pm0.18)\bullet10^4$ (M^{-1} s^{-1}) and the dissociation rate constant $k_d = 1.86(\pm0.53)\bullet10^{-4}$ (s^{-1}), resulted in a $\log K_{Fe^{3+}L}^{Cond} = 17.81$ ($\log K'_{FeL} = 7.81 \pm 0.05$). In this study, a single model ligand was studied and under the range of concentration used in the kinetic study a 1:1 complex was assumed, which reduces the hypothesis of the study (Croot and Heller, 2012). The k_f values determined in natural surface waters with multiple detection windows and simultaneous experiments with radiotracers and CSV (Croot and Heller, 2012) ranked from k_f $3.24\bullet10^5$ (M^{-1} s^{-1}) to $6.92\bullet10^5$ (M^{-1} s^{-1}), similar to other model Fe ligands (Witter et al., 2000), including ligands with different stoichiometry with Fe (not only 1:1). Dissociation rate constants were also similar to other values in open ocean (Croot and Heller, 2012). The k_d values ranked from $6.31\bullet10^{-5}$ (s^{-1}) to $2.75\bullet10^{-4}$ (s^{-1}). Croot et al. (2011) divided the natural ligands into two groups: strong (Ls) and weak (Lw), commonly named L1 and L2 (and L3) type ligands.

GA is able to complex Fe(III) in solution (Table 3) as well as other polyphenols such as (±) catechin acid, sinapic acid and gallic acid (González et al., 2019). In GA, the complex would involve both carboxylate group and phenolic groups as donors. GA is a weak ligand and it can explain why GA is exudated by phytoplankton in natural seawater and seawater enriched with high metal concentration (López et al., 2015; Rico et al., 2013). The formation rate revealed that the Fe (III)-GA complex is relatively rapid ($k_f = 1.19(\pm0.18)\bullet10^4$ (M^{-1} s^{-1})) but slower than other ligands (Table 3) from the same family of polyphenols compounds (k_f in the range of 3.1–$4.2\bullet10^5$ (M^{-1} s^{-1}); González et al., 2019). However, the dissociation rate constant is very low ($k_d = 1.86(\pm0.53)\bullet10^{-4}$ (s^{-1})) compared with other single ligands (Table 3). The log K_{FeL}^{Cond} determined from the kinetic approach resulted in 7.81 ± 0.04 while for other polyphenols like (±) catechin, sinapic acid and gallic acid ranked from 8.86 to 9.01. This can be related to the participation of the carboxylic acid group in the complexation process and the presence of the hydroxyl group in *para* position.

Therefore, Fe(III)-GA can be considered a weak ligand in marine environments. However, the role of GA on the Fe(III) biogeochemistry should be considered because of its ability to keep Fe in solution for longer, where the $t_{1/2}$ in solution for Fe′ was increased.

Table 3 shows other studied ligands in seawater in terms of Fe(III)-binding capacity (Witter et al., 2000). These ligands, protoporphyrin IX, phaeophytin, apoferritin, phytic acid, alterobactin A and B, ferrichrome and desferrioxamine, are strong ligands (L1-type of ligands) with a k_f ranging from $0.93\bullet10^5$ to $19.6\bullet10^5$ M^{-1} s^{-1}, and where the dissociation rate constant ranged from $0.05\bullet10^{-6}$ to $15.8\bullet10^{-6}$ s^{-1}. The data collected from literature for natural waters show a high variability (from $0.31\bullet10^{-6}$ to $39\bullet10^{-6}$; Luther et al. (1997) and Witter and Luther. (1998)), which attests to the huge diversity of ligands in water. In the case of other polyphenol compounds, the stoichiometry can be from 1:1 to 1:3 metal-to-ligand complexes (Andjelković et al., 2006; Fazary et al., 2008; Powell and Taylor, 1982; Strlič et al., 2002) whereas the pH, ionic strenght and concentrations of both chemicals are relevant in the interest of properly identifying the stoichiometry. Recently, the stoichiometry of other polyphenols in seawater such as (±)-catechin, sinapic acid and gallic acid were measured (González et al., 2019) indicating the importance of the experimental conditions and the combination of analytical techniques in order to properly define the Fe-organic ligand complexes.

Polyphenols ability to complex Fe can be attributed to the presence and the amount of *ortho*- and hydroxyl groups (Khokhar and Apenten, 2003; Moran et al., 1997). As has been previously reported (González et al., 2019; Santana-Casiano et al., 2010, 2014), the interaction between Fe(III) and organic moieties cannot be only understood by attending to the role of the hydroxyl groups as other ligands such as carboxylic moieties, can be involved. In fact, an outer sphere.

complex has been proposed between GA and Hematite (α-Fe_2O_3) above pH 5 and increasing with pH, involving both carboxylate groups as acceptors from waters of hydration or protonated surface sites and phenolic groups as donors to surface (hydr)oxo groups (Hanna and Quilès, 2011). Moreover, the presence of major ions in seawater, such as Mg^{2+} and Ca^{2+} can play an important role on the interactions between Fe(III) and organic ligands.

Ultimately, marine cells such as *P. tricornutum* or *D. tertiolecta* exudate GA in order to respond to different physico-chemical conditions (López et al., 2015; Rico et al., 2013). These cells are able to produce GA to complex Fe(III) in solution and, also reduce Fe(III) to Fe(II), making possible its assimilation for longer periods. The terrestrial supply of polyphenols have also been studied (Blazevic et al., 2016; Krachler et al., 2005, 2010, 2012, 2015, 2016, 2019; Orlowska et al., 2016, 2017a, 2017b, 2016; Rathgeb et al., 2017). Therefore, GA has to be considered as an organic functional group in the pool of marine organic ligands (López et al., 2015; Rico et al., 2013; Santana-Casiano et al., 2014), and also within the humic pool formed by phenolic, benzoate, phthalate and salicylate groups (Capelle et al., 1996).

5. Conclusions

The present manuscript studied the role of gentisic acid (GA) on the Fe(III) reduction in seawater and NaCl-$NaHCO_3$ solutions as a function of different physico-chemical conditions. GA is a phenolic compound previously reported as a part of the organic exudates produced by marine microalgae and, then in the pool of organic ligands in natural waters. In this current investigation, it has been demonstrated that GA in a ratio of 1:10 Fe(III):GA produced an important Fe(III) reduction to Fe(II) in seawater achieving 4.3 nM of Fe(II) at pH 7.00 and 0.42 nM at pH 8.01, for 60 min of reaction. In addition, the reduction rate constant was always higher in seawater than in NaCl-$NaHCO_3$ medium for the whole pH range (7.00–8.01). The dependence of the log *K* with pH, both in seawater and NaCl-$NaHCO_3$ solutions, reflected the higher control of major ions in seawater on the Fe(III) reduction process affecting the availability of hydroxyl and carboxylic ligands for Fe(III). Accordingly, at pH lower than 7.5, Mg^{2+} and Ca^{2+} accelerate the formation of Fe(II), while the presence of SO_4^{2-}, K^+, F^- and Sr^{2+} decreased the reduction rate constant in respect to NaCl-$NaHCO_3$ solutions.

The reduction of Fe(III) to Fe(II) by GA was characterized by the formation of Fe(III)-GA complexes with a formation rate constant of $k_f = 1.19(\pm0.18)\bullet10^4$ (M^{-1} s^{-1}), a dissociation rate constant of $k_d = 1.86(\pm0.53)\bullet10^{-4}$ (s^{-1}), and conditional complexation constant of $\log K_{Fe^{3+}L}^{Cond} = 17.81 \pm 0.05$, that can be associated to a complex involving the ortho-carboxylic and hydroxy ligands in the GA.

The presence of GA in seawater allows that Fe will have higher permanence times because GA is both able to reduce Fe(III) to Fe(II) and form a weak complex (L3-type) with Fe(III), making Fe more bioavailable for phytoplankton.

Declaration of Competing Interest

None.

Acknowledgments

This work has been funded by ATOPFe (CTM2017-83476-P) project given by the Ministerio de Economía y Competitividad from Spain. Veronica Arnone's participation was funded by the PhD grant (PRE 2018-084476).

References

Alcalde, B., Granados, M., Saurina, J., 2019. Exploring the antioxidant features of polyphenols by spectroscopic and electrochemical methods. Antioxidants 8, 523.

Andjelković, M., Van Camp, J., De Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., Verhe, R., 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chem. 98, 23–31. https://doi.org/10.1016/j.foodchem.2005.05.044.

Blazevic, A., Orlowska, E., Kandioller, W., Jirsa, F., Keppler, B.K., Tafili-Kryeziu, M., Linert, W., Krachler, R.F., Krachler, R., Rompel, A., 2016. Photoreduction of terrigenous Fe-humic substances leads to bioavailable iron in oceans. Angew. Chem. 128, 6527–6532.

Brown, J.E., Khodr, H., Hider, R.C., Rice-Evans, C.A., 1998. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. Biochem. J. https://doi.org/10.1042/bj3301173.

Bruland, K.W., Lohan, M.C., 2003. Controls of trace metals in seawater, Treatise on Geochemistry, ed. Elderfield, H.

Capelle, S., Pellegrini, S., Cotelle, P., Imbenotte, M., Pommery, J., Catteau, J.P., 1996. Autoxidation of gentisic acid in aqueous media. Water Res. 30, 1299–1303. https://doi.org/10.1016/0043-1354(95)00287-1.

Croot, P.L., Heller, M.I., 2012. The importance of kinetics and redox in the biogeochemical cycling of iron in the surface ocean. Front. Microbiol. 3, 1–15. https://doi.org/10.3389/fmicb.2012.00219.

Croot, P.L., Johansson, M., 2000. Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2- thiazolylazo)-p-cresol (TAC). Electroanalysis 12, 565–576. https://doi.org/10.1002/(Sici)1521-4109(200005)12:8<565::Aid-Elan565>3.0.Co;2-L.

Croot, P.L., Heller, M., Schlosser, C., Wuttig, K., 2011. Utilizing radioisotopes for trace metal speciation measurements in weawater. Radioisot. - Appl. Phys. Sci. 247–278. https://doi.org/10.5772/23182.

De, A., Dey, D., Das, A., Kole, N., Biswas, B., 2018. Gentisate-1, 2-dioxygenase activity by an iron (II)-phenanthroline complex. J. Chem. Sci. 130, 26.

Devireddy, L.R., Gazin, C., Zhu, X., Green, M.R., 2005. A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell 123, 1293–1305.

Devireddy, L.R., Hart, D.O., Goetz, D.H., Green, M.R., 2010. A mammalian siderophore synthesized by an enzyme with a bacterial homolog involved in enterobactin production. Cell 141, 1006–1017.

Fazary, A.E., Taha, M., Ju, Y.-H., 2008. Iron complexation studies of gallic acid. J. Chem. Eng. Data 54, 35–42.

Feng, T.C., Cui, C.Z., Dong, F., Feng, Y.Y., Liu, Y.D., Yang, X.M., 2012. Phenanthrene biodegradation by halophilic Martelella sp. AD-3. J. Appl. Microbiol. 113, 779–789. https://doi.org/10.1111/j.1365-2672.2012.05386.x.

Galato, D., Ckless, K., Susin, M.F., Giacomelli, C., Ribeiro-do-Valle, R.M., Spinelli, A., 2001. Antioxidant capacity of phenolic and related compounds: correlation among electrochemical, visible spectroscopy methods and structure–antioxidant activity. Redox Rep. 6, 243–250. https://doi.org/10.1179/135100001101536391.

Gerringa, L.J.A., Rijkenberg, M.J.A., Wolterbeek, H.T., Verburg, T.G., Boye, M., de Baar, H.J.W., 2007. Kinetic study reveals weak Fe-binding ligand, which affects the solubility of Fe in the Scheldt estuary. Mar. Chem. 103, 30–45. https://doi.org/10.1016/j.marchem.2006.06.002.

Gledhill, M., Buck, K.N., 2012. The organic complexation of iron in the marine environment: a review. Front. Microbiol. 3, 1–17. https://doi.org/10.3389/fmicb.2012.00069.

González, A.G., Cadena-Aizaga, M.I., Sarthou, G., González-Dávila, M., Santana-Casiano, J.M., 2019. Iron complexation by phenolic ligands in seawater. Chem. Geol. 511, 380–388.

González-Dávila, M., Santana-Casiano, J.M., Millero, F.J., 2005. Oxidation of iron (II) nanomolar with H_2O_2 in seawater. Geochim. Cosmochim. Acta 69, 83–93. https://doi.org/10.1016/j.gca.2004.05.043.

Granger, J., Price, N.M., 1999. The importance of siderophores in iron nutrition of heterotrophic marine bacteria. Limnol. Oceanogr. 44, 541–555.

Hanna, K., Quilès, F., 2011. Surface complexation of 2,5-dihydroxybenzoic acid (gentisic acid) at the nanosized hematite-water interface: an ATR-FTIR study and modeling approach. Langmuir 27, 2492–2500. https://doi.org/10.1021/la104239x.

Harpel, M.R., Lipscomb, J.D., 1990. Gentisate 1,2-dioxygenase from Pseudomonas. Purification, characterization, and comparison of the enzymes from Pseudomonas testosteroni and Pseudomonas acidovorans. J. Biol. Chem. 265, 6301–6311.

Hudson, R.J.M., 1989. The Chemical Kinetics of Iron Uptake by Marine Phytoplankton.

Hudson, R.J.M., Morel, F.M.M., 1989. Distinguishing between extra-and intracellular iron in marine phytoplankton. Limnol. Oceanogr. 34, 1113–1120.

Hudson, R.J.M., Morel, F.M.M., 1990. Lron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions. Limnol. Oceanogr. 35, 1002–1020.

Hudson, R.J.M., Morel, F.M.M., 1993. Trace metal transport by marine microorganisms: implications of metal coordination kinetics. Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 129–150.

Hudson, R.J.M., Covault, D.T., Morel, F.M.M., 1992. Investigations of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes. Mar. Chem. 38, 209–235. https://doi.org/10.1016/0304-4203(92)90035-9.

Hutchins, D.A., Witter, A.E., Butler, A., Luther, G.W., 1999. Competition among marine phytoplankton for different chelated iron species. Nature 400, 858–861. https://doi.org/10.1038/23680.

Hynes, M.J., O'Coinceanainn, M., 2004. The kinetics and mechanisms of reactions of iron (III) with caffeic acid, chlorogenic acid, sinapic acid, ferulic acid and naringin. J. Inorg. Biochem. 98, 1457–1464. https://doi.org/10.1016/j.jinorgbio.2004.05.009.

Jahagirdar, D.V., 1974. Potentiometric studies of Fe(III) complexes of some substituted salicylic acids. J. Inorg. Nucl. Chem. 36, 2388–2390.

Jones, J.G., Poole, J.B., Tomkinson, J.C., Williams, R.J.P., 1958. The relationship between proton dissociation constants and the stability constants of complex ions. J. Chem. Soc. 2001–2009.

Joshi, R., Gangabhagirathi, R., Venu, S., Adhikari, S., Mukherjee, T., 2012. Antioxidant activity and free radical scavenging reactions of gentisic acid: in-vitro and pulse radiolysis studies. Free Radic. Res. 46, 11–20.

Kalinowska, M., Mazur, L., Regulska, E., Korczak, A., Świderski, G., Lewandowski, W., 2016. Calcium complex of 2, 5-dihydroxybenzoic acid (gentisic acid): synthesis, crystal structure, and spectroscopic properties. J. Coord. Chem. 69, 2415–2421.

Kalyanaraman, B., Ramanujam, S., Singh, R.J., Joseph, J., Feix, J.B., 1993. Formation of 2, 5-dihydroxybenzoic acid during the reaction between singlet oxygen ($1O_2$) and salicylic acid: analysis by ESR oximetry and HPLC with electrochemical detection. J. Am. Chem. Soc. 115, 4007–4012.

Khokhar, S., Apenten, R.K., 2003. Iron binding characteristics of phenolic compounds: some tentative structure-activity relations. Food Chem. 81, 133–140. https://doi.org/10.1016/S0308-8146(02)00394-1.

Krachler, R., Jirsa, F., Ayromlou, S., 2005. Factors Influencing the Dissolved Iron Input by River Water to the Open Ocean.

Krachler, R., Krachler, R.F., von der Kammer, F., Süphandag, A., Jirsa, F., Ayromlou, S., Hofmann, T., Keppler, B.K., 2010. Relevance of peat-draining rivers for the riverine input of dissolved iron into the ocean. Sci. Total Environ. 408, 2402–2408.

Krachler, R., von der Kammer, F., Jirsa, F., Süphandag, A., Krachler, R.F., Plessl, C., Vogt, M., Keppler, B.K., Hofmann, T., 2012. Nanoscale lignin particles as sources of dissolved iron to the ocean. Glob. Biogeochem. Cycles 26.

Krachler, R., Krachler, R.F., Wallner, G., Hann, S., Laux, M., Recalde, M.F.C., Jirsa, F., Neubauer, E., von der Kammer, F., Hofmann, T., 2015. River-derived humic substances as iron chelators in seawater. Mar. Chem. 174, 85–93.

Krachler, R., Krachler, R.F., Wallner, G., Steier, P., El Abiead, Y., Wiesinger, H., Jirsa, F., Keppler, B.K., 2016. Sphagnum-dominated bog systems are highly effective yet variable sources of bio-available iron to marine waters. Sci. Total Environ. 556, 53–62.

Krachler, Regina, Krachler, Rudolf, Valda, A., Keppler, B.K., 2019. Natural iron fertilization of the coastal ocean by "blackwater rivers.". Sci. Total Environ. 656, 952–958.

Kuma, K., Nishioka, J., Matsunaga, K., 1996. Controls on iron(III) hydroxide solubility in seawater: the influence of pH and natural organic chelators. Limnol. Oceanogr. 41, 396–407. https://doi.org/10.4319/lo.1996.41.3.0396.

Kurkdjian, A., Guern, J., 1989. Intracellular pH: measurement and importance in cell activity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 271–303. https://doi.org/10.1146/annurev.pp.40.060189.001415.

Kuzma, M., Kovács, N., Sziva, L., Maász, G., Avar, P., Perjési, P., 2018. Oxidation of hydroxy-and dihydroxybenzoic acids under the udenfriend's conditions. An HPLC study. Open Med. Chem. J. 12, 13.

Lack, L., 1959. The enzymic oxidation of gentisic acid. Biochim. Biophys. Acta 34, 117–123.

Liu, X., Millero, F.J., 2002. The solubility of iron in seawater. Mar. Chem. 77, 43–54. https://doi.org/10.1016/S0304-4203(01)00074-3.

Liu, Z., Lanford, R., Mueller, S., Gerhard, G.S., Luscieti, S., Sanchez, M., Devireddy, L., 2012. Siderophore-mediated iron trafficking in humans is regulated by iron. J. Mol. Med. 90, 1209–1221.

Lodovici, M., Guglielmi, F., Casalini, C., Meoni, M., Cheynier, V., Dolara, P., 2001. Antioxidant and radical scavenging properties in vitro of polyphenolic extracts from red wine. Eur. J. Nutr. 40, 74–77. https://doi.org/10.1007/PL00007386.

López, A., Rico, M., Santana-Casiano, J.M., González, A.G., González-Dávila, M., 2015. Phenolic profile of *Dunaliella tertiolecta* growing under high levels of copper and iron. Environ. Sci. Pollut. Res. 22, 14820–14828. https://doi.org/10.1007/s11356-015-4717-y.

Luther, G.W., Wu, J., Johnson, K.S., Gordon, R.M., Coale, K.H., Luther, G.W., Wu, J., Johnson, K.S., Gordon, R.M., Coale, K.H., 1997. What controls dissolved iron concentrations in the world ocean? — a comment. Mar. Chem. 57, 173–179. https://doi.org/10.1016/S0304-4203(97)00046-7.

Maldonado, M.T., Price, N.M., 2001. Reduction and transport of organically bound iron by *Thalassiosira oceanica* (*Bacillariophyceae*). J. Phycol. 37, 298–309.

Miller, C.J., Rose, A.L., Waite, T.D., 2012. Hydroxyl radical production by H_2O_2-mediated oxidation of Fe(II) complexed by Suwannee River fulvic acid under circumneutral freshwater conditions. Environ. Sci. Technol. 47, 829–835.

Millero, F.J., 1986. The pH of estuarine waters. Limnol. Oceanogr. 31, 839–847. https://doi.org/10.4319/lo.1986.31.4.0839.

Millero, F.J., 1996. In: Raton, Boca (Ed.), Chemical Oceanography. CRC Press.
Millero, F.J., Sotolongo, S., Izaguirre, M., 1987. The oxidation kinetics of Fe(II) in seawater. Geochim. Cosmochim. Acta 51, 793–801.
Millero, F.J., Yao, W., Aicher, J., 1995. The speciation of Fe(II) and Fe(III) in natural waters. Mar. Chem. 50, 21–39. https://doi.org/10.1016/0304-4203(95)00024-L.
Mira, L., Fernandez, M.T., Santos, M., Rocha, R., Florêncio, M.H., Jennings, K.R., 2002. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic. Res. 36, 1199–1208. https://doi.org/10.1080/1071576021000016463.
Moran, J.F., Klucas, R.V., Grayer, R.J., Abian, J., Becana, M., 1997. Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: Prooxidant and antioxidant properties. Free Radic. Biol. Med. 22, 861–870. https://doi.org/10.1016/S0891-5849(96)00426-1.
Morel, F.M.M., Price, N.M., 2003. The biogeochemical cycles of trace metals in the oceans. Science (80-.). https://doi.org/10.1126/science.1083545.
Orlowska, E., Roller, A., Wiesinger, H., Pignitter, M., Jirsa, F., Krachler, R., Kandioller, W., Keppler, B.K., 2016. Benzoic hydroxamate-based iron complexes as model compounds for humic substances: synthesis, characterization and algal growth experiments. RSC Adv. 6, 40238–40249.
Orlowska, E., Enyedy, É.A., Pignitter, M., Jirsa, F., Krachler, R., Kandioller, W., Keppler, B.K., 2017a. β-O-4 type dilignol compounds and their iron complexes for modeling of iron binding to humic acids: synthesis, characterization, electrochemical studies and algal growth experiments. New J. Chem. 41, 11546–11555.
Orlowska, E., Roller, A., Pignitter, M., Jirsa, F., Krachler, R., Kandioller, W., Keppler, B. K., 2017b. Synthetic iron complexes as models for natural iron-humic compounds: synthesis, characterization and algal growth experiments. Sci. Total Environ. 577, 94–104.
Porwal, S.K., Furia, E., Harris, M.E., Viswanathan, R., Devireddy, L., 2015. Synthetic, potentiometric and spectroscopic studies of chelation between Fe(III) and 2,5-DHBA supports salicylate-mode of siderophore binding interactions. J. Inorg. Biochem. 145, 1–10. https://doi.org/10.1016/j.jinorgbio.2014.12.010.
Powell, H.K.J., Taylor, M.C., 1982. Interactions of iron (II) and iron (III) with gallic acid and its homologues: a potentiometric and spectrophotometric study. Aust. J. Chem. 35, 739–756.
Rahaman, R., Munshi, S., Banerjee, S., Chakraborty, B., Bhunia, S., Paine, T.K., 2019. Dioxygen reactivity of iron (II)–gentisate/1, 4-dihydroxy-2-naphthoate complexes of N4 ligands: oxidative coupling of 1, 4-dihydroxy-2-naphthoate. Dalton Trans. 48, 16993–17004.
Rathgeb, A., Causon, T., Krachler, R., Hann, S., 2017. From the peat bog to the estuarine mixing zone: common features and variances in riverine dissolved organic matter determined by non-targeted analysis. Mar. Chem. 194, 158–167.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
Regulska, E., Kalinowska, M., Wojtulewski, S., Korczak, A., Sienkiewicz-Gromiuk, J., Rzączyńska, Z., Świsłocka, R., Lewandowski, W., 2014. Theoretical (in B3LYP/6-3111++ G** level), spectroscopic (FT-IR, FT-Raman) and thermogravimetric studies of gentisic acid and sodium, copper (II) and cadmium (II) gentisates. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 132, 713–725.
Reid, R.T., Live, D.H., Faulkner, D.J., Butler, A., 1993. A siderophore from a marine bacterium with an exceptional ferric ion affinity constant. Nature 366, 455–458.
Richardson, D.R., Lane, D.J.R., Becker, E.M., Huang, M.L.-H., Whitnall, M., Rahmanto, Y. S., Sheftel, A.D., Ponka, P., 2010. Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. Proc. Natl. Acad. Sci. 107, 10775–10782.
Rico, M., López, A., Santana-Casiano, J.M., González, A.G., González-Dávila, M., 2013. Variability of the phenolic profile in the diatom *Phaeodactylum tricornutum* growing under copper and iron stress. Limnol. Oceanogr. 51, 144–152. https://doi.org/10.4319/lo.2013.58.1.0144.
Rose, A.L., Waite, T.D., 2003. Effect of dissolved natural organic matter on the kinetics of ferrous iron oxygenation in seawater. Environ. Sci. Technol. 37, 4877–4886. https://doi.org/10.1021/es034152g.
Roy, E.G., Wells, M.L., King, W.D., 2008. Persistence of iron(II) in surface waters of the western subarctic Pacific. Limnol. Oceanogr. 53, 89–98.
Rue, E.L., Bruland, K.W., 1995. Complexation of iron (III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping. Mar. Chem. 50, 117–138. https://doi.org/10.1016/0304-4203(95)00031-L.
Saeki, K., Hayakawa, S., Isemura, M., Miyase, T., 2000. Importance of a pyrogallol-type structure in catechin compounds for apoptosis-inducing activity. Phytochemistry 53, 391–394. https://doi.org/10.1016/S0031-9422(99)00513-0.
Santana-Casiano, J.M., González-Dávila, M., Millero, F.J., 2005. Oxidation of nanomolar levels of Fe (II) with oxygen in natural waters. Environ. Sci. Technol. 39, 2073–2079. https://doi.org/10.1021/es049748y.
Santana-Casiano, J., González-Dávila, M., González, A., Millero, F., 2010. Fe (III) Reduction in the presence of catechol in seawater. Aquat. Geochem. 16, 467–482. https://doi.org/10.1007/s10498-009-9088-x.
Santana-Casiano, J.M., González-Dávila, M., González, A.G., Rico, M., López, A., Martel, A., 2014. Characterization of phenolic exudates from *Phaeodactylum tricornutum* and their effects on the chemistry of Fe(II)-Fe(III). Mar. Chem. 158, 10–16. https://doi.org/10.1016/j.marchem.2013.11.001.
Sarthou, G., Bucciarelli, E., Chever, F., Hansard, S.P., González-Dávila, M., Santana-Casiano, J.M., Planchon, F., Speich, S., 2011. Labile Fe(II) concentrations in the Atlantic sector of the Southern Ocean along a transect from the subtropical domain to the Weddell Sea gyre. Biogeosciences 8, 2461–2479. https://doi.org/10.5194/bg-8-2461-2011.
Sawyer, D.T., Sobkowiak, A., Roberts, J.L., 1995. Electrochemistry for Chemists. Wiley.
Shaked, Y., Lis, H., 2012. Disassembling iron availability to phytoplankton. Front. Microbiol. 3, 1–26. https://doi.org/10.3389/fmicb.2012.00123.
Shaked, Y., Kustka, A.B., Morel, F.M.M., Erel, Y., 2004. Simultaneous determination of iron reduction and uptake by phytoplankton. Limnol. Oceanogr. Methods 2, 137–145.
Shaked, Y., Kustka, A.B., Morel, F.M.M., 2005. A general kinetic model for iron acquisition by eukaryotic phytoplankton. Limnol.Oceanogr. 50, 872–882. https://doi.org/10.4319/lo.2005.50.3.0872.
Spiegel, M., Kapusta, K., Kołodziejczyk, W., Saloni, J., Żbikowska, B., Hill, G.A., Sroka, Z., 2020. Antioxidant activity of selected phenolic acids–ferric reducing antioxidant power assay and QSAR analysis of the structural features. Molecules 25, 3088.
Sroka, Z., Cisowski, W., 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. 41, 753–758. https://doi.org/10.1016/S0278-6915(02)00329-0.
Strlič, M., Radovič, T., Kolar, J., Pihlar, B., 2002. Anti- and prooxidative properties of gallic acid in Fenton-type systems. J. Agric. Food Chem. 50, 6313–6317. https://doi.org/10.1021/jf025636j.
Sunda, W., Huntsman, S., 2003. Effect of pH, light, and temperature on Fe–EDTA chelation and Fe hydrolysis in seawater. Mar. Chem. 84, 35–47.
Taylor, A.R., Brownlee, C., Wheeler, G.L., 2012. Proton channels in algae: reasons to be excited. Trends Plant Sci. 17, 675–684. https://doi.org/10.1016/j.tplants.2012.06.009.
Theis, T.L., Singer, P.C., 1974. Complexation of iron(II) by organic matter and its effect on iron(II) oxygenation. Environ. Sci. Technol. 8, 569–573.
Town, R.M., Filella, M., 2000. Dispelling the myths: is the existence of L1 and L2 ligands necessary to explain metal ion speciation in natural waters? Limnol. Oceanogr. 45, 1341–1357. https://doi.org/10.4319/lo.2000.45.6.1341.
Udenfriend, S., Clark, C.T., Axelrod, J., Brodie, B.B., 1954. Ascorbic acid in aromatic hydroxylation. J. Biol. Chem. 208, 731–738.
van den Berg, C.M.G., 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2 II. Experimental procedures and application to surface seawater. Mar. Chem. 11, 323.
van den Berg, C.M.G., 1995. Evidence for organic complexation of iron in seawater. Mar. Chem. 50, 139–157.
Velika, B., Kron, I., 2012. Antioxidant properties of benzoic acid derivatives against superoxide radical. Free Rad. Antioxidants 2, 62–67.
Wan, X., Xiang, W., Wan, N., Yan, S., Bao, Z., Wang, Y., 2018. Complexation and reduction of iron by phenolic substances: implications for transport of dissolved Fe from peatlands to aquatic ecosystems and global iron cycling. Chem. Geol. 498, 128–138. https://doi.org/10.1016/j.chemgeo.2018.09.019.
Witter, A.E., Luther, G.W., 1998. Variation in Fe-organic complexation with depth in the northwestern Atlantic Ocean as determined using a kinetic approach. Mar.Chem. 62, 241–258.
Witter, A.E., Hutchins, D.A., Butler, A., Luther, G.W., 2000. Determination of conditional stability constants and kinetic constants for strong model Fe-binding ligands in seawater. Mar. Chem. 69, 1–17. https://doi.org/10.1016/S0304-4203(99)00087-0.
Wu, J.F., Luther, G.W., 1995. Complexation of Fe(III) by natural organic-ligands in the Northwest Atlantic-Ocean by a competitive ligand equilibration method and kinetic approach. Mar. Chem. 50, 159–177.
Wu, Y., Xiang, W., Fu, X., Yan, S., Su, J., Liu, J., Bao, Z., 2016. Geochemical interactions between iron and phenolics originated from peatland in Hani, China: implications for effective transport of iron from terrestrial systems to marine. Environ. Earth Sci. 75, 336. https://doi.org/10.1007/s12665-015-5189-6.
Yano, K., Arima, K.E.I., 1958. Metabolism of aromatic compounds by bacteria. J. Gen. Appl. Microbiol. 4, 241–258.